Foreword

Dear Colleagues

It was with the great pleasure we welcomed you to the 7th IOBC conference on Integrated Fruit Production at Avignon in 2008.

The programme for this meeting was exceptionally rich and diverse and contained many interesting and exciting contributions. The numbers of offered oral and poster presentations (>200) was quite exceptional and well beyond our expectations. It demonstrated that Integrated Plant Protection in Fruit Crops remains an area of strong interest in the WPRS and beyond. We greatly appreciated the strong representation at the conference by delegates from the USA and Canada. We are sorry that we were not been able to accommodate all the offered oral papers in the programme. This has meant that we requested >30 offered oral papers to be presented as posters. Furthermore, the number of posters was so great that 2 separate poster sessions were necessary.

This was the 4 yearly meeting of our whole IOBC Working Group which comprises 5 sub-groups (pome fruit arthropods, pome fruit diseases, soft fruits, stone fruits and IFP guidelines). The decision to have parallel sessions for pests and diseases was taken to ensure that both entomologists and pathologists attended this conference.

Our working group, formerly named the ‘Orchards Working Group’ is near to celebrating its 50th anniversary. Our group was a pioneer in Integrated Pest and Disease Management and has come a long way from its historical roots. We welcomed Ernst Boller, Honorary member of the IOBC, and a person with a strong interest in and with good links with our past, who gave a historical review of our Working Group’s origins, founder members and activities.

The IOBC council provided substantive funds to support this conference and these funds mainly to support the attendance of 8 students and young persons starting their career at this conference who otherwise would have been unlikely to attend. We hope that their attendance at this conference will cement their interest in our subject.

Finally, we are especially indebted to our colleagues from INRA Avignon who hosted this conference.

Jerry Cross (Convenor), Claudio Ioriatti, Christian Linder, Fabio Molinari, Carlo Malavolta, Jesus Avilla, Arne Stensvand, Benoît Sauphanor.

Conference Scientific Committee
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Integrated Production: where is it and where is it going?

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IOBCwprs Commission “Integrated Production: Guidelines and Endorsements”

Abstract: The objective of this presentation is to give an overview of the present status of Integrated Production worldwide, from a conceptual rather than from a quantitative approach, and to present some ideas for discussion on the future of IP. There is no doubt that Integrated Production systems are now well established worldwide, and implemented and applied by growers, from the “old Europe”, where the first attempts began in the 1970s, to the “new world”, with Brazil probably being the best example of a very dynamic organization. Brazilian IP guidelines and projects, based on IOBC guidelines, do not only cover most of the crops grown, but also animal production. In many European countries, IP has become the minimal standard for an agriculture that is considered sustainable enough to be eligible for state subsidies. However, are IP-based commodities certified and sold as such and known by the consumer? The answer to this question might be “not enough”. IP has always had a problem of communication. The implementation of IP has not only enhanced the sustainability of agricultural cropping systems (but, as yet, not of animal husbandry), but has also facilitated the acceptance of techniques and processes that increase human health, food safety, and respect for environmental and ethical issues. These achievements, however, are not perceived by the public. Recently, the impact on production of controlling systems developed by private stakeholders, such as GlobalGAP, has been very strong. Is there a need and a future for IP under these circumstances? From the scientific point of view, it is clear that the answer is positive, as IP will continue to be a driving force to improve cropping systems. From the commercial point of view, the answer is not so clear, unless it becomes clear to the consumer in the future that IP is a label of total quality, not only taking into account extrinsic or intrinsic quality, but also environmental, social and ethical quality.

Integrated Production, Present status, Perspectives
Disease management in organic apple orchards is more than applying the right product at the correct time.

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Abstract: The relative importance of diseases of apple varies with cultivar, management, time, and climate. Many aspects of the cropping system influence the development of diseases and offer possibilities for management. The choice of the variety determines the disease management for the lifetime of the orchard. As apple scab is the dominant disease, the choice to plant commercially attractive Vf resistant and low susceptible varieties is a logical step in more arid production regions. In 2008, Vf resistant varieties made up 30.8 % of the Dutch organic apple production, and 10.7 % of the European organic apple production. Cultural practices affect the growth and nutrient status of the tree, and therewith directly and indirectly influence the susceptibility to diseases. Sanitation measures are common practise for most organic fruit growers and help to make other measures more effective by reducing infection inoculums. Hot water treatment is embraced as an effective technique to reduce losses by storage diseases. Despite all preventive measures, disease control in organic orchards at an economically feasible level still largely depends on the application of fungicides. Decision support systems like RIMpro are an important tool for growers to optimize the application of fungicides. Measures that allow reduction of fungicidal applications on key diseases can lead to the development of a secondary disease complex that can cause severe losses when not managed effectively. In research, advisory and practical decision making and disease management in organic orchards should always be seen in the perspective of the management of the total growing system. With all factors that contribute to disease management optimized, we are able to successfully implement new materials and methods that may not be as effective as common fungicides in themselves, but add to the effectiveness of the disease management system as a whole. This total system approach makes organic fruit growing what it is.

Apple, Organic production, Disease management, Vf resistance
Designing cropping systems to achieve Integrated Fruit Production goals

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Abstract: Orchards encounter strong protection problems, because of both the demand of high standards of visual quality that requires an intensive use of pesticides, and the current adaptation of pests and diseases to those pesticides. Facing these problems while preserving production and quality and being attentive to the preservation of the environment, supposes designing cropping systems for integrated fruit production. In this contribution, we expose the two ways of this design: expert-based and model-based. Then, we point out the areas of research that should be strengthened to design IFP cropping systems on sound bases. The first is the analysis of current protection practices. The second is the study of crop-pest-enemies interactions under the influence of crop and pest management. The third is landscape studies. We conclude on the multidisciplinary nature of research for IFP purposes.

Key words: IFP, cropping system, cultural practice, expert-based design, model-based design

Introduction

To face the environmental problems caused by the intensive use of chemical pesticides in orchards (Aubertot et al, 2005), the reasoning of chemical interventions has been the subject of thinking and research for many years. However, the corresponding progress in the eighties were rapidly countered by the adaptation of pests and diseases (resistance to pesticides, to biological control agents, to resisting cultivars), which is favoured by the perenniality of orchard systems and the concentration of fruit crops in production basins. Moreover, fruit crops are subjected to high quality standards, and the pest/disease pressure is likely to influence many quality traits. The definition of IFP by IOBC in 2002 considers that the “priority to ecologically safer methods” should not impair the quality of the production on which its economical value relies. Reaching such objectives requires thinking together production and protection, considering the crop as a whole, i.e. a set of plants, soil, pests/diseases and their enemies and a network of interactions between them, how this ‘crop as a whole’ changes under the influence of various management choices, and finally designing cropping systems beyond protection systems.

In the following lines, we summarize the two ways of cropping system design as they emerge from the works of agronomists (Doré et al, 2006), and point out the areas of research that should be strengthened to design IFP cropping systems on sound bases.

Two ways of designing cropping systems

The first way is what can be called “expert-based design” because the core of the design is expert knowledge on the ‘crop as a whole’ (see above). Amongst possible experts, research workers, advisers, and stakeholders are able to provide complementary viewpoints and information. The central idea is to formalise a cropping system prototype to be tested in the field. Basically, this is the prototyping methodology (Vereijken, 1997) adapted by Lançon et al (2007; but see also Debaeke et al, 2009). After Lançon et al (2007), four main steps can be
defined for expert-based design. First, the main constraints, i.e. limiting factors such as a high pressure of a given pest or manpower, and the broad classes of criteria to be examined to assess the performance and costs of the prototype, have to be defined. Second, a theoretical prototype, as well as assessment indicators, is elaborated. The prototype may combine “If-Then-Else” and simpler rules (Debaeke et al, 2009). According to the choices at step 1, the indicators may concern the agronomic and economic performances and the sustainability of the system. Since the nineties, many agri-environmental indicators have been developed to assess the adverse effects of cropping systems (Bockstaller et al, 2009), and biological groups are also consistent indicators especially with respect to pest management (e.g. Simon et al, 2007). The third step is on station evaluation of the prototype which becomes, at this time, an experimental prototype suffering local adaptations. The fourth and last step is that of on farm evaluation of what has become, following the previous steps, a farmer prototype. Of course, the methodology is not just top-down and linear and it requires many adjustments and loops (Debaeke et al, 2009).

Model-based design is the second way of designing cropping systems. The core of the design is a model representing the ‘crop as a whole’ (see above). Of course, it is a prerequisite. Designing the model means, since exhaustiveness is impossible, a lot of choices about the constraints, the elements of the cropping system to consider, and the output criteria. We shall not develop these aspects to rather concentrate on the model use. The central idea is create or select in silico (using the model and a computer and the diagnosis of appropriate experts during simulations!) prototypes of innovative cropping systems to be adjusted, then tested during on station and on farm evaluation stages and triggering loops just as in the case of the expert-based design. This relies on a dialogue between a model and a virtual cropping system defined as a set of rules (see above; “If-Then-Else” rules entail a closer interaction between the model and the virtual cropping system than “Do-That” rules that just provide an input to the model). Searching solutions (“good” cropping systems) means looking for the “good” parameters of the rules, e.g. the threshold for spraying or releasing biological agents. The search space can be very wide (as in Tixier et al, 2008 with a model of plant-soil-nematode interactions in banana) or narrowed to satisfy a set of constraints (as in Loyce et al, 2002a with a model of ethanol wheat encompassing the effect of fungal diseases). Then, multi-criteria analysis is the key tool to assess the simulated results of the cropping systems. Depending on the number of possible solutions, it can belong to the family of decision-aid methods (Sadok et al, 2008) that is convenient to compare and rank a limited number of solutions (see Loyce et al 2002a,b for an application), or to the family of multi-objective optimisation, with exact algorithms (e.g. dynamic programming) or heuristic methods (e.g. evolutionary algorithms: see deVoil et al, 2006 for an application), depending on the complexity of the problem and model. In all cases, and with or without criteria aggregation, the question of weighing the different criteria to rank or optimise solutions is crucial. The case studies of Loyce et al (2002b) and Tixier et al (2008) showed the consequences of different sets of weights on the appreciation of “good” cropping systems. IFP is a good case study to search trade-offs between competing objectives such as fruit quality and environmental preservation by using such sets of weights.

**Which areas of research to strengthen to design IFP cropping systems?**

The first area is the analysis of current practices. How to imagine new ways of doing without referring to what is currently done? The objects of this analysis are the diversity and internal consistency of practices, the objective reasons (van Mele and van Lenteren, 2002) as well as the constraints, conceptions and influences behind the technical systems (Nesme et al, 2006). Also, the potential for changing should be analysed by examining not only the conditions for
adoption of new systems, but also the existing trajectories (Lamine and Bellon, 2009).

The second area is the study and modelling of crop-pest-enemies interactions under the influence of cropping systems, including cultural methods and chemical or biological protection treatments. This network of interactions is at the core of expert or model-based design of cropping systems. Some joint studies of production and health performances have shown the interest of innovative management methods for both fruit quality and pest/disease control. In the peach-brown rot case study, new tree training and regulated deficit irrigation promoted sugar in fruit and storage potential and lowered the disease incidence (Mercier et al, 2008). Research on the underlying mechanisms revealed the major contribution to host susceptibility of cuticular cracking, which follows fruit growth and generates entry sites for the fungus, and the way of modulating it by irrigation and fruit thinning (Gibert et al, 2007). Similarly, studies on peach-green aphid interactions have shown that moderate winter pruning can contribute to aphid control because favouring “rosettes” (short shoots) at the expense of long axes preferred by aphids (Grechi et al, 2008).

The third area is that of landscape studies. Though pests, diseases and their enemies spread over the landscape, till now few studies have considered this, especially for fruit crops and pests (but see Ricci et al, 2009). Detailed data should supply the controversial question of the effects of landscape complexity on pests, beneficials and natural pest control (Bianchi et al, 2006). Landscape traits, including not only those dealing with allocation of crops and non-crops, but also distribution of crop management practices (Ricci et al, 2009), open the way to extend the cropping system concept. Accordingly, landscape design is the new paradigm of landscape ecology (Nassauer and Opdam, 2008), and the above-cited methods of cropping system design should apply to landscape design as well.

Conclusion

Designing cropping systems and landscapes to implement IFP requires several things that are illustrated by contributions in this issue. First, a set of methodologies to perform system experiments, models and multicriteria analysis. Second, a network of stations and pilot farms to evaluate prototypes emerging from expert or model-based proposals. Third, knowledge on fruit crops “as a whole”, the interactions between their components and the upscaling at the landscape level. This means that multidisciplinary is the key word of the approach.

References


Developing a protocol and a marketing niche for Eco Apples in NY State

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Abstract: In 2007, Cornell University, University of Massachusetts, Red Tomato\textsuperscript{TM} (a nonprofit produce marketing corporation), and the IPM Institute of North America, Inc. received a 2-year grant to develop a protocol for producing and marketing “Eco Apples\textsuperscript{TM}” in the Northeast. Red Tomato’s mission is connecting farmers and consumers through marketing, trade and education and a belief in family-farms, and a locally-based, ecological, fair trade food system. The goal is to create a market niche for “Eco Apples\textsuperscript{TM}” that will result in premium prices and access to high-quality markets such as Whole Foods and Trader Joe’s. Red Tomato’s apple sales grew from $130,000 in 2004 to $1.9 million in 2008. The program grew from 6 New England growers with 475 acres in 2005 to 635 acres and 9 growers in 2008. Participating growers complete a self-assessment, pay an annual certification fee and submit scouting and pesticide application records. The protocol is adjusted annually by Red Tomato employees, participating growers, and university personnel. Pesticides are classified into 3 categories: Green, use with justification; Yellow, use when Green materials are not available or effective; and Red, do not use. In 2007 and 2008, pest control in Eco Apple orchards was generally as effective as that in growers’ standard blocks. Economic costs and returns to participating growers have not yet been calculated.

Keywords: IPM, reduced risk pesticides, marketing apples, Red Tomato\textsuperscript{TM}, Eco-Apple\textsuperscript{TM}

Introduction

Organic apple production is nearly impossible in the Northeastern United states because the complex of insect and disease pests is much more formidable than in many other apple production areas of the world. However, recent studies in a multi-state RAMP (Risk Avoidance and Mitigation Program) grant have shown that Northeastern apple growers can adequately control insect pests and diseases using IPM programs that utilize only reduced risk pesticides, which are less toxic to humans and safer for the environment. However, these programs are more expensive than growers’ standard control programs using conventional pesticides. Apple growers in this region would be more likely to adopt these types of IPM programs if they could receive premium prices for apples grown using these techniques to help offset increased costs of materials, and sampling and monitoring programs. In 2007, Cornell University, the University of Massachusetts, and Red Tomato\textsuperscript{TM}, a private non-profit corporation, started a research program to determine if a multi-state market niche could be created for Northeastern apples grown under IPM programs using reduced risk pesticides that would provide growers with premium prices and market access that is similar to that currently utilized by organic fruit.

Red Tomato was founded in 1996 by Michael Rozyne and is based in Canton, MA. It is a
non-profit corporation that is funded through grants and donations. This organization connects farmers with markets and consumers with fresh fruits and vegetables. Red Tomato’s mission is "connecting farmers and consumers through marketing, trade, and education, and through a passionate belief that a family-farm, locally-based, ecological, fair trade food system is the way to a better tomato." This corporation has developed a marketing concept in which “Eco Apples™” are equal to organic apples in prices and access to high quality market outlets. Eco Apples are grown using ecological farming methods by family farms in the Northeastern US. The prototype program was developed in 2003 by Red Tomato in conjunction with the University of Massachusetts, two commercial growers, and New England Fruit Consultants. This group analyzed pest management options and economics, and developed marketing procedures. The goals of this Eco Apple project are: 1) To eliminate or minimize the use of organophosphate and carbamate insecticides; 2) To eliminate or minimize the use of potentially carcinogenic fungicides; 3) To develop a marketing plan that increases revenues sufficient to offset the increased costs of an intensive pest management programs that uses reduced risk pesticides. The program has been refined and expanded from 2004-08.

Red Tomato has gradually been expanding sales of Eco Apples since the program began. The following marketing benchmarks have been reached by the company: 2004, Eco Apple sales of $130,000; 2005, sales of $400,000; 2006, sales of $600,000; 2007, sales of $1.3 million; 2008, sales of $1.9 million. The acreages and numbers of growers has also expanded from 475 acres of apples and 6 growers in 2005-06 to 635 acres and 9 growers in 2008. The primary markets for Red Tomato are upscale specialty stores such as Whole Foods, Trader Joe’s and a collection of independent chains throughout the Northeast.

Materials and methods

Tom Green, Director of the IPM Institute of North America, is responsible for the classification of pesticides that can be used by growers in the program. Pesticides are classified according the their overall hazard rating. "Green" pesticides can be used with justification. "Yellow" pesticides can be used, with justification, when Green list or other alternatives are not adequate, and those pesticides on the "Red" list cannot be used in the program. Pesticides are classified within the program according to the following criteria: acute toxicity to wildlife, fish and birds; acute toxicity to humans; possible/likely/probable carcinogen; reproductive/developmental toxin; toxicity to pollinators, natural enemies, secondary pests; toxicity to wildlife; suspected endocrine disruptor; broad-spectrum pesticide; resistance risk; potential or known groundwater contaminant. Examples of insecticides classified as Green in the program are: Assail (acetamiprid), Dipel (Bacillus thuringiensis, Surround (kaolin clay), oil, Spintor (spinosad), mating disruption, and Esteem (pyriproxyfen). Insecticides classified as Yellow are: Proclaim (emamectin benzoate); Provado (imidacloprid); Intrepid (methoxyfenozide); Sevin (carbaryl) – for thinning only); Calypso (thiacloprid) – only for plum curculio; and Asana (esfenvalerate) – only for rosy apple aphid, as a special case in Pennsylvania. Some examples of Red insecticides are: Guthion (azinphosmethyl) – acute toxicity, AChE inhibitor, broad spectrum; Apollo (clofentezine) – possible carcinogen, suspected endocrine disruptor); Savey (hexythiazox) – possible carcinogen, moderate aquatic toxicity; Rimon (novaluron) – acute toxicity; Warrior (lambda cyhalothrin) acute toxicity, beneficials, endocrine disruptor; Actara (thiamethoxam) – likely carcinogen (although now placed in the Yellow category following an EPA review of further toxicology data).

Participating growers in the project are located in the Northeast, with 2 in New Hampshire, and 1 each in Vermont, Massachusetts and Connecticut. Currently, NY has 4
participating farms with 62 acres. Two of the NY participating growers are wholesale marketers, and 2 are direct marketers. In New England, the 5 farms are participating with a total of 500 acres. The NE cooperators include both direct and wholesale producers.

Results and discussion

During the two seasons of the project, pest control has been similar to standard growers’ conventional programs both in NY and in New England. New England growers have always obtained higher levels of clean fruit than those in NY. Two of the NY orchards (Stone Ridge and Ten Eyck) had lower percentages of clean fruit at harvest than some of the other blocks at harvest in 2007, because of ineffective scab control programs. Disease control in all NY blocks was much better in 2008.

Table 1. Percent clean fruit at harvest in participating Eco Apple Grower orchards.

<table>
<thead>
<tr>
<th>State/Orchard</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vermont / Sunrise</td>
<td>95.3</td>
<td></td>
</tr>
<tr>
<td>Connecticut / Lyman</td>
<td>96.2</td>
<td></td>
</tr>
<tr>
<td>New Hampshire / Alyson</td>
<td>93.1</td>
<td></td>
</tr>
<tr>
<td>Massachusetts / Clark</td>
<td>90.1</td>
<td></td>
</tr>
<tr>
<td>Vermont / Scott</td>
<td>90.4</td>
<td></td>
</tr>
<tr>
<td>New York Orchards</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple Acres</td>
<td>89.8</td>
<td>97.2</td>
</tr>
<tr>
<td>Stone Ridge</td>
<td>73.5</td>
<td>-</td>
</tr>
<tr>
<td>Ten Eyck</td>
<td>83.0</td>
<td>82.2</td>
</tr>
<tr>
<td>Truncali Home</td>
<td>87.4</td>
<td>88.2</td>
</tr>
<tr>
<td>Truncali Bingham</td>
<td>-</td>
<td>90.5</td>
</tr>
<tr>
<td>Knight</td>
<td>-</td>
<td>96.5</td>
</tr>
</tbody>
</table>

It has been somewhat difficult to directly determine the prices of fruit that growers have sold within the program, but growers were surveyed to determine how they perceived the benefits of an association with Red Tomato. The growers generally agreed that the prices they received were better than that from other apple brokers and that they received the prices promised. They also believed that they had a good relationship with Red Tomato, and that communication in the project was quite transparent. The growers felt that Red Tomato has been understanding when they were unable to reach set targets, and consequently they would not be interested in going elsewhere. They recognized that Red Tomato is interested in working with smaller growers and because of that they didn’t have to market entire tractor-trailer loads of apples. Other brokers were cited as being erratic in their dealings with growers, even when high quality produce was being marketed. Growers were relieved that Red Tomato was doing the “footwork” with potential markets so that growers were not pressured into selling prematurely. They appreciated Red Tomato’s contacts and their ability to deal with logistics. Finally, the growers were satisfied with the added value of the Eco Apple brand, and the dependability of the market even in this niche arena.

Despite the relative success of this short-term project, there are still problems and potential limitations to developing a value-added marketing concept such as the “Eco Apple” approach. It is very important for grower participants in this type of program to obtain both
access to selective markets and also premium prices. Unfortunately, many selective marketers tend to continually raise the bar of certain criteria that growers must follow to gain access to their markets and sometimes do not raise prices to reimburse growers for additional effort and expenses. If these types of programs are to succeed in the future, it will be necessary to attempt to persuade market outlets to pay premium prices to reimburse growers and, if necessary, to pass on the additional expenses to consumers. Also, in this project, some key new reduced risk pesticides are not approved for use in the current protocol. Certainly, in moving forward in classifying pesticides, it is necessary to be sufficiently flexible to allow the use of certain products in this program that might not be perfect for each classification category, but still have an overall profile that is better than standard materials that are currently used by growers. Even though this project has been gradually expanding, both in terms of sales, and numbers of growers and acreage, it is still a relatively small operation. Often it is inherently difficult to occupy a specialized market niche and expand a program so that large numbers of growers can participate. Currently this project focuses primarily on growers marketing to wholesale outlets, and benefits for growers directly marketing apples in local farm stands have not yet been optimized. Direct-market growers often have long-term clients that already trust their current pest management practices and appreciate the quality of their products. Therefore, the only benefit from participating in a program such as Eco Apple marketing for small direct marketers might be in attracting new customers. However, in adopting a niche marketing program, the direct marketer must put the whole farm in the project to avoid negative perception by his consumers of any non-certified fruit that might be grown on the farm. These growers also run the risk of eroding confidence of long-term customers who might perceive that the adoption of a new program indicates that previous practices were not adequately protecting consumers and the environment.

Finally, it is important to continue to promote this project in such a way that other powerful interest groups such as growers using conventional pesticides and organic growers are not threatened by this style of niche marketing. Organic growers have traditionally been opposed to production practices that use non-organic pesticides in spite of their desirable toxicity, selectivity, and safety to the environment. Often, conventional growers have a deep-seated fear that the promotion of a niche market for Eco Apples implies that conventional growing practices are detrimental to the environment and unhealthy for the consumer. Consequently, they feel that promotion of such niche markets could lower the demand and price for their conventional products.

Acknowledgements

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References

Eco Apple Protocol can be viewed on the Red Tomato Eco Apple home page: http://www.redtomato.org/ecoapples.html
Analyzing the results of a biodiversity experiment: Enhancing parasitism of *Platynota idaeusalis* (Lepidoptera: Tortricidae)

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Abstract: A common goal of conservation biological control is to enhance biodiversity and increase abundance and effectiveness of predators and parasitoids thus increasing sustainability of pest management. Although many studies report an increase in abundance of natural enemies, it has been difficult to document increases in rates of biological control. To enhance parasitism of the leafroller, *Platynota idaeusalis* (Tortricidae), alternate food was provided by interplanting peaches with extrafloral nectaries into apple orchards. Laboratory studies showed that the presence of peach extrafloral nectar increased longevity and parasitism rates by *Goniozus floridanus* (Bethylidae), the dominant parasitoid in West Virginia, USA. In orchard studies we found the total number of Hymenopteran parasitoids was higher on peach trees than on adjacent apple trees. Abundance of Hymenoptera was also significantly higher on the side of traps facing away from rather than toward peach trees, indicating attraction to peach trees producing extrafloral nectar. However, total parasitism rates of *P. idaeusalis* by all species of parasitoids were not affected by the presence of peach extrafloral nectar in any field studies. Insect injury to fruit at harvest showed that fruit from orchards with interplanted peach trees had less injury from San Jose scale (*Quadraspidiotus perniciosus*) and stink bugs (Pentatomidae) than fruit from an apple monoculture control orchard. Although interplanting with peach trees did not result in detectable increased biological control, the experiment did have beneficial results for pest management. By collecting data on the response of an ecosystem service (e.g., fruit quality) we were able to document a reduction in damage of two pests by the interplanting of peach trees with extrafloral nectaries into apple orchards. This demonstrates the need for a more holistic approach to evaluating habitat manipulation experiments. Without information on the response of yield quality we would have concluded that the experimental addition of peaches had no effect on insect pest damage. But, the yield data showed that pentatomids and scales responded with decreased damage, through an as yet undetermined mechanism, from the experimental manipulation. A cost:benefit analysis of the habitat manipulation is needed before recommendations can be made. It appears promising, however, that the sustainability of apple ecosystems can be enhanced by increasing its biodiversity with the addition of peach trees with extrafloral nectaries.

Key Words: apple, conservation biological control, *Goniozus floridanus*, Bethylidae, habitat manipulation, extrafloral nectar, ecosystem service
Genetic modification of apple to control diseases:

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Abstract: Apple scab is controlled by a high number of fungicide applications. Fireblight control is difficult and in some situations and up to three Streptomycin applications are necessary. The application of such pesticides is highly questioned because of their potential environmental impact and residues. Classical breeding has produced scab resistant cultivars and in the near future also fireblight resistant cvs. However, their popularity is limited as the traditional market dominant cvs have quality characteristics for producers, storage and consumers difficult to equal, and contrary to most other crops, apples are recognized as a cultivar, e.g. Gala, Golden Delicious, and not as a crop e.g. Bananas. In order to maintain the cultivar, single genes coding for enzymes and other proteins which can inhibit or at least reduce the development of scab and fireblight can be introduced by DNA-technology. A large range of foreign genes e.g. encoding lysozymes from bacteriaphages, fungi and animals have been used and in some cases reduce fireblight and/or scab susceptibility. Pathogen derived genes or pathogen induced promotors may also contribute. In all cases, all of the incorporated genes and control sequences are foreign and the marker genes needed for the selection of the transformed cells are antibiotic (e.g. nptII) or herbicide resistance genes (Bar). However such transgenic plants are currently unacceptable in Europe, especially as apple is mostly a fresh consumed product and consumers are highly sensitive to the issue. Even if legislation would permit such transgenic apple cultivars, no producer will take the risk of not being able to sell his product. Moreover, his personal profit includes the reduction of the number of treatments. Objection of the consumers, opinion makers and sometime policy makers are very broad, ranging from ethical issues (we should not manipulate genes in a way which nature does not, e.g. across natural barriers) to potential risks of outcrossing, vertical gene transfer and others. Therefore an approach which delivers to plant only genes (including promotors and terminators) originating from a crossable donor plant avoids most of the product oriented objection and could be an interesting alternative to transgenesis. This, however, does not eliminate the general objections to the technology itself. Such plants are defined as cisgenic. To create a cisgenic plant, firstly the apples own resistance genes and promoter sequences need to be cloned, and, secondly, a technology which eliminates the selection genes needs to be implemented. Both are currently available. We introduced HcrVf2, one of the open-reading-frames present in the genomic region introgressed in Malus x domestica from Malus floribunda 821, conferring Vf resistance against scab into the cvs. Gala and Elstar. The gene is constitutively expressed at a high level under the control of its own promoter and gives full resistance to an equal level and interaction as the Vf resistance introgressed by classical breeding. For the development of cisgenic plants, marker genes are necessary as they are for the development of transgenic plants. However, a system of post selection elimination of the marker genes has been implemented in strawberry and is currently applied to apple. A further system is reported to deliver ‘marker gene free’ in tomato and tobacco plants. We are currently testing the two systems and developing a third, all using, as a target, the HcrVf2 gene with its own promoter. The final result will be a plant of the target cv. into which the HcrVf2 has been introduced by DNA-recombinant technology corresponding to the definition of cisgenic. Concurrently, we are identifying further scab resistance genes and fireblight resistance regions with the final scope of obtain a cisgenic
apple cv. with fireblight resistance and scab resistance based on several functional different resistances. Plants of popular cvs. with resistance to the two diseases can contribute to a reduction of environment contamination and fruit residues avoiding the major critics against transgenic plants.

*Cisgenic, Transgenic, Malus*
Dispersal estimates of codling moth fertilized females in a French farm based on kinship assessments

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Abstract: Until now, population dynamics of Lepidoptera pests were mainly inferred from the monitoring of adult males using pheromone traps. Here, we analysed the dynamics of dispersal of codling moth fertilized females, which is more closely connected with the agronomic attacks. The dispersal of the fertilized females was estimated using genetic inferences of full-sibs among their offspring. We collected 6824 larvae using geo-referenced band traps in nine orchards (differing in host-plants and insecticide practices) from an experimental farm (90 ha) for five generations (2003-2006). Heterogeneity in the densities of larvae was mainly explained by inter-generation (twice higher for the diapausing larvae generation) and inter-orchard (50 times higher in untreated apple orchards) differences. A sub-sample of 1064 individuals was genotyped with a set of 13 microsatellite loci for kinship inferences. Three hundred forty pairs of individuals were unambiguously determined as full-sibs. Ninety-six % of the full-sibs were collected within orchards, either on the same tree or on relatively distant trees. The remaining 4% pairs of full-sibs were collected at all the inter-orchard distances (80 to 700 m) including different host-plants. These results confirm the relatively sedentary behaviour of the codling moth females in spite of their ability to disperse over very long distances and to lay their eggs on different host-plants.

Keywords: Cydia pomonella, population dynamic, kinship inferences, dispersal, microsatellite

Introduction

Understanding the population dynamics of insect pests is an important issue for improvement of their control, notably in the context of insecticide reduction. This is particularly true for the codling moth, the major insect pest in apple and pear orchards, which is responsible for most insecticide treatments in European and North American orchards. Despite intensive insecticide pressures, codling moth can have locally dramatic impact on fruit production, notably because of the development of insecticide resistances (Reyes et al 2007). Damage caused by the codling moth is due to fruit perforation by its larvae, which prevent fruit commercialisation. The whole larval development occurs within the fruit. Fifth instar larvae pupate on the tree where they develop either directly or after overwintering. Consequently, the distribution of the codling moth larvae represents the distribution of the laying sites. We can assess the dispersal of the laying of each female analysing the spatial distribution of its offspring. This dispersal is more connected with the agronomic attacks than that of males, which is generally measured. So far, there have been almost no direct measures of female dispersal, notably for long distance flight, due to the lack of suitable trapping method, such as pheromone trap used for males (Mani & Wildbolz 1977). Here, genetic inferences of full-sib pairs using microsatellite loci among geo-referenced larvae samples were performed to assess the dispersal of fertilized females in the field.
Materials and methods

Sampling sites and trapping
Samplings were performed in nine different orchards at Gotheron INRA research station, France. These orchards include different host plants (apple, pear, walnut and apricot) for the codling moth and they were managed with different phytoprotection practices (non-treated, organic and conventional). Samplings were performed in autumn 2003, 2004, 2005 and 2006, and in spring 2005 in order to collect samples across two successive generations in 2005. Fifth instar larvae were caught in corrugated cardboard traps wrapped around tree trunks. Five to 45 traps were set per orchard depending on their characteristics (Table 1).

Table 1: Orchard characteristics

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Species</th>
<th>Variety</th>
<th>Protection</th>
<th>Number of trees</th>
<th>Area (m²)</th>
<th>Number of traps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Apple</td>
<td>Multiple</td>
<td>Non-treated</td>
<td>37</td>
<td>1575</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Apple</td>
<td>Smoothee</td>
<td>Organic</td>
<td>233</td>
<td>2500</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Apple</td>
<td>Multiple</td>
<td>Organic</td>
<td>313</td>
<td>4800</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>Apple</td>
<td>Multiple</td>
<td>Conventional</td>
<td>169</td>
<td>2500</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Apple</td>
<td>Multiple</td>
<td>Conventional</td>
<td>718</td>
<td>10500</td>
<td>45</td>
</tr>
<tr>
<td>6</td>
<td>Apple</td>
<td>Golden</td>
<td>Conventional</td>
<td>123</td>
<td>2300</td>
<td>30</td>
</tr>
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<td>7</td>
<td>Pear</td>
<td>Multiple</td>
<td>Conventional</td>
<td>485</td>
<td>5000</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Walnut</td>
<td>Franquette</td>
<td>Conventional</td>
<td>370</td>
<td>21600</td>
<td>35</td>
</tr>
<tr>
<td>9</td>
<td>Apricot</td>
<td>Multiple</td>
<td>Non-treated</td>
<td>14</td>
<td>230</td>
<td>5</td>
</tr>
</tbody>
</table>

Genetic inferences
A total of thirteen microsatellite loci (Cp1.60, Cp1.62, Cp2.39, Cp2.129, Cp2.131, Cp3.169, Cp3.180, Cp4.129, Cp4.S, Cp5.24, Cp5.M, Cp6.32, Cp6.46) were scored for 1064 individuals. Primers of microsatellite loci had been identified from a partial genomic library of C. pomonella (Franck et al 2005). Amplifications were carried out in 10 µL reaction volumes containing 10 mM Tris-HCl, pH 9, 50 mM KCl, 200 µM each dNTP, 0.4 µM each primer, 1.5 mM MgCl₂, 0.5 units Taq DNA polymerase, 0.1 mg/ml BSA with two µL of DNA template. The reverse primer for each pair was labeled with 700 or 800 IRDye™. We visualized PCR products on 6.5% polyacrylamide denaturing gels on a Licor 4200 automatic DNA sequencer using Saga™ software (Li-Cor).

Kinship assignments were performed between individual pairs from each generation based on likelihood ratio tests (Queller & Goodnight 1989). We tested the hypothesis that pairs were full-sibs versus the alternative hypothesis they were unrelated based on the alleles they shared at each locus. For several loci, the likelihood ratio is the product of likelihood ratios at each locus. The sum of the decimal logarithms of the likelihood ratios over all the loci (lodscore) was the statistic used to compare the individual pairs. We used lodscore threshold values high enough to consider that the number of unrelated pairs misclassified as full-sibs in our data set was null at each generation.

Estimates of the dispersal of fertilized females
Since larvae do not disperse among trees during their development, the geographic distribution of full-sibs provide estimates of the dispersal of their mother. The dispersal curb of these fertilized females was estimated based on the distribution of the geographic distances between full-sibs pairs. This distribution was compared to a random distribution of the full-sibs obtained by re-sampling the same number of distances as the number of observed full-
sibs pairs in all distances between all pairs of individuals genotyped at each generation.

Results

Codling moth trapping
We collected a total of 6824 larvae over the five generations. Table 2 shows the mean number of captures per tree. The densities of larvae captured per tree were on average higher for the second generation than the first generation. Differences among orchards were mainly explained by higher densities of captures in orchard 1, the untreated apple orchard and lower densities of capture in conventional orchards, in particular orchard 5. We also collected very small numbers of codling moths in orchard 9, a non-treated apricot orchard.

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Number of larvae per tree</th>
<th>min</th>
<th>mean</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.7</td>
<td>52.3</td>
<td>92.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>1.3</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>0.9</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>0.8</td>
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</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
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<td>0.7</td>
<td>1.7</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>0.4</td>
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<td>8</td>
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<td>9</td>
<td>0.0</td>
<td>0.9</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Estimation of the dispersal of fertilized females
The threshold lodscore value taken into account to estimate the laying dispersal was seven at each generation. At this threshold level, a total of 340 pairs were classified as full-sibs among the 1064 individuals genotyped. The distribution of the full-sib pairs significantly differed from a random distribution at almost all the classes of distances. Twenty-three % of the full-sibs were found on the same tree. The mean distance of the dispersal curve was 35 m, the median 80 m and its mode 20 m. The inter-orchard dispersal of the laying was around 4%. It did not depend apparently on the distances between orchards or on host-plants. The longest distances of dispersal were recorded between orchards 5 and 7 (691 m) and between orchards 1 and 6 (698 m). No inter-orchard dispersal was observed among the samples collected in spring 2005, which may represent different migratory behavior between the first and the second generations. The distribution of the distances between full-sibs was also heterogeneous within the orchards. With regard to the random distribution of the distances between full-sibs, we observed an excess of sibs on the same tree and at the longest intra-orchard distances.

Discussion
In this study we found that codling moth females frequently laid their eggs on the same tree. However, in around 3/4 of the cases they laid their eggs on different trees. They usually laid their eggs within the same orchard, but they were also capable of long distance flights between orchards between two sequences of egg-laying. Our results extend previous results based on mark-release-recapture methods established using pheromone or bait traps to measure male and female dispersals, respectively. Captures of marked males with pheromone traps indeed suggested that male dispersal occurs over relatively short distances, but that rare
cases of long distance dispersal are possible -- up to 11 km (Mani & Wildbotz 1977) in agreement with the high flight ability of the pest (Schumacher et al 1997) and the low genetic differentiation among populations (Franck et al 2007). Mark-release-recapture experiments performed with alimentary baited traps (Steiner 1940) also indicated that females disperse over relatively short distances (mean 200 m, up to 650 m). Measured dispersal distances depend largely on the experimental protocol used and on the landscape characteristics. Using kinship analyses to determine dispersal overcame some difficulties posed by classical protocols such as the possible impact of baits on dispersal, the possibly different behaviour of laboratory strains as compared to wild populations, or the artificial environment used to measure flight ability. Nevertheless, our results are probably highly dependent on the landscape in which dispersal was estimated. First, the maximum distance between two sampling points was 856 m, which made it impossible to measure larger dispersal distances. Second, hedgerows or large amounts of open field are supposed to act as barriers to dispersal for codling moth (Tyson et al 2007) and the windbreaks surrounding the orchards may limit movement between orchards in this study. Third, the small area of each orchard in this study may explain the relatively low distances between two sequences of egg-laying since females rarely disperse between orchards.

Mating disruption is now frequently used to control codling moth in apple and pear orchards. The flight of fertilized females within the area under mating disruption has been suggested as being one cause of the lack of efficiency of this control method, in particular at the border of the area under mating disruption. Here, half of the fertilized females flew more than 80 m. This indicates that, for mating disruption to be efficient, mating disruption area should be separated from non-disrupted orchards by a few hundred meters. The minimum orchard area under mating disruption should be larger than the typical French recommendation of 3 ha.

Acknowledgement

We are very grateful to Karine Morel, Freddy Combes and Jean-François Toubon for their assistance in collecting codling moth larvae. We thank Benoît Sauphanor for his helpful comments in defining the project. The project was funded by INRA (department “Santé des Plantes & Environnement”) and the ECOGER program “Ecco des vergers”.

References

Observations on the phenology of codling moth in untreated orchards in the Netherlands and Belgium

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Abstract: Effective control of codling moth, Cydia pomonella, requires a good knowledge of the periods of egg laying and hatching of the larvae. Observations were done in a large number of untreated apple orchards in the Netherlands and Belgium to get an insight in the egg laying behaviour. At regular intervals all codling moth damaged fruits were collected from marked plots in insecticide-free orchards. Larvae were removed from the fruits and the age of each larva was determined from its length and the width of the head capsule. For the individual larvae their approximate date of egg deposition was back-calculated from temperature records. In this way, frequency distributions of egg laying and hatching of successful codling moth larvae in local populations could be generated. Egg laying and subsequent egg hatch showed patterns with distinct peaks. Egg laying in different regions showed similar patterns within years. The consequences of these patterns for effective codling moth control strategies will be discussed.

Codling moth, Cydia pomonella, Apple, Phenology
Differences among *Cacopsylla melanoneura* Förster (*Homoptera: Psyllidae*) insight from molecular markers

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**Abstract:** The psyllid *Cacopsylla melanoneura* (Förster) is one of the vectors of ‘*Candidatus Phytoplasma mali*’, the causal agent of apple proliferation disease (AP). In northern Italy, overwintering adults of *C. melanoneura* can be found both on apple (*Malus domestica* L.) and on hawthorn (*Crataegus monogyna* L.) from the end of January. Eggs are laid on the two host plants around March and the neanids complete their development at the end of April. Around mid-June the new generation adults move to shelter plants. Adults of the new generation can be found on conifers (especially *Picea abies* L.) at high altitudes from the end of the summer to the winter. The presence of AP phytoplasma was assessed by PCR in overwintering adults collected on the three host plants (apple, hawthorn and Norway spruce). The genetic variations among populations of *C. melanoneura* collected on the different host plants and in different localities were analyzed using microsatellites markers developed for *C. melanoneura* and COI sequences. ‘*Candidatus Phytoplasma mali*’ was found in most of *C. melanoneura* populations with differences in the percentage and titre. Data obtained from microsatellite analyses indicate differences among populations, which could explain the differences in the efficiency of acquisition and transmission of AP phytoplasma by the different populations.

*Psyllids, Apple, Hawthorn, Microsatellites, DNA markers*
Whole-farm infestation trends and management programs for obliquebanded leafroller in apples

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Abstract: Because of an incomplete understanding of the role of habitat, alternate hosts, and adult movement in NY fruit infestations by obliquebanded leafroller (OBLR), we wished to obtain a temporal and spatial picture of summer larval re-infestation patterns. In 2007, an unrealistically aggressive early season spray program was used to eradicate overwintered larvae on 3 commercial farms (17–32 acres) with a history of OBLR injury. Summer generation adults were monitored using a network of pheromone traps located at different orchard strata on all 4 ordinal sides. Weekly terminal and fruit samples were taken at each station. Although large numbers of adults were caught, larval terminal infestations and fruit damage remained low in all blocks. There were no substantial differences in adult catches, larval infestations or fruit damage in the different orchard strata. This suggested the utility of developing a sampling plan for the summer OBLR generation based on fruit damage rather than the traditional sampling of larvae on growing terminals. In 2008, methods above were repeated on 6 farms (20–40 acres) and participating growers agreed to leave small plots untreated with no sprays against summer larvae until the first damaged apple was observed. These small plots and at least two other areas being treated with standard programs were sampled 2 times/week until damage was detected. After a recommended spray, sampling continued but additional sprays were not recommended unless fruit damaged exceeded 1.5%. Pesticide spray recommendations were followed in only 6 of the 12 total sample-based plots, owing to cutbacks in some growers' pest management programs resulting from early season hail damage to the crop. Nevertheless, fruit damage at harvest showed no significant differences between the sample-based program and the grower standard preventive program, either in total percent damage or in any USDA grade categories (X-Fancy, Utility, and Cull). A partial budget analysis will be conducted to determine grower returns in the standard vs. research plots.

Keywords: obliquebanded leafroller, Choristoneura rosaceana, pheromone trap monitoring, larval infestation, fruit damage inspection, apple

Introduction

Obliquebanded leafroller (OBLR) is the most challenging fruit-feeding, or direct pest, of apple in New York State. Its life cycle is out of sync with that of most other orchard pests, as it overwinters as a partially grown larva and begins feeding immediately in the spring as temperatures warm. This necessitates extra pesticide applications specifically targeting summer populations. Additionally, OBLR has developed resistance to many conventional pesticides (e.g., OPs and pyrethroids), so effective management requires the use of selective, specialty materials (such as B.t., IGRs, microbials, etc.). Even so, it is still very difficult to completely eliminate damage from this pest. In high-pressure situations, 1–3% fruit damage at harvest is common. It has the capacity for long flight, plus other behavioral traits that render it unsuitable for pheromone mating disruption.

Furthermore, there has always been an incomplete understanding of the role of such factors as habitat, alternate hosts, adult movement, natural enemies, etc. in the incidence of fruit infestation. For instance, overwintered larvae can often be found in unmanaged or feral
sites, but the subsequent summer populations are negligible. This is essentially the opposite of the situation seen in commercial orchards. Larval sampling is not always a reliable indicator of population pressure. In previous studies, we attempted to eradicate the overwintered generation in 5-A plots in order to eliminate summer population damage. Despite obtaining extremely low larval numbers at bloom and during the July sampling period, fruit damage at harvest occurred as before.

In 2007, a study was set up on 3 commercial farms (18–33 A, total size) with a history of OBLR injury. To eradicate the overwintered larvae, an unrealistically aggressive early season spray program was used. Summer generation adults were monitored using a network of pheromone traps located at different orchard strata: edge, mid-interior and center on all 4 ordinal sides (N, S, E, W). Weekly terminal- and fruit-infestation samples were taken at each station. Although large numbers of adults were caught in all the orchards, subsequent larval terminal infestations and fruit damage remained low during the season in all blocks. There were no substantial differences in adult catches, and larval infestations or fruit damage in the different orchard strata. Results suggested that large-scale or whole-farm OBLR management might reduce damage from summer generation OBLR more effectively than treating individual blocks or small plots; sampling of fruit might better determine initial timing and treatment need than the traditional practice of timing preventive sprays for 1st egg hatch and then sampling terminals for larvae.

Materials and methods

In 2008, trials were set up in 20–40-A plots on 6 commercial farms having a history of OBLR infestation in Wayne, Saratoga and Onondaga Counties of New York State: Jerry Knight (Burnt Hills), Apple Acres (Lafayette), Todd Furber (Sodus), Van Fleet (Wolcott), Debadts (Sodus), and G&S Orchards (Macedon). Management of the overwintered generation was accomplished by a grower-applied spray of Proclaim at the petal fall stage, to attempt to reduce populations of overwintering OBLR to very low levels. Pheromone traps were deployed in the orchards and checked weekly to determine when the summer brood of OBLR emerged. Participating growers agreed to apply standard treatments against the summer generation of larvae in most of the originally treated area, but to leave two small plots untreated with no sprays to be applied against summer larvae until the first damaged apple was observed. The two small research plots and two other areas being treated with standard programs were sampled separately during each sampling session.

Weekly samples were taken for larval infestations at each farm starting 28 May; 50 foliar terminals were examined for OBLR larvae from each of 20 trees located in each of the four main quadrants of each farm plot, for a total of 4000 samples per site. This was repeated the next week, and beginning the week of 23 June, the sampling focused on both terminals (200 per quadrant per farm) and developing fruits (1000 per quadrant per farm). This sampling protocol was followed until the weeks of 10 August and 18 August, when only fruits were sampled. After a spray was recommended in any of the small research plots, sampling continued, and additional sprays were not recommended unless fruit damage exceeded 1.5%. On Sept. 8–12, fruit damage was compared just before harvest in the research and standard plots, by examining 500–1500 fruits randomly sampled from each treatment at each farm, depending on how many separate plantings or varieties constituted each of the treatment plots. These were sorted into USDA grade categories based on presence and extent of OBLR summer feeding damage found. Results were transformed by arc-sine square-root and subjected to analysis of variance and Fisher's protected lsd test for means separations ($P = 0.05$).
Results and discussion

The pheromone traps caught peak numbers of adults during the period from 13–26 June, with the highest catches recorded at the Furber and Knight sites (Fig. 1). The first flight subsided by the beginning of August, and the second flight was tracked until 18 August, and had peak numbers considerably lower than those of the first brood.

![OBLR Captures in Pheromone Traps 2008](image)

Fig. 1. Pheromone trap catches in commercial orchards receiving insecticide applications for control of obliquebanded leafroller on a sample-based vs. standard protective schedule.

Initial sampling of clusters and foliar terminals after petal fall indicated that the Proclaim treatments were generally successful, and almost no live OBLR larvae were found in examining 1,000 samples per plot at each site. Terminal infestations by the first summer brood started to be detected by the final week in June, however, reaching maximum levels of 0.8–3.3% starting with the 8 July samples, although in some sites (Furber, Knight) persisting until early August. Fruit damage from this brood was first detected in samples taken on 26 June (Knight), 8 July (Apple Acres, Van Fleet), 14 July (DeBadts), 29 July (Furber) and 4 August (Craft). Maximum levels noted were generally between 0.3–0.5%, except at Knight, where 2.0% was recorded on 10 August.

Growers made their own decisions about the need for pesticide sprays to control the summer generation of OBLR larvae, taking into account the information provided to them after each of our weekly fruit damage samples. Because a number of early summer hail events had caused significant fruit damage in most NY orchards this season, many growers elected to cut back on their management sprays to economize on fruit that was not expected to be worth as much at harvest. Consequently, OBLR programs in 2008 were not necessarily representative of what would normally have been implemented. For example, at Apple Acres, no OBLR sprays were applied all summer, as the grower used only organophosphates as a low-maintenance program. At Van Fleet, Warrior plus Dipel was applied on 8 July, but all plots were treated without regard for the presence or absence of damaged fruit in the samples examined by that date. Similarly, Delegate was used in directed OBLR management sprays at the other sites, although not necessarily on timings corresponding to our sampling-based information. Overall, only 6 of the 12 total Sampling-based Management Program plots received sprays according to the fruit damage information gathered from our samples.

Harvest fruit evaluations revealed generally low levels of damage caused by the summer
generations of OBLR larvae, ranging from 0.3–2.6% in the Sample-based program, and 0.0–1.9% in the Preventive program (Table 1). No significant differences between the management programs were found in these values or in any USDA Grade category. A partial budget analysis eventually will be conducted to determine grower returns in the standard vs. research plots, using estimates of pesticide program costs and packout returns for each of the farms where these trials were conducted.

Table 1. Percent larval fruit damage and USDA grades in plots where OBLR was managed using a preventive vs. a sample-based spray decision program, NY.

<table>
<thead>
<tr>
<th>Farm</th>
<th>OBLR Mgt Program</th>
<th>% Fruit damage</th>
<th>USDA Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OBLR Mgt Program</td>
<td></td>
<td>X-Fancy</td>
</tr>
<tr>
<td>Apple Acres</td>
<td>Preventive</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Craft</td>
<td>Preventive</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>DeBadts</td>
<td>Preventive</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Furber</td>
<td>Preventive</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Knight</td>
<td>Preventive</td>
<td>1.5</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>2.6</td>
<td>0.6</td>
</tr>
<tr>
<td>VanFleet</td>
<td>Preventive</td>
<td>1.9</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Sample-based</td>
<td>1.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

(No significant differences between management programs in any damage category.)

These results suggest that sampling the fruit for damage might be a better way to determine the treatment need and initial timing than our traditional practice of timing preventive sprays for 1st egg hatch and then sampling foliar terminals for larvae. If fruit damage actually does tend to occur relatively uniformly anywhere throughout these larger blocks, then it should be possible to sample any representative portion of a whole-farm planting as a basis for developing a fruit damage threshold to optimize the economics of making OBLR management decisions on this scale.

**Acknowledgements**

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Population dynamics of *Anarsia lineatella* and their relation to crop damage in northern Greece IPM peach orchards: towards the development of EIL

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Extended Abstract

The peach twig borer *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) is one of the major economic pests of stone fruits worldwide (Balachowsky et Mesnil 1935, Jones 1935, Bailey 1948, Summers 1955, Balatchowsky 1966, Damos and Savopoulou-Soultani 2008a). In Northern Greece, a great variety of peach (*Prunus persica*) cultivars (early, middle and late ripening, table and industrial) are cultivated and exported worldwide, so that peach production is considered to be essential for the economy. Efforts are made to improve pest control using Integrated Pest Management (IPM) in order to achieve high standards in products. In northern Greece, *A. lineatella* has 3 or usually 4 generations per year depending on prevailing temperatures (Damos and Savopoulou-Soultani 2007). During the past few years *A. lineatella* has been increasingly damaging to peach cultivation and, along with *Grapholitha molesta* (Lepidoptera: Tortricidae), they have been key targets for implementation of effective control strategies in terms of IPM in northern Greece. Larvae feed primarily on buds and tender shoots of the host tree after emergence from hibernacula, where they overwinter as larvae (Balachowsky et Mesnil 1966, Damos and Savopoulou-Soultani 2008b). Sustainable crop productions try to minimize energy input flows in agroecosystems in order to maintain a long term benefit for all enabling parts: agroecosystems, biodiversity, farmers, consumers, as well as society in general. As far as specific plant protection strategies are concerned, it is common sense that the application of insecticides would also lead to better pest management and to lower production cost only when economically justified. However, early warning and forecasting systems, which are indispensable in pest management strategies, have not been fully developed for *A. lineatella* in peach orchards. What is not clear is the population density of these species sufficient to cause economic injury to the plant. Under this framework, field studies were conducted for three successive years (2005, 2006 and 2007) in peach orchards of northern Greece in order to examine relationships between densities of *A. lineatella* populations and peach (*Prunus persica*) yields. Moreover, natural populations of *A. lineatella* were observed in order to assess crop response to the presence of the pest. Field population dynamics of *A. lineatella* were evaluated by using indirect measures (i.e. adult moth flight using pheromone traps), while injury on plant and fruit damage were estimated by absolute measures (i.e. counting injury on shoots as well as on fruits caused by larvae during the season). Regression analysis was used, first to determine if injury could be predicted from *A. lineatella* males captured on pheromone traps and second, if early shoot flagging caused by larvae of the first generation was correlated with fruit damage of the forthcoming generations (Knight and Croft 1987, Knight and Hull 1989, Savopoulou et al. 1989). Numbers of moths captured on sex pheromone traps and fruit damage varied during the 3 years of observation, ranging from 50 to 250 individuals per trap (*F*=5.563, df=2.11, *P*<0.05 and *F*=50.299, df=2.11, *P*<0.05, for first and second flight respectively). Shoot flagging and fruit damage was significantly lower in 2006 when compared to 2005 and 2007 (*F*=2.772, df=2.11, *P*<0.05 and *F*=14.809, df=2.11, *P*<0.05, for shoot flagging and fruit damage respectively). Mean shoot strike injury ranged from 5-15%, while fruit damage levels ranged from 5-10% during the three years of observation. According to the linear model, the increase in moth density...
during the first flight should result in a significant reduction in yield \((y=0.436x+10.22, R^2=0.635, P<0.05)\). Regression of male moths captured during the second flight and observed yield loss was also significant \((y=0.5231x+17.204, R^2=0.792, P<0.05)\). Moreover, according to the linear model derived by counting the number of shoot strikes, during the first observation period, a forthcoming yield loss can be estimated \((y=27.389x-6.304, R^2=0.711, P<0.05)\). Finally, a significant relationship was also observed between the numbers of second generation larvae and yield loss \((y=163x, R^2=0.890, P<0.05)\). The slope from the above regression can be used in the calculation of EIL and the fixed ET (Higley and Pedigo 1993, 1996). Results suggest that relative damage on fruits caused by \textit{A. lineatella} can be estimated using either male trap captures or by observing early shoot flagging symptoms. Additionally, pesticides should not be applied if population of \textit{A. lineatella} causing damage is lower than management cost. Developing a relationship between pest abundance and damage to crops is essential for the calculation of EIL leading to informed management decisions.

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An update on brown spot of pear

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Abstract: Brown spot of pear is caused by the fungus *Stemphylium vesicarium* (Wallr.) Simmons. (f. asc. *Pleospora allii*) and cause severe economic losses in pear-growing areas in Europe. Pear cultivars differ in susceptibility to the disease, being the most affected Abate Fetel, Passe Crassane, Alexandrine and Conference. Disease control is achieved with several fungicides (mainly dithiocarbamates, dicarboximides and strobilurins) at fixed spray schedules from petal fall to preharvest, and often 10 to 24 treatments are needed. A forecasting system has been developed (BSPcast) and validated as an advisory tool to schedule fungicide sprays, maintaining efficacy of disease control as in the fixed spray schedule, but with savings of fungicide treatments from 20 to 70%. However, disease control is still insufficient under high inoculum pressure or favorable conditions. Since the fungus overwinters as *P. allii*, in fallen infected leaves or fruits, factors affecting pseudothecia development were studied to refine control programs addressed to decrease the primary inoculum. A forecast model (PAMcast) which related the proportion of mature pseudothecia to cumulated degree-days was developed and validated in field trials, and used to forecast the development of the ascigerous state that ends in the subsequent spring with the release of ascospores. Control of the primary inoculum is critical for management of brown spot of pear because a reduction of levels or a delay in its production decrease considerably disease intensity in the subsequent year. Biological, chemical, and mechanical methods for decreasing overwintering inoculum of *P. allii* and disease intensity have been evaluated, and different efficacies have been obtained. Future research should be focused to key stages of the biological cycle, quantitative specific analysis of inoculum, and novel control methods, including biological control, in order to develop an efficient integrated system for disease management.
Basis for new strategies in integrated control of brown spot of pear 
 (*Stemphylium vesicarium*, teleomorph *Pleospora allii*)

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Abstract: Brown spot of pear is caused by the fungus *Stemphylium vesicarium* (Wallr.) Simmons, and produces high economical losses in several pear-growing areas in Europe including Spain, Italy, France, The Netherlands, Belgium and Portugal. The management of the disease is based on protective fungicides applied at fixed schedule or according to the BSPcast model. But, the efficacy in disease control is limited, especially when disease pressure is high. In order to reduce the disease pressure, additional methods focused to reduce the inoculum may be incorporated in the integrated control. To characterize the inoculum, populations of *S. vesicarium* from different pear orchards in Girona (Spain) were characterized for their pathogenical activity. Additionally, the dynamics of *S. vesicarium* inoculation under natural conditions were determined. On the other hand, *S. vesicarium* overwinters on pear in fallen infected leaves or fruits as pseudothecia of its teleomorph *Pleospora allii*, the relationship between disease levels at leaf fall and the production of pseudothecia was determined.

Keywords: Inoculum, pathogenical activity, potential dose

Introduction

Brown spot of pear (*Pyrus communis* L.) is a disease caused by the fungus *Stemphylium vesicarium* (Wallr.) E. Simmons that produces important economic losses in several fruit tree growing areas of Europe including Spain, Italy, France, The Netherlands, Portugal and Belgium (Montesinos et al. 1995a; Rossi et al., 2005a; Llorente and Montesinos, 2006a). The control of brown spot of pear is based on the application of protective fungicides and requires a high number of fungicidal sprays during the pear growing season according to a fixed schedule (every 7 to 15 days) or timed according to the BSPcast forecasting system (Llorente et al., 2000). The efficacy of control using the BSPcast guided schedule of fungicide sprays is similar to the fixed schedule and provides 30% savings in fungicide sprays. But, the efficacy in disease control is limited, especially when the disease pressure is high. *S. vesicarium* overwinters as pseudothecia of its teleomorph *Pleospora allii* (Rabenh.) Ces. & De Not. on the ground in the orchard on fallen infected leaves and fruits of pear (Llorente and Montesinos, 2004) or on dead herbaceous plants growing in the grass sward (Rossi et al., 2005a; Rossi et al., 2008). In order to improve the integrated control of brown spot of pear, strategies and methods oriented to decrease the overwintering inoculum have been tested. Mechanical methods based on pear leaf removal and application of biocontrol agents based on *Trichoderma* spp. reduced the number of ascospores released and the effect on brown spot of pear progress was significant. These sanitation practices may be incorporated in an integrated brown spot management system but the use of methods to reduce the inoculum of *P. allii* did not improve the efficacy in disease control of current fungicides applied according to the BSPcast model. The general objective of this work was to increase the efficacy of brown spot of pear control using new strategies to improve the reduction of the inoculum. These strategies must be focused at
reduction of both kinds of inoculum (Pleospora allii and Stemphylium vesicarium), not only
the overwintering inoculum. With this purpose the determination of the inoculum potential is
critical. The inoculum potential is a function of the density of the pathogen, but also of the
virulence of this pathogen. The objectives were to determine i), the dynamics of $S$. vesicarium
inoculation under natural conditions, ii) the characterization of natural $S$. vesicarium / $P$. allii
populations for their pathogenical activity and iii) to establish the relationship between the
levels of disease at leaf fall ($S$. vesicarium) and the production of overwintering inoculum ($P$. allii).

Material and methods

Determination of the dynamics of $S$. vesicarium inoculations under natural conditions
Two trials were performed in 2006 and 2007 in Girona (Spain). Pear plants of the cultivar,
Conference, were transported to an experimental pear orchard naturally affected by brown
spot from May to September. Then, these plants remained during 10 days under field
conditions with the objective to trap the inoculum of $S$. vesicarium. After this period, the
plants were returned to the laboratory and, with the purpose to induce the infection, these
plants were incubated for 24 hours with wetness under 22.5 °C. Thereafter, plants were dried
and placed in a controlled environment chamber for the expression of symptoms for 4 to 6
days and, finally, disease incidence (% affected organs) and severity (affected area in a leaf)
was evaluated. Also, the BSPcast model was used, considering the meteorological data of the
orchard, to forecast the level of infection risk (Montesinos et al. 1995a; Llorente et al., 2000).

Characterization of natural $S$. vesicarium / $P$. allii populations for their pathogenicity
A collection of more than 100 $S$. vesicarium and $P$. allii isolates was obtained from different
sources in 27 pear orchards in Girona (Spain) during 2007 and 2008. Periodically, samples
were collected from infected and symptomless pear leaves and fruits, non-hosts, such as,
herbaceous plants, pear leaf debris from the soil and air inoculum by using spore traps.
Cultures of fungi obtained from these samples where grown on PDA or V8 medium. Then
colonies were examined under an optical microscope and those morphologically similar to
$S$. vesicarium were isolated. Finally, monoconidial cultures were grown and identified as $S$. vesicarium according to Simmons (1965, 1989). The pathogenicity of isolates was evaluated
by inoculation of immature pear fruits (Conference, Abate Fetel and Passe Crassane cv.) and
young pear leaves (Conference cv.) (Montesinos et al., 1995b).

Relationship between the disease levels at leaf fall ($S$. vesicarium) and the production of
overwintering inoculum ($P$. allii)
Six trials were performed during 2004, 2005 and 2006 in two pear orchards (Passe Crassane)
in Girona (Spain). These orchards were naturally affected by brown spot. Just before leaf fall
pear leaves and the disease level was determined (lesions/cm² of leaf). From October to May
leaves were analyzed (more than 200 leaves/trial) by using an optical microscope and the
number of pseudothecia/lesion and number of lesions/cm² of leaf were determined

Results and discussion

Determination of the dynamics of $S$. vesicarium inoculations under natural conditions
In the three years, the inoculation under natural conditions in the two orchards was produced
from May to September, but especially during July and August (Fig. 1). In most of days the
high levels of infection risk predicted by BSPcast model agreed with the periods with a high
level of inoculation. But, the dynamics also showed that in some periods the inoculation was
produced in non-favourable environmental conditions. Studies focused to determine the viability of this inoculum are necessary. Conversely, at the beginning of the epidemics, fungicide treatments may be unnecessary because the population of pathogen is very low. In order to avoid these unnecessary treatments, information related to the inoculation must be incorporated in brown spot management.

Figure 1. Disease severity in a pear orchard with natural pathogen inoculations during 2006 (above). Each point corresponds to the infections produced by the \textit{S. vesicarium} inoculum deposited on previous period. Bars correspond to mean standard error. Infection risk predicted by BSPcast is also presented (below), the level of action threshold (CR$\geq 0.4$) is indicated.

Characterization of natural \textit{S. vesicarium} / \textit{P. allii} populations for their pathogenicity
Isolates showed different patterns of disease progress. The 22\% of isolates were non-pathogenic, explained by the saprophytic ability of \textit{S. vesicarium}. Among the pathogenic ones, 18\% showed a slow disease progress and 10\% of isolates showed slow disease progress at the beginning, but fast at the end. Most isolates showing these disease patterns were obtained from pear leaf debris, indicating that they probably need a period of latency before infection. Finally, in 50 \% of isolates the disease progress corresponded to those obtained from infected organs. The non-pathogenic group included 60\% of air isolates, 70\% of non-host isolates, 15\% of leaf debris isolates and 6\% of pear infection isolates. Therefore, the direct measurements of inoculum quantity on air using a volumetric spore traps may overestimate the real pathogenic population.

It was observed that pathogenic \textit{S. vesicarium} isolates were able to produce mycelium, conidia and pseudothecia on infected pear leaves. But non-pathogenic \textit{S. vesicarium} isolates were also able to produce mycelium, conidia and pseudothecia on dead pear leaves. It was determined that inoculum was also produced in other places out of the orchard. This information must be incorporated in control strategies focused to decrease the inoculum production (Llorente, et al., 2008).
Relationship between the levels of disease at leaf fall (S. vesicarium) and the production of overwintering inoculum (P. allii)

The relationship between the disease level of brown spot at leaf fall and the production of P. allii pseudothecia was linear ($R^2: 0.959$), with a mean of 1.3 pseudothecia/lesion. At leaf fall, the number of lesions/leaf may be evaluated and these data used to determine the inoculum potential of P. allii in the next spring. According to results presented above, the majority of this inoculum may be pathogenic, so the information related the level of P. allii inoculum must be incorporated in brown spot disease management to establish the appropriate strategy.

Acknowledgements

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References


Drought and oxidative stress determine the sensitivity of the pear to Brown spot infections

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Abstract: A survey among Belgian fruit growers carried out in 2006 revealed that Brown Spot is not equally spread in Belgium. The absence of Brown Spot is linked to the presence of loam. It is thought that the specific drainage properties of a loam soil are responsible for the absence of Brown Spot. An epidemiological study carried out in 2005 and 2006 supports this idea. In this study different orchards with a wide range of infection intensities were examined. An analysis of the soils in those orchards revealed that soil drainage conditions play a role in determining the sensitivity of the tree for Stemphylium infections. During the growth season of 2005, 2006 and 2007 actual Stemphylium infection risk was determined by means of window treatment experiments and fruit encapsulating experiments. An in-depth analysis of the occurrence of the actual Stemphylium infection risk moments revealed a close relation with the occurrence of drought stress during these growth seasons. The sensitivity of the pear towards brown spot infections is not only linked to drought stress, but also to high radiation, ozone and temperature. In the 2008, chlorophyll fluorescence measurements were performed to determine the relative importance of the different factors that contribute to the oxidative stress on pears during the growing season. Non photochemical quenching (NPQ) was used as a measure of oxidative stress damage and protection against this type of stress. A correlation analysis indicates that ozone is probably the largest contributor to oxidative stress damage on pear.

Brown spot, Oxidative stress, Drought
Evaluation of ascospore maturity models to estimate seasonal ascospore discharge of pear scab (*Venturia pirina*)

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Abstract: Estimates of ascospore maturity generated by models developed for *Venturia pirina* in Victoria, Australia (V-NV, V-SV), Oregon, USA (S), or for *Venturia inaequalis* in New Hampshire, USA (NH-1 and NH-2) were compared to observed ascospore release of *V. pirina* in 21 site/yr combinations. When plotted against degree-days, the lag phase and slope of all model estimates differed from observed release. The S model and V-SV model fit well with the data from Southern Victoria, while the data from Norway, Belgium and most years from Northern Victoria show a lag phase in the beginning of the season that was not present in the two models. In particular, data from the high-rainfall region of southern Victoria showed more variation between years than the other sites. Identifying the precise biofix (bud break) to initiate degree-day accumulation for the NH-2 model was problematic at both Australian sites, as regions with warm winters and minimal chilling exhibit protracted bud break. Linear regressions generated similar R² values for the various models in many cases, but where differences were noted they more often favored the most recent model developed for *V. inaequalis* (NH-2). The NH-2 model also provided the most accurate estimates of 95% ascospore depletion (a key event in many disease management programs) for Norway, Belgium, and the higher rainfall areas of southern Victoria. Although developed for use in management of apple scab, the NH-2 model appears a reasonably accurate tool for predicting the release of ascospores by the pear scab pathogen, in particular in regions with moderate rainfall and colder winters.

Keywords: Apple scab, biofix, bud break, epidemiology modelling

Introduction

Pear scab, caused by *Venturia pirina* Aderhold, is an important disease of pear worldwide (Shabi, 1990). The fungus survives primarily as pseudothecia in leaf litter in the orchards, or as mycelium on shoots and buds. Ascospores are released from infected leaf litter by rain or heavy dew beginning around bud break and for 3 to 4 months thereafter, with a peak in release between early bloom and petal fall. If rain is continuous, most ascospores will be released during daylight hours (Villalta, 2001). Many of the most common methods of studying development of pseudothecia (squash mounts, spore traps, etc) are too tedious and time consuming for use on more than a limited basis in advisory programs for disease management. Thus, for both *V. pirina* and the related pathogen *V. inaequalis*, degree-day driven mathematical models of ascospore maturation have been developed. Such environmentally-driven models have several advantages for advisory programs. They can be inexpensively applied over broad areas and can provide site-specific estimates of ascospore maturity and release if coupled with on-site weather observations. Furthermore, if driven by
forecasted weather, the models can provide true forecasting of ascospore maturity and release. However, although several such models exist, none have been evaluated for \textit{V. pirina} outside of the geographical region in which the specific model was developed. Our objective in the present study was to internationally evaluate several existing models developed for \textit{V. inaequalis} and \textit{V. pirina} to estimate the cumulative maturity and release of ascospores by \textit{V. pirina}.

Materials and methods

Spore trapping data of \textit{V. pirina} from Norway (7 years), Belgium (5 years), and Australia (5 years from 2 sites) were used to evaluate the models. For all sites, Burkard 7-day recording volumetric spore traps (Burkard Manufacturing Co Ltd., Rickmansworth, Hertfordshire, UK) were installed during the primary inoculum season. Heavily infected, overwintered pear leaves surrounded the spore traps. Number of ascospores recorded was adjusted for the volume of air sampled and recorded as spores per m$^3$ air.

Electronic data loggers provided hourly records of precipitation, temperature, RH, and leaf wetness. Temperature and RH were measured 1.5 to 2 m above ground in weather shelters or radiation shields. The first model of Villalta \textit{et al.} (2001) based on data from southern Victoria (V-SV) uses all degree days, accumulated from first ascospore. The second is based on data from northern Victoria (V-NV), and uses degree days accumulated only on days with $\geq 0.2$ mm rain, starting from first ascospore. For the New Hampshire model ((NH-1; Gadoury \textit{et al.}, 1982), bud break is used as a biofix, and either all degree days were counted, or degree day accumulation was halted when the number of rain-free days exceeded 7 (NH-2; Stensvand \textit{et al.} 2005). For the model (S) described by Spotts \textit{et al.} (2000), all degree days from detection of the first ascospore were included. All models are shown in Fig. 1. The different degree days (base 0°C) were calculated based on daily average temperatures and put into the models described above.

Overwintered pear leaves collected at bud break, heavily infected with pear scab, were used to simulate seasons of ascospore discharge in the lab. Samples were stored at 20°C, and ascospores were collected at regular intervals (2 times per week). Eight samples from 3 different countries were tested, including 1 from France, 3 from Belgium and 4 from Norway in the period between 2002 and 2006.

The percentages estimated by the models were regressed against observed (trapped) number of ascospores, and results from the lab tests. The concordance correlation coefficient (CCC; Lin, 1989) is shown for each pair of observed versus predicted values, and the slope and intercept of the regression line were tested for equality to 1 and 0, respectively, by Students $t$-test.

Results and discussion

Table 1 shows the regression equation and CCC of numbers of discharged/mature spores estimated by the different models regressed against actual number of spores trapped. The results show that the distributions of the data points differed between the models. This variation is correlated with what the models use as biofix, and how degree days were accumulated. The NH-models use bud break as biofix and, especially for the data from Australia, the date of bud break seemed not to agree that well with first ejected ascospore. Identifying the precise biofix (bud break) to initiate degree-day accumulation can be problematic, as regions with warm winters and minimal chilling exhibit protracted bud break. Overall, the NH- models give a good prediction of when the season started and ended, as shown in Table 1.
The V-NV model gave a good prediction of both the start and the end of the season. But when accumulating degree days only on days with more than 0.2 mm precipitation, the early lag phase seemed to be eliminated in most years, and the model underestimated the percentage of spores released in the middle of the season.

The S-model and V-SV model both overestimate the number of spores released in the beginning of the season. Both models fit well with the data from southern Victoria, while the data from Norway, Belgium and most years from northern Victoria show a lag phase in the beginning of the season. The reason why this lag phase was only seen in some places may be caused by a combination of amount of rain and how the biofix was determined.

Table 1. Linear regression\textsuperscript{a} of observed versus predicted number of ejected/mature ascospores of \textit{Venturia pirina}.

<table>
<thead>
<tr>
<th>Spore trap data from\textsuperscript{b}</th>
<th>Models evaluated</th>
<th>NH-1</th>
<th>NH-2</th>
<th>V-SV</th>
<th>V-NV</th>
<th>S</th>
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<tr>
<td>Norway</td>
<td>y = 0.99x + 2.65</td>
<td>y = 1.05x - 12.3</td>
<td>y = 0.72x + 31.2</td>
<td>y = 1.11x - 15.36</td>
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<td>y = 0.99x + 1.83</td>
<td>y = 0.77x + 27.2</td>
<td>y = 0.94x + 6.84</td>
<td>y = 0.70x + 24.32</td>
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<td>b = 1, P = 0.056</td>
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<td>y = 0.94x - 2.94</td>
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<td>y = 1.01x - 6.59</td>
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\textsuperscript{a} Results from linear regressions of observed versus predicted values, shown as concordance correlation coefficients. The slope and intercept of the regression line was tested for equality to 1 and 0, respectively, by Students \textit{t}-test (\(P\leq0.05\) = significant difference)

\textsuperscript{b} Spore trap data from one site in Norway, two sites in Australia (southern and northern Victoria) and one site in Belgium.
Figure 1. Graphic illustration of the models evaluated

References

Developing an effective trap and lure to monitor Lygus rugulipennis

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Abstract: Lygus rugulipennis, the European tarnished plant bug (Miridae), is an important pest of strawberries, raspberries and cucumbers causing malformation of fruit. Ordinarily mirids are controlled with sprays or chlorpyrifos. However, increasing demand for zero residues fruit and the eradication of effective pesticides from IPM programmes is rendering crops more susceptible to attack from mirids. The overall aim of this project is to develop a long-lived, practical lure, attractive to these species, in order to monitor populations so that effective spray timings and spray applications can be made to control the pests in fruit crops. Male L. rugulipennis are attracted to traps baited with live virgin females. Volatiles produced by virgin female L. rugulipennis have been identified as, hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal and these elicit electroantennographic (EAG) responses from males in analyses by linked gas chromatography–electroantennography (GC-EAG). Using ratios similar to those produced by the female at the time of ‘calling’, when males are attracted to females, we have demonstrated the attractiveness of the volatiles to male L. rugulipennis in the field. We also tested a number of home-made and commercially available traps for monitoring mirids. Green cross-vane funnel traps were the most effective and practical of those tested.

Key words: capsid, European tarnished plant bug, Lygus rugulipennis, mirid, pheromone, raspberry, strawberry

Introduction

Mirid pests (capsids), are common and important pests of many horticultural crops. Crop damage caused by Lygus rugulipennis is sporadic and unpredictable resulting in severe economic losses at low population densities, particularly in strawberry (Cross, 2004). In conventional crops it is controlled by sprays of broad-spectrum insecticides (Garthwaite & Thomas, 2001), organophosphorus insecticides being the most effective and frequently used. However, there is a reduction in available organophosphorus insecticides, and neonicotinoids and other modern insecticide groups are only partially effective or ineffective against this pest. Insecticides used in organic crops are inadequate and of short persistence. Because of this mirid pests present a bottle neck in the development of IPM programmes. In addition, insect sampling methods (sweep-net or beating-tray sampling) are time consuming and unsuitable for use by growers.

Traps baited with virgin females of L. rugulipennis (Innocenzi, et al., 1998; Glinwood, et al., 2003) have been shown to attract conspecific males, but attraction to a synthetic lure has never been demonstrated. Sex pheromone identification in mirids has been hampered by the abundant defensive secretions, of which, certain compounds can function both as components of the pheromone and as defensive secretions (cf. Blum, 1981, 1996; Groot, et al., 2001 Zhang et al., 2007). Previous research on L. rugulipennis (Cross & Hall, 2003) identified three female-specific pheromone components, hexyl butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal. This paper outlines studies aimed at monitoring mirid pests, by engineering
an effective lure with the correct ratio and release rate of the female sex pheromone compounds and enhancing trap catch by effective trap design.

Materials and Methods

**Field trial 1 – based on daytime entrainments**
The previously estimated ratio of HB:E2HB:KA released by a single *L. rugulipennis* female, based on whole day entrainments, was 1.5:1.0:0.08 (Innocenzi et al., 2003). In 2007 field trials showed that female *L. rugulipennis* were attracting males in the morning. Based on volatile collections (entrainments) from female *L. rugulipennis* at 3 times of day (daytime, evening, night) a small scale randomised block experiment comparing catches of capsids in white cross vane funnelled bucket traps, baited with the newly calculated blend, was set up in the weed field at EMR. The two treatments were microcapillary reservoir lures containing: 1) 10% 3-component mix (HB: E2HB:KA) in sunflower oil, release rate approx. 1 µg h⁻¹, and 2) sunflower oil only. There were 10 replicates of each treatment positioned alternately and spaced 10 m apart. Numbers, sex and species of mirid were recorded.

**Field trial 2 – trap design**
To test the efficacy of different trap designs for capturing mirids, small scale randomised block field experiments comparing trap designs were set up in the weed field, at EMR. Green, 20 x 20 cm, delta traps with a sticky base were compared to bucket traps with 250 ml water and a drop of detergent added. The traps were positioned at weed flower height. Mature virgin females were contained in a cage (hair roller with gauze around the outside and a lid at either end, holding the gauze in place) with damp paper to maintain humidity and a section of bean as food. A cage was placed into the top (lid) of each trap. Cypermethrin was sprayed onto the surface of the sticky base at 0.35 l ha⁻¹ for treatment C. The clear delta trap was made of vinyl sheets held together with a paper binder (Table 1).

A second field trial compared green delta traps, clear delta traps, sticky stake traps and white cross vane funnelled bucket traps (Agrisense) with commercially available pre-mounded different coloured cross vane bucket traps (Agralan) (Table 2). There were 4 replicates of each trap, spaced more than 10 m apart. Traps were checked and females changed every week. Numbers, sex and species of mirid were recorded. Count data for all trials were Log₁₀ transformed for ANOVA.

### Table 1. Trap designs used in first trial, 27 June – 11 July.

<table>
<thead>
<tr>
<th>Code</th>
<th>Trap</th>
<th>Capture device</th>
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</thead>
<tbody>
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<td>A</td>
<td>Green delta</td>
<td>original sticky base</td>
</tr>
<tr>
<td>B</td>
<td>Green delta</td>
<td>‘new’ base</td>
</tr>
<tr>
<td>C</td>
<td>Green delta</td>
<td>original base with cypermethrin sprayed on</td>
</tr>
<tr>
<td>D</td>
<td>Green delta</td>
<td>original sticky base + ecotac</td>
</tr>
<tr>
<td>E</td>
<td>Cross vane funnel bucket trap</td>
<td>Water with detergent</td>
</tr>
<tr>
<td>F</td>
<td>Cross vane funnel bucket trap</td>
<td>lambda-cyhalothrin cross veins + water with detergent</td>
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<td>G</td>
<td>Clear delta trap</td>
<td>Sticky base</td>
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Table 2. Trap designs used in second trial, 27 August – 1 September.

<table>
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</thead>
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<td>Green delta</td>
<td>sticky base with Ecotac</td>
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<tr>
<td>I</td>
<td>Clear delta trap</td>
<td>sticky base with Ecotac</td>
</tr>
<tr>
<td>J</td>
<td>White cross vane funneled bucket (Agrisense)</td>
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<td>K</td>
<td>Premoulded green cross vane funnel bucket trap (Agralan)</td>
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<tr>
<td>L</td>
<td>Premoulded white cross vane funnel bucket trap (Agralan)</td>
<td>water with detergent</td>
</tr>
<tr>
<td>M</td>
<td>Premoulded yellow cross vane funnel bucket trap (Agralan)</td>
<td>water with detergent</td>
</tr>
<tr>
<td>N</td>
<td>Sticky stake trap</td>
<td>Ecotac</td>
</tr>
</tbody>
</table>

Results and Discussion

Field trial 1 – based on daytime entrainments
Significantly more male *L. rugulipennis* males were captured in traps baited with pheromone lures compared to the control ($P=0.018$, LSD=0.2009, Fig. 1). Numbers of female *L. rugulipennis* were not significantly higher in the pheromone treated baited traps ($P=0.502$, LSD=0.1465, Fig. 3), indicating that the new blend of compounds, based on daytime collections of volatiles, was functioning as a female sex pheromone. Numbers of capsids had begun to decline in the field by this time so further work is needed to confirm these results.

Figure 1. Numbers of male and female *Lygus rugulipennis* trapped (27 August – 1 October) using the new pheromone blend based on daytime entrainments.
Field trial 2 – trap design

Although there were no significant differences between trap types ($P=0.360$, LSD$=0.1934$), the funnel traps (white or llambda-cyhalothrin cross vanes) captured more male *L. rugulipennis* than the green delta sticky trap. However, the llambda-cyhalothrin cross vane trap only caught mirids in the 4th (final) week of the experiment (Fig. 2). There were significantly more mirids captured in the green cross vane funnelled bucket traps compared to the green or clear delta traps ($P=0.121$, LSD$=0.7541$, Fig. 3). Green traps (sticky cylinders) were also effective at capturing *Lygus* males in trails by Blackmer, *et al*., (2008).

Acknowledgements

Thanks to Defra, Horticultural Development Company, GlaxoSmithKline Blackcurrant Grower’s Research Fund, GlaxoSmithKline, East Malling Trust, East Malling Ltd., Agrisense, Cucumber Growers Association, K G Growers Ltd. and Donald J Moor for funding this project (HortLINK project HL0184). We owe gratitude to the Project Co-ordinator, Mr Tom Maynard and other researchers on the project, Ms L Amarrawardana (NRI) and Mr A L Harris, (EMR).

References

Interactions among predatory insects in strawberry production

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Abstract: A range of pest species are important in strawberry and can cause serious damage to the plants and the fruit. Several species of thrips are found on strawberry, and western flower thrips, Frankliniella occidentalis, is particularly difficult to control as it is resistant to most available insecticides. The strawberry aphid, Chaetosiphon fragaefolii, is a virus-vector and honeydew produced by the aphids also causes fruits to become sticky and unmarketable. Feeding by the capsid Lygus rugulipennis on developing fruits causes severe malformation of the fruit. Many predatory insects found in strawberry plantations consume a range of prey species and can thus contribute to biocontrol of pests. However, the availability of ‘alternative’ prey species may affect the degree of control the predators exert over particular pest species. In this project we examined the interactions among aphid, capsid and thrips and predatory arthropods in strawberry, to provide the basic information needed to optimise the biological components of pest management systems, thus reducing pesticide use. In laboratory experiments to determine the biocontrol potential of predators, 1st instar C. carnea larvae and adult female O. laevigatus consumed similar numbers of 3rd instar C. fragaefolii. 1st instar C. carnea consumed fewer F. occidentalis than did O. laevigatus adults. C. carnea and O. laevigatus consumed similar numbers of 1st instar L. rugulipennis. The potential of C. carnea and O. laevigatus to significantly reduce numbers of C. fragaefolii, F. occidentalis and L. rugulipennis when each pest was presented alone was demonstrated in laboratory experiments. However, when combinations of predators were present, biocontrol of pest species was reduced in some cases due to predator interactions.

Frankliniella occidentalis, Chaetosiphon fragaefolii, Lygus rugulipennis, Biocontrol
Developing Integrated Pest Management programmes for protected strawberry crops in Southern France

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Abstract: Integrated Pest Management (IPM) strategies which have been effective in the UK were tested in tunnel grown strawberry crops at Hortis Aquitaine, Southern France, from March to October 2008. The cost and pest control effect of two IPM strategies were compared in separate tunnels, one equipped with a misting system and the other not. Thrips, Frankliniella occidentalis, were effectively controlled by either Amblyseius cucumeris combined with Orius laevigatus in the misted tunnel or A. swirskii and Orius laevigatus in the non-misted one. Neither tunnel required chemical intervention against thrips, and control was very good compared to chemical programmes. Spider mites, Tetranychus urticae, were effectively controlled in both tunnels by Phytoseiulus persimilis together with a single treatment of hexythiazox (Nissorun®). Four different aphid species occurred in the trials. Aphidius colemani achieved some control of Aphis gossypii but Aphidoletes aphidimyza failed to establish and a single pirimicarb (Pirimor G®) was used. Further trials are recommended to develop effective aphid control. The use of selective chemicals in the IPM programmes allowed the invasion of naturally occurring predators which helped control pests. All pests were effectively controlled in the IPM tunnels and fruit quality was good. The numbers of chemical treatments were significantly reduced in comparison to an adjacent tunnel where pests were controlled using insecticides. In this tunnel, pest numbers increased rapidly and ten insecticide treatments were required over two months to achieve some control. Different rates and timings were proposed to ensure an economic programme for growers.

Key words: Biological Control, protected strawberries, France, thrips, spider mites, aphids, Integrated Pest Management.

Introduction

Strawberry growing systems are very varied in France, from open field to crops on hanging gutters under glass. In the South West, the main production region, there are around 1500 ha of strawberries, including approximately 350 ha on hanging gutters. Growers tend to be small with an average of 1.5 ha of strawberries, grown mainly under tunnels. Most growers distribute to specific wholesalers via their "grower organizations". A lack of chemicals is forcing them to move towards IPM (Trottin-Caudal et al, 2002). Fewer products are registered in strawberries and registered products are becoming less effective due to resistance. Consumer pressure for pesticide-free produce also has a major influence on growers.

In 2008, as part of the BCP Certis European wide trials programme, IPM strategies that have been effective in the UK (Sampson, 2007) were tested in tunnel-grown everbearer strawberry crops at Hortis Aquitaine, in the South of France. Thrips, aphids and spider mites are the most common and damaging pests. The small-scale trial compared the cost and effectiveness of two IPM strategies in two tunnels; tunnel A, equipped with a misting system and tunnel B, without. The objective of this trial was to see whether UK strategies achieve cost effective pest control in South West France and to determine whether Amblyseius swirskii
establishes and achieves thrips control in the warmer and drier conditions found there.

Materials and methods

The trial was carried out in two 200m² tunnels, each planted on 18th March 2008, with six rows of everbearer strawberries (cvs. Mara des Bois, Charlotte and Cirafine) grown in peat on hanging gutters. Only tunnel A was equipped with a misting system.

The timing and numbers of pest control treatments are detailed in Table 1. The generalist aphid predator *Aphidoletes aphidimyza* was released preventatively against all aphid species and the parasitoid *Aphidius colemani* was released once *Aphis gossypii* was observed. *Phytoseiulus persimilis* was released once *Tetranychus urticae* was observed. Thrips predators were released preventatively.

Table 1. The timing and numbers of pest control treatments.

<table>
<thead>
<tr>
<th>Pest</th>
<th>Date(s)</th>
<th>Beneficials/spray</th>
<th>Tunnel A (misted)</th>
<th>Tunnel B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrips</td>
<td>09/04/08</td>
<td><em>Hypoaspis miles</em></td>
<td>150/m²</td>
<td>150/m²</td>
</tr>
<tr>
<td></td>
<td>16/04/08</td>
<td><em>Amblyseius cucumeris</em> (sachets)</td>
<td>1/2 linear m</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16/04/08</td>
<td><em>Amblyseius swirskii</em> (sachets)</td>
<td>-</td>
<td>1/2 linear m</td>
</tr>
<tr>
<td></td>
<td>14/05/08, 18/06/08</td>
<td><em>Orius laevigatus</em></td>
<td>1.25 /m² x 2</td>
<td>1.25 /m² x 2</td>
</tr>
<tr>
<td></td>
<td>29/05/08, 16/07/08</td>
<td><em>Amblyseius cucumeris</em> (sprinklers)</td>
<td>125 /m² x 2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>29/05/08, 16/07/08</td>
<td><em>Amblyseius swirskii</em> (sprinklers)</td>
<td>-</td>
<td>50 /m² x 2</td>
</tr>
<tr>
<td>Aphids</td>
<td>09/05/08 to 09/07/08</td>
<td><em>Aphidoletes aphidimyza</em></td>
<td>2.5 /m² x 7</td>
<td>2.5 /m² x 7</td>
</tr>
<tr>
<td></td>
<td>20/05/08 to 09/07/08</td>
<td><em>Aphidius colemani</em></td>
<td>1.25 /m² x 5</td>
<td>1.25 /m² x 5</td>
</tr>
<tr>
<td></td>
<td>27/06/08</td>
<td><em>Pirimor G®</em> (pirimicarb)</td>
<td>Spot treatment</td>
<td>Spot treatment</td>
</tr>
<tr>
<td>Spider mites</td>
<td>14/05/08 to14/07/08</td>
<td><em>Phytoseiulus persimilis</em></td>
<td>2.5 /m² x 3</td>
<td>2.5/m²x2, 5/m²</td>
</tr>
<tr>
<td></td>
<td>04/07/08</td>
<td><em>Nissorus® (hexythiazox)</em></td>
<td>1.25/m², 5/m²</td>
<td>7.5/m² x 2</td>
</tr>
</tbody>
</table>

In each tunnel, 25 plants were selected at random and monitored weekly. One flower and two leaves (one old, one young) were selected per plant and the numbers of thrips and beneficials counted. Aphids, spider mites and whiteflies were recorded using a scale: 0 : 0 individual ; 1 : 1 to 3 individuals ; 2 : 4 to 10 individuals ; 3 : 11 to 30 individuals ; 4 : >30 individuals. Presence and absence of all species was recorded on cores, flowering stems and white fruits.

Results and discussion

**Thrips control**

Thrips and predator numbers are shown in Figures 1 (Tunnel A) and 2 (Tunnel B). Thrips numbers were higher in the drier, non misted tunnel with *A. swirskii*, but both *Amblyseius cucumeris* and *Amblyseius swirskii* established in the separate tunnels to control thrips and prevent fruit damage. *A. swirskii* built up more rapidly than *A. cucumeris*, confirming that lower release rates are required. *Orius laevigatus* took longer to establish than expected. This was probably because there were no flowers and few thrips at the time of the first release, leading to starvation or dispersion. It is probably advantageous to wait for flowering before *Orius* release so that they can survive by feeding on pollen.

*Hypoaspis miles* were not recovered after release. In the U.K., *Hypoaspis* is released in glasshouse crops to reduce sciarid fly and thrips numbers. However they are not usually
needed in shorter season tunnel grown crops. Further trials will be done without *Hypoaspis*, to reduce costs.

Predators successfully controlled this major pest without the need for insecticides. No damage was observed on fruits, which were of good quality. In contrast, in an adjacent tunnel where no predators were released, three insecticide treatments were required to control thrips and the crop had to be stopped early in mid July due to pest damage and poor quality plants.

**Figure 1.** Thrips and beneficial populations in the misted tunnel A, with *A. cucumeris*.

**Figure 2.** Thrips and beneficial populations in the unmisted tunnel B, with *A. swirskii*.

**Aphid control**
The most common aphid species were *Macrosiphum euphorbiae*, *Rhodobium porosum*, *Aphis gossypii* and *Aulacorthum solani*. Despite natural enemy releases, aphid numbers built up during May and June, and a spray with pirimicarb was required (Figure 3). The pattern was similar in both tunnels, so only Tunnel B is shown. Although *Aphidius colemani* established, it is host specific and did not control the potato aphids (*Macrosiphum* and *Aulacorthum* spp.). *Aphidoletes aphidimyza* was released to control these species, but no larvae were recovered. This was probably because night temperatures were exceptionally low in 2008. Minimum temperatures remained < 10°C in April and < 15°C in May. A failure to establish was also observed at nearby growers, where *Aphidoletes* usually establishes. The fungicides used in these trials (penconazole, myclobutanil, azoxystrobin, thiophanate-methyl) should only have had a limited impact on *Aphidoletes* establishment. From July, natural enemy numbers increased rapidly and all aphid species were controlled for the rest of the crop. Aphid control was aided by natural invasions of lacewings (*Chrysoperla*) and hoverflies (*Syrphids*).
Figure 3. Aphid and natural enemy numbers in the non-misted Tunnel B.

Spider mite control
Spider mites were first seen at the beginning of May. They increased more rapidly in the non-misted tunnel B, affecting 50% of plants (12% with 11-30 mites per plant) when numbers peaked in early July, compared to 14% of plants (2% with 11-30 mites per plant) in the misted tunnel A. *Phytoseiulus persimilis* established and reduced spider mite numbers in both tunnels, but a single spray with hexythiazox (Nissorun®) was necessary to reduce numbers. In this trial, *Phytoseiulus* was released into hot-spots, but experience suggests that blanket treatments would achieve better control. This will be trialed in 2009.

Cost of control
The total costs were 1.4 € /m² and 1.6 € /m² for the *A. cucumeris* and *A. swirskii* strategies respectively. These costs could be reduced by 0.5 € /m² by removing *Hypoaspis miles* and reducing the *Orius* and *Aphidoletes* releases. The resulting 0.9 € /m² to 1.1 € /m² would be acceptable for growers.

The trials demonstrated that thrips and spider mites can be controlled effectively and economically in S. France using biological control agents. Together with *O. laevigatus*, both *A. swirskii* and *A. cucumeris* established and controlled thrips without the need for chemical intervention. *P. persimilis* controlled spider mites with the aid of a single insecticide treatment although blanket rather than spot treatments should improve control. The numbers of chemical treatments were reduced from ten to two, in comparison to an adjacent tunnel where plants were grown without biological control. Further trials are needed to fine tune the programme and to improve biological control against the range of aphid species present.

References
Alternative methods to reduce storage decay in organic apple production; time of harvest and calcium applications

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Abstract: In Norway, organic apple growers only have sulphur available as a fungicide. When organically grown apples are stored, growers must thus rely entirely on alternative means to reduce the amount of storage decay. It is known that harvest time and calcium content may affect fruit rots in apple. The effect of harvest time on storage decay was assessed during three years. After storage there was a clear increase in fruit decay from the earliest to the latest picking times, both recorded as total decay and for the important storage diseases bitter rot (caused by *Colletotrichum acutatum*) and lenticell rot (caused by either *Phlyctaena vagabunda* or *Cryptosporiopsis curvispora*). In mean of three years apples of cv. Aroma harvested 2 or 1 week prior to normal harvest time, at normal harvest or 1 or 2 weeks afterwards and stored for three months in a ventilated cold store, had 6, 14, 35, 33, and 35% bitter rot, respectively. Similar numbers for lenticell rot (in mean of two years) were 6, 10, 11, 16 and 24%, respectively. Applications of calcium at different times prior to harvest reduced the amount of storage decay in some trials, but not consistently.

Keywords: Bitter rot, *Colletotrichum acutatum*, *Cryptosporiopsis curvispora*, lenticell rot, *Phlyctaena vagabunda*

Introduction

The most important storage diseases in apple in Norway are bitter rot, caused by *Colletotrichum acutatum*, and lenticell rot, caused by either *Phlyctaena vagabunda* or *Cryptosporiopsis curvispora*. In organic apple production in Norway sulphur is the only available fungicide, and alternatives are needed in order to control storage decay. Harvest time is known to influence development of storage decay. Late harvested apples of a cultivar generally develop more fruit rot than earlier harvested apples of the same cultivar. However, results obtained in experiments have varied depending on cultivar tested, location, year and diseases present (Gulanduzzi et al., 2005; Landfald, 1981, Neri et al., 2005; Valiuskaite et al., 2006; Wilkinson & Sharples, 1967). In England, development of lenticell rot on apples inoculated with *Gloeosporium perennans* (*Cryptosporiopsis curvispora*) was promoted by delayed harvest time (Edney, 1964). It has been shown that applications of calcium may prevent fruit decay due to fungal diseases. Apples treated three times with calcium chloride in the growing season developed less bitter rot (*Colletotrichum* spp.) after storage than if not treated (Biggs, 1999). The main cultivar in organic and in convention apple growing in Norway is Aroma. Cv. Aroma is scab tolerant but very susceptible to storage decay. The objectives of the present experiments were to evaluate the effects of harvest time and calcium applications on storage decay in organically grown apples of cv. Aroma.
Materials and methods

Harvest time
During 2004-2006 apples of cv. Aroma were picked over a five week period in an organic orchard. There were regular treatments with sulphur against apple scab in spring and early summer, but no other treatments against storage rots. Harvest started 2 weeks prior to optimal harvest date according to prognoses from the advisory service in the area. Fruits were then harvested 1 week before normal harvest time, at normal harvest, or 1 or 2 weeks after normal harvest. Each time three replicates of 65 apples in each were picked at different locations in the orchard. Sixty apples were laid separately in boxes and stored in a ventilated cold store (4°C). The other 5 apples from each replicate were used to measure fruit quality; fruit firmness, background and cover colour, sugar content, and starch content. Streif indexes (Streif, 1996) were calculated as firmness/(starch*sugar). Monthly (four times) during storage the fruits were assessed for symptoms of storage diseases. Each time five apples were taken out from each replicate for measurement of fruit quality as mentioned above, but now also including titratable acid content. February 1 the remaining 45 apples were taken out of cold store and assessed for storage diseases after 2 weeks at 20°C.

Calcium experiments
In 2005 three or six applications with calcium chloride was compared with an apple scab spray programme with dithianon (Delan WG, BASF Agro BV, Arnhem, The Netherlands) in a conventional field where other fungicide applications ceased at time of bloom (May 23). In 2006, applications with sulphur in spring and early summer against scab were combined with three applications with either calcium hydroxide (350g of 95% CaOH /100L water) or calcium chloride (500g of 77% CaCl2/100L) against storage diseases. Controls were either sulphur applications against scab in spring and early summer or unsprayed. Experiments were carried out in an organic orchard. Both years the apples were picked at time of optimal harvest according to fruit quality measures and stored for 4 months at 4°C and 2 weeks at 20°C. Quality and disease assessments were done as in the harvest time experiment.

Results and discussion

Harvest time
Harvest started between September 12 and 20, from 1656 to 1794 degrees days after full bloom all three years. Background colour increased from first to last picking in the first two years. In the third year the background colour was not measured at the last picking, and no difference was found from first to fourth picking. Cover colour was significantly higher on the last picking compared to the first all three years. Difference in calculated Streif-index at harvest from first to last picking was 0.04, 0.13 and 0.22 in 2004, 2005 and 2006, respectively (Fig. 1). At end of storage no or only minor differences in fruit quality parameters were detected, and apples from all picking times had fruit quality within the limits for eating quality at end of storage (data not shown). Data for monthly assessments of Streif-index during the storage period are not included here.

In mean of three years fruit decay at end of cold storage was 3 % for the first picking and 20% for apples picked 2 weeks after normal (Fig. 2). Apples picked one or two weeks after normal harvest time developed significantly more decay than apples picked at normal harvest time or before (Fig. 2). Most of the decayed apples at that time could still have been used for juice production, because the lesions were small. Total fruit decay after two weeks at room temperature in mean of the three years was 18 and 64% on apples from first and last picking, respectively (Table 1). There was a gradual increase in total fruit rot from the first to the last
picking date. Incidence of bitter rot was significantly higher on apples picked after and at normal picking time compared to the two earliest harvests. For lenticel rot there was a significant difference between the first and the last picking time. Organically grown apples are rarely stored over long time, because of the risk of storage decay (Maxin et al., 2005). These trials show that such apples can be stored over several months in a normal cold store if they are picked early enough, but also that they may have to be consumed relatively quickly after they are brought out of storage.

**Calcium experiments**
Different calcium treatments performed over two years did not give a consistent effect against storage decay. Experiments will be carried out in several more orchards, and number and timing of applications need to be further investigated before we can recommend calcium sprays as a management strategy against storage diseases.

**Acknowledgements**

The Norwegian Ministry of Agriculture and Food supported the experiments financially. Jan Ove Nes kindly let us pick apples in his orchards. Thanks also to technicians at Ullensvang and Njøs research stations for valuable assistance.

**References**

Table 1. Fruit decay in organically grown apples of cv. Aroma stored 4 months at 4°C and 2 weeks at 20°C. Mean of three years with trials; three replicates of 40 apples for each harvest time; 2 or 1 week before normal harvest time, at normal harvest, or 1 or 2 weeks after normal harvest.

<table>
<thead>
<tr>
<th>Time of picking as related to normal harvest time</th>
<th>Total fruit rot (%)</th>
<th>Bitter rot (%)</th>
<th>Lenticell rot (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks before</td>
<td>18.3 d</td>
<td>6.1 b</td>
<td>4.2 b</td>
</tr>
<tr>
<td>1 week before</td>
<td>32.8 c</td>
<td>13.6 b</td>
<td>8.6 ab</td>
</tr>
<tr>
<td>Normal</td>
<td>46.4 b</td>
<td>33.3 a</td>
<td>7.8 ab</td>
</tr>
<tr>
<td>1 week after</td>
<td>57.5 ab</td>
<td>35.0 a</td>
<td>9.2 ab</td>
</tr>
<tr>
<td>2 weeks after</td>
<td>63.9 a</td>
<td>33.3 a</td>
<td>15.6 a</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 1. Streif-values at harvest of organically grown apples of cv. Aroma harvested over 5 weeks in 2004-2006. Mean of three years of trials; three replicates of 5 apples for each harvest time; 2 or 1 week before normal harvest time, at normal harvest, or 1 or 2 weeks after normal harvest.

Figure 2. Incidence (%) of fruit decay in organically grown apples of cv. Aroma harvested over 5 weeks and stored for 4 months at 4°C; assessments made directly after the apples were brought out of cold storage. Mean of three years with trials; three replicates of 40 apples at each harvest time; 2 or 1 week before normal harvest time, at normal harvest, or 1 or 2 weeks after normal harvest.
Sources of inoculum of Colletotrichum acutatum in cherry and apple

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Abstract: Colletotrichum acutatum causes bitter rot (often named anthracnose) in cherry and apple. It is the most important fruit decay in sour cherry in Norway and may give severe losses also in sweet cherry and apple. We have found the fungus in all fruit and berry crops grown commercially in the country and on many ornamentals and a few weeds. Single spore isolates frequently developed the ascigerous stage of the fungus (Glomerella acutata) in culture, but it was not detected on apple or cherry plant material. If still attached to the tree, fruits and fruit stalks of sour cherry infected the previous year produced conidial inoculum throughout the entire following season. Also newly infected sour cherry flowers produced conidial inoculum until harvest. Up to 80% of the fruit spurs on sweet cherry had buds infected with C. acutatum in spring. Apple buds also contained the fungus, but to a much lower extent. More than 90% of the sweet cherry leaves could be infected with C. acutatum around harvest in heavily infected orchards. Symptoms on leaves never appeared in the orchards. We also found such asymptomatic leaf infections in apples. Most of the inoculum seemed to be present on the fruit trees themselves. However, initial inoculum in newly established, disease free plantings may be introduced from older fruit trees, ornamentals and weeds in or in close vicinity to the orchards.

Key words: Anthracnose, bitter rot, Glomerella acutata

Introduction

Anthracnose, often named bitter rot, may cause complete yield loss in sour cherry (Prunus cerasus) in Norway, but severe losses have also occurred in sweet cherry (P. avium) and apple (Malus domestica). The disease is caused by the ascomycete Glomerella acutata (imperfect stage: Colletotrichum acutatum). The sexual stage has only been documented once in nature, on highbush blueberry in Norway (Talgø et al., 2007). The fungus may attack all fruit and berry species and is commonly found in many ornamental and weed species. In cherry, bitter rot develop fruit symptoms very late, often just prior to or even after harvest, and its ability to ruin the crop after harvest may have negative implications for the marketing (Børve & Stensvand, 2008). In warmer fruit growing regions bitter rot typically develops during mid to late summer in apple (Crusius et al., 2002; Everett et al., 2006; Sutton, 1990), while in Norway it is mostly known as a storage disease although it may appear prior to harvest (Børve et al., 2008). The present work took place over several years to investigate how C. acutatum overwinters and what inoculum sources are present under Norwegian orchard conditions. Some of these findings have been presented elsewhere (Børve & Stensvand, 2006; 2007; 2008; Børve et al., 2008; Stensvand & Børve 2006, Stensvand et al., 2006; 2008).

Materials and methods

Various cherry and apple plant material was collected and either incubated directly in saturated air at 20-25°C for 1 to 3 weeks (dormant leaf and flower buds, old infected fruits and fruit stalks, flowers, fruits, shoot pieces) or treated with paraquat (Cook 1993, EPPO
Results and Discussion

*C. acutatum on different plant species*
In Norway, we have detected *C. acutatum* in all fruit and berry species grown commercially in the country, i.e. apple, pear (*Pyrus communis*), sweet cherry, sour cherry, plum (*Prunus domestica*), strawberry (*Fragaria × ananassa*), raspberry (*Rubus idaeus*), blackberry (*Rubus fruticosus*), black currants (*Ribes nigrum*) and highbush blueberry (*Vaccinium corymbosum*). Furthermore, we have found it on the following genera of ornamentals: *Aesculus*, *Alnus*, *Forsythia*, *Ilex*, *Juglans*, *Laurocerasus*, *Maespilus*, *Magnolia*, *Populus*, *Rhododendron*, *Sorbus* and *Tilia*. Finally, the fungus has been detected in the following genera of herbs/weeds: *Geum*, *Rumex*, *Taraxacum* and *Urtica*. It has been most damaging in sweet and sour cherry, apple and strawberry.

*Glomerella acutata*
Approximately 10% of our single spore isolates have developed perithecia, the ascigerous stage of the fungus in culture. Most of these were originally isolated from apple. When looking at genetic variation among isolates, we also see that a group of isolates, mainly from *M. domestica*, showed large variability, indicating that sexual reproduction also occurs in nature. We have not detected the perfect stage of the fungus on apple or cherry plant material. We thus anticipate that the predominant inoculum source is conidia.

*Buds*
We have detected *C. acutatum* in bud scales of sweet and sour cherry and apple. When investigating 13 different site/cultivar/year combinations in commercial and research sweet cherry orchards, we found that a mean of 43% (varied from 4 to 80%) of the fruit spurs contained one or more buds with *C. acutatum*. In 13 site/cultivar/year combinations in sour cherry, we found a mean incidence of 37% (varied from 5 to 72%) flower and leaf bud infections. In apple, the frequency of infected buds was much lower, but it was detected in 7 of 17 site/cultivar/year combinations. Nine (1.3%) of a total of 689 apple flower buds were infected.

*Old fruits and fruit stalks*
Old infected fruits and fruit stalks were collected in late autumn and either placed on the ground in autumn or spring or left hanging in the trees. From time of bud break until after harvest, fruits and fruit stalks were collected at two-week intervals and investigated for quantity of conidia of *C. acutatum*. Experiments took place over three years. By mid June there were very few conidia left in plant material on the ground. For fruits and fruit stalks left hanging in the trees, a substantial quantity of conidia was formed beyond harvest. If not removed during harvest or pruning, old fruits and fruit stalks may remain attached for a long time into the next growing season.

*Flowers and green leaves*
Newly infected flowers of sour cherry produced conidia until harvest. Green leaves of sweet cherry, sour cherry and apple all contained asymptomatic infections of *C. acutatum*. In three different sweet cherry orchards observed over three years, a mean of 45 and 34%, respectively, of leaves on fruit spurs and vegetative shoots contained *C. acutatum*. There was a gradual build-up of both incidence and severity on sweet cherry leaves during two months.
prior to harvest. In the most severely attacked orchard more than 90% of the leaves were infected.

**Wood**

We observed infections in one year old wood tissue in all three fruit crops.

**Potential inoculum sources**

Old fruits, fruit stalks, wood, bud shells and green leaves all have asymptomatic infections and may be easily overlooked as important sources of inoculum. They all produce inoculum in close vicinity to the new susceptible tissue. A conidium only needs to travel a few millimetre or centimetre from the source to the new tissue. Thus inoculum from within the trees and the orchards are probably the most important source of inoculum in orchards where the fungus is already established. For newly established disease free orchards, outside sources like old orchards of all fruit species, berry crops, many ornamentals and weeds should be considered potential inoculum sources.

**References**


Early season control of storage rots of apple

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Abstract: Fungal rots cause significant losses in stored apples. Until recently rotting in stored apples was controlled primarily in the UK by post harvest fungicide drenches. This practice is no longer acceptable because of the likely presence of a fungicide residue in the fruit, which, although usually below the MRL, is not acceptable to consumers. In addition, Nectria and other rots are poorly controlled by post-harvest fungicide drenches. Alternative approaches for control of Nectria rots are based on identifying rot risks. Fruits are stored only short term whenever a high risk is predicted. Alternatively, protectant fungicides are applied in July/August, which may also lead to detectable residues in fruit. The results of limited orchard trial in the 1990s, however, indicated that application of carbendazim during blossom and petal-fall significantly reduced the incidence of Nectria rot in store. The mechanism for this is not understood but could be due possibly to the reduction in Nectria inoculum from cankers or to the protection of fruit at a key infection stage. The purpose of this work was to understand this mechanism and examine whether other potential rots (e.g, Gloeosporium or Botryosphaeria) could also be controlled similarly. Orchard trials were also established to identify alternative fungicides to carbendazim. Effective control of Nectria and other rots by application of fungicides at blossom and petal fall would also minimise the risk of residues in fruit at harvest.

Apple, canker, Nectria galligena, storage rot, fungicide
Utilization of Mating Disruption and Codling Moth Granulosis Virus (CpGV) in Commercial Apple Orchard in Pennsylvania, USA.

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Abstract: During the last five years, codling moth, *Cydia pomonella* L., reestablished itself as the dominant direct fruit pest in most apple orchards in Pennsylvania, USA. Together with the Oriental fruit moth, *Grapholita molesta* (Busck), and the eastern USA leafroller complex, the codling moth has become the driving force for insecticide treatments applied in orchards. When the codling moth developed resistance to older insecticides, it forced growers to seek new methods to control this pest and adopt newer methods such as mating disruption or bio-rational compounds to provide adequate control. Although both tactics have been used for a long time in organic orchards, no experience existed in conventional orchards in Pennsylvania. Therefore, a multi-year project was initiated to evaluate such methods in conventional orchards where both methods were incorporated into standard pest control practices. During three consecutive seasons, various rates and combinations of the codling moth granulosis virus (CpGV) and mating disruption were utilized in orchards and provided excellent control of internal fruit feeders, even when CpGV was applied as alternate row middle applications. CpGV laboratory and field bioassays conducted on apples and nectarines revealed a toxicity of the codling moth granulosis virus against neonates of Oriental fruit moth.

Key words: fruit pests, biological control, Oriental fruit moth,

Introduction

During the last five years, the codling moth (CM), *Cydia pomonella* L., reestablished itself as the dominant fruit pest in most apple orchards in Pennsylvania, USA. Together with the Oriental fruit moth (OFM), *Grapholita molesta* (Busck), the codling moth has become the driving force for insecticide treatments applied in orchards. Since 1998, multiple loads of apples destined for the processing markets were rejected by USDA inspectors for the presence of CM and OFM in the fruit (Krawczyk 2006).

The main reasons cited for this continuous pest outbreak include insecticide resistance (i.e., organo-phosphates), the loss and/or restrictions on insecticides due to the Food Quality Protection Act, or issues related to the efficient applications of insecticides (Krawczyk 2006). In order to overcome some of these issues, new approaches were extensively evaluated during the last few years. One management tactic that has succeeded in controlling CM and OFM throughout the U.S. while minimizing the use of insecticides is mating disruption (MD) using synthetic sex pheromones to control pest insects (Hull and Krawczyk 2006).

Another novel technology that has become available for both conventional and organic growers to manage CM is a naturally occurring CM granulosis virus (CpGV) commercially available in two products – Cyd-X® (Certis USA) and Carpovirusine® (Arysta LifeScience). Recently, CpGV became widely utilized in many conventional orchards. Once the larva ingests the virus, the occlusion bodies are dissolved. The virus penetrates the gut lining, replicates itself and then spreads to other organs (Lacey and Shapiro-Illan 2008).

In this report we discuss the results of field evaluations of the CM granulosis virus and
mating disruption products in commercial orchard settings in Pennsylvania, USA from 2005 to 2007. Also, due to the continuous pressure in Pennsylvania orchards from OFM, we present results of laboratory/field bioassays evaluating the effect of CpGV on this pest.

Materials and methods

Field trials 2005-2007
An apple orchard near Arendtsville, PA was selected for this study. Prior to the study, the orchard had a history of problems with internally fruit feeding Lepidopteran larvae, primarily CM but also OFM. The orchard was divided into three blocks with continuous, although yearly changing, treatments. In 2005, Block 1 (about 4.9 ha) was treated with Cyd-X, at the rate of 150 ml/ha/side for both broods (total 11 applications, 1650 ml per season) in combination with Isomate CM TT (CBC America) at the rate of 500 dispensers/ha; Block 2 (about 4.5 ha) was treated with Isomate CM TT plus conventional insecticides; and Block 3 (2.8 ha) was treated with conventional insecticides only. In the 2006 season, Block 1 was treated with a low rate of Cyd-X (37.5 ml/ha/side) for both broods of CM (total 10 applications; 375 ml per season) and an application of dual dispensers Isomate CM/OFM TT (CBC America) (500 dispensers/ha); Block 2 was treated with a higher rate of Cyd-X (75 ml/ha/side) for both broods of CM (10 applications, 750 ml/season) and Isomate CM/OFM TT; and the Block 3 was treated with a conventional program. In the 2007 season, Block 1 received Cyd-X at the rate of 75 ml/ha/side applied for the second brood of CM (2 applications; 150 ml/season) and Isomate CM/OFM TT; Block 2 received Cyd-X for both broods of CM (5 applications; 375 ml/season) also in combination with Isomate CM/OFM TT; and Block 3 was treated with a grower’s choice insecticide program.

All applications were applied with an airblast sprayer calibrated to deliver 472 liters of solution. All Cyd-X and insecticide sprays were made as alternate row middle (ARM) applications and the amount of Cyd-X applied is listed as the amount/ha/side of the tree. The Isomate dispensers were hung in the top one-third of the trees within 10 days of CM biofix. The trees in Block 1 and 2 were 2.5-3.5 m high; in Block 3 were about 3.5-4 m in height.

Various combinations of large plastic Delta-style pheromone traps (Pherocon VI) baited with either CM 1X Long-Life® lures, or CM-DA combo lures, or CM 10X Megalures® (Trece Inc., Salinas, CA) were deployed in each block. All CM traps were hung in the top 20% of the tree canopies, about 70 meters apart. For OFM monitoring, two Delta-style pheromone traps with Trece OFM Long-Life® lures were also hung in the center of each block. OFM traps were hung at a height of 2 m above the ground. Traps for each species were placed in the orchard before expected biofixes. Traps were monitored weekly.

Numbers of CM injured fruit were estimated based on in situ evaluations within each treatment block during the mid-season and at harvest. At each sampling date a group of 100 or 200 apples were examined on 25-50 trees per block. A total of 4000 to 5000 apples were examined per treatment during each visual search. All larvae found within injured fruit were identified to species.

Field/laboratory bioassays: Three apple and three nectarine trees were sprayed to the point of drip with a solution of Carpovirusine prepared at the equivalent rate of 1 liter/ha using a Solo backpack sprayer at a pressure of 60-80 psi. Control trees were sprayed with water. Fruit from each treatment were collected at 2 hours, 3 days and 7 days after application. Treated fruit were placed in 473 ml clear plastic containers and 5 neonates of CM or OFM were placed on each fruit. At each collection time, 15 fruit were used per treatment (75 larvae). Mortality readings were conducted at 7-8 days after the placement of larvae on fruit recording the number of entries per fruit and then by searching for live larvae.
Results and Discussion

During each year of the project the combination of CpGV and mating disruption treatments provided effective control of CM and OFM (Table 1). During the first year of the project the treatment of Isomate CM TT plus insecticides (Block 2) had the fewest fruit injured by CM. The treatment of Isomate CM TT plus Cyd-X (Block 1) was the next most effective treatment. All of the larvae found during the harvest were CM. From the 2006 study, we found that both Cyd-X and Isomate CM/OFM TT combination treatments were the most effective treatments. The conventional insecticide block was again the least effective treatment. The treatment of Cyd-X at the 37.5 ml/ha rate and MD in 2006 was intentionally placed in the block that had the lowest level of CM fruit injury in 2005. From the 2007 study, we concluded that the combination of five ARM Cyd-X sprays for first and second broods along with MD was the most effective treatment for minimizing injury from CM and OFM. The treatment of only two ARM Cyd-X sprays for 2nd brood CM along with MD was also very effective, although a slightly higher amount of fruit injury was found. The conventional insecticides for the control of CM and OFM continued to be less effective. (Table 1).

Table 1. Efficacy of CM granulosis virus CpGV (Cyd-X) and Isomate CM/OFM TT against codling moth and Oriental fruit moth in a PA apple orchard during 2005-2007.

<table>
<thead>
<tr>
<th>Year/block</th>
<th>% injured fruit</th>
<th>Retrieved CM/OFM larvae*</th>
<th>Mean no. of CM adults **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mid-season</td>
<td>harvest</td>
<td></td>
</tr>
<tr>
<td>2005/B1</td>
<td>0.00 a</td>
<td>0.43 b</td>
<td>6/0</td>
</tr>
<tr>
<td>2005/B2</td>
<td>0.00 a</td>
<td>0.04 a</td>
<td>0/0</td>
</tr>
<tr>
<td>2005/B3</td>
<td>0.00 a</td>
<td>1.52 c</td>
<td>17/0</td>
</tr>
<tr>
<td>2006/B1</td>
<td>0.00 a</td>
<td>0.00 a</td>
<td>0/0</td>
</tr>
<tr>
<td>2006/B2</td>
<td>0.00 a</td>
<td>0.18 a</td>
<td>1/0</td>
</tr>
<tr>
<td>2006/B3</td>
<td>0.03 a</td>
<td>0.43 b</td>
<td>3/0</td>
</tr>
<tr>
<td>2007/B1</td>
<td>0.00 a</td>
<td>0.15 a</td>
<td>1/1</td>
</tr>
<tr>
<td>2007/B2</td>
<td>0.00 a</td>
<td>0.03 a</td>
<td>0/0</td>
</tr>
<tr>
<td>2007/B3</td>
<td>0.40 b</td>
<td>1.08 b</td>
<td>8/0</td>
</tr>
</tbody>
</table>

Mean percent of injured fruit within the same column for the same year followed by the same letter are not significantly different (Fisher’s Protected LSD, $P \leq 0.05$). * – Number of live CM/OFM larvae retrieved during the harvest fruit evaluations. ** - Average cumulative seasonal number of CM adults collected in traps baited with a CM 1X lure in each block.

After three years of CpGV and MD use in this orchard to manage both CM and OFM we found very encouraging results for using these two tactics in combination to control these two pests even when applying CpGV as ARM applications. Block 2 over the three-year period received the most applications of Cyd-X (2005 – 11 ARM applications; 2006 – 10 ARM applications, 2007 – 5 ARM applications). This treatment regime has continually reduced CM and OFM adult populations over time while maintaining very low levels of fruit injury. The treatment regime for CM and OFM in Block1 also continued to reduce the adult populations of these two pests over time while maintaining excellent fruit protection. In 2005, Block 1 received MD and insecticides during the season – no CpGV, but in 2006 and 2007 it received MD and ARM applications of Cyd-X under a different rate and timing regime. In 2006, this block received 10 ARM applications of Cyd-X, and in 2007, this block received only 2 ARM applications of Cyd-X during the second brood of CM.

The bioassays evaluating the effect of CpGV on both OFM and CM and the persistence
of the virus in the orchard revealed high efficacy of an applied field rate on both pest species (Tab.2). While the toxic effect of CpGV on CM neonate larvae was expected, mortality of OFM was unexpected, especially at the standard field rate. On both fruits, apple and nectarine, the OFM larvae exposed to field aged residues of CpGV exhibited mortality compared to the control treatment. In another study, not reported here, no mortality of OFM was observed after exposure to CpGV residue on peach fruit (Krawczyk, unpub. data).

Table 2. Survival of CM and OFM neonate larvae after exposure to field aged residues of CpGV (Carpovirusine) during field/laboratory bioassays.

<table>
<thead>
<tr>
<th>Pest</th>
<th>Crop</th>
<th>Treatment</th>
<th>1 DAT***</th>
<th>3 DAT</th>
<th>7 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cydia pomonella</td>
<td>Apple</td>
<td>Control</td>
<td>3.40 a</td>
<td>3.67 a</td>
<td>3.47 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CpGV</td>
<td>0.93 a</td>
<td>1.73 b</td>
<td>2.53 c</td>
</tr>
<tr>
<td>Grapholita molesta</td>
<td>Apple</td>
<td>Control</td>
<td>3.00 a</td>
<td>3.40 a</td>
<td>3.47 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CpGV</td>
<td>0.47 a</td>
<td>0.67 a</td>
<td>1.47 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nectarine Control</td>
<td>2.80 a</td>
<td>3.01 a</td>
<td>2.93 a</td>
</tr>
</tbody>
</table>

Means within the same row followed by the same letter are significantly different (Fisher’s protected LSD, \( P \leq 0.05 \)). * - mortality reading at 7 days after larval placement on treated fruit; ** - CpGV applied at a equivalent field rate of 1 liter/ha in 937 liters of solution (Carpovirusine); *** - DAT – days after treatment.

The results from these studies show that CpGV and MD can be successfully integrated in different ways to combat annual CM and OFM pest problems, especially where resistance to a number of conventional insecticides is present or pest populations are high.

Acknowledgments

The authors would like to thank Mr. Brian Knouse for allowing us to use his orchard to conduct this study and his cooperation with making the necessary applications. We also would like to thank Certis USA, Arysta Lifescience, CBC America and State Horticultural Association of Pennsylvania for their support of this project.

References

Pheromone-Based Management Strategies for the Dogwood Borer, *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae)

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Abstract: The dogwood borer is a serious wood boring pest of apple in eastern North America. The increased severity of dogwood borer infestations in apple orchards is similar to that of the apple clearwing moth, *S. myopaeformis* Brkh, which became serious pest of apple following the introduction of size-controlling rootstocks in Europe. These rootstocks promote the formation of burr knots on exposed portions of the rootstock and on the trunk and scaffold limbs. Burr knots are an excellent food resource for *S. myopaeformis* larvae and also serve as the primary point of infestation by dogwood borer. Historically, the organophosphate insecticide chlorpyrifos has been the material growers have relied upon for control of dogwood borer in apple orchards. However, interest in promoting more sustainable management practices and recent restrictions and cancellations of organophosphates within the USA highlight the importance of developing alternative management tactics for this pest. Our recent identification of the sex pheromone, 88:6:6 v/v/v (**Z**,**Z**)-3,13 octadecadienyl acetate (ODDA): (**E**,**Z**)-2,13-ODDA: (**Z**,**E**)-3,13-ODDA, and a behavioral antagonist of dogwood borer, (**E**,**Z**)-3,13-ODDA, provided us with the opportunity to evaluate pheromone-based management strategies such as mass trapping and mating disruption. We evaluated the potential of pheromone-based mass trapping of males to reduce dogwood borer infestations and evaluated an antagonist-based pheromone blend for disruption of dogwood borer mate-finding in commercial apple orchards in North Carolina, Virginia, and West Virginia. We removed large numbers of males from orchards at all locations from high and low density mass trapping plots over two years. However, infestation in high and low density mass trapping plots was not reduced to the level of chlorpyrifos-treated plots. The most promising approach for pheromone-based management of dogwood borer appears to be mating disruption. An antagonist-based dispenser deployed at a rate of 250/ha effectively disrupted mate-finding by male dogwood borer. In plots with mating disruption dispensers, captures in pheromone-baited traps were virtually eliminated and no males were captured in traps baited with virgin females. We are currently evaluating the efficacy of disruption formulations for dogwood borer based on the sex pheromone blend and the antagonist.
Volatiles initiate egg laying in common green lacewings

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Abstract: Adults and larvae of the common green lacewing Chrysoperla carnea feed on many insect pest species and are important predators in biological control of many crop plants. Previous work has shown that adults are attracted to chemicals occurring in the scent of flowers, and that the presence of aphids on crop plants enhances oviposition by adults. In the present study, the effect of a three compound blend of phenylacetaldehyde, acetic acid and methyl salicylate was tested for its effect on oviposition by C. carnea in two areas in Norway. In both 2007 and 2008 a significantly higher number of C. carnea eggs were laid inside delta traps with the ternary blend compared to control traps. From 16 May to 15 June 2007 a total of 110 and 177 eggs were found inside 5 baited delta traps in each of two orchards in Western Norway. No eggs were found in control traps. Similar results were obtained in one orchard in Eastern Norway. When lures with the ternary blend were attached directly to the tree, the number of eggs did not significantly increase. Use of attractive volatiles to enhance egg laying, and to increase biological control by lacewings are discussed.

Key words: Green lacewing, attractive volatiles, oviposition, biological control

Introduction

Larvae of the common green lacewing Chrysoperla carnea feed on many insect pest species, and are one of the most important predators in biological control for many crop plants (Senior & McEwen 2001). The common green lacewing hibernates as adults and start feeding and egg laying after 2-3 nights of compulsory migration flight in early spring (Duelli 1980). Previous work has shown that adults locate habitats and food sources by responding to semiochemicals. L-tryptophan, a component of some artificial honey dews, increased concentration of adult C. carnea when sprayed onto tree canopies (McEwen et al. 1994). Ballal & Singh (1999) found in a wind tunnel study that for both males and females, C. carnea flight towards sunflower was significantly higher compared to the control. In trap tests in Hungary phenylacetaldehyde was found to be attractive to both male and female C. carnea (Tóth et al. 2006). Further, Reddy (2002) found that lacewings were more attracted to plants damaged either by mites or mechanically damaged plants compared to healthy plants in wind tunnel studies.

Previous works also indicate that lacewings use volatiles to locate oviposition sites. A significant difference in number of eggs laid by C. carnea was found between different host plants in a study by Ballal & Singh (1999). Kungel & Cottrell (2007) found that adult C. rufilabris laid significantly more eggs in aphid infested pecan compared to uninfested pecan, and that food spray incorporating both protein and carbohydrates increased numbers of lacewing eggs in an area.

Augmentative release of lacewing larvae is a well known control method against many insect pest species (Easterbrook et al. 2006). Lacewing eggs and larvae are produced and sold by several companies. However, experiments with augmentative release as a control method
show varying results (Michaud 2001, Easterbrook et al. 2006). The use of volatiles that initiate and increase number of eggs laid might be a more powerful and cheaper control method compared to augmentative release of lacewings. Based on previous work (Tóth et al. 2006) we have investigated the effect of a ternary blend of volatiles on oviposition in C. carneae.

Material and methods

Trials were conducted in two areas in Norway in both 2007 and 2008. A ternary blend of phenylacetaldehyde, acetic acid and methyl salicylate, previously reported to be attractive to adult lacewings (Tóth et al. 2006), was tested as attractant for green lacewing oviposition. A bait composition of 100 mg of each compound was loaded onto a 1 cm piece of dental roll (Celluron®, Paul Hartmann Ag. Heidenheim, Germany), which was put into a polyethylene bag (ca 1.0 x 1.5 cm) made of 0.02 mm linear polyethylene foil. Lures were placed in delta traps, and each trap was placed in a single tree 1.5 – 2.0 m above ground.

In 2007, 5 delta traps with lures and 5 delta traps with no lure (control) were placed in each of two sweet cherry orchards in Western Norway on 16 May. The distance between traps was at least 10 m. Traps in orchard 1 were removed on 12 September, and traps in orchard 2 were removed on 15 June. In order to not kill too many adults, sticky plates in two of the traps in orchard 1 were removed 15th June. Lures in delta traps in orchard 1 were changed once during this period. Traps were inspected for eggs and adults every week. In an apple orchard in eastern Norway 5 traps with lures and one control trap were checked for eggs and adults from 3 June until 8 August. Traps were checked 10 times during this period.

In 2008, trials were set up in two orchards, sweet cherry and plum, in Western Norway. Delta traps with lures, without lures and delta traps with lures and sticky bases were compared for effect on number of eggs deposited. In addition, lures were also attached to branches to evaluate whether the same effect was found when placed directly on a tree. Treatments are as follows: 1) delta trap with lure, 2) delta trap with lure and sticky plate, 3) delta trap with sticky plate (delta trap control), 4) one dispenser on each tree, 5) four dispensers on each tree and 6) no dispenser (dispenser control). The number of eggs in different treatments was compared as follows; counting the number on 10 evenly distributed branches on each of the treatments with lures on the tree and the control tree (treatment 4-6), counting the number of eggs on branches with lures (treatment 4 and 5) and counting number of eggs inside traps (treatment 1-3).

In Eastern Norway, five traps with lures and five control traps placed in an apple orchard were compared for effect on deposition of eggs during the season.

Data from trials in Western Norway in 2008 were analysed by analysis of variance (GLM ANOVA) (SAS Institute Inc. 2005). For variables that explained a significant part of the variance, Tukey’s test was used to analyse differences between means (Zar 1984).

Results

The ternary blend clearly affected the number of lacewing eggs deposited in both areas and all fields. Eggs were found inside delta traps baited with the ternary blend in all fields, both areas and both years. At most a total number of 118 and 71 eggs were found at one recording on five traps in each of the fields in Western Norway in 2007 (Figure 1). Similar results were found in Eastern Norway in both 2007 and 2008 (Figures 2 and 3). In 2008 the number of eggs found in delta traps was lower compared to 2007 in Western Norway; at most, 12 eggs were found at one recording in eight traps.

No eggs were found in control traps in Western Norway. In Eastern Norway a total of
two and three eggs were found in control traps in 2007 (one trap) and in 2008 (five traps), respectively.

Figure 1. Total number of eggs and adults *C. carnea* in five delta traps at each recording in field 1 and field 2 in Western Norway in 2007.

Figure 2. Total number of eggs and adults *C. carnea* in 5 delta traps at each recording in one field in Eastern Norway in 2007.
In 2008 the effect of placement of dispensers were investigated. No effect of neither field nor treatment on number of eggs deposited on 10 evenly distributed branches with one, four or no dispensers was found ($F = 0.91$, df = 3, $P = 0.45$). Further, no effect of field was found when the number of eggs deposited in delta traps and on branches with lures was analyzed ($F = 0.22$, df = 1, $P = 0.64$), however a clear effect of treatment was found ($F = 13.79$, df = 4, $P < 0.0001$). Significantly more eggs were found inside traps with lures compared to the other treatments (Figure 4). No significant effect of placement of lure in the tree canopy on number of eggs deposited was found.

![Figure 3. Total number of C. carnea eggs in 5 delta traps at each recording in one field in Eastern Norway in 2008](image)

![Figure 4. Mean number of eggs on branches with lures attached to it on trees with one lure (South side) or 4 lures (South, East, West and North) and traps with either lure, lure and sticky base or only sticky base (control). Both fields combined (n = 8).](image)
Discussion

Because of their great ability to prey on small insects and mites and high reproductive potential, lacewing larvae have a high potential in biological control (Klingen et al. 1999, Corrales & Campos 2004, Easterbrook et al. 2006). Augmentative release of lacewing larvae or eggs increases the population of this predator. However, a method that clearly increases oviposition by naturally occurring adult lacewings in an area might be an alternative to augmentative release. Tóth et al. (2006) show that phenylacetaldehyde is attractive to adult lacewings and suggest that this component might be part of the chemical information C. carnea use to locate feeding sites. Our study confirms this, and also show that a combination of phenylacetaldehyde, methyl salicylate and acetic acid placed in delta traps increase adult oviposition by more than 150 times during the season.

Our data indicate that delta traps are more attractive as oviposition sites compared to leaves; we found significantly more eggs deposited in traps compared to branches with lures attached to it. The shelter provided by the delta trap may increase the time adults spend to oviposit, or may reflect the innate preference to place eggs on sheltered places.

For volatiles that are attractive to ovipositing females to be used in biological control, the presence of food for the newly hatched larvae is essential. Mean longevity of C. carnea larvae with no food or with only leaves was about 1.5 days in laboratory studies (Hoddle & Robinson 2004). In orchards with annual aphid or mite problems, attractive volatiles initiating oviposition might give successful biological control.

In the future we want to find a suitable design for using volatiles for attraction and oviposition of lacewings. Where and how lures should be placed in tree canopies must be investigated. Further, the possible effect of the ternary blend on biological control on pest population development must be investigated.

References


Sucrose as an apple tree resistance inducer against *Cydia pomonella* L.

Sylvie Derridj1, François Moulin2, Eric Ferre3, Hubert Galy3, Arnaud Bergougnoux4, Ingrid Arnaud5, Jacques Auger5.

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Abstract: The studies of plant insect relationships are necessary for research of new control methods. We showed that the soluble carbohydrates and sugar alcohols exuded on the leaf surface influence *Cydia pomonella* L. egg-laying and neonate larval behaviour. The metabolite pattern and quantities can explain apple tree resistance to egg-laying. The plant resistance can be obtained by modifying the pattern with spraying sucrose solutions on apple tree. This was done in several orchards and varieties, over three years alone and/or in association with chemical or biological controls.

The spraying of 100 ppm sucrose or 10 ppm did not differ, and the addition of sucrose to treatments, leads to increase the practical efficacy and the ABBOTT one. The practical efficacy = (% of damage on the treatment reference - % of damage with sucrose addition)/% of damage on the treatment reference, was 30% over three years and several varieties. These results open a research field on pesticide alternatives and on improvement of biological controls. Enhancement of this technology should be obtained by studies of dose effects, duration and time period efficacy. Knowledge of genes concerned in this induction would be helpful for resistance selection.

Key words: pests, apple tree, leaf surface, sugars.

Introduction

On the leaf surface there are primary metabolites coming from photosynthesis which pass through the cuticle. Their ratios and quantities (ng/cm²) are linked to the molecules and to the permeability of the cuticle (Stammitti et al 1999) which is plant species specific. These metabolites can give informations on leaf physiology, stage and also plant species (Fiala et al., 1990). We have shown that soluble carbohydrates and/or sugar alcohols influence host acceptance and egg laying after alighting (Derridj et al., 1989, Lombarkia and Derridj, 2002) and also neonate locomotion on leaf surface on two Lepidoptera (*Ostrinia nubilalis* and *Cydia pomonella*). Females detect these molecules by gustatory sensillae present on their legs and their ovipositor. We have related the ratio between three soluble carbohydrates and three sugar alcohols to susceptibility and resistance to *C. pomonella* egg laying (Lombarkia and Derridj, 2008).

Foliar spraying on maize of low quantities (1 to 10mg per liter) of sucrose have a systemic effect on the plant and reduced *O. nubilalis* egg laying. We used this result to reduce damages on apple of an other lepidopteran, *C. pomopnella*. The experiments in semi-field conditions showed that a sucrose treatment of 10 ppm (10mg/L) changed the leaf surface composition of apple trees and the egg distribution of *C. pomonella* within the tree twenty days after the treatment.

Here we relate orchard experiments (garden, experimental and open fields) carried out over 3 years in several countries and geographical regions from France and from Europe. The
single effect of sucrose could be estimated in open fields and in association with chemical and biological insecticide treatments in all the situations.

Material and methods

Insect rearing
Experiments were performed in orchards. In the north of France they were carried out throughout the season during two insect generations. In the south of France, in Greece and Italy they began after the first flight at the beginning of male captures of the second generation. These studies were conducted up to harvest through the two last generations of the insect.

Sugar and insecticide treatments
Sucrose used was from SIGMA (S 1888, 99.5% purity) and used at 10ppm (1g per 100 liters) dilution. It was sprayed every twenty days throughout the season on the whole tree in the north of France from March to September.

The sucrose and fructose treatments were applied in tank mix with several insecticides according to the trial: Phosmet 50%, Malathion 440g/L, Phosalone 500g/L, Chlorpyrifos ethyl 480g/L and the bio-insecticide granulovirus Carpovirusine.

Apple tree varieties:
In France several varieties were evaluated experimentally: Golden Delicious, JonaGold, New Jonagold, Gloster, Melrose and PinkGold and in Greece and Italy: Mondial Gala and, in one orchard, Golden Delicious.

Orchards of experimentation
Experimentations began in 2005 and continued until 2008. In France, the orchard garden was located in Versailles at the Potager du Roi (five varieties, half rows of about 14 trees were treated with insecticide and sucrose vs insecticide alone, one or two rows per variety), in La Morinière an experimental orchard with two varieties, four rows treated with sugar and carpovirusine and six with carpovirusine, arranged in 4 blocks of 3 trees was used.

ANADIAG open field studies took place in commercial orchards in France, Greece and Italy from 2006 to 2008. The experimental design used in all the trials was a randomized complete block with four blocks, the size of the plots varied between 2 and 4 trees depending on the tree sizes, assessments were only performed on the central part of each plot.

Damage estimation: Damage on apples was estimated at harvest. The mean of practical efficiency = (% of damage on the treatment reference - % of damage with sucrose addition)/% of damage on the treatment reference, was calculated over three years. In the potager du Roi each tree is considered as a unit, all apples from each tree and from the soil were observed, their numbers varied with the variety and pruning from 22+/−8 for Melrose to 204.5 +/-41 for Golden. At La Morinière 500 fruits were sampled from trees per treatment for each variety and apples from the soil were also sampled.

In the ANADIAG trials the damage were also assessed at harvest on both retained and fallen fruit. The ABBOT efficacy= (100xT0-Tt)/T0, T0= % of infested fruits in control plots, Tt= % of infested fruits in treated plots, was based on the % of infested fruits at harvest. Depending on the trials between 1.02% and 44.35% of infested fruit were assessed at harvest.

Results and discussion

ABBOTT efficacy and sucrose single effect
ANADIAG experiments in open fields permit the evaluation of the effect of sucrose alone at 10 and 100 ppm vs untreated trees. The sugar alone reduced C. pomonella damages (Table1). The mean ABBOTT efficacies throught the trials conducted with sucrose alone were 42.69%
+/- 12.22 at 10ppm and 37.93% +/- 17.38 at 100ppm dose rates. The mean ABBOTT efficacy value reached by the fructose at 10ppm was 37.21% +/- 14.58. Neither significant dose rate nor sugar type effects have been detected in any of the trials. No significant difference between the insecticide treatments and the sugar applied alone has been detected in six trials out of seven.

The insecticide Abbott efficacy was increased of about 10% by the addition of sucrose. The effect was not additive and probably in the formulation of commercial product substances with similar activity as sucrose may be present.

Table 1. Mean Abbott efficacy levels (Efficacy level in percent vs. Untreated control damage level) for sucrose, insecticides (chemical and bio-insecticides), and sucrose + insecticides.

<table>
<thead>
<tr>
<th></th>
<th>07/FR RY</th>
<th>07/ITA015</th>
<th>06/FR EL</th>
<th>08/GR ML</th>
<th>07/GR11</th>
<th>07/FR GA</th>
<th>08/ITA ML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>(1.02) a</td>
<td>(1.75) a</td>
<td>(22.26) a</td>
<td>(24.93) a</td>
<td>(37.58) a</td>
<td>(40.67) a</td>
<td>(44.35) a</td>
</tr>
<tr>
<td>Fructose 10ppm</td>
<td>36 a</td>
<td>33.35 a</td>
<td>19.52 b</td>
<td>60.04 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose 10ppm</td>
<td>38.1 b</td>
<td>31.34 a</td>
<td>37.98 b</td>
<td>63.33 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose 100ppm</td>
<td>61.9 c</td>
<td>21.23 a</td>
<td>30.66 bc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insecticide</td>
<td>80.95 c</td>
<td>54.63 a</td>
<td>36.6 b</td>
<td>46.53 cde</td>
<td>41.55 bc</td>
<td>78.9 c</td>
<td></td>
</tr>
<tr>
<td>Insecticide + Sucrose</td>
<td>92.86 c</td>
<td>45.14 a</td>
<td>47.12 b</td>
<td>50.66 de</td>
<td>61.34 d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter do not significantly differ (P<0.05, Student-Newman-Keuls test performed in each trial individually).

Data in brackets corresponds to the % of infested fruits in the untreated control at harvest.

Means followed by the same letter do not significantly differ (P<0.05, Student-Newman-Keuls test performed in each trial individually).

Data in brackets corresponds to the % of infested fruits in the untreated control at harvest.

**Practical efficacy of sucrose added to chemical and biological treatments**

When gathering all the data from the different geographic regions, treatments, varieties and throughout the 3 years, the correlation between the proportions (%) of apple damaged treated with insecticides and treated with insecticide amended with sucrose shows a linear curve (figure 1). The distinction between the doses 10 and 100 ppm of sucrose did not show any different relationship. On the basis of the equation of the correlation we can calculate the general practical efficicacy = (% of damage on the treatment reference - % of damage with sucrose addition)/% of damage on the treatment reference. It is on the order of 30%. There is no link between the efficacy of sucrose and the potential for damage (susceptibility) of the variety, but variety is the main source of variation. Jonagold and NewJonagold were particularly and constantly unaffected by the sucrose treatment.
Figure 1. Correlation between apple damage treated with insecticide and insecticide + sucrose at 10 and 100 ppm in experiments over three years and three European countries. Correlation equation conducted on the mean practical efficacy of 30% = (% of damage on the treatment reference - % of damage with sucrose addition)/% of damage on the treatment reference, due to addition of sucrose to different insecticides.

We induced plant protection against *C. pomonella* by spraying sucrose in tiny quantities. The phenomenon seems both general and reproducible. The chemical analysis of the metabolites on the leaf surface of apple trees (Ferrè *et al.*, 2008) which showed that the sucrose treatment mostly induced changes (quantities and ratios) in metabolites; the signals for the insect for host acceptance and egg laying are changed. It should present the advantage of avoiding plant damage due to neonate larvae. Knowledge about the mechanisms induced will open new directions for research in crop protection.

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To Josep Blay, Stefano Bergaglio, Sotiris Pantazis respectively Directors of Anadiag Iberica, Anadiag Italia et Anadiag Hellas for their participation to the open field studies.
ENSP of Versailles all people who harvested apples at the Potager du Roi.

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Attractiveness of mixtures of pheromone and host plant volatiles to *Cydia molesta* (Busck) (Lepidoptera: Tortricidae)

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**Abstract:** In the Oriental Fruit Moth (*Cydia molesta*) the role of the female pheromone blend has been very well studied in the behaviour of males, but only a little in the behaviour of females. On the other hand, the role of host plant volatiles has been recently studied in the behaviour of females, but it has not yet been studied with respect to male behaviour. From these recent studies, a blend of five host volatiles - three green leaf volatiles and two aromatics - has been shown to affect female behaviour. In this work we studied the effect of the five host volatiles on the behaviour of *C. molesta* males. By doing wind tunnel experiments we have demonstrated that there is an effect of host plant volatiles on male behaviour but only when mixed with a sub-optimal dose of pheromone. No effect was found when the blend was tested alone. A variety of responses were found when one compound was removed from the five host volatile blend, or when the host plant volatile was placed alone. Nonetheless, male landing was always higher when exposed to the mixtures than with the pheromone alone. We could also see that when the aromatic compounds were removed from the blend, male landing was lower and no difference was found when removing any of the green leaf volatiles. The best landing response was achieved when the sub-optimal dose of pheromone was mixed with the complete blend of five host volatiles that is known to affect the behaviour of females.

*Cydia molesta, Wind tunnel, Plant volatiles, Pheromone*
Improving the effectiveness of mating disruption for tree fruit pests

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Abstract: Over the past five years, we have been exploring ways to achieve mating disruption of tortricid moth pests of fruit superior to that provided by current formulations. Different release devices, distributions, and active ingredients may be called for, depending upon the mechanism(s) of disruption to which a particular pest species or population size is most vulnerable. Several lines of evidence indicate that competition between pheromone dispensers and females is the primary mechanism of communicational disruption of tortricid moths in the field, especially for hand-applied formulations. From a practical standpoint, the best disruption will be achieved when dispensers are highly attractive and numerous point sources are distributed uniformly within the orchard. Wax formulations applied at high point source densities have provided outstanding disruption of some key fruit pests, including Oriental fruit moth. However, achieving a very high level Codling moth (CM) disruption has proved more challenging. Recent efforts to develop more effective and economical disruption formulations for CM have been guided by a series of experiments conducted in replicated plots consisting of large field cages constructed over 12 apple trees. A series of experiments using various types of dispensers revealed that attraction alone was insufficient for achieving a high level of disruption. Outstanding results were only achieved when CM males were prevented from making multiple orientations to pheromone sources. The high cost of mating disruption is often cited as a major impediment to broader adoption of the tactic. Attract-and-kill technologies offer the possibility of a cost-effective option for CM disruption. The economics of point-source dispensers could be improved through more efficient use of the precious active ingredient.

IPM, Codling moth, Mating disruption, Apple, Pheromone
Assessing efficacy of mating disruption in apple orchards by release and recapture of males in net-cages

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Abstract: Codling moth *Cydia pomonella* (L.) is regarded as a major pest of pome fruit worldwide. The implementation of mating disruption for its control has been increasing during the last two decades (Witgall et al., 2008; Angeli et al., 2007). Due to increased regulatory restrictions of conventional insecticides and other environmental issues, in some fruit growing districts mating disruption is now deployed on well over 50% of the pome fruit area and it is considered an integral part of pest management programs for this species (Thomson et al., 2009). The evaluation of the efficacy of the commercial formulation used for mating disruption appears to be a relevant factor for further support the use of this technique. In this work we evaluate the use of net-cages (Doye & Koch, 2005) as a field method for the evaluation of the efficacy of mating disruption. A pheromone treated plot and an untreated area were provided with four 2 mc net-cages each equipped with a trap. No plants were included in the cage. Codling moth males were released in the cages and caught in unbaited or female baited delta trap. In each of the four cages, a fixed number of males (5, 10, 15, and 20) were released with the aim to evaluate the effect of male density on trap catch. Release of males was replicated three times. As a general result, the number of males captured in the female baited traps was dependent on the number of released males in the cage (Linear regression, R^2=0.996; ANOVA, P<0.001). A different pattern was, however, observed between the untreated and the pheromone treated plot. While in the control plot the captures could be represented by a linear curve with no apparent saturation (R^2=0.959), in the pheromone plot we observed a logarithmic trend with a tendency to saturation (R^2=0.968). Although the treatment did not affect the number of males caught in the blank traps (ANOVA, P>0.05), a higher proportion of males was trapped with the lowest male dose in the control plot indicating that this dosage may be strongly biased by accidental captures. The efficacy of the pheromone formulation was calculated as catch inhibition by comparing the fraction of males caught by female baited traps in the treated and control plots. The inhibition of captures due to the treatment was 68% with a release of 20 males and 96% with the dose of 5 males. From these preliminary results it appears that a dose of 20 males per cage is necessary to highlight behavioural differences of searching males due to a pheromone treatment. Further research on factors such as trap architecture, presence of plants in the cage and volume of the cage may be of help for the optimization of this method.

Key words: codling moth, *Cydia pomonella*, field test, pheromone.

References


Recent progress in sanitation practices to manage apple scab

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Abstract: Sanitation practices to control apple scab, caused by *Venturia inaequalis* (Cke.) Wint., are aimed at reducing the primary inoculum, with the expectation that there will be an approximate reduction in primary scab, but the reduction in primary scab may be much less than expected. Two recent publications are reviewed that allow an analysis of the major factors that influence the relationship between the reduction in ascospores trapped and leaf litter (the source of ascospores) and the reduction in primary scab on spur leaves and on older leaves and fruit in sanitized compared to non-sanitized plots. Suggested guidelines for sanitation trials based on the analyses are presented.

Key words: apple scab, *Venturia inaequalis*, sanitation, IPM, integrated control

Introduction

Several sanitation methods reduce the primary inoculum and, as a consequence, increase fungicide efficacy to control scab and have the potential to reduce the fungicide dose to control scab (MacHardy, 1996). However, few scab management programs have successfully incorporated these methods into practice, even in organic orchards where high ascospore dose and the application of less efficacious fungicides approved for organic apple production often result in unacceptable levels of scabbed fruit. Sanitation trials are conducted to garner additional support for including sanitation in scab management programs, but the results may show great disparity between expected and actual reductions in scabbed leaves and fruit. This disparity may be interpreted as a failure of sanitation to meet its expected effect on the pathogen and the leaf litter, but the results may be deceptive due to factors that lessen or mask the actual reductions in ascospores and the leaf litter. Two recent publications are analyzed to explain why it is important to consider these factors when designing sanitation trials and interpreting trial results.

Investigation 1

Holb (2006), in Hungary, investigated the potential of four sanitation treatments to reduce the leaf litter, trapped ascospores and scab on spur clusters, older leaves and fruit (Table 1). There was general agreement between the reductions in leaf litter density (LLD) and trapped ascospores and significant reductions in scab depending on year, cultivar susceptibility and sanitation treatment, but the reductions were often considerably less than expected. For example, the LLD and ascospores trapped were reduced 56 to >95% in three of the sanitation treatments, but significant reductions in scabbed leaves and fruit were only 18-37%. Most noticeably, reductions in spur scab were markedly less than expected in the leaf removal/plastic foil plots in which trapped ascospores and the leaf litter were reduced more than 95%. Several factors associated with increased disparity between reductions in ascospores and leaf litter and reductions in scab incidence (Table 2) help explain the results.

1. High predicted potential ascospore dose (PAD): PAD (ascospore/m² orchard floor) ranged from 923 to 3,549 in the two integrated orchards and from 54,598 to 164,047 in the two
organic orchards.

2. Numerous infection periods: 14 and 37 infection periods in the two organic orchards and 20 and 35 infection periods in the two integrated orchards from mid-Mar. to mid-Oct. in 2003 and 2004, respectively.

3. Scab susceptible and moderately scab susceptible cultivars planted in each orchard.

4. Non-sanitation control plots and unsuccessful sanitation plots, i.e., plots not significantly different from control plots, in each orchard.

Table 1. Selected data from recent publications that investigated the relationship between reductions in leaf litter and trapped ascospores and reductions in scab incidence.

<table>
<thead>
<tr>
<th>Sanitation practice</th>
<th>LLD</th>
<th>Trapped ascospores</th>
<th>% Reduction in Scab incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spur leaves</td>
</tr>
<tr>
<td>Holb, 2006</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collect fallen leaves 56-79</td>
<td>56-79</td>
<td>56-79 (I) 61-65 (O)</td>
<td></td>
</tr>
<tr>
<td>Collect fallen leaves + straw mulch 72-92</td>
<td>72-92</td>
<td>72-92 (I) 72-79 (O)</td>
<td>18-37% significant reduction compared to non-sanitized plots.</td>
</tr>
<tr>
<td>Collect fallen leaves + plastic foil cover</td>
<td>&gt;95</td>
<td>&gt;95</td>
<td>18-37% significant reduction compared to non-sanitized plots.</td>
</tr>
<tr>
<td>Straw mulch 24-38</td>
<td></td>
<td></td>
<td>No significant difference</td>
</tr>
<tr>
<td>Holb, 2007†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Remove fallen leaves 83-89</td>
<td>83-89</td>
<td></td>
<td>42-47</td>
</tr>
<tr>
<td>Disc cultivation 50-58</td>
<td>50-58</td>
<td></td>
<td>7-26</td>
</tr>
<tr>
<td>Shred leaves 43-50</td>
<td>43-50</td>
<td></td>
<td>23-36</td>
</tr>
<tr>
<td>Cover orchard floor with plastic foil &gt;99</td>
<td>&gt;99</td>
<td>56-68</td>
<td>17-25; 20-27*</td>
</tr>
</tbody>
</table>

*1 = integrated orchard management; O = organic orchard management.

2No significant difference in LLD among treatments; plastic foil was the only treatment with leaf scab significantly different from non-sanitized control.

3Each trial was significantly different to non-sanitized control plots depending on year, cultivar susceptibility and orchard management program.

42002 and 2003 data.

Investigation 2

A second study by Holb (2007) evaluated the relationship between the reduction in LLD caused by four sanitation treatments applied to the leaf litter and the reduction in primary (spur cluster) scab and scab on older leaves and fruit in two organic orchards (Table 1). Removing fallen leaves mechanically in autumn reduced the LLD 83-89% by mid-Apr, but spur leaf scab was reduced only 42-47%. Similar discrepancies between reductions in LLD and spur leaf scab occurred with the other sanitation treatments. Several factors listed in Table 2 help to explain the discrepancies.

1. High ascospore dose indicated by ~14% and ~23% spur scab in both organic orchards in 2002 and 2003, respectively, after the fourth infection period.
3. Many early season infection periods: 9 and 8 infection periods at one organic orchard and 8 and 6 infection periods at the second organic orchard from 1 Mar until 25 May in 2002 and 2003, respectively.

3. Small scale sanitized and non-sanitized plots and highly susceptible cultivars Jonagold and Mutsu (in addition to the moderately susceptible test cultivars Jonathan and Elstar) in each orchard may have resulted in inter-plot transport of ascospores.

Table 2. Factors that alter the relationship between the percent reduction in ascospores and LLD caused by sanitation and the percent reduction in primary scab, compared to non-sanitized plots.

<table>
<thead>
<tr>
<th>Conditions favoring close relationship</th>
<th>Conditions increasing disparity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sanitation had been employed three or more years</td>
<td>1. No previous sanitation program</td>
</tr>
<tr>
<td>2. No post infection fungicides that inhibit the pathogen¹</td>
<td>2. Inhibitory post infection fungicides</td>
</tr>
<tr>
<td>3. One sanitation treatment only in an orchard</td>
<td>3. Non-effective sanitation treatments and non-sanitized control plots in the same orchard</td>
</tr>
<tr>
<td>4. Low PAD²</td>
<td>4. High PAD</td>
</tr>
<tr>
<td>5. Test orchard/plot is &gt;15 m from an untreated or severely scabbed orchard</td>
<td>5. Test orchard/plot is &lt;15 m from an untreated or severely scabbed orchard</td>
</tr>
<tr>
<td>6. Conditions unfavorable for conidia overwintering on shoots and buds</td>
<td>6. Conditions that favor conidia overwintering on shoots and buds</td>
</tr>
<tr>
<td>7. Few primary infection periods</td>
<td>7. Many, primary infection periods</td>
</tr>
<tr>
<td>8. Low/moderate scab susceptible cultivars in the sanitized orchard/plot</td>
<td>8. High scab susceptible cultivars in the sanitized orchard/plot</td>
</tr>
<tr>
<td>9. Large plot size</td>
<td>9. Small plot size</td>
</tr>
<tr>
<td>10. Conditions favorable for earthworms and antagonistic microorganisms</td>
<td>10. Conditions favorable for earthworms and antagonistic microorganisms</td>
</tr>
<tr>
<td>11. Effective fungicide program</td>
<td>11. Ineffective fungicide program</td>
</tr>
</tbody>
</table>

¹Infections stopped by post infection fungicides that do not kill the fungus may become active in autumn resulting in development of the sexual stage and ascospore production after leaf fall that will not be counted in an autumn assessment of scab lesions to predict PAD.

²PAD (potential ascospore dose): ascospores per m² orchard floor.

Guidelines for sanitation trials

In broad terms, primary scab in a sanitized plot is the product of several factors: actual ascospore dose (predicted PAD - reduction in PAD caused by sanitation), number, timing and severity of primary infection periods, level of cultivar susceptibility to scab and neighboring sources of ascospores. Earthworms and microorganisms that remove or degrade the leaf litter or are antagonistic to the pathogen also reduce PAD, but their effects are not easily measured so are not included here. PAD is the most important measureable factor to consider in evaluating the performance of a sanitation practice because it can vary greatly from orchard to orchard and from year to year in an orchard. Holb (2007), for example, found that in plots treated with straw mulch (alone or in combination with leaf collection), the greater the PAD the lower the effect on trapped ascospores.

Sanitation will always increase fungicide efficacy to control scab and, thus, is recommended for every orchard, especially organically managed orchards for reasons...
stated above. Integrating sanitation with strategies to reduce the recommended fungicide dose to control scab depends largely on ascospore dose, and it is clear that sanitation applied for this purpose will fail in some orchards. For example, the PAD assessed by Holb (2007) was ~164,000 in one organic orchard and 923 in one integrated (traditionally managed) orchard. Assuming that the straw mulch/plastic foil treated plots reduced trapped ascospores by 80%, the adjusted ascospore dose in those plots would be ~32,000 and 164 ascospores per m² orchard floor in the integrated and organic orchards, respectively. Sanitation would increase fungicide efficacy in both orchards, but the 32,000 PAD in the organic orchard would still be well above the threshold for reducing early season fungicide dose, whereas the 164 PAD in the integrated orchard would be well within the low-risk threshold category for considering a reduction in fungicide dose (MacHardy, 1996, 2000).

Summary

Sanitation will be most successful in achieving its expected effect on primary scab when only one sanitation practice is tested, PAD is low, few, less severe primary infection periods occurred, the orchard is planted with low to moderate scab susceptible cultivars and has been well managed for scab (i.e., overwintering conidia on wood and buds unlikely), and there is no potentially high source of ascospores within 20 m. A commercial orchard will not have all of these ideal conditions, so each factor must be considered in selecting a sanitation method and deciding how to best utilize sanitation in the scab management program.

References


Fungicide Sprays During the Window of Germination, A Special Tool for Fungicidal Control of Apple Scab in Organic and Integrated Apple Production

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Abstract: The most frequent factors responsible for failures in controlling apple scab (Venturia inaequalis) are the amount of fungal inoculum, poor strategy and timing of fungicide spray applications and the intrinsic, incomplete efficacy of the fungicides. Despite the progress made in apple scab control fungicides remain a highly underestimated risk because their less than 100% efficacy in the field is not gradable enough to match the enormous variation of inoculum, resulting in a high risk of poor control as the inoculum increases. The application of more than one fungicide spray per infection period is the only effective way of adapting the efficacy of scab control to high inoculum levels. A protectant is applied shortly before rain and a curative compound after the rain event if a severe infection has built up. The curative compound controls the spores which passed the protectant fungicide resulting in a significant increase of efficacy compared to just the protectant before the rain. When curatives are not available, a protectant may be applied during the window of germination, a time period when the ascospore release of the day has almost terminated but no, or just a few, spores have infected. The time window is determined using the simulation software RIMpro in conjunction with the weather forecast. This method has been introduced in organic fruit production (OFP) at Lake Constance area in 2002 and has improved the results of primary scab control to, or above, the level obtained in IFP. After having become standard in OFP, the method is also used in IFP after the detection of wide spread resistance to Anilinopyrimidines at Lake Constance area in 2005.

Key words: Apple scab, double spray, window of germination, organic fruit production, inoculum related control

Introduction

When analysing failures of control of apple scab (Venturia inaequalis, Cooke, Wint.) during the primary season in commercial fruit farms, there are four major reasons:

i) until recent years the fungal inoculum did not play any role for the control strategy which was very much focused on the sole use of fungicides. Although the potential ascospore dose in commercial orchards may vary by a factor of up to 10^6, this difference in risk is still only marginally reflected by the present control strategies, indicating a lack of dynamic in the response. The result is that in relation to the generaleal level of scab attack in an individual year in a certain region, success usually is poor in high inoculum orchards.

ii) the common response following a year with high scab attack is the combination of a search for new fungicides or mixtures which gave good results in fungicide trials (not strategy trials), more frequent sprays and probably also higher dose rates and spray volumes.

iii) missing the incorporation of the development of the host into the timing of fungicide sprays. Since a protectant fungicide may cover a rosette leaf area expansion of no more
than approximately 80% (Knaus, 2001) which may be exceeded within 2 days, common fungicide spray intervals of 7 to 10 days during this period give a good imagination of the poor adaptation of the fungicide spray schedules to the development of the host.

**Table 1**: The theoretical effect of the inoculum on the scab attack

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Inoculum</th>
<th>Ascospores landed on leaves/m² orchard floor</th>
<th>Efficacy of the fungicide %</th>
<th>Lesions/m² of orchard floor</th>
<th>Efficacy required for 1 lesion/m² of orchard floor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>low</td>
<td>100</td>
<td>99</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>medium</td>
<td>1,000</td>
<td>99</td>
<td>10</td>
<td>99.9</td>
</tr>
<tr>
<td>3</td>
<td>high</td>
<td>10,000</td>
<td>99</td>
<td>100</td>
<td>99.99</td>
</tr>
</tbody>
</table>

iv) although scab control has been very much focused on the use of fungicides, it has been overlooked, that under field conditions fungicides have an intrinsic, incomplete efficacy below 100%. In the theoretical calculations (Table 1) it becomes clear, that the success of fungicide sprays is very closely related to the inoculum. To obtain the same attack in orchard # 2 and orchard #3 as in orchard #1 given in the example in Table 1, the fungicide theoretically needs to be 10 x and 100 x, respectively, more effective as in orchard #1 which is impossible with any of the classic “concepts” like new products, reduced spray intervals, increased dose rates and water volumes or adjuvants. Because the efficacy of a fungicide cannot be increased as required to compensate for a high inoculum, the scab attack may reach an unacceptable level although the fungicide worked properly.

**Increasing fungicidal efficacy**

In recent years much progress has been made by integrating the reduction of the inoculum through sanitation practices into the control strategy. Also sophisticated simulations of the behavior of the host and the pathogen have been developed, which in combination with weather forecast data allow real predictions. With these tools and new information technologies problems caused by poor timing of fungicide sprays can mostly be eliminated.

Despite all this progress the fungicides have remained a highly underestimated risk because of their incomplete efficacy in the field which in their classic use is not gradable enough to match high inoculum levels. The only way for a substantial increase is a double treatmant on the same infection, which has been proven by Trapman (pers. comm., 1996) in a field trial (Figure 1). Trapman applied different dose rates of a protectant fungicide before any rain event during the primary season and in a second set of blocks additionally to the protectant sprays three applications of a curative fungicide after the three severe primary infections. In a third block only three curative treatments after the severe infections were carried out.

The additional curative sprays increased the total efficacy of the fungicide strategy by a factor of 5.6 compared to the pure protectant fungicide strategy. But even this improvement is not high enough to compensate for a high inoculum shown in Table 1 for orchard #3 where an increase of a factor of 100 is required to compensate the high inoculum relative to orchard #1. This demonstrates again the importance of the sanitary treatments.

The purpose of these double sprays is to kill the ascospores that have survived the protectant fungicide, thus accumulating efficacy yielding in a lower scab attack in high inoculum orchards (Figure 2). This strategy has been recommended by the author at Lake Constance area since the early 1990's and has become standard in the area in 1996, after resistance to DMIs was detected and the Anilinopyrimidines were registered. After 10 years of use when in 2005 wide spread resistance to Anilinopyrimidines has been detected at Lake
Constance area, this strategy could no more be applied since no more curative compounds without resistance had been available.

Scab Control Strategies:
The Effect of Double Treatments

When true curative fungicides are not available as in organic fruit production or in case of resistance, there exists an alternative for using the potential of double treatments. When following the development of an infection in a simulation program like RIMpro (Figure 2) the ascospore release is triggered by threshold levels of light and rain. Assuming the rain event starts at night and continues throughout the whole day ascospore release peaks around noon time and finishes in the evening. As the spores are released throughout daylight (Figure 2; white peaks), a fraction lands on leaves, increasing in number with a similar pattern as the release, and starts germinating. Under optimum temperature and continuous leaf wetness, the germination process ends after approximately 6 hours. The number of primary structures inside the leaf tissue increases according to the pattern of the ascospore release, in reality...
being modified by temperature and leaf wetness (Figure 2, black sigmoid line).

In Figure 3 a time gap is shown from the end of spore release on May 5 until the first ascospore has established a stable structure in the leaf on May 6. During this time window of 13 hrs all the ascospores that landed on the leaves are under germination. Although highly modified by actual weather conditions, this time window occurs at any day with an ascospore release. Protectant fungicides, which kill germinating spores as long as no stable primary structure inside the leaf is established, can be applied as a regular cover spray prior to rain but also between the moment the last ascospore of the day landed on the leaf has started germination until the moment when the first ascospore of the day landed on the leaf establishes a stable primary structure inside the host. Applied at increasing time after the first ascospores have infected the leaf, the efficacy of the protectant will decrease and has no more effect when all germinating spores have completed infection prior to the time of application. This use of protectant fungicides is closely related to the Mills Warning System aiming at the application of lime sulfur and sulfur sprays and dusts also in the rain event during which an infection was expected (MacHardy, 1996). As with the curative fungicides the protectant fungicides are applied as a second treatment after a regular cover spray put on before the onset of rain events during which a heavy ascospore release is expected.

A greenhouse test was conducted to prove that the activity of protectant fungicides applied in the window of germination is not negatively affected by rain during the application. For testing various protectants, potted and untreated trees were inoculated with $10^5$ conidia ml$^{-1}$ and kept wet with 2 mm of artificial rain for 5 hrs to allow sufficient germination. After 5 hrs of rain fungicides were applied during the rain. Immediately after the application, artificial rain was intensified to 10 mm within 30 min to wash off the products. The trees were kept moist for 20 hrs at 18°C after the inoculation and then under dry conditions until lesions appeared. The results (Table 2) indicate a significant inhibiting effect. Even sulfur at a concentration of 0.2%, equivalent to a dose rate of 2 kg/ha, inhibited the development of the lesions completely. Trifloxystrobin and Mancozeb also showed complete inhibition. Lime sulfur and Dithianon yielded very good activity while the two Captan-formulations displayed the lowest activities.
Table 2: Efficacy of protectant fungicides applied into the window of germination during rain. Different letters indicate a significant difference according to the Dunn multiple comparison test (p<0.05).

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Attack (%)</th>
<th>Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>6.8 a</td>
<td>-</td>
</tr>
<tr>
<td>Captan WDG (0.125%)</td>
<td>2.2 ab</td>
<td>68</td>
</tr>
<tr>
<td>Captan WP (0.12%)</td>
<td>0.5 b</td>
<td>93</td>
</tr>
<tr>
<td>Dithianon (0.05%)</td>
<td>0.2 b</td>
<td>97</td>
</tr>
<tr>
<td>Lime sulfur (1.5%)</td>
<td>0.1 b</td>
<td>99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Attack (%)</th>
<th>Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur (0.2%)</td>
<td>0.0 b</td>
<td>100</td>
</tr>
<tr>
<td>Sulfur (0.5%)</td>
<td>0.0 b</td>
<td>100</td>
</tr>
<tr>
<td>Trifloxystrobin (0.01%)</td>
<td>0.0 b</td>
<td>100</td>
</tr>
<tr>
<td>Mancozeb (0.2%)</td>
<td>0.0 b</td>
<td>100</td>
</tr>
</tbody>
</table>

Implementation

The use of protectant fungicides in the window of germination during peak ascospore releases of apple scab in high inoculum orchards has become standard in the past few years as a tool to improve the efficacy of regular protectant fungicides. Because of the mainly weaker fungicides available in organic fruit production the method significantly improved scab control during the primary season, which has been impressively demonstrated in the years 2007 - 2009 where primary scab control was as good, or better, in high inoculum organic orchards compared to blocks on integrated fruit farms.

A worst case example of the method is presented in Figure 4, which shows the peak ascospore release in 2006 during a 5 day rain event with up to 70% of the season’s total release. In the graph the white line represents the ascospore releases and the black line the progress of the infection. The three most important windows of germination are marked with the bright arrows and the corresponding time periods by the squares underneath. All growers have put on a regular protectant fungicide as a basic protection on April 25 or in the morning of April 26, before the rain event started in the afternoon. Most organic growers and a few IP-growers put on at least the first two of the three recommended sprays during the respective windows of germination and by doing so could keep their high inoculum blocks almost scab free. The infection incidence ranged from 0 - 2% infected shoots at the end of primary season.

Additionally the graph shows three incorrect responses of other growers. A first group which did not want to apply a second spray in their high inoculum blocks on the same day waited until the next morning when they put on a protectant. As can be seen by the left crossed arrow, this spray was too late for the release of the first day because these spores already had completed infection indicated by the high RIM-values. This spray was also too early for the release on the same day (April 27), because this fungicide was already washed off before the spore release started in the early afternoon. A second group waited until Friday and put on a spray in the morning, which also gave poor results in high inoculum blocks because it was again too late for the previous release and too early for the following release on the same day. Finally a third group applied an Anilinopyrimidine on Saturday, April 29 or Sunday, April 30, but these sprays also did not give satisfying results because of the widespread resistance to this chemical group.
The method was introduced in organic fruit production at Lake Constance area in 2002 and has improved the results of primary scab control to or even above the level obtained in IFP. After having become standard in organic fruit production, the method is also used in integrated fruit production since after the detection of widespread resistance to Anilinopyrimidines at Lake Constance area in 2005 no more curative compounds are available.

References:


Assessment of fungicide protection strategies in experimental apple orchards

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Abstract: In order to protect apple trees against scab, powdery mildew and post-harvest diseases, a large number of fungicides are applied in apple orchards from green-tip stage to harvest. To satisfy society’s demand to decrease the number of plant protection treatments, innovative protection strategies were assessed over four years in experimental orchards. In the case of apple scab, fungicide protection management takes the primary inoculum level and the means for reducing this inoculum, as well as the cultivar susceptibility, into account, in order to define a climatic risk level (according to Mills) as the intervention threshold. The decision to use fungicides against powdery mildew is based on the assessment of disease levels present in the orchard (use of a percentage threshold of leaves infected with powdery mildew). The application of these decision rules makes it possible to reduce the number of fungicide applications against scab and/or powdery mildew by more than 50%, while keeping these two diseases under control. In organic farming systems, the cultivar most susceptible to scab had scab damage on fruits despite careful reduction of the inoculum at fall and a large number of fungicide treatments during the season. No fungicide protection treatment for post-harvest diseases was applied in organically grown orchards nor in the low-input system for the two cultivars considered not to be highly susceptible to these diseases. These different protection strategies are assessed in terms of disease control and economic costs.

Key-words: apple orchard, disease, fungicide, protection strategy, sanitation practice, cultivar susceptibility

Introduction

The control of apple fungal diseases in French orchards is mainly based on chemical control. To satisfy society’s demand to decrease the number of plant protection treatments, it is necessary to reduce the high number of fungicide applications in apple orchards. An orchard system trial was designed (Simon et al., this volume) in order to test several pest and disease management regimes that were based on patterns of decision rules.

The aims of this study were (i) to test integrated disease control strategies, based on the combination of validated methods (cultivar resistance, sanitation, restricted chemical control), and (ii) to carry out a multi-criteria (agronomic, economic) assessment of these integrated strategies.

Materials and methods

Experimental site

The experimental orchard was planted in January 2005 at the INRA experimental unit of Gotheron. Located in Southern France in the middle Rhône Valley, the area has a continental climate with summer Mediterranean influences. The specific disease context on the experimental site is the absence of fireblight and of Vf-virulent strains. Lastly, copper
applications against Nectria canker are not necessary at fall.

**Experimental design**

The experimental design includes 3 orchard systems with different weed, pest and disease management regimes (Simon *et al.*, this volume). The frames for disease management are:
- **Supervised (SV)**: «supervised chemical control of diseases»,
- **Low-input (LI)**: «reduction of the use of fungicides»,
- **Organic (OG)**: «no synthetic fungicide, reduction of the use of mineral fungicides ».

Three apple cultivars were planted in each of these three systems:
- **Smoothee 2832T®**: susceptible to scab, powdery mildew and *Gleosporium* spp. diseases;
- **Melrose**: low-susceptibility to scab and *Gleosporium* spp. diseases, susceptible to powdery mildew;
- **Ariane**: scab resistant (*Vf* gene), susceptible to powdery mildew, low-susceptibility to *Gleosporium* spp. diseases (Giraud *et al.*, 2006).

The combination of systems and cultivars creates a nine plots (each of 0.37 ha) experimental design. All the plots have been planted on the same rootstock (PI80) and at the same planting density. Except for plant protection strategies, the plots have the same cultural practices. Only the OG system differs for fertilization (organic inputs) and thinning (hand-thinning only). Orchard and tree training aim at lowering disease incidence: low planting density (5 m between row by 2 m within row) and centrifugal training favouring canopy aeration and growth stop in summer (Simon *et al.*, 2006).

**Sanitation practices and fungicide strategies**

Sanitation practices and fungicide strategies that were applied are presented in table 1 and 2.

For scab management, the leaf ploughing in / removal sanitation practice consists in the removal of litter leaves from the alley with a lawn sweeper combined with leaf ploughing in within the row with a cultivator (Gomez *et al.*, 2007). Olivier proposals consist in the use of a Mills curve as a threshold for fungicide applications (Olivier, 1986). For example, for a low-susceptibility cultivar, with a low autumn inoculum, the threshold is the severe Mills risk during the period of high ascospore ejections (Brun *et al.*, this volume). The *Vf* fungicide scab strategy consists in the protection of moderate and severe Mills risks during the period of high ascospore ejections. The standard one consists in the protection of any risk of contamination during the primary period of ascospore ejections (Table 1). At the end of the primary contamination period, fungicide applications are stopped unless more than 1% shoots are scabbed (except in OG Melrose, OG Smoothee® and LI Melrose where the threshold is 2% of scabbed leaves).

<table>
<thead>
<tr>
<th>Smoothee®</th>
<th>Supervised</th>
<th>Low-input</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sanitation practice</strong></td>
<td>Optional leaf shredding*</td>
<td>Ploughing in /removal</td>
<td>Ploughing in /removal</td>
</tr>
<tr>
<td><strong>Fungicide strategy</strong></td>
<td>Standard</td>
<td>Standard</td>
<td>Standard</td>
</tr>
<tr>
<td>Melrose</td>
<td><strong>Sanitation practice</strong></td>
<td>Optional leaf shredding*</td>
<td>Ploughing in /removal</td>
</tr>
<tr>
<td><strong>Fungicide strategy</strong></td>
<td>Standard</td>
<td>Olivier proposals</td>
<td>Olivier proposals</td>
</tr>
<tr>
<td>Ariane</td>
<td><strong>Sanitation practice</strong></td>
<td>Systematic leaf shredding</td>
<td>Ploughing in /removal</td>
</tr>
<tr>
<td><strong>Fungicide strategy</strong></td>
<td><em>Vf</em> strategy</td>
<td><em>Vf</em> strategy</td>
<td><em>Vf</em> strategy</td>
</tr>
</tbody>
</table>
For powdery mildew management (Table 2), the removal of infected shoots is realised at pruning in winter. Between green tip and bloom, the removal of primary infected buds is realised at performing centrifugal tree training (artificial extinction procedure). The fungicide strategy is based on Audemard et al. (1993) proposals (SV system) or on simplified Audemard et al. proposals (LI and OG systems). After blooming, leaf assessments of powdery mildew (5 unrolled leaves at the top of 100 shoots) are carried out every 14 days and/or when the previous fungicide application is no more effective against the disease. When the treatment threshold is reached, one fungicide treatment is applied, followed by a second one when it is no more effective (Table 2). The protection against powdery mildew stops at the end of the growth period of shoots (generally at the end of June).

Before harvest, fungicide applications against storage disease are applied in the SV system and in LI Smoothee® only.

Table 2: Disease management strategies against powdery mildew

<table>
<thead>
<tr>
<th>Sanitation practices Winter</th>
<th>Supervised</th>
<th>Low-input</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Removal of infected shoots at pruning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between green tip and bloom</td>
<td>No bud removal</td>
<td>Removal of primary infected buds</td>
<td></td>
</tr>
<tr>
<td>Fungicide strategy Before bloom</td>
<td>(Audemard et al., 1993)</td>
<td>(simplified Audemard et al. proposals)</td>
<td></td>
</tr>
<tr>
<td>Systematic fungicide application</td>
<td>No fungicide application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After bloom</td>
<td>&lt; 2% infected leaves: no fungicide application</td>
<td>&lt; 5% infected leaves: no fungicide application</td>
<td></td>
</tr>
<tr>
<td>From 2 to 5%: sulphur application</td>
<td>≥ 5% infected leaves: synthetic fungicides application</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5% infected leaves: sulphur fungicides</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and discussion

From 2005 to 2008, a good efficacy of the fungicide strategy against powdery mildew was observed for all of the plots. From 2007 to 2008, there was no development of post-harvest diseases at storage. From 2005 to 2008, no scab protection was necessary after the primary period of ascospore ejections (generally at end-May) except for OG Smoothee® which required sulphur applications during summer 2007 and 2008. At harvest 2006 and 2007, there were no scabbed fruits except 0.3% in the OG Smoothee® plot in 2007. But, in 2008, 8%, 4% and 1% of scabbed fruits were observed in OG Smoothee®, OG Melrose and LI Melrose, respectively. The year 2008 was an exceptional rainy year with 33 Mills risks (among which 8 severe ones) observed from green tip stage to harvest.

The annual mean number of fungicide applications (2006-2008 period) ranged from 5 (LI Melrose) to 18 (OG Smoothee®) (Figure 1).

For the French context, the annual mean cost of disease protection (including input, labour, and machinery) ranged from 380 €/ha (SV Ariane) to 1200 €/ha (OG Smoothee®).

These results show that the combination of validated management strategies against apple diseases make it possible to strongly reduce the number of fungicide applications while keeping diseases under control and protection costs at an acceptable level.
Figure 1. Annual mean number of fungicide applications per target disease according to decision rules.

References


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Brown Rot Disease Development and Management Perspectives in Organic Apple Orchards

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Abstract. Brown rot of apple, caused by Monilinia fructigena, is a serious disease in organic orchards especially if preceded by severe fruit injuries caused by codling moth. Therefore, the aims of this three-year study were first, to monitor and analyze summer disease development of brown rot in time; second, to investigate environmentally friendly disease control approach against brown rot; and third, to develop an overall brown rot management strategy for organic apple production. Brown rot monitoring showed that epidemics started 3 to 4 weeks earlier on the ground than in the tree, then continuously increased up to harvest. Analyses of disease progress curves showed that the three-parameter logistic function gave the best fit to brown rot over four non-linear growth functions. Data analyses demonstrated an overall description of fruit rot development by relative rate of disease increase (β), area under disease progress curve (AUDPC), and final disease incidence (Yf). Yf in the tree was highly correlated with incidence on dropped fruit on the orchard floor, showing strong evidence of vertical inoculum movement from the orchard floor to the tree. Based on this result, efficacy of fruit drop removal on fruit rot incidence was studied in integration with Bacillus thuringiensis treatments against codling moth and/or reduced use of sulphur fungicide compounds. Treatments with an integrated control approach resulted in a significantly lower fruit rot incidence on all cultivars compared with general brown rot management schedules. The above epidemiological and control results were incorporated into a novel brown rot management strategy for organic apple orchards.

Keywords: organic production, brown rot, apple, epidemiology, management strategy
Repco results on the control of scab in organic apple cultivation

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Abstract: Apple scab, caused by *Venturia inaequalis*, is mainly controlled by sulphur and copper containing products in organic cultivation of apple. It is EU policy to phase out the use of copper products. Therefore, the aim of the EU project Repco was to find alternatives for copper in organic cultivation of apple. Three years of field experiments were done on the scab susceptible cultivar Jonagold. We report on the efficacy of potassium bicarbonate and yucca-extracts in comparison with sulphur and copper. Applications were made according to the RimPro warning system and the weather forecast during the ascospore season. Then all plots were treated weekly with sulphur till harvest. The efficacy of the treatment schedule of 6 to 7.5 kg potassium bicarbonate plus 4 kg sulphur as a tank mix was as effective as 0.2 kg copper in two years and even as effective as 0.5 kg copper in a third year of experiments. Similarly, the treatment schedule of 7.5 l yucca extract plus 4 kg sulphur as a tank mix was as effective as the copper schedules. It is concluded that both potassium bicarbonate and yucca extract both in combination with sulphur can replace copper treatments to control apple scab in organic cultivation.

*Apple scab, Copper, Organic culture, Potassium bicarbonate, Venturia inaequalis, Yucca extract*
Effect of Cladosporium cladosporioides H39 on conidia production of Venturia inaequalis under orchard conditions

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Abstract: New methods for control of apple scab during summer epidemics are needed for organic farming, since the use of copper fungicides will be restricted in the future. The fungal antagonist Cladosporium cladosporioides H39, pilot-formulated as a water dispersible granule, was applied in an apple orchard during summer 2008. Applications of C. cladosporioides H39 significantly reduced conidia production by the apple scab pathogen Venturia inaequalis by up to 67 %. Applications of sulphur were less effective with a maximum reduction of V. inaequalis conidiation by 27 %.

Key words: apple scab, biological control, Cladosporium cladosporioides, Venturia inaequalis, sporulation

Introduction

Multiple fungicide applications for control of apple scab (Venturia inaequalis) are common in world-wide apple production (MacHardy, 1996). Fungicides based on copper or sulphur are currently used in organic farming (Holb et al., 2003). The use of copper will be restricted in the European Union in the near future (European Commission, 1991). Biological control may be an alternative to the use of fungicides.

Research on biological control of apple scab until now has mainly focussed on reduction of the primary inoculum in overwintering leaves (Andrews et al., 1983; Carisse et al., 2000). New methods are needed to reduce the spread of the disease during the summer season when the disease is progressing in multiple cycles of infection and sporulation, especially in organic farming. Such summer epidemics are driven by conidia of V. inaequalis produced in colonies of V. inaequalis developing biotrophically on infected leaves (MacHardy et al., 2001).

The fungal antagonist Cladosporium cladosporioides H39, isolated from a V. inaequalis colony on an apple leaf, has been selected for its potential to suppress conidiation of V. inaequalis on apple seedlings (Köhl et al., 2009). Applications of conidia of the antagonist C. cladosporioides H39, pilot-formulated as water dispersible granules (WG), reduced conidia production of V. inaequalis under orchard conditions in experiments carried out in 2006 and 2007 conditions. The objective of the experiment carried out in 2008 was to confirm these results in another season and to obtain first insights into the best timing of antagonist applications after predicted infection periods for V. inaequalis.

Material and methods

Production of conidia of H39
For applications in the orchard, conidia of C. cladosporioides H39 were produced in a Solid-State Fermentation (SSF) system by PROPHYTA Biologischer Pflanzenschutz GmbH, Germany. The harvested conidia were formulated as a water dispersible granule (WG). For the preservation of product quality, the final product was stored at 4 °C. Viability of dried
conidia was determined on malt extract agar (1 g malt extract l⁻¹) before the beginning of the field experiment. Conidia incubated for 24 hours at 20 °C with germ tubes longer than half of the minimum diameter of a conidium were considered to be viable.

**Orchard assay**

The experiment was carried out in the organically managed orchard at Applied Plant Research, Randwijk, The Netherlands on 8 year-old trees cv Jonagold. The aim of the experiment was to control the summer epidemic of apple scab by antagonist applications. Therefore, it was essential to allow an initiation of an epidemic in the orchard during the primary season. Weather conditions during the primary season of 2008 favoured scab development and many scab symptoms were found in the orchard by the beginning of June. A severe hail event seriously damaged the orchard on June 22. On June 24 and 26, Topsin-M (a.i. 500 g thiophanate methyl l⁻¹, Certis Europe B.V., Maarsen, The Netherlands) was applied at of rate of 140 ml per 100 litre water (applied at 1000 l ha⁻¹) on the entire orchard and the neighbouring orchards to prevent wound infections by European fruit tree canker (*Nectria galligena*). No further fungicide treatments were carried out to reduce the progress of the scab epidemic before or during the experiment. During the following weeks, trees produced abundant new shoots with new leaves. Such newly formed leaves, not damaged by hail nor reached by the fungicide sprays, were used in the experiment.

The experiment was arranged in a design with 6 blocks, with 2 blocks in the same tree row. Each block consisted of 3 plots, each with 4 trees. Between plots, 2 untreated trees served as a buffer. The following 3 different treatments were randomly allocated to the plots: (1) untreated as control; (2) conidial suspension of pilot-formulated H39 (2 x 10⁶ viable conidia ml⁻¹; 2 l per plot). The spray additive Trifolio S-forte (Trifolio-M GmbH, Lahnau, Germany) was added to the suspension at a rate of 2 ml l⁻¹ to improve the spray layer on the leaf surface. Biweekly applications of H39 were carried out on July 22, July 24, July 28, July 31, August 05, August 07, August 11, and August 18. (3) The third treatment consisted of weekly application of sulphur (Thiovit-Jet, Syngenta Crop Protection B.V., Roosendaal, The Netherlands; a.i. 80 % sulphur) at a rate of 0.4% (400 gram per 100 litre water at 1000 l ha⁻¹). Sulphur treatments were applied on July 22, July 28, August 5, and August 11.

The number of scabbed leaves and the number of scab spots per leaf were assessed for the leaves of 10 shoots of each of the 4 trees per plot before the experiment started on June 11. In total 227 to 239 leaves were examined per plot. On September 19, scab symptoms were assessed on 177 to 232 leaves per plot which had been produced after the beginning of the experiment. Disease severity (% leaf surface covered with scab symptoms) was estimated using following classes: 1: No scab; 2: 1 – 10 % coverage; 3: 11 – 50 % coverage; and 4: 51 – 100 % coverage. A severity index was calculated using the formula:

\[
DS = \frac{(0 x N_1 + 1 x N_2 + 2 x N_3 + 3 x N_4)}{N_{total}} \times 100
\]

In which \(N_1\), \(N_2\), \(N_3\), and \(N_4\) is the number of leaves grouped in the classes 1, 2, 3, and 4, respectively, and \(N_{total}\) is the total number of leaves assessed per plot.

Conidia production of *V. inaequalis* was assessed on susceptible young leaves developed during the course of the experiment on 3 sampling dates. Sampling dates were chosen so that sets of susceptible leaves present during a predicted infection period were sampled approximately 5 weeks after the infection period. The Mills table, based on leaf wetness duration and temperature, was used to predict infection periods. The second youngest just unfolded leaf was labelled 1 to 3 days after a predicted infection period on a set of 3 twigs belonging to the same tree in each plot. The period between predicted infection and first
application of *C. cladosporioides* H39 as well as the number of sprays and period of protection by sprays differed with sampling date (Figure 1). After 35 days, the 2 leaves just unfolded at the date of labelling and the next 2 younger leaves, unfolded after labelling but expanded during the course of the experiment, were sampled, resulting in a sample consisting of 12 leaves per plot. Sampling dates were August 22 (of leaves labelled on July 18), August 26 (of leaves labelled on July 22), and September 04 (of leaves labelled on August 1). The 12 leaves per sample were pooled and put into 250-ml glass bottles. Within 2 h, 100 - 150 ml (depending on leaf mass) of tap water with 0.01 % Tween 80 was added and bottles were shaken with a flask shaker at 700 OCS min⁻¹ for 10 min. From the obtained suspensions, sub-samples of 6 ml were stored at -18 °C. The concentration of conidia of *V. inaequalis* was determined for each suspension with the aid of a haemocytometer. The leaf surface of all leaves per sample was measured with an area meter.

![Figure 1. Predicted infection periods, dates of spray applications of *Cladosporium cladosporioides* H39 and sulphur, and sampling dates (L: Labelling of new leaves; S: Sampling).](image)

**Results and discussion**

Before the experiment started, scab incidence on leaves did not differ for plots belonging to the different treatments. The mean incidence was 81.7 % in plots used as control, 84.6 % for plots later treated with H39, and 84.3 % for plots later treated with sulphur. Also the number of leaf spots per leaf did not differ statistically, so that it can be assumed that scab development was similar in the different plots before the experiment started.

On leaves sampled on August 22, August 26 and September 04 from untreated trees 35,242 (11,447 to 74,870), 30,242 (18,926 to 59,497) and 32,533 (19,698 to 46,350) conidia of *V. inaequalis* cm⁻² leaf surface were produced on average (backtransformed means, range for 6 replicates in brackets) (Figure 2). On leaves of trees treated with *C. cladosporioides* H39, the number of spores was statistically significantly lower with 11,499 conidia of *V. inaequalis* cm⁻² leaf surface (67 % reduction based on backtransformed values) on the first sampling date and 15,139 conidia cm⁻² leaf surface (50 % reduction) on the second sampling date. For the last sampling date, no significant effect was observed for applications of *C.*
**Cladosporioides** H39 with 21,163 conidia cm\(^{-2}\) leaf surface (35 % reduction) on treated leaves. Applications of sulphur resulted in a reduction of the number of conidia of *V. inaequalis* produced per cm\(^{-2}\) leaf surface by 2, 16, and 26 % for the different sampling dates (Figure 2). Scab severity, assessed on September 19, one month after the last treatment with H39, was 2.2 for the control treatment, but statistically significantly lower with 1.8 for H39-treated plots (Figure 3). In sulphur treated plots, scab severity was 2.0 which did not differ significantly from the other treatments.

![Figure 2](image1.png)

**Figure 2.** Effect of treatments with conidia of *Cladosporium cladosporioides* H39 or sulphur on conidia production of *Venturia inaequalis*. Bars of the same sampling date with a common letter do not differ significantly (LSD-test; \(\alpha = 0.05\)).

![Figure 3](image2.png)

**Figure 3.** Effect of treatments with conidia of *Cladosporium cladosporioides* H39 or sulphur on scab severity. Bars with a common letter do not differ significantly (LSD-test; \(\alpha = 0.05\)).

During the orchard experiment environmental conditions resulted in a high number of infection periods and scab development was favoured. Also, the exceptional development of new shoots after the hail event supported the summer epidemic. Under such a severe disease pressure treatments with sulphur often are not sufficient to achieve disease control as was found in our experiment. Treatments with the antagonist H39 reduced conidia production of *V. inaequalis* under such severe conditions, confirming results from orchard experiments carried
out in 2006 and 2007. The strongest effect was found for the first sampling date, when treatments with H39 started after the predicted infection, but were continued until 4 days before sampling. For the third sampling date, multiple treatments with H39 had been carried out before the predicted infection period but the last treatment had been applied 17 days before sampling. In this situation, conidia production of *V. inaequalis* was reduced by 35%. Since the effect of the antagonist may also depend on environmental factors which differed before the different sampling dates, more data are needed from repeated orchard experiments before conclusions on optimum timing of antagonist applications can be drawn.

For the first time scab severity has been assessed after treatments with the antagonist H39. The reduced scab severity at the high scab level observed in the orchard demonstrated that *C. cladosporioides* H39 has a high potential to control scab epidemics.

**Acknowledgements**

This work is funded by the European Commission (Project No 501452; REPCO) and the Dutch Ministry of Agriculture, Nature and Food quality. We thank B. Heijne and R. H. N. Anbergen, Applied Plant Research, and M. Trapman, Bio Fruit Advies, for fruitful discussions and U. Eiben, Prophyta Biologischer Pflanzenschutz GmbH, for encouraging collaboration.

**References**


**Biological control strategy of codling moth with entomopathogenic nematodes in organic and conventional farming**

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**Abstract:** The emergence of resistant codling moth strains to the *Cydia pomonella* Granulosis Virus is a threat to control this pest in organic farming. The research of new biocontrol agents is a high stake to propose alternative solutions to farmers.

On one hand, the efficacy of two entomopathogenic nematode species (*Steinernema feltiae* and *Steinernema carpocapsae*) was evaluated using different exposure methods, against various life stages of the codling moth. In order to simulate the exposure of larvae in apple, young apples were soaked in solutions of various concentrations of each nematodes species at several dates after the sting of 1st instar larvae. This study was completed in 2008 with a test under natural conditions. 5th instar larvae in cocoons were exposed within cardboard strips on which nematode solutions were sprayed under laboratory conditions to check the importance of temperature on the control of codling moth with entomopathogenic nematodes. This test under laboratory conditions was completed with a spray application on the ground in an orchard, where 5th instar larvae in cardboard strips had been buried. *S. feltiae* has caused a higher mortality on larvae in apples under laboratory and natural conditions. On fifth instar larvae and at temperature <20°C, the mortality rate was higher with *S. feltiae* (55%) than with *S. carpocapsae* (40%). The application on orchard soil confirmed this.

On the other hand, the toxicity of several plant protection products used in orchard has been evaluated using the method developed by the IOBC working group on “Pesticides and Beneficials”. Three insecticides including Carpovirusine® and two fungicides have been evaluated. The carpovirusine exhibited the lowest toxicity level among the tested products. The main life history parameter of nematodes affected by the tested products was fecundity. However, nematode mortality and infectivity were not reduced significantly.

These trials allow consideration of integration of entomopathogenic nematodes in a codling moth control strategy, with foliar and ground application. The selected nematodes species would be *S. feltiae*. As a “cruiser” it has significantly controlled the target stages of the codling moth under natural conditions. This organism can be used in parallel with other plant protection products of orchard farming taking care of the contact duration and of the exposure level.

Key-words: entomopathogenic nematodes, *Steinernema feltiae*, *Steinernema carpocapsae*, codling moth, *Cydia pomonella*, side effects, plant protection products.

**Introduction**

The codling moth *Cydia pomonella* (L.) (Lepidoptera : Tortricidae) is able to develop resistance against chemical and organic plant protection products. Entomopathogenic nematodes have shown an interesting efficacy under natural conditions against codling moth diapausing larvae (Lacey et Unruh, 1998 ; Unruh et Lacey, 2001 ; Lacey et al., 2006). Nematodes efficacy against others codling moth stages has been studied as well as their susceptibility to plant protection products which are commonly used in apple growing.

**Materials and Methods**

Organisms used

The nematodes used for these tests were formulated as packages of 50 million infective
juveniles. Two species were used in each test: Steinernema carpocapsae (Weiser) and Steinernema feltiae (Filipjev).

Codling moths were supplied by the zoology experimental unit of the INRA Magneraud (France, 17) as eggs and as diapausing larvae reared on an artificial diet.

**Entomopathogenic nematodes against codling moth larval stage in apple under laboratory conditions**

Using the method of Charmillot (1994) two neonate larvae were set up at T0 on apples (3-5 cm in diameter from the variety Gala harvested in Saturargues (France,34)). Several dates after the set up (T0 + 1, 3, 6, 10, and 14 d) 40 apples were soaked in a nematodes solution at a corresponding rate of 100 million nematodes /ha (Charmillot et al., 1994). Apples were kept under controlled conditions (25±2°C, 75±15% HR). Codling moth larvae stage and mortality was assessed 48 hours later. The observation of the damage on the apples was done following the method of Baggiolini and Grob (ECB method N°18, AFPP, 1987).

**Entomopathogenic nematodes against codling moth larval stage in apple under natural conditions**

In an apple orchard (Gala variety, 1988, France, 34), seven treatments were performed: a water treated control and three rates of each nematodes species (5, 15.81 and 50 nematodes /cm2). The orchard was artificially infested with codling moth black head stages distributed homogeneously in the orchard. Each replicate corresponded to an elementary plot of twelve apple trees (72 m2). Apples of the 6 central trees were infested. Three replicate plots have been set up per treatment group.

A counting to assess the damages and the level of infestation was performed three days after the set up of the eggs. The damage observed was at the level 1a-2a (following the scale of Bagglioni and Grob), caused by codling moth first and second larval stages. The application was performed at a volume of 500L/ha. Mortality was assessed 7 days later. Temperature conditions during the exposure period (07/06/2008 to 17/06/2008) were between 9.8°C and 29.2°C.

**Entomopathogenic nematodes against codling moth diapausing larval stage under laboratory conditions**

Using the method developed by Lacey and Unruh (1998), diapausing larvae of codling moth were exposed through corrugated cardboard strips where they had spun their cocoons, at a rate of 10 entomopathogenic nematodes/sq.cm and at the following temperature conditions: 15, 20, 25, and 30 °C. 4 replicates of 10 larvae were established for each treatment group.

**Entomopathogenic nematodes against codling moth diapausing larval stage under natural conditions**

In an apple orchard (Gala variety, 1988, France, 34), seven treatments were performed: a water treated control and three rates of each nematodes species (5, 15.81 and 50 nematodes /cm2). The orchard was artificially infested with codling moth diapausing larvae in corrugated cardboard strips in the soil (2 cm depth). Ten larvae were placed in each strip of corrugated cardboard. Each replicate corresponded to an elementary plot of 12 apples trees (72 m2). Codling moth larvae were placed at the bottom of the 6 centered trees. Three elementary it is unclear what you mean by ‘elementary plots’. Please revise. plots have been set up per treatment group.

The strips were collected two days later and brought back to the laboratory to be observed seven days after the application.
Side effects of plants protection products on entomopathogenic nematodes

Side effects of selected PPP used in apple growing were studied using the IOBC method of Peters (2003).

Table 1: List of the PPP tested

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Use</th>
<th>Active substance</th>
<th>Recommended rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carpovirusine ®</td>
<td>Insecticide</td>
<td>CpGv</td>
<td>1 L/ha</td>
</tr>
<tr>
<td>Suprême ®</td>
<td>Insecticide</td>
<td>Acetemipride</td>
<td>250 g/ha</td>
</tr>
<tr>
<td>Syllit ®</td>
<td>Fungicide</td>
<td>Dodine</td>
<td>169 g/ha</td>
</tr>
<tr>
<td>Teppeki</td>
<td>Insecticide</td>
<td>Flonicamide</td>
<td>140 g/ha</td>
</tr>
<tr>
<td>Topaze</td>
<td>Fungicide</td>
<td>Penconazole</td>
<td>250 g/ha</td>
</tr>
</tbody>
</table>

Results and discussion

Entomopathogenic nematodes against codling moth larval stage in apple under laboratory conditions

Correspondence between the soaking date, the level of damage and the codling moth stage in the apples is shown in Table 2. Mortality results are presented in Table 3.

Table 2: Larval instars of the codling moth at the nematode infestation time (t = 0 sting of the neonate larvae)

<table>
<thead>
<tr>
<th>Soaking date</th>
<th>Codling moth stage</th>
<th>Location in the apple and damage observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>t +1 L1</td>
<td>Some mm under the skin / 1a</td>
<td></td>
</tr>
<tr>
<td>t +3 L2</td>
<td>Some cm under the skin / 2a</td>
<td></td>
</tr>
<tr>
<td>t +6 L2 – L3</td>
<td>Pips nearly reached / 3a</td>
<td></td>
</tr>
<tr>
<td>t +10 L3 – L4</td>
<td>Tunnel to the pips / 3a, 4 a</td>
<td></td>
</tr>
<tr>
<td>t +13 L4 – L5</td>
<td>Pips eaten, larvae in the center of the apple / 4 a</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Mortality of codling moth larvae in the apples soaked after several time after the infestation of the larvae

<table>
<thead>
<tr>
<th>Soaking date</th>
<th>Control*</th>
<th>S. carpocapsae*</th>
<th>S. feltiae*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T+1</td>
<td>5.40 b</td>
<td>13.70 b</td>
<td>42.08 a</td>
</tr>
<tr>
<td>T+3</td>
<td>10.80 b</td>
<td>33.81 a</td>
<td>49.01 a</td>
</tr>
<tr>
<td>T+6</td>
<td>5.00 b</td>
<td>29.64 a</td>
<td>36.73 a</td>
</tr>
<tr>
<td>T+10</td>
<td>16.07 b</td>
<td>46.48 ab</td>
<td>75.00 a</td>
</tr>
</tbody>
</table>

* Numbers followed by the same letter within a column are not significantly different at the significance threshold of 5% following the results of the Newman and Keuls multiple mean comparison test performed after the analysis of variance.

S. feltiae efficacy is greater on L1 and L3-L4 codling moth stages. As a “cruiser”, this species is more effective on less motile hosts (Kaya and Gaugler, 1993) compared with S. carpocapsae which is an ambusher and which does not move to meet its host. The difference between host-searching behaviors of these nematodes species can explain this phenomenon.
Entomopathogenic nematodes against codling moth larval stage in apple under natural conditions

![Graph showing mortality of larvae collected in apple after the exposure under natural conditions](image)

Figure 1: Mortality of larvae collected in apple after the exposure under natural conditions (numbers followed by the same letter are not significantly different at the threshold of 5%)

The rate of nematodes used has a significant effect on the mortality of codling moth larvae in apple.

Table 4: Results of the probit analysis (lethal doses and confidence interval at the threshold of 5%, resistance ratio) of tests under natural conditions on larvae in apples

<table>
<thead>
<tr>
<th>Nematodes species</th>
<th>LR10 Nematodes/sq.cm</th>
<th>LR50 Nematodes/sq.cm (CI 95%)</th>
<th>LR90 Nematodes/sq.cm</th>
<th>RR50</th>
<th>RR90</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. carpocapsae</td>
<td>0.16</td>
<td>143.70 (45.82-8752499)</td>
<td>123416.65</td>
<td>2.11</td>
<td>67.74</td>
</tr>
<tr>
<td>S. feltiae</td>
<td>2.55</td>
<td>68.10 (29.19-40.45)</td>
<td>1821.99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LR = lethal rate, 95% of confidence interval  
CI = confidence interval  
RR = resistance ratio, 95% of confidence interval.

No good dose response effect could be determined for S. carpocapsae from the probit analysis. S. carpocapsae is less effective than S. feltiae on codling moth larvae in fruits. To obtain 90% larval mortality a 68-fold rate of S. carpocapsae has to be used compared with S. feltiae. This confirmed what was already observed under laboratory conditions.

Side effects of plants protection products on entomopathogenic nematodes

Table 6: Impact of the tested products on Steinernema carpocapsae
The only life history parameter of entomopathogenic nematodes which has been affected was the fecundity.

These studies revealed an interesting potential of entomopathogenic nematodes to control codling moth larvae at several stages including stages located in fruits. Moreover, in short term strategies, this method could be compatible with conventional and organic farming as low impact on nematodes mortality or infectivity of PPP has been reported.

Acknowledgements

We thank E-Nema and Arne Peters for the supply of nematode strains to perform the test.

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Mass releases of Trichogramma minutum to control the obliquebanded leafroller, Choristoneura rosaceana, (Lepidoptera: Tortricidae) in apple orchards

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Abstract: Control of the obliquebanded leafroller (OBLR) represents a challenge for apple growers because all stages of this multivoltine pest can be simultaneously present on apple fruits and leaves during summer. In order to establish a new control strategy that targets the pest eggs, we evaluated the impact of repeated mass releases of the egg parasitoid, Trichogramma minutum, on OBLR populations and damage compared to conventional (chemical) and control treatments. Approximately, 1 million egg parasitoids/ha/week were released during 11 weeks in high-density plots of commercial apple orchards. More than 80% of sentinel egg masses were parasitized in the release plot from the second week after the first release. Sentinel egg masses in trees in which T. minutum were released were not more frequently parasitized than those placed at mid-distance between two trees but the number of parasitized eggs/egg masse differed significantly between those trees. The impact of treatments was evaluated by sampling 100 annual shoots and 200 apples per treatment plot. An average of 8.7 larvae per sampling unit was observed in the release plot and was not significantly different from the chemical treatment (11 larvae) and the control (10 larvae) plots. Damage made by OBLR larvae on apples was similar between treatments but damage made by total tortricids was significantly lower in the release (1.8%) and the chemical control (2.1%) plots than in the control (3.1%) plot. Results suggest that mass releases of Trichogramma minutum should be used with a complementary control measure to significantly reduce OBLR population and damage.

Mass release, Egg parasitoid, Trichograms, Biological control, Oblique banded leafroller
Assessing the role of Syrphidae in the suppression of woolly apple aphid in Virginia, USA

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Abstract: The fate of individual woolly apple aphid colonies on the branches of potted apple trees deployed in an experimental and a commercial orchard or held in a screened cage was recorded at 2-day intervals over 14 days from late May to early June, 2008, in Virginia, USA. Colonies on trees in the orchards either became extinct or were severely disrupted by predation by day 14. Two syrphid species, *Heringia calcarata* and *Eupeodes americanus* were the predominant arthropod predators recorded in colonies. Colonies on trees in the cage showed no decline, despite the presence of large numbers of the parasitoid, *Aphelinus mali*.

Key words: *Eriosoma lanigerum*, predation, hover flies

Introduction

Although the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) is common and widespread in apple orchards in eastern North America, its populations reach economically important levels only infrequently and unpredictably. In most seasons, a guild of arthropod natural enemies consisting mainly of the parasitoid, *Aphelinus mali* (Haldeman) and several species of Syrphidae (Brown and Schmitt 1994) appear to provide levels of biological control sufficient to prevent outbreaks. Occasionally however, outbreaks of the aphid occur, sometimes on a regional scale, causing significant injury.

Short and Bergh (2005) showed that the eggs of the three most prominent hover fly predators of woolly apple aphid, *Heringia calcarata* (Loew), *Eupeodes americanus* (Weidemann) and *Syrphus rectus* Osten Sacken, can be readily differentiated, based on the surface sculpturing on the exochorion of the egg. Using this method to separate species, Short and Bergh (2004) found that eggs of *H. calcarata* were recovered only from colonies of woolly apple aphid and that eggs of *E. americanus* and *S. rectus* were recovered from colonies of woolly apple aphid, spirea aphid and rosy apple aphid occurring in the same apple orchards in Virginia, USA. Larval *H. calcarata* fed preferentially and survived best on woolly apple aphid, compared with spirea aphid or rosy apple aphid (Short and Bergh 2004). In combination, these data led Short and Bergh (2004) to conclude that *H. calcarata* is a specialized predator of woolly apple aphid in the apple ecosystem. Bergh and Short (2008) sampled eggs from woolly apple aphid colonies on the branches of potted apple trees deployed in an experimental apple orchard for 48-h intervals weekly from early April through late September from 2003-2005. Eggs of *E. americanus* were always present earliest, showing a pronounced peak in April or May, a decline in numbers from June through August and a minor secondary peak in September. Eggs of *H. calcarata* appeared somewhat later, typically in May and were present throughout the season. Eggs of *S. rectus* were least abundant and did not show a consistent pattern of abundance among the three seasons.

Our working hypothesis is that regulation of woolly apple aphid populations, which typically peak in June in the mid-Atlantic states (Brown and Schmitt 1994), occurs through
the effects of natural enemies relatively early in the season and that disruption of these effects can lead to damaging outbreaks later. As one of several first steps toward understanding the temporal dynamics of the relationship between the pest and its enemies and the relative role of each biological control agent, we examined the fate of individual woolly apple aphid colonies on potted apple trees deployed in apple orchards.

Material and methods

‘Gala’ apple trees on M.26 rootstock were grown in 19 liter plastic pots and infested with woolly apple aphids from colonies on other potted trees. When relatively small, discrete colonies had formed on the branches of the trees, they were transported to a research orchard at the Virginia Tech station and to a commercial orchard nearby. On 25 May, 2008, 5 trees were placed in holes in the ground beneath the canopy of randomly selected, mature trees in each orchard so that the soil was flush with the rim of the pot containing the tree. Five trees were also placed in a 1.8 x 1.8 x 3.6 m screened field cage at the research station. Five colonies on each tree were flagged and numbered. Beginning on 27 May and at 2-d intervals through 8 June, the status of each colony was rated by visual observation, according to the following scale: 5) colony in pristine, undisturbed state, 4) colony showed some signs of disturbance but was mostly intact, 3) colony showed about 50% of original integrity, 2) colony showed about 25% of original integrity, 1) colony nearly completely eliminated but contained a few live aphids, 0) colony was extinct. At each 2-d interval, the number of syrphid eggs near each colony was counted, using a 16x lens. As well, hover fly larvae and other predators in each colony were counted and the presence or absence of adult *A. mali* was noted. After 14 days (on 8 June), a short section (about 3 cm) of branch containing each colony was pruned and placed individually in small, clear plastic cups. In the laboratory, the number of hatched and unhatched eggs of each syrphid species in each colony was counted and recorded under a microscope. Each colony was then returned to the covered, clear plastic cup and held in a controlled environment chamber at 25°C and a 14:10 light:dark regime. At intervals over about 3 weeks, adult *A. mali* that had emerged from each colony were counted and removed from each cup.

Results and discussion

Female syrphids oviposited in 44% of colonies on trees in each orchard during the first 48 hours of exposure. By day 14 (8 June), 96% and 100% of colonies on trees in the commercial and research station orchards, respectively, contained ≥1 hatched and/or unhatched egg, while no hover fly eggs were recovered from colonies on trees in the screened field cage. During the study, hover fly larvae were observed in 52% of colonies in each orchard. The only other predators recorded were lacewings; their eggs and larvae were recorded from 24% and 8% of colonies in the research station orchard.

Examination of excised colonies on day 14 revealed that eggs of *H. calcarata* comprised 60.8% and 79.6% of eggs on trees in the research station and commercial orchards respectively. Eggs of *E. americanus* comprised 39.2% and 13.9% of those in the research station and commercial orchards, respectively, while eggs of *S. rectus* represented 6.5% of those from the commercial orchard. No unhatched eggs were found in colonies on trees in the research station orchard while a few unhatched eggs of *H. calcarata* were found in the commercial orchard. The mean total number of hatched and unhatched eggs per colony recorded on day 14 ranged from 1.4 to 2.6 and from 2.0 to 5.4 on trees in the research station and commercial orchard, respectively. *H. calcarata* eggs were the only eggs recorded from 38% and 50% of colonies at the research station and commercial orchards, respectively. Only
E. americanus eggs were found in 24% of colonies at the research station. S. rectus eggs were never found by themselves. Both H. calcarata and E. americanus eggs were found in individual colonies on trees at the research station (33%) and commercial orchard (32%). Both H. calcarata and S. rectus eggs were found in 5% of colonies at the commercial orchard and eggs of all three species were found in 5% and 14% of colonies at the research station and commercial orchard, respectively.

Recovery of adult A. mali from the excised colonies revealed significantly higher numbers of parasitoids per tree from colonies on caged trees (358.6 ± 24.2 SE) than from colonies on trees in the research station (2.6 ± 1.7 SE) or commercial (36.6 ± 13.1 SE) orchards.

Colony decline was not observed on trees held in the screened field cage and the average colony status rating remained at 5 (undisturbed, pristine state) for the duration of the study. In fact, many colonies showed an increase in size, although this was not quantified. An unknown predator, likely birds or an insectivorous mammal caused a very rapid decimation of many colonies on trees in the research station orchard on day 4 of the study and consequently it was not possible to adequately interpret the effects of arthropod natural enemies on colony collapse in that orchard. In the commercial orchard, the decline of colonies was first noted on day 8 (2 June), and the average colony rating on each tree showed a continuous reduction through 8 June, at which time the average rating was 2.2, 0.2, 0.2, 1.6 and 0.6 for trees 1 through 5, respectively.

These data reveal that, under the conditions of this study, hover fly larvae appeared to be responsible for the collapse of woolly apple aphid colonies. While H. calcarata was most numerous, it is likely that both it and E. americanus played an important role in colony decline. H. calcarata larvae are smaller than those of E. americanus and while the voracity of E. americanus larvae feeding on woolly apple aphid has not yet been determined, it is likely that they consume more aphids than does H. calcarata. The common observation of multiple eggs per species in a given colony and multiple species per colony concurs with our previous findings (Bergh and Short 2008) and raises questions about the interactions among the predatory syrphid species. Intraguild predation by larvae of one species on another or intraspecific cannibalism of younger larvae by older ones may influence the dynamics of woolly apple aphid biocontrol, particularly considering the generalist versus specialist habits of the different guild members. Very importantly, our confirmation that A. mali was present in colonies on trees in both orchards suggests strongly that aphid predation by hover flies is influencing the population dynamics of A. mali by predation on parasitized aphids, by reducing the number of aphid hosts, or both. The large numbers of A. mali recovered from colonies on trees in the field cage, in combination with our findings that the parasitoid did not influence colony status during the study, shows that the role of A. mali in early season population suppression of woolly apple aphid needs to be reassessed.

While these data reveal the role of syrphid larvae on the suppression of established, albeit relatively small, woolly apple aphid colonies introduced into apple orchards, they do not provide needed information on the role of natural enemies in aphid suppression during the earlier stages of colony establishment and growth. Hover fly females are known to select host aphid colonies based on a number of criteria, one of which is colony size. Preferential response to and selection of colonies, based on their size, may differ for the specialist and generalist species, but remains undocumented. Furthermore, although A. mali may not be as discriminating as some of the predators, its activity is more adversely affected by cool temperatures than its host. As well, Bergh and Short (2008) showed that the syrphid predators are active earlier in the season than when A. mali is considered to be an important natural enemy. We intend to implement studies similar to that reported here, involving deployment of potted apple trees known to support root colonies of woolly apple aphid. By deploying trees
early in the season, before the aphids have established arboreal colonies, and by following the establishment, growth and fate of those colonies through time, we expect to significantly improve our understanding of the relative roles of the members of this natural enemy guild and the influence of disturbing them at critical periods in the growing season.

References


Habitat and prey preferences of the two predatory bugs Anthocoris nemorum (L) and A. nemoralis (Fabricius) (Anthocoridae: Hemiptera-Heteroptera)

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Abstract: The annual occurrence and distribution of the predatory bugs Anthocoris nemorum and A. nemoralis between apple, pear and herbal vegetation was assessed. In the laboratory anthocorid prey preference was assessed in two-choice experiments with key pests of apple and pear including pear psyllid, apple psyllid, green apple aphid, rosy apple aphid and red spider mites. Anthocorids were the dominant early season predatory bugs, co-occurring with spiders. Anthocoris nemorum dominated in apple, while A. nemoralis dominated in pear. A. nemorum was also common in herbal vegetation, especially in midsummer. Anthocorid numbers were correlated with numbers of collembola, psyllids and aphids in apple, and with numbers of psyllids in pear. A. nemoralis preferred pear psyllid to green apple aphid, while A. nemorum preferred green apple aphid. Both species preferred psyllids to spider mites. In the two years studied, A. nemorum had two generations proving that it can be bivoltine under Danish climate conditions. In the mid summer the higher density of annual vegetation, simultaneous with lower density in trees, suggests that herbal vegetation may maintain A. nemorum in orchards at times of low prey numbers in the trees. Habitat and prey preferences of the two anthocorid species identify A. nemorum as a biological control agent of special importance in apple, whereas A. nemoralis is of importance in pear.

Anthocoris nemorum, Anthocoris nemoralis, Voltinism, Orchard, Psyllids, Cacopsylla pyri, Cacopsylla mali, Aphis pomi, Dysaphis plantaginea, Panonychus ulmi, Preference, Behaviour
Does windborne pollen mediate the effects of pesticides on predatory mites?

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Abstract: Generalist predatory mites belonging to the family Phytoseiidae can persist in European apple orchards when prey is scarce by feeding on pollen and other alternative foods. It has been reported that grass management can affect pollen availability on apple leaves with implications for phytoseiid persistence. The use of pesticides is a major factor affecting phytoseiid abundance in apple orchards. In this study we compared the effects of a number of pesticides on populations of Kampimodromus aberrans in two apple orchards with a different grass management, i.e. a high or a low grass mowing frequency. Reducing grass mowing frequency resulted in higher predatory mite numbers probably because of a higher pollen availability on apple leaves. A laboratory study was planned to demonstrate the role of pollen availability in mediating interactions between pesticides and phytoseiids.

Phytoseiidae, Pollen, Pesticides, Kampimodromus aberrans, Grass management
A new view of the sooty blotch and flyspeck fungal complex on apples

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Abstract: Fungi in the sooty blotch and flyspeck (SBFS) complex blemish the epicuticular wax layer of apple fruit. Recent studies combining molecular techniques with morphological characterization revealed that the SBFS complex is far more diverse than previously realized. Surveys of orchards in 14 eastern U.S. states in 2000 and 2005 uncovered 62 SBFS species in five taxonomic orders. Orchards with fungicide-spray programmes had lower diversity in their SBFS assemblages than non-sprayed orchards. Some SBFS species occurred in almost all orchards, whereas other species were regional in distribution or were found in only one or two orchards. Collaborations with other laboratories have revealed patterns of SBFS diversity in Germany, Serbia and Montenegro, Brazil, China, Florida, and Costa Rica, and have led to the discovery of many new species. Using an RFLP method for HaeIII digests of rDNA, we found distinctive banding patterns for 14 genera and species. With our library of RFLP banding patterns and ITS and LSU sequences, we documented consistent phonological patterns among SBFS species in timing of colony appearance on apples, and identified several new reservoir host species. These tools have the potential to further clarify SBFS ecology, etiology, and taxonomy. In adapting a SBFS warning system from the Southeast U.S. for use in the Midwest, we found that cumulative hours of relative humidity greater than 97% was more accurate than cumulative hours of leaf wetness in predicting the initial appearance of SBFS colonies on apples.

IPM, Sooty blotch and flyspeck, Genetic diversity, Biogeography, Disease-warning systems
Fireblight research: Warming up to new ideas and solutions.

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Laboratoire de production fruitière intégrée de l’IRDA, Mont-Saint-Bruno, Québec

Abstract: Fire Blight (caused by *Erwinia amylovora*) remains a big concern in apple production regions around the world. Every three years, researchers meet for an international workshop dedicated to this disease. In 2007, the Portland (Oregon) meeting brought forward recent findings in pathogen biology, genomics, host-pathogen interactions and disease management. This talk will attempt to link the important findings reported at the meeting and see how novel detection techniques, reduction in host susceptibility, and new disease control methods can impact future disease management at the farm level.

*Fire Blight, Disease management*
The complex life history of a predator: sibling species, variability of side-effects and enigmatic disappearances of the earwig

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Abstract: The common earwig (Forficula auricularia), plays an important role in reducing summer pest pressure. However, large inter-orchard and even inter-annual variation in earwig densities jeopardizes biocontrol reliability. To boost populations of univoltine earwigs we need a more detailed knowledge on presence, life history and interactions with orchard management. Detailed population monitoring and experimentation revealed some critical issues for biocontrol: (1) F. auricularia consists of two different phylogenetic species with different reproductive strategies (timing of egg-laying and number of broods). Which type inhabits an orchard determines population development and recovery potential after catastrophic events. (2) Earwig populations show high variability in responses to specific orchard management. Repetitive field trials aiming to determine side-effects of insecticide treatments and mechanical weeding showed wide ranges of effects. This is due to exposure level and rigidity of the earwigs rather than migration or mobility. To determine true side-effects, long-term (up to 1 month) monitoring is essential. (3) Earwig populations crash at two critical periods. Losses of nesting females during hibernation are very high, a factor most important in limiting population development. A second loss of substantial amounts of earwigs occurs at the moult from 4th instar nymphs to adults, a phenomenon that is perhaps linked to intraspecific competition. Breaking down the complexity of earwig populations in orchards into smaller components provides insights on how to increase populations and biocontrol efficacy of earwigs.

Earwig, Forficula auricularia, Pests, Orchard, Populations
Side effects of pesticides on the European earwig Forficula auricularia L. (Dermaptera: Forficulidae)

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Abstract: The European earwig Forficula auricularia L. (Dermaptera: Forficulidae), a generalist predator in organic and integrated orchards, can contribute to the biological control of woolly apple aphid and pear sucker only when populations are numerous. As earwigs have a single generation per year, a potential side effect of crop protection is likely to influence population dynamics and size. Therefore we studied the effect of 31 plant protection products sprayed at registered dose rates on larvae and adult earwigs using a standardised laboratory test. Earwigs were exposed to fresh dried residue on bean leaves for 5 days and then transferred to rearing units (with additional, untreated food and water) under controlled conditions for another 30 days. Lethal and sub lethal effects were assessed during the entire test period. As the earwigs were collected in Belgian and Dutch orchards populations of both countries were tested separately and exchanged between institutes for independent test validation. Results revealed that 20 compounds proved to be harmless and 5 slightly harmful. The remaining products were moderately harmful till toxic of which some induced abnormal behaviour. We selected 5 of these (abamectine, indoxacarb, spinosad, thiacloprid and flufenoxuron) for dose response testing and demonstrate that registered dose rates of some products hover between harmless and harmful.

Forficula auricularia, Insecticides, Beneficial
Impact of four insecticides on the European earwig, Forficula auricularia L., in an apple orchard

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² Faculty of Agronomy “Eliseu Maciel”(FAEM), Federal University of Pelotas - UFPel, Pelotas, RS - Brazil

Abstract. The European earwig Forficula auricularia (Dermaptera: Forficulidae) is an important predator of psyllids and aphids, including the woolly apple aphid. Resurgence of the latter pest is often connected to the use of pesticides which harm earwigs. A field test was carried out in 2008 with four new-generation insecticides (thiacloprid, spinosad, indoxacarb and flonicamid) used in apple production, to study their effects on earwig populations. Earwigs are nocturnal and hide in shelters during the day. We installed bamboo tubes as artificial shelters at the end of May, for sampling purposes. Once the shelters were clearly occupied by earwigs, and when earwigs were in the 4th instar, the insecticides were applied (4 replicates of 7 trees per plot); control plots were left untreated. The numbers of earwigs in the shelters of 5 trees per plot were assessed for up to 10 weeks post-application, by knocking the earwigs out of the tubes, collecting them in a plastic bag and photographing them for later counts from the digital images. Immediately afterwards, the earwigs were released back to the appropriate tree. All of the insecticides caused significant reductions (Henderson & Tilton method) in the earwig numbers as compared with control populations. Within two weeks post-application reductions were most pronounced for indoxacarb with a maximum of 76%, followed by thiacloprid with 60 %, spinosad with 59 % and flonicamid with 48 %. Six weeks post-application, the population effects were still about −50 % for indoxacarb and thiacloprid, and were reduced to about −30% for flonicamid and spinosad.

Keywords: Forficula auricularia, insecticides, integrated plant protection, side effects

Introduction
During the last years an increase in abundance of woolly apple aphid (Erisoma lanigerum Hausmann) (Hemiptera: Aphididae) populations has been observed in apple orchards in Germany and neighbouring countries. The European earwig Forficula auricularia L. (Dermaptera: Forficulidae) is known to be an important predator of E. lanigerum as well as of other aphids and psyllids (e.g. Mueller et al., 1988; Nicholas et al., 2005; Lahusen et al., 2006, Höhn et al. 2007, Helsen & Simonse, 2006). The question is, if outbreaks of woolly apple aphids might be connected to a reduction of the earwig population due to unintended effects of modern insecticides used against main orchard pests, especially against codling moth (Cydia pomonella)(Lepidoptera: Tortricidae). We chose four modern insecticides for a field test on side-effects on the earwig.

Material and Methods

Experimental orchard
We used an orchard from the field site of our institute in Dossenheim: variety ‘Golden Delicious’ on M 9, planted in 1994 as spindle bush trees.
**Experimental design**
The trial was established as a randomized complete block with four replicates per treatment, seven trees per replicate and the five inner trees used for sampling. The blocks were separated by an untreated apple row. For the assessment of the earwigs, shelters were made from three bamboo tubes glued together. The bamboo tubes had a length of 20 cm, and an inner diameter of ~ 1.6 cm. The shelters were fixed to the tree trunk in vertical position using a wire and with contact to at least one branch. The tubes were open at the lower end and closed by the node at the upper end, thus being well protected against rain. One shelter was installed per tree. The installation of the shelters took place three weeks before treatment. A pre-treatment count was carried out 10 days before the treatment. After treatment counts took place on 7, 14, 28, 42 and 72 days after treatment. With regard to methodical aspects, we also compared shelters made from 1, 2 and 3 bamboo tubes.

To assess the earwigs, the shelters were carefully demounted and the earwigs knocked out of the shelter into a plastic bag painted with a black grid. The plastic bag was carefully spread in a box and photographed for later count of the earwigs from the digital image. Immediately afterwards, the earwigs were released back to the appropriate tree and the shelters re-installed. At each assessment date, 30 to 70 earwigs were collected for the determination of the development stage in the laboratory.

**Insecticides**
Four new-generation insecticides were tested (Table 1). The insecticides were applied with a spray gun connected to a commercial sprayer. The application rate was calibrated and measured with a water meter (4.2 litre of spray solution were applied per replicate; the trees were treated from both sides of the row). The application took place on 17.06.2008.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Active ingredient</th>
<th>g or ml/ha per 1 m canopy height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calypso</td>
<td>480 g/l thiacloprid</td>
<td>100</td>
</tr>
<tr>
<td>SpinTor</td>
<td>480 g/l spinosad</td>
<td>150</td>
</tr>
<tr>
<td>Steward</td>
<td>300 g/kg indoxacarb</td>
<td>85</td>
</tr>
<tr>
<td>Teppeki</td>
<td>500 g/kg flonicamid</td>
<td>70</td>
</tr>
</tbody>
</table>

**Statistical Analysis**
Data were analysed with the Simulate Procedure, SAS 9.1 (proc mixed). Efficacy values were calculated according to Henderson & Tilton (1955).

**Results**
The shelters were quickly accepted by the earwigs. Two weeks after the installation high numbers were caught (162 on average per shelter) (Fig. 1). With regard to the developmental stages, on 13th June 2008, four days before the application of the insecticides, 97 % of the earwigs were L4, 3 % were adults. With the first check after the treatment onwards, only adults were present.

All insecticides caused significant reductions in the earwig numbers compared to the untreated control (Fig. 1). Within two weeks post-application reductions according to Henderson & Tilton were most pronounced for indoxacarb with a maximum of 76 %, followed by thiacloprid with 60 %, spinosad with 59 % and flonicamid with 48 %. Six weeks post-application, the population effects were still about –50 % for indoxacarb and thiacloprid,
and were reduced to about –30% for flonicamid and spinosad (Table 2). Reductions caused by indoxacarb were mostly significantly worse compared with the other insecticides.

![Graph of earwig population reduction](image)

**Fig. 1. Mean number of earwigs per shelter before and after the treatments.**

**Significant differences:** Before treatment: *P*<0.05 for control compared to thiacloprid and indoxacarb; 7 DAT: *P*<0.0001 for control compared to all treatments and Indoxacarb compared to flonicamid; 14 DAT: *P*<0.01 for control compared to all treatments and indoxacarb compared to thiacloprid and flonicamid; 28 DAT: *P*<0.0001 for control compared to indoxacarb, indoxacarb compared to flonicamid and *P*<0.05 for indoxacarb compared to spinosad; 42 DAT: *P*<0.01 for control compared to thiacloprid and indoxacarb, *P*<0.05 for indoxacarb compared to spinosad and flonicamid.

![Graph of earwig population in different shelters](image)

**Fig. 2. Mean number of earwigs in different shelter sizes, made from 1, 2 or 3 tubes.**

**Significant differences:** 11.6.08: *P*<0.01 for b2 compared to b1 and b3; 14.7.: *P*=0.02 for b2 compared to b3; 26.08: *P*=0.02 for b2 compared to b3.

**Shelter sizes**

The different shelter sizes were comparable in trapping success (Fig. 2). There were no consistent significant differences between the sizes.

**Table 2. Reduction of earwig population according to Henderson & Tilton (%) and IOBC classification (1=harmless (<25%), 2=slightly harmful (25-50%), 3=moderately harmful (51-90%), 4=severely harmful (90-100%)).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Henderson &amp; Tilton</th>
<th>IOBC Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Spinosad</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Flonicamid</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

114
75%); 4=harmful (>75%) (Hassan 1994), (DAT=Days After Treatment).

<table>
<thead>
<tr>
<th>Henderson &amp; Tilton (%)</th>
<th>IOBC Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT 7</td>
<td>DAT 14</td>
</tr>
<tr>
<td>Flonicamid</td>
<td>48.3</td>
</tr>
<tr>
<td>Spinosad</td>
<td>58.7</td>
</tr>
<tr>
<td>Thiacloprid</td>
<td>60.5</td>
</tr>
<tr>
<td>Indoxacarb</td>
<td>70.6</td>
</tr>
</tbody>
</table>

**Discussion**

With the small plot design it was possible to detect significant toxicity effects caused by the insecticides. The most severe effect was observed with indoxacarb, followed by thiacloprid and spinosad. Flonicamid was the only insecticide where the reduction stayed below 50%. The decrease in population reduction after 4 weeks and later in our field study is probably due to movement of the earwigs between the plots, especially from untreated areas into treated plots. If a study requires longterm observations (e.g. in the case of insect growth regulators) bigger plot sizes are recommended. Toxicity to earwigs have also been reported by Lahusen et al. (2006) and Höhn et al. (2007) for neonicotinoids and by Cisneros et al. (2001) for spinosad. As the local earwig population produce only one generation per year, the observed impact is serious. Concerning shelter size, all three sizes can be recommended for use.

**Acknowledgments**

We thank Dr. E. Moll, JKI Kleinmachnow, and Dr. D. Stephan, JKI Darmstadt, for their support in the statistical evaluation with SAS.

**References**


SAS, Statistical Analysis System, version 9.1
Control of the woolly apple aphid (*Erisoma lanigerum* Hausm.) by releasing earwigs (*Forficula auricularia* L.) and support oil applications

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5Julius-Kühn Institut Darmstadt, Institut für biologischen Pflanzenschutz, Heinrichstr. 243, D-64287 Darmstadt

Abstract: The woolly apple aphid (*Erisoma lanigerum* Hausm.) has been recognized as a serious pest in organic fruit growing where it may cause severe economic damage due to a lack of control strategies. Based on preliminary results a research project funded by the Federal Office for Agriculture and Food, Germany runs from 2007 to 2009 in cooperation with different research facilities in Germany to develop an on-farm strategy to control the woolly apple aphid in organic fruit growing. Earwigs (*Forficula auricularia* L.), as natural predators of woolly apple aphids, climb the trees when they turn into L3-Larvae in the end of May/beginning of June. By then the population of woolly apple aphid may reach high infestation levels. To control the woolly apple aphid until the earwigs appear in the trees oil applications were made in addition to the release of earwigs. We present preliminary results of the first and second year of the project’s field trials. They showed good efficacies for applying oil preparations by brush in the first year. The efficacy of releasing earwigs is inconsistent and depended on the infestation intensity. In the second year the trials have been expanded by a comparison of oil application by spraying and by brush in combination with releasing earwigs. On high infestation levels the oil application by brush proved to be more effective.

Key words: woolly apple aphid, *Erisoma lanigerum* Hausm., earwig, *Forficula auricularia* L., oil

Introduction

Several experiments trying to regulate the woolly apple aphid (*Erisoma lanigerum* Hausm.) with the parasite *Aphelinus mali* Hald. were performed in recent years but did not produce satisfactory results for a practical fruit growing strategy (Hetebrügge et al. 2006). The common earwig (*Forficula auricularia* L.) is endemic and widespread throughout Central Europe. It is a nocturnal omnivore feeding on animals and plant materials. Earwigs are therefore important natural antagonists of the woolly apple aphid, and they should be encouraged as beneficial organisms in organic orchards. Field trials were set up to investigate woolly apple aphid control by releasing earwigs. In addition, the application of oil was evaluated as a supporting measure to retard woolly apple aphid population growth prior to the release of earwigs. The earwigs overwinter in the ground and the females lay their eggs and raise the brood in nests in the ground. Therefore we investigated if the mechanical soil management typical for organic orchards affects the earwig population.

The project’s trials have been established in cooperation with several partners: At the “DLR Rheinpfalz, Kompetenzzentrum Gartenbau” in Ahrweiler (West Germany), with the “Ökoobstbau Norddeutschland Versuchs-und Beratungsring e.V.” (ÖON) in Jork (“Altes Land”, North Germany), and at the “Kompetenzzentrum Obstbau Bodensee” (KOB)
Additionally round-robin tests in practical growing situations were started in 2007 under coordination of the “Beratungsdienst Ökologischer Obstbau” (Bodensee, South Germany). The health status of the earwigs from all sites were examined in the laboratory of the “Julius-Kühn-Institut” (JKI) in Darmstadt. Considering the complexity of the project, we focus only on selected results of the field trial in this publication.

Material and methods

In three different orchards trials with five different variants (Tab. 1) were established in 2007. In variants 2 and 3 different numbers of earwigs, which differed between the three sites depending on their availability, were released in the orchards in June 2007 (Tab. 1). In variants 4 and 5 additionally two different oil preparations, Promanal Neu and TS-forte, respectively, were applied undiluted by brush in April 2007 (between BBCH 57 - 65) when the colonies just started to produce wool (Tab. 1). A brush whose bristles were cut in half to exploit also the mechanical effect was used to apply the oils. Rolls of corrugated board (Ahrweiler, Altes Land) and coffee filters with wood wool (Bodensee) were hung into the trees and were monitored after a few days to determine the earwig population before the release of new earwigs in the orchards. The hideouts were left in the trees throughout the whole season and were used to estimate the population monthly by counting the contained individuals. The infestation with woolly apple aphid colonies was also determined monthly by investigating the infestation in cm² per tree.

Table 1: Overview of variants and number of released earwigs at all sites in 2007

<table>
<thead>
<tr>
<th>variant ID</th>
<th>title</th>
<th>number of released earwigs per tree</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ahrweiler</td>
</tr>
<tr>
<td>1</td>
<td>control</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>earwig 1</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>earwig 2</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>TS-forte + earwig 1</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Promanal Neu + earwig 1</td>
<td>50</td>
</tr>
</tbody>
</table>

In 2008 the trials were expanded by a comparison between the oil application by brush and application by spraying (variants shown in Table 2). Pipes made of bamboo were used as hideouts in the field trial sites at Ahrweiler and Altes Land. The assessments of woolly apple aphid infestation, the monitoring of the hideouts to investigate the earwig population and the oil application by brush were the same as in 2007. The spraying was done in March before the colonies began producing wool. To examine the influence of mechanical soil management the orchard where the trials were set up in 2007 were used. One row was covered with plastic foil (Maypex) while the other row was treated conventionally with mechanical soil management.

Table 2: Overview of variants and number of released earwigs at all sites 2008

<table>
<thead>
<tr>
<th>variant ID</th>
<th>title</th>
<th>Number of released earwigs per tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>earwig 1</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>earwig 2</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>spraying + earwig 1</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>brushing + earwig 1</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>brushing</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>spraying</td>
<td>0</td>
</tr>
</tbody>
</table>
Results and Discussion

Although the infestation in the plots of variants 2 and 3 started at a lower level than the control plot an influence of the earwigs as a single measure against the woolly apple aphid colonies was significant in the orchard at Lake Constance in 2007. As Fig. 1 shows the increase of the infestation level in the control continued until mid July while the increase in the plots of variant 2 and 3 was stopped after releasing the earwigs in mid June. The efficacies (Henderson & Tilton calculation) were 42% for 50 earwigs in variant 2 and 52% for 100 earwigs in variant 3. The combined treatments of oil and earwigs increased the efficacies to 83% for TS-forte plus earwigs and to 95% for Promanal Neu plus earwigs.

![Woolly Apple Aphid Infestation in Different Variants at Bodensee in 2007](image)

Fig. 1: Woolly apple aphid infestation in the different variants at Bodensee in 2007

In 2008 the woolly apple aphid colonies, especially in the orchard in Ahrweiler, increased to a massive infestation until the end of June. Here a decreasing effect to the woolly apple aphid colonies by earwigs did not manifest in the orchard (Fig. 2). A single adult earwig may devour up to 120 aphids per night (Lohrer 2008). We observed a slight correlation between the number of earwigs and the infestation level of the trees so we assume that the earwigs migrated to trees with high infestation levels. Despite the high eating potential and the presence of earwigs in highly infested trees the amount of released earwigs seemed not to be sufficient to control the massive infestation in Ahrweiler. In addition, we observed a high number of other beneficial organisms, such as ladybeetles, *Aphelinus mali* and the larvae of lacewings and hoverflies which all feed on woolly apple aphid. Oil application by brush showed better efficacies compared to spraying. Brushing allows a more targeted application and exploits additional mechanical effects. Efficacies were 24% for spraying and 70% for the application by brush independent of releasing earwigs.
To determine the influence of the mechanical soil management typical for organic orchards we sampled the earwig population in two rows of Jonagold one of which was covered with a plastic foil (Maypex). The results after one testing season do not show significant difference between the number of earwigs in the trees of the covered and uncovered rows. Earwigs can make their soil nests up to 20 cm below the surface or between the roots of grass and herbs in the alleyways so that the mechanical soil management may not affect them.

**Conclusion**

Earwigs climb the trees in the beginning of June (Gobin et al. 2006) so that there is the same lack of woolly apple aphid control in spring as is known for *Aphelinus mali* Hald. Furthermore the trials showed that earwigs as a single measure against the woolly apple aphid are not sufficient to keep the woolly apple aphid at reasonable levels. Therefore an additional oil application seems to be indispensable. We found higher efficacies for the oil application by brush than by spraying for high infestation levels. The mechanical soil management in organic orchards seems to have no negative influence on the earwig population (one year results). The project will be continued in 2009 to confirm the trial results.

**References**


Population modelling of the European earwig as a decision tool for orchard management

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Abstract: Earwigs, Forficula auricularia (L.) (Dermaptera: Forficulidae) are beneficial predators in apple and pear orchards where they are capable of maintaining several pest species below economic thresholds. Earwigs thus play an important role in integrated fruit orchards and are essential in organic top fruit cultures. Numbers of earwigs show large interannual variations in densities in both organic and IPM orchards, this limits their practical use. All practical attempts for re-establishing earwig populations have failed. These problems indicated that a theoretical approach was necessary. In order to develop strategies for increasing earwig populations we have built a population model. This enables the prediction of earwig phenology throughout the season while a sensitivity analysis allows us to identify key factors and critical periods in the earwigs’ life cycle. The European earwig is a complex of two sibling species. The timing of oviposition, before and after winter respectively, is a big difference in life history characteristics between these species. A day-degree model was constructed and validated with existing field data from several European and non-European populations. Results show remarkable differences between regions regarding both oviposition strategies. Oviposition timing can cause either large variation in earwig phenology or not. First sensitivity analyses reveal that the numbers of nests during winter have a very big impact on the population in relation to spring or summer survival. However more knowledge about the interactions between species and limiting and regulating processes is required for developing specific and effective orchard management strategies. Such work is currently underway.

Population dynamics, Day-degree model
Codling moth insecticide resistance management in North Carolina apples

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Abstract: In recent years the codling moth has become the major pest of apples in North Carolina. The emergence of this pest coincided with the widespread adoption of insect growth regulators and neonicotinoids as primary control tools. A resistance monitoring program was conducted in 2006 and 2007 that used a novel 16-well plasticware containing lyophilized codling moth diet that was rehydrated with insecticide solutions to assay neonates. Resistance was detected to the IGR’s methoxyfenozide and novaluron, and the neonicotinoid acetamiprid. In 2008, codling moth resistance management programs were initiated that relied on the use of mating disruption and targeted applications of two new insecticides, spinetoram and rynaxypyr. Codling moth damage in commercial orchards declined to its lowest levels in recent years, and overall insecticide use was also reduced.

Cydia pomonella, Insecticides, Mating disruption
A new isolate overcoming *Cydia pomonella* resistance to Granulovirus: improvement of its efficiency by selection pressure on resistant hosts.

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³ GRAB. Agroparc, 84914 Aviron Cedex 9, France
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Abstract: Since 2004, some codling moth (*Cydia pomonella*) populations resistant to the Mexican isolate of *Cydia pomonella* granulovirus (CpGV-M) were detected in different organic orchards in Western Europe. A resistant laboratory colony of codling moth (RGV) was built by introgression of the resistance character in a susceptible laboratory colony (Sv). The resistance of the RGV colony to the CpGV-M came over 60,000-fold when compared to the susceptible laboratory colony (to Sv). To overcome this resistance, the efficiency of CpGV isolates from various origins was investigated. Two of them (I12 and NPP-R1) presented an increased activity on RGV larvae. NPP-R1 reduces the resistance factors of RGV to 7-fold and 46-fold at the LC₅₀ and LC₉₀. Genetic characterization showed that NPP-R1 is a mixture of at least two prevalent genotypes, one of them being similar to CpGV-M. The 2016-r8 isolate obtained from eight cycles of selection of NPP-R1 on RGV larvae had a sharply reduced proportion in the CpGV-M genotype and an increased efficiency on RGV. Carpovirusine samples were formulated with these isolates for field experiment. Results from Germany, Italy and France gave promising results, showing that the 2016-r8 isolate is a good candidate to control CpGV-M resistant codling moth populations.

Key words: *Cydia pomonella*, granulovirus, CpGV, resistance, selection, genetic diversity

Introduction

The baculovirus family is divided in two genus, the nucleopolyhedrovirus (NPV) and the granulovirus (GV). In contrast with large genetic and molecular knowledge on nucleopolyhedrovirus (NPV), up to recently limited information was available on the genetic variability of granulovirus isolates. The prototype of the genus, the *Cydia pomonella* granulovirus (CpGV) has been extensively studied, and three main genotypes were identified, namely, the Mexican type (CpGV-M), from the original isolate, the English type (CpGV-E) and the Russian type (CpGV-R) (Crook et al., 1985). Baculoviruses are widely used for the control of insect pests. The use of a single purified genotype that can be characterized in deep versus the use of a natural virus isolate has been object of debate. In the case of CpGV, as no variability was detected in the natural isolate originated from Mexico (Tanada, 1964) , this question has not been addressed. All the virus preparations used in Europe contain this isolate. In 2004, codling moth populations resistant to the virus preparations were detected in Germany and in France. Since then, a more careful analysis of other virus isolates confirmed that they are often composed by more than one genotype (Rezapanah et al., 2008) and they
possess different biological properties, as some are able to replicate in the CpGV-M resistant hosts. Here we present the analysis of a virus isolate, NPP-R1, able to replicate in such resistant host, its genetic heterogeneity and the importance of these genotypes for the final efficacy of the virus in the field.

**Material and Methods**

The CpGV-M strain, usual active ingredient in the commercial formulations was systematically included as a control for the activity. The natural virus isolate NPP-R1 strain is able to partially overcome the resistance in the laboratory colonies. The NPP-R1 isolate was also subjected to selection through successive passages on RGV insects as described previously (Berling et al., 2009). The eighth passage (NPP-R1.8) was used for the 2008 field trials.

All virus isolates were amplified on resistant insects from the original inoculums, to constitute a stock for the test production. Mass production was made on susceptible insects in the same conditions as the industrial production of Carpovirusine®. The different virus isolates were formulated by Natural Plant Protection in the same way as the Carpovirusine®, at a final concentration of 1x10¹³ OBs/L, and used at 1 L/ha.

These formulated virus preparations were called Carpovirusine 2000 for the standard formulation using CpGV-M, Carpovirusine R1 for the NPP-R1 formulation, and Carpovirusine R1.8 for the NPP-R1.8 formulation. Each of these formulations was tested for its ability to control both Sv and RGV larvae in standard bioassays.

**Results and Discussion**

The RGV colony originates from larvae collected in an orchard where CpGV-M was not able to control codling moth. It was selected to genetic homogeneity for the resistance character and for its homogeneous genetic background. CpGV-M LC₅₀ for the susceptible laboratory colony (Sv), that has the same genetic background as RGV is 24 OB/µl. In contrast, for RGV, it is 1.38x10⁶ OB/µl. That is, a factor of 60 000. The NPP-R1 isolate performed as well as CpGV-M on Sv larvae (LC₅₀ = 25.8), but was able to replicate on RGV (LC₅₀ =166) (Table 1). Although the pathogenic effect is relatively high it does not reach the same level on both hosts.

<table>
<thead>
<tr>
<th></th>
<th>Min CL₅₀ (GV/µl)</th>
<th>Max</th>
<th>Min CL₉₀ (GV/µl)</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>CpGV-M/Sv</td>
<td>14,1</td>
<td>35,9</td>
<td>177</td>
<td>512</td>
</tr>
<tr>
<td>CpGV-M/RGV</td>
<td>3.17×10⁻⁵</td>
<td>4.91×10⁶</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>NPP-R1/Sv</td>
<td>14.5</td>
<td>39.9</td>
<td>197</td>
<td>703</td>
</tr>
<tr>
<td>NPP-R1/RGV</td>
<td>91,2</td>
<td>278</td>
<td>5.95×10⁻³</td>
<td>3.80×10⁴</td>
</tr>
</tbody>
</table>

Restriction enzyme length polymorphism analysis revealed that NPP-R1 is composed of at least two genotypes, one similar to CpGV-M and the second, characteristic of NPP-R1, called CpGV-R1 type. CpGV-M alone was not able to produce a pathogenic effect on RGV. As there was a difference in the LC₅₀ for NPP-R1 in Sv and RGV, cycles of replication in both hosts
have been set up to analyse the possible modification of the relative proportions of each genotype in the two alternative hosts. Four successive cycles of replication of NPP-R1 in RGV larvae conducted to the reduction to undetectable levels of the CpGV-M like genotype, and to an increase of the efficacy of the virus isolate. In contrast, no modification was observed when NPP-R1 was amplified on Sv larvae neither in the proportion of the genotypes nor in their biological activities. Both the LC₅₀ and the LC₉₀ on RGV were reduced when such cycles of replication were carried out (Figure 1). Up to the ⁴ᵗʰ passage (NPP-R1.4), this change might be related to the reduction of the CpGV-M component, but both LC₅₀ and LC₉₀ decreased after the following passages. Selection of a better adapted genotype is obviously taking place, even if no modification has been detected using RFLP analysis (data not shown). The different in the shape of both curves can be noticed, LC₉₀ changes faster than LC₅₀ in the first passages, suggesting again the effect of the CpGV-M like genotype in the global efficacy.

![Figure 1. Evolution of the LC₅₀ and LC₉₀ of NPP RI isolate on susceptible (Sv) and resistant (RGV) larvae after successive passages on RGV larvae.](image)

Probably the codling moth populations that host virus infections have variable susceptibility to the virus if there is not a high external pressure, like repeated virus treatments. In such conditions, the genetic diversity of the virus would be an advantage, as the CpGV-M component clearly has higher activity on susceptible larvae, and the CpGV-R1 type performs better on resistant larvae. However, if both genotypes act in a completely independent way, it could be expected the disappearance of CpGV-M in only one passage through RGV larvae. This does not happen. It can be concluded that both genotypes interact during their replication in RGV. Their behaviour in Sv is more difficult to analyse as each genotype alone is able to replicate in this host.

In contrast with NPVs, a single OB in GVs carries only one virion, and consequently, only one genotype. Maintain of the genetic diversity implies that multiple infection is also the rule on GVs, even in the LD₅₀ in the laboratory has been estimated to be lower than five OB (Sheppard and Stairs, 1977). In biological control conditions, most of the host population must be killed for an acceptable reduction of damages, implying a systematic multiple infection.

Large plot field trials with NPP R1 were conducted in 2007 in orchards where resistance was previously confirmed (Berling et al., 2009b). Although good control of codling moth population was not achieved, a significant reduction of the overwintering larvae was obtained. 2008 field trials were carried out using the virus isolate obtained after eight passages, called NPP R1.8. Again, the number of larvae collected in the corrugated cardboard traps decreased in the plots treated with Carpovirusine R1.8 compared to those treated with CpGV-M.

In locations with no resistance occurred, both CpGV-M and NPP R1.8 reduced the
overwintering populations (Table 2). In locations with resistance, the reduction is only significant in parcels treated with Carpovirusine R1.8. In addition, the number of infected larvae collected is lower in parcels treated with the latter, suggesting that the final issue of the infection is the quick death of the larvae, while with CpGV-M, some infected larvae survive at least to the last larval stage, and might produce adults. Would these adults carry a persistent infection similar to that observed for NPV (Cory and Myers, 2003)? And what will be the importance of this vertical transmission in the regulation of the populations? Specific studies will be required to address these questions.

The results obtained by allowing a continuous adaptation of a genetically variable virus isolate to a host clearly confirmed the potential of this method. As more insect generations per year can be obtained in laboratory conditions than in the field (about 10 compared to 3), it can be expected that any new host resistance could be used to select appropriate virus isolates as far as a continuous survey of codling moth field populations could be implemented.

Table 2. Mean number of larvae collected in corrugated cardboard traps in the parcels treated with CpGV-M or NPP-R1.8 formulated as Carpovirusine (Carpovirusine 2000 and Carpovirusine R1)

<table>
<thead>
<tr>
<th>Host population</th>
<th>Virus formulation</th>
<th>Infected</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>Untreated</td>
<td>0.5</td>
<td>6.5</td>
</tr>
<tr>
<td>(Spinimbeco,</td>
<td>C2000</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Italy)</td>
<td>R1.8</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Resistant</td>
<td>Untreated</td>
<td></td>
<td>3.3</td>
</tr>
<tr>
<td>(St Aubin,</td>
<td>C2000</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>France)</td>
<td>R1.8</td>
<td>0</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Acknowledgments

This work was supported by the French Research Agency (ANR- 06-RIB-003-02) and by NPP. Marie Berling received a fellowship from the Ecole des Mines d’Alès.

References


Resistance Management: A Global Industry Response from the Insecticide Resistance Action Committee

Andrea Bassi
Insecticide Resistance Action Committee (IRAC)

Abstract: IRAC was formed in 1984 to provide a co-ordinated crop protection industry response to prevent or delay the development of resistance in insect and mite pests. The main aims of IRAC are firstly to facilitate communication and education on insecticide resistance and secondly to promote the development of resistance management strategies in crop protection and vector control so as to maintain efficacy and support sustainable agriculture and improved public health. It is IRAC’s view that such activities are the best way to preserve or regain the susceptibility to insecticides that is so vital to effective pest management. In general, it is usually easier to proactively prevent resistance occurring than it is to reactively regain susceptibility. IRAC is an inter-company organisation that operates as a Specialist Technical Group under the umbrella of CropLife International. IRAC is also recognised by The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations as an advisory body on matters pertaining to resistance to insecticides. The group’s activities are coordinated by the IRAC Executive and Country or Regional Committees with the information disseminated through conferences, meetings, workshops, publications, educational materials and the IRAC website (www.irac-online.org). The Executive Committee supports resistance management project teams and also provides a central coordination role to regional, country and technical groups around the world. Insecticide resistance remains one of the greatest challenges in modern agriculture and public health pest management, and it is crucial that it is tackled effectively. Indeed, resistance is everyone’s problem and by working together, insecticide resistance can be successfully managed. IRAC is playing a major role in this effort.

IRAC, Resistance management, IPM, IRM, Vectors, Public health
Molecular aspects of QoI and DMI fungicide resistance in NY populations of the apple scab pathogen Venturia inaequalis

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Cornell University, Department of plant pathology and plant-microbe biology, Geneva, NY, 14456

Abstract: Apple producers in the northeastern US are strongly reliant on sterol demethylation inhibitor (DMIs) and Quinone outside inhibitor (QoIs) fungicides to manage yearly epidemics of apple scab. DMI resistance in NY populations of Venturia inaequalis has been observed for several years, but the mechanisms of resistance are not completely understood. Similar to what was described previously, 32 NY V. inaequalis isolates representing a range of DMI sensitivities had anomalous insertions containing promoters upstream of the CYP51A1 gene. Unlike previous reports, several baseline sensitive isolates lacked inserts altogether, while highly resistant isolates provided indications of larger previously uncharacterized insertions. At the range of DMI sensitivities tested, a clearer pattern for this mechanism of DMI resistance is beginning to emerge. In 2007, we detected five isolates in a western NY orchard displaying the qualitative resistance phenotype to QoI fungicides. On sequencing the target site region in the cytochrome b gene, we found that all five isolates had the G143A target site mutation associated with QoI qualitative resistance in Europe. The mitochondrial mutation appeared to be at a homoplastic state on QoI-amended media. However, after three successive transfers on non-QoI-amended media over the course of four months, two of the five isolates reverted to the wildtype genotype, raising questions as to mutation stability in the absence of selective pressure.

Sterol demethylation inhibitor, Quinone outside inhibitor, Fungicide resistance, Apple scab, Venturia inaequalis
Practical aspects of QoI and DMI fungicide resistance in Northeastern US populations of the apple scab pathogen Venturia inaequalis

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Abstract: Sterol demethylation inhibitor (DMIs) and quinone outside inhibitor (QoIs) fungicides are essential for managing apple scab and other early season apple diseases in the northeastern United States. Moreover, a second generation of DMI fungicide chemistries is on the verge of being released for apple disease management in the US. Shifts toward DMI and QoI resistance have been observed in Northeastern US populations of Venturia inaequalis over the past five seasons as use of these fungicide chemistries continues. In 2007 & 2008, we surveyed a minimum of 25 commercial, 4 research, and 3 baseline apple orchards for sensitivity to myclobutanil (DMI), trifloxystrobin (QoI), and dodine (guanidines). We found that all of the commercial orchards were strongly shifted above baseline sensitivity to myclobutanil and trifloxystrobin. We also found that more than 75% of the orchards had a myclobutanil sensitivity level reduced beyond the point in which we achieved apple scab control in our research orchard with DMI-resistant V. inaequalis populations. Interestingly, several orchards have dodine sensitivities approaching that of V. inaequalis populations from baseline orchards. Field testing of DMI and QoI fungicides in DMI-resistant and QoI-shifted orchards suggests that the new chemistries could overcome practical resistance in varieties less susceptible to apple scab, but not in highly susceptible varieties that contribute to high levels of V. inaequalis inoculum. However, it remains to be seen if dodine resistance will quickly re-emerge during a season of renewed use.

Sterol demethylation inhibitor, Quinone outside inhibitor, Fungicide resistance, Apple scab, Venturia inaequalis
Can *Venturia inaequalis* populations show a reduced sensitivity to a multisite fungicide? The case study of Captan in French orchards.

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**Abstract:** Since 2000, the control of apple scab, which is mainly based on chemicals in French orchards, has faced several cases of control failure. One of the causes of this situation could be the emergence of a reduced sensitivity of *V. inaequalis* to multisite fungicides. As multisite fungicides are not known to induce resistance in fungal pathogens, such a possibility has not been investigated. Between 2002 and 2006, different experiments showed an in vivo reduced efficiency (27.6 to 48 % on incidence and severity of the disease) of Captan for the control of a *V. inaequalis* population from an orchard in which this fungicide failed to control scab. This efficiency was lower than that for Mancozeb (96.5 to 100 %) on the same population, and lower than the efficiency of Captan on other populations less exposed to the fungicide. The variability in sensitivity to Captan of *V. inaequalis* strains collected in 5 orchards differently exposed to Captan was assessed in vitro, and a significant difference of ED₅₀ values, which ranged between 5.2 and 51.9 mg/l, was displayed. These results show consistent elements, but not clear evidence of a reduced sensitivity of *V. inaequalis* to multisite fungicides. They support the need for applied and basic research on this question.

**Key words:** apple scab, chemical control, fungicide resistance.

**Introduction**

In 2008, after 2 years of favourable climatic conditions for the development of the disease, scab remains the main problem in French apple orchards, despite protection strategies based on intensive chemical control. Numerous fungicide treatments were applied following a strategy of preventive and curative treatments to flank the main contamination periods. In fact, since 2000, the control of apple scab has faced several cases of failure. The high susceptibility of the most planted cultivars, weather conditions and the emergence of resistance to strobilurin and anilinopyrimidine fungicides probably contributed to this situation. However, another hypothesis, namely the emergence of a reduced sensitivity of *Venturia inaequalis* to multisite fungicides, could explain some failure cases. As multisite fungicides are not known to induce resistance in fungal pathogens, such possibility has not been investigated. Here we present results obtained within the frame of work aimed at testing this hypothesis.

**Materials & Methods**

This work started with the study of a case of unexplained treatment failure reported in a French orchard (orchard 1). Unexplainable scab problems had been reported for 4 years in this isolated 10 ha orchard, where 2 protectant fungicides, captan and mancozeb, had been used
for 40 years. In the 5 years preceding our work, this orchard received 50 captan and 31 mancozeb treatments. More than half of captan treatments were eradicant treatments, applied at double rate. For the tests made in vivo, the efficiency of captan and mancozeb was tested on the inoculum from orchard 1 and from an INRA experimental orchard (orchard 2), which received, during the same period, 26 captan and 4 mancozeb applications (no eradicant treatment). The tests were done in a growth chamber, on apple seedlings from the cross Golden Delicious x Granny Smith. The 2 fungicides were applied 48 or 72 h before inoculation (preventive applications) at French full (1.5 and 1.6 kg/ha) and half registered rate, on 12 plantlets for each treatment. At treatment date, the last unrolled leaf was labelled. The incidence and severity of the disease were assessed 21 days after the inoculation. The protocol of the test and the scale for severity assessment (from 1 to 7) are described in Parisi et al. (1993). For the in vitro tests, monoconidial strains from 5 other French orchards were assessed by a classical mycelium growth test in the presence of 8 doses of captan. The results were expressed as the mean ED 50 of the 13 to 16 strains tested per orchard. The orchards had different exposures to captan (in brackets):
- Orchard 3: untreated plot in a moderately treated farm (low)
- Orchard 4: abandoned for many years (low)
- Orchard 5: commercial orchard treated with captan and problems of scab control (moderate)
- Orchard 6: intensively treated experimental orchard (high)
- Orchard 7: organic orchard, never exposed to captan but treated with copper and sulphur for 20 years (not exposed)

For all the tables, letters indicate homogeneous groups (H.g.) (ANOVA, LSD test, \( P=0.05 \)).

Results and Discussion

The efficiency of captan to control the inoculum from orchard 1 was low (Table 1). No significant difference was found between the incidence of the disease on the control (sprayed with water) and the treated plants. For the severity, the efficiency of the full dose treatment was 48 %. Conversely, the efficiency of mancozeb, tested in the same conditions, was very good (Table 1). The comparison of the efficiency of these 2 fungicides on 2 inoculums with different exposure to captan showed that the low efficiency of captan on the inoculum from orchard 1 was reproducible (Tables 2 and 3) while this efficiency was higher, but partial, on the inoculum from orchard 2 (50 to 58.7 % on incidence and severity, respectively). Mancozeb displayed a good efficiency on these 2 inoculums (Tables 2 and 3).

Table 1. Efficiency of preventive treatments with captan and mancozeb on incidence and severity of apple scab (inoculum from orchard 1)

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Incidence</th>
<th>Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orchard 1</strong></td>
<td>% scabbed leaves</td>
<td>H.g.</td>
</tr>
<tr>
<td>Water</td>
<td>22.6</td>
<td>a</td>
</tr>
<tr>
<td>Captan ( \frac{1}{2} ) reg. rate</td>
<td>18.4</td>
<td>a</td>
</tr>
<tr>
<td>Captan reg. rate</td>
<td>15.7</td>
<td>ab</td>
</tr>
<tr>
<td>Water</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>Mancozeb ( \frac{1}{2} ) reg. rate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mancozeb reg. rate</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Efficiency of preventive treatments with captan and mancozeb on the incidence of apple scab (inoculum from orchards 1 and 2).

<table>
<thead>
<tr>
<th>Inoculum :</th>
<th>Orchard 1</th>
<th>Orchard 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence</td>
<td>Incidence</td>
</tr>
<tr>
<td></td>
<td>% scabbed leaves</td>
<td>H.g.</td>
</tr>
<tr>
<td>Water</td>
<td>29</td>
<td>ab</td>
</tr>
<tr>
<td>Captan ½ reg. rate</td>
<td>24</td>
<td>abc</td>
</tr>
<tr>
<td>Captan reg. rate</td>
<td>21</td>
<td>bc</td>
</tr>
<tr>
<td>Mancozeb ½ reg. rate</td>
<td>6</td>
<td>d</td>
</tr>
<tr>
<td>Mancozeb reg. rate</td>
<td>1</td>
<td>e</td>
</tr>
</tbody>
</table>

Table 3. Efficiency of preventive treatments with captan and mancozeb on the severity of apple scab (inoculum from orchards 1 and 2).

<table>
<thead>
<tr>
<th>Inoculum :</th>
<th>Orchard 1</th>
<th>Orchard 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Severity</td>
<td>Severity</td>
</tr>
<tr>
<td></td>
<td>Mean score labelled leaf</td>
<td>H.g.</td>
</tr>
<tr>
<td>Water (control)</td>
<td>5.6</td>
<td>a</td>
</tr>
<tr>
<td>Captan ½ reg. rate</td>
<td>3.1</td>
<td>bc</td>
</tr>
<tr>
<td>Captan reg. rate</td>
<td>3.5</td>
<td>b</td>
</tr>
<tr>
<td>Mancozeb ½ reg. rate</td>
<td>0.5</td>
<td>d</td>
</tr>
<tr>
<td>Mancozeb reg. rate</td>
<td>0.17</td>
<td>d</td>
</tr>
</tbody>
</table>

The in vitro test showed significant differences between the log mean ED 50 of the strains from the different orchards (Table 4). The strains from orchards less exposed to captan were more sensitive, while the moderately and highly exposed orchards had the less sensitive strains, with one exception: the strains from the organic orchard (not exposed) showed the highest value of ED 50 (Table 5).

Table 4. Mean ED50 of strains from 5 French orchards differently exposed to captan

<table>
<thead>
<tr>
<th>Orchard</th>
<th>Captan selective pressure</th>
<th>Nb of strains</th>
<th>Mean ED 50</th>
<th>Log mean ED 50</th>
<th>Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Low</td>
<td>16</td>
<td>13,8</td>
<td>2,6 a</td>
<td>7,7-19,6</td>
</tr>
<tr>
<td>4</td>
<td>Low</td>
<td>13</td>
<td>14,7</td>
<td>2,6 a</td>
<td>5,2-31,5</td>
</tr>
<tr>
<td>5</td>
<td>Moderate</td>
<td>16</td>
<td>20,5</td>
<td>2,9 b</td>
<td>12,5-41,5</td>
</tr>
<tr>
<td>6</td>
<td>High</td>
<td>14</td>
<td>31</td>
<td>3,3 e</td>
<td>12,8-50,6</td>
</tr>
<tr>
<td>7</td>
<td>Not exposed (organic)</td>
<td>14</td>
<td>33,7</td>
<td>3,4 e</td>
<td>15,1-51,9</td>
</tr>
</tbody>
</table>

The results suggest that an intensive exposure to captan can lead to a decreased sensitivity of the treated *V. inaequalis* population, with a significant decrease in the efficiency of the fungicide, as observed in orchard 1. However, two years without treatments permitted the recovery of a high efficiency of the fungicide (Table 5).

Table 5. Efficiency of preventive treatments with captan and mancozeb on incidence and severity of apple scab.
severity of apple scab (inoculum from orchard 1 after 2 years without captan applications)

<table>
<thead>
<tr>
<th>Orchard 1</th>
<th>Incidence</th>
<th>H.g.</th>
<th>Efficiency</th>
<th>Severity</th>
<th>H.g.</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% scabbed leaves</td>
<td></td>
<td></td>
<td>Mean score labelled leaf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (control)</td>
<td>17.1 a</td>
<td>a</td>
<td>5.4 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captan ½ reg. rate</td>
<td>6.4 c</td>
<td>62.6</td>
<td>2.1 c</td>
<td></td>
<td>61.1</td>
<td></td>
</tr>
<tr>
<td>Captan reg. rate</td>
<td>2.6 de</td>
<td>85</td>
<td>1.2 ed</td>
<td></td>
<td>77.8</td>
<td></td>
</tr>
<tr>
<td>Mancozeb ½ reg. rate</td>
<td>9.0 bc</td>
<td>47.4</td>
<td>2.6 b</td>
<td></td>
<td>51.8</td>
<td></td>
</tr>
<tr>
<td>Mancozeb reg. rate</td>
<td>0 e</td>
<td>100</td>
<td>0 e</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

The in vitro test confirmed that a differential sensitivity to captan exists in French orchards, and seems related to the exposure to the fungicide. The organic orchard, initially supposed to have a sensitive population because not exposed to captan, showed a population similar to that of a highly exposed orchard. This suggests that exposure to a multisite fungicide can induce a decrease in sensitivity to a multisite fungicide belonging to another family.

It was not possible to collect a population never exposed to captan or any other multisite fungicides to obtain consistent data on the baseline sensitivity of *V. inaequalis* to captan, so, this preliminary work cannot bring the proof that multisite fungicides can also induce population modifications which could explain recently reported cases of treatment failure. As resistance to captan has been reported for *Botrytis cinerea* and *Macrophomina phaseolina* (Anitha et al., 1989, Barak et al., 1984, Diánez et al., 2002) and cannot be excluded for *V. inaequalis*, we need further investigations to test this hypothesis. The pathosystem apple/*V. inaequalis* is a good support for this study, as the multisite exposure of the fungal populations can be very high in some old orchards.

In France, taking into account these results and the difficulties of controlling the disease, the recommendations are to apply no more than 3 treatments per year with each of these multisite families.

**Acknowledgments**

We thank the technicians and researchers of different French regions for sending us *V. inaequalis* inoculum. A special thought for Roger Orts (CTIFL La Morinière) for his help.

**References**


Validation of an apple scab fungicide spray action threshold to help reduce Captan residue levels on Fruits.

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Abstract: Although most fungicide applications targeting apple scab aim to control primary infections in spring, sprays are also routinely applied during the summer to avoid any potential fruit infection. The objective of this project was to validate an action threshold for summer sprays based on the incidence of summer foliar scab that could help refine the spray approach thus minimizing the presence of fungicide residues on harvested fruit. The experiment was carried from 2006 to 2008 in a McIntosh/M9 orchard with a planting distance of 3.65m x 1.25m. Replicated plots of 40 trees were set up with different scab levels, all within the range of that observed in well maintained orchards. This was done by skipping either one or two treatments in early spring or based on the inoculum level present from the previous year. There were 2 plots per inoculum level and per treatment and 6 or 7 blocks depending on year for a total of 36 to 42 plots. Treatments were: no summer fungicide application, current grower standard, sprays based on the proposed threshold of 5 scab-infested leaves per 100 shoots. Although fruit scab at harvest often remained low in plots with foliar scab levels below threshold, fruit scab observed after 12 weeks of storage was consistently at commercially unacceptable levels.

Apple scab, Maturation Model, Spray timing
Breeding high quality disease resistant apple varieties

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Abstract: Breeding for high quality apples combined with excellent agronomic features and durable disease resistance is a highly relevant approach for sustainable production systems. This includes multi-disease resistance against the most important apple problems: scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*). A promising strategy to develop apple cultivars with durable multi-disease resistance is the pyramiding of major genes. The presence of pyramided resistance can be detected by marker-assisted selection. For many known apple scab resistance genes, molecular markers are available. We focus on new achievements for breeding scab and mildew resistant and fire blight tolerant apple cultivars.

Key words: apple breeding, marker-assisted selection, fire blight, disease resistance

Introduction

To achieve durable disease resistance several functionally different resistances against the same pathogen can be combined. This approach can be followed thanks to molecular markers. Marker-assisted selection (MAS) facilitates and accelerates the selection of novel cultivars. Markers linked to the *Vf*, *Vh2*, *Vh4*, *Vbj* and other apple scab resistance genes (reviewed in Gessler et al. 2006) and the *Pl1*, *Pl2*, *Pld* and *Plw* mildew resistance (Markussen et al. 1995; Seglias and Gessler 1997; James and Evans 2004) are available. To breed for fire blight resistant apple cultivars, genetic variation in the breeding material and in Swiss and international apple germplasm collections and in wild species is exploited.

Fire blight reached epidemic proportions in Switzerland in 2007 and other parts of Europe as well (Duffy et al., 2007). Early, warm weather was conducive for fire blight infections during a long synchronous bloom, exposing many more blossoms than usual to the causal pathogen, *Erwinia amylovora*. The use of antibiotics is not a desired sustainable approach for horticultural production in Europe. Among the options for alternative management strategies there is scope for breeding fire blight resistant apple cultivars by exploiting genetic variation in the germplasm and by using markers associated with resistance QTLs (Szalatnay et al. this volume). For fire blight almost no major resistance genes have so far been found. However, Peil et al. (2007) assumed a major resistance gene for fire blight on linkage group 3 of *Malus x robusta 5*. QTLs for fire blight resistance in the apple progeny ‘Discovery’ x ‘Fiesta’ have been mapped and molecular markers linked to the ‘Fiesta’ linkage group 7 major QTL have been developed (Khan et al. 2006)

Material and Methods

Crosses were performed to pyramid genes related to disease resistance. Potential parents are tested for the presence or absence of the expected resistance genes and crosses designed accordingly. Seeds of the progenies are stratified for two month at 2°C in humid sand and raised in the greenhouse in spring. At the 4-leaf stage they are inoculated with a liquid scab suspension of 350,000 conidia per ml. Seedlings are kept under high humidity and at a temperature of 18-20°C. Evaluation of the scab symptoms is carried out after 2 weeks using the scale of Chevalier
et al. (1991). The phenotypic screening of promising advanced selections for relative fire blight tolerance was conducted in the quarantine glasshouse at ACW. Scion material was grafted onto M9 rootstock. Trees were planted in early spring in plastic deep-pots 60 cm from Stuewe & Sons (Corvallis, US) with a length of 35.5 cm and a diameter of 7 cm and grown in the glasshouse for several weeks prior to inoculation. For each variety, 6 to 10 replicate trees were inoculated by puncturing the distal tip of shoots 15-30 cm long with a syringe containing an E. amylovora suspension of 10^6 cfu/ml of strain FAW 611. Spreading of disease symptoms was evaluated in weekly intervals over three weeks by measuring the expansion of the necrotic lesion from the shoot tip in relation to the total shoot length.

Molecular analysis for scab and mildew resistance genes was performed with potential parents for the 2008 crosses and with selected progenies according to methods described by Frey et al. (2004). The molecular SCAR-markers AE 10-375 and GE 8019 flanking the ‘Fiesta’ linkage group 7 major QTL for fire blight tolerance and developed by Khan et al. (2006) were used to characterize the advanced selections.

Results and discussion

Phenotypic and molecular scab screening

Figure 1 shows the segregation of four seedling progenies in different scab resistance and susceptibility classes. In progeny 1 (‘Ariane’ x ‘Fuji’) only the Vf scab resistance is present in one parent. The expected ratio of 50% susceptible (class 0-3b) and 50% susceptible progeny plants (class 4) is obvious. In progeny 2 both parents carry the Vf resistance and the expected ratio of 75% resistant to 25% susceptible plants was perfectly detectable. 25% of the progeny should carry Vf in a homozygous state. Combining Vh2 and Vf (progeny 3) resulted in a slightly higher share of resistant plants compared to progeny 2. In progeny 4, 3 different genes were involved and with this genetic setup only 12.5 % of the progeny individuals should be susceptible, corresponding perfectly well with the phenotypic scoring. In progenies 3 and 4 no plants in classes 0 (no symptoms) and 1 (pin point pits) were detected.

Table 1 presents advances achieved at ACW in breeding selections with pyramided scab resistance combined with mildew resistance. Marker analysis allows us to verify expected genetic constitution. For hybrids 16102 and 16208 molecular analysis has shown that instead of two expected scab resistance genes, three scab resistance genes were present: Vf, Vh2 and Vh4. According to Bus et al. (2005), the Russian apple R 12740-7A carries three major resistance genes: Vh2, Vh4 and Vr. Additionally, the genotypes 16102 and 16208 carry Pl1 and Pl2 mildew resistance, respectively. However, selection 16254 did not carry the expected
P11 resistance. Fruit quality is reasonably good in these selections. Selection for tree and fruit characteristics will continue in this material in order to find the genotypes with the best overall performance.

Table 1: Molecular analysis of hybrids carrying pyramided resistance against scab with at least two different genes (Vf, Vh2, Vh4) and a mildew resistance (P11 or P12); + = marker present, - = marker absent * = SSR marker, **SCAR marker).

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Cross</th>
<th>Genes expected</th>
<th>Marker for</th>
<th>Fruit quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vf</td>
<td>Vh2*</td>
</tr>
<tr>
<td>16208</td>
<td>FAW 8259 x FAW 11561</td>
<td>Vf, Vh2, P12</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16254</td>
<td>Ariwa x Reka</td>
<td>Vf, Vh2, P11</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16102</td>
<td>Ariwa x Regia</td>
<td>Vf, Vh4, P11</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Phenotypic fire blight screening

Glasshouse screening of advanced selections with a shoot inoculation test for fire blight resistance highlighted considerable differences among selections (Figure 2). Genotypes such as FAW 8159, FAW 14995 and FAW 14959 displayed low susceptibility to fire blight. They are promising as parents and as cultivars. Two individuals of the progeny ‘Florina’ x ‘Nova Easygro’ (F x N 3535 and F x N 3529) displayed good resistance as well, as can be expected considering the known fire blight tolerance of the parents. ‘Gala’ was used as susceptible reference cultivar.

Figure 2: Fire blight glasshouse test with advanced selections and cultivars evaluated 1, 2 and 3 weeks after inoculation (LL1, LL2, LL3). The genotypes carrying the flanking markers AE
and GE for the QTL identified on LG7 of ‘Fiesta’ are indicated with arrows (Nb of plants in brackets, bars represent standard deviation).

**Molecular analysis**

Analysis of 38 potential parents and advanced selections revealed seven genotypes amplifying both the SCAR-markers AE 10-375 and GE 8019. They should carry the QTL allele identified on linkage group 7 of ‘Fiesta’ conferring increased resistance to fire blight. Among the 22 genotypes displayed in Figure 2, 4 carried the markers AE and GE flanking the fire blight resistance QTL. Obviously they were less susceptible to the disease than the other genotypes.

Besides parents, progeny plants also were analysed for the presence of the above mentioned fire blight QTL. The cross Enterprise x FAW 11546 revealed 58 out of 102 plants carrying both the AE and GE markers, respectively. The parental variety Enterprise carries both markers in a heterozygous state and FAW 11546 carries only the GE marker. The same segregation pattern, close to 50:50, was detected with progeny plants of the cross FAW 11567 (Vh2, Vh4, AE-, GE+) x FAW 12556 (Vf, PlD, AE+, GE) where 13 out of 29 plants carried AE and GE. Only one plant carried the pyramided resistances of Vf, Vh2, Vh4, PlD and AE+GE. Vf, Vh2 and Vh4 confer scab resistance and PlD resistance to powdery mildew. Plants carrying pyramided resistances will be preferentially selected.

**Conclusions**

Progress was achieved in breeding new apple varieties with durable disease resistance and high fruit quality. We consider the strategy to pyramid resistance factors towards the same pathogen and to integrate scab, mildew and fire blight resistance in the same genotype as being a promising approach.

**References**


Recent advances in epidemiology of strawberry powdery mildew

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Abstract: Cleistothecia on leaves of deciduous perennials are often dispersed before leaf fall to other substrates. In contrast, strawberry leaves remain attached during winter, and cleistothecia of Podosphaera macularis remained attached to these leaves. Release of overwintered ascospores was coincident with renewed plant growth, and pathogenicity of ascospores was confirmed. Upper and lower surfaces of emergent leaves were similarly susceptible, but upper surfaces were obscured by folding in emergent leaves. Emergent leaves exposed to airborne inoculum developed severe infection of the lower surface, but not the obscured upper surface. Emergent leaves acquired ontogenic resistance during unfolding, and the upper leaf surface thereby escaped infection. We found no evidence that the pathogen survives winters in New York, USA or Norway within crown tissue. Plants stripped of infected leaves remained mildew-free when forced after overwintering, while mildew colonies commonly developed on emergent leaves of plants not stripped of mildewed leaves. Unsprayed plots established using mildew-free plants either remained asymptomatic or developed only traces of powdery mildew during one growing season, even when located within 100 to 150 meters of severely diseased plots. In summary, our results suggest the following: (i) sanitation, use of disease-free plants, and eradicative treatments could contribute greatly to management of strawberry powdery mildew; (ii) cleistothecia represent a functional source of primary inoculum; and (iii) the common observation of higher mildew severity on lower leaf surfaces may reflect escape of the upper epidermis due to the combined effect of leaf folding and rapid acquisition of ontogenic resistance.

Powdery mildew, Small fruit diseases, Strawberry diseases, Epidemiology, Ontogenic resistance, Cleistothecia
Integrated protection of table-grape from powdery mildew in Southern Italy

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2 Dipartimento di Protezione delle Piante e Microbiologia applicata, Università degli Studi di Bari - Via Amendola, 165/A 70126 Bari (Italy)

Abstract Powdery mildew (Erysiphe necator Schw.) is one of the most severe diseases of grapevine wherever the crop is grown, especially under hot and dry climate like that occurring in the Mediterranean area. Two field trials were conducted on table-grape in Southern Italy in 2007 and 2008, to evaluate the effectiveness of different spray schedules based on the following fungicides: boscalid, either alone or in mixture with kresoxim-methyl; metrafenone; myclobutanil, either alone or in mixture with sulphur or meptyldinocap; penconazole; proquinazid; pyraclostrobin+metiram; quinoxyfen, either alone or in mixture with sulphur; sulphur; tebuconazole; trifloxystrobin. The climatic conditions during both the trials were particularly favourable to the pathogen, so that prevalence values of 97-100% of infected bunches in the untreated plots were reached at the end of both trials. Under such disease-conducive conditions, all the tested spray schedules always allowed a statistically significant reduction of disease incidence as compared to the untreated check. In particular, the best results were obtained when kresoxim-methyl+boscalid, pyraclostrobin+metiram, proquinazid or quinoxyfen had been applied during the periods of highest disease pressure.

Keywords: powdery mildew, table-grape, IPM

Introduction

Powdery mildew, caused by Erysiphe necator Schw., is one of the most common and severe diseases of grapevine wherever the crop is intensively grown, and especially under hot and dry climate, like that occurring in the Mediterranean area. It costs millions dollars annually to vine growers, due to crops losses and the intensive usage of fungicides for its control.

Nowadays, several classes of fungicides are available for the control of powdery mildew, although the use of some of them like DMIs and QoIs are challenged with the risk of acquired resistance. Decreased effectiveness of DMIs due to acquired resistance has been reported in Portugal (Steva et al., 1988, 1989b), France (Steva et al., 1989a), Italy (Garibaldi et al., 1990), California (Ogawa et al., 1988; Gubler et al., 1996; Ypema et al., 1997) and New York State (Erickson and Wilcox, 1997). In 2007, following the monitoring activity carried out by the Fungicides Action Resistance Committee (FRAC), the presence of E. necator populations resistant to QoIs was confirmed in Eastern Austria and in Hungary. Cases of resistance were also detected, in a few locations, in the Czech Republic and Slovakia (http://www.frac.info/frac/index.htm).

The management of resistance includes the setting up of spray programmes with an appropriate alternation of fungicides with different mode of action. In the last several years, new fungicides with novel modes of action have been authorised for the use on grapevine in Italy against powdery mildew and offer new opportunities for crop protection and more
appropriate resistance management. The fungicides currently allowed in Italy on grapevine against powdery mildew are listed in Table 1.

Table 1. Fungicides and microbial antagonists allowed in Italy against grapevine powdery mildew.

<table>
<thead>
<tr>
<th>Class</th>
<th>Active ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anilides</td>
<td>Boscalid</td>
</tr>
<tr>
<td>Benzophenones</td>
<td>Metrafenone</td>
</tr>
<tr>
<td>Dinitrophenoles</td>
<td>Meptyldinocap</td>
</tr>
<tr>
<td>DMI</td>
<td>Cyproconazole, difenoconazole, fenbuconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole, triadimenol</td>
</tr>
<tr>
<td>Hydroxypyrimidines</td>
<td>Bupirimate</td>
</tr>
<tr>
<td>Phenoxyquinolines</td>
<td>Quinoxyfen</td>
</tr>
<tr>
<td>Qols</td>
<td>Azoxystrobin, kresoxim-methyl, pyraclostrobin, trifloxystrobin</td>
</tr>
<tr>
<td>Quinazolinones</td>
<td>Proquinazid</td>
</tr>
<tr>
<td>Spiroketalamines</td>
<td>Spiroxamine</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Sulphur</td>
</tr>
<tr>
<td>Microbial antagonists</td>
<td>Ampelomyces quisqualis</td>
</tr>
</tbody>
</table>

The present paper reports the results of two field trials, carried out on table-grape in Southern Italy in 2007-2008, aimed at evaluating the effectiveness of spray schedules based on different fungicides for the control of powdery mildew.

Materials and methods

Both trials were carried out in an arbour vineyard cv. Victoria located in Puglia (Southern Italy), in an area where table-grape growing is very common, and climatic conditions are highly favourable to powdery mildew.

The experimental design of 4 randomised blocks, with plots of 8-10 plants surrounded by 1- row untreated border area, was adopted in both the trials. Sprays were carried out at 8-11 d intervals, depending on disease pressure and fungicide persistence. Sprays after véraison were aimed at preventing late infections on the rachis. Fungicides were applied with motorised knapsack sprayers delivering a water volume of 1,000 l ha⁻¹.

The tested fungicides, employed either alone or in alternation in spray programmes, are listed in Table 2.
Table 2. Fungicides and formulate rates employed in the trials.

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Formulates (% a.s.)</th>
<th>Company</th>
<th>Formulate rates (g or ml/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kresoxim-methyl+boscalid</td>
<td>Collis SC (18,2+9,1)</td>
<td>Basf Crop Protection</td>
<td>400</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>Vivando SC (42.37)</td>
<td>Basf Crop Protection</td>
<td>250</td>
</tr>
<tr>
<td>Pyraclostrobin+metiram</td>
<td>Cabrio Top WG (5+55)</td>
<td>Basf Crop Protection</td>
<td>1500</td>
</tr>
<tr>
<td>Boscalid</td>
<td>Cantus WG (50)</td>
<td>Basf Crop Protection</td>
<td>1200</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>Systhane 4.5 Plus EO (4.5)</td>
<td>Du Pont De Nemours</td>
<td>1250</td>
</tr>
<tr>
<td>Proquinazid</td>
<td>Talendo EC (20.53)</td>
<td>Du Pont De Nemours</td>
<td>250</td>
</tr>
<tr>
<td>Sulphur</td>
<td>Kumulus Tecno WG (80)</td>
<td>Basf Crop Protection</td>
<td>5000</td>
</tr>
<tr>
<td>Penconazole</td>
<td>Topas 10 EC EC (10.2)</td>
<td>Syngenta Crop Protection</td>
<td>200</td>
</tr>
<tr>
<td>Trifloxystrobin</td>
<td>Flint WG (50)</td>
<td>Bayer CropScience</td>
<td>150</td>
</tr>
<tr>
<td>Quinoxyfen</td>
<td>Arius SC (22.58)</td>
<td>Dow AgroSciences</td>
<td>300</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>Folicur SE (4.35)</td>
<td>Bayer CropScience</td>
<td>2300</td>
</tr>
<tr>
<td>Meptyldinocap+myclobutanil</td>
<td>GF 1831 L (11.3+4.8)</td>
<td>Sipcam</td>
<td>1250</td>
</tr>
<tr>
<td>Quinoxyfen+sulphur</td>
<td>Macho SC (3.6+46.7)</td>
<td>Sipcam</td>
<td>1600</td>
</tr>
</tbody>
</table>

Symptom severity on berries and rachis was assessed by evaluating 250-300 bunches per plot. An empirical scale with 8 classes of infection [0 = healthy bunch; 1 = bunch with 1-5% infected berries (ib) or 1-5% infected rachis surface (irs); 2 = 6-10 ib or % irs; 3 = 11-15 ib or % irs; 4 = up to 25% ib or irs; 5 = 26-50% ib or irs; 6 = 51-75% ib or irs; 7 = 76-100% ib or irs] was used to calculate, in addition to the disease prevalence (percentage of infected bunches), the disease severity and its weighted mean value, according to McKinney (1923), by using the following formulas:

\[
\text{Disease severity} = \frac{\sum v \cdot f}{n}
\]

\[
\text{McKinney’s Index} = \frac{\sum v \cdot f}{N \cdot X} \cdot 100
\]

where: \(v\) = numerical value of each class of the empirical scale; \(f\) = frequency of bunches in each class; \(n\) = number of infected bunches; \(N\) = number of observed bunches; \(X\) = the highest class value of the empirical scale.

All data, were transformed in arcsine square root percent according to Bliss (1937), were submitted to ANOVA; mean values were separated by Duncan’s Multiple Range Test (Duncan, 1955).

The spray programmes tested in the trials are shown in Table 3 and 4.
Results

**Trial A (2007)**

Weather conditions were very favourable to *E. necator* and the first symptoms appeared in the first week of June in untreated plots. On 12 June, the infections were observed on 47% of bunches of untreated plants, with a McKinney’s index of 13% (Table 3). Thereafter, the disease pressure increased markedly so that, on 27 June, all bunches showed symptoms on berries in untreated plots, with a McKinney’s index value of 85%. On 20 July, 7 days after the last application, a further increase of the disease was recorded on untreated plants, and the McKinney’s index reached 91%.

Under such disease-conducive conditions, all the tested spray schedules always induced a statistically significant reduction of the disease incidence, as compared to the untreated check. In particular, the best results were obtained when the earliest sprays (beginning and end of blossoming), before symptom appearance, had been carried out with kresoxim-methyl+boscalid or pyraclostrobin+metiram, which showed the high preventive effectiveness of such fungicides. Applications with kresoxim-methyl+boscalid for the whole duration of the trial and the alternation of kresoxim-methyl+boscalid and metrafenone yielded a disease control significantly higher as compared to the sole usage of metrafenone, in the presence of the highest disease pressure.

**Trial B (2008)**

The trial was conducted in the same vineyard as Trial A. For this trial, two early applications with sulphur were carried out before the beginning of blossoming on all the plots, except the untreated one, because of the high levels of infections occurred during the previous year and of the high susceptibility of the cultivar to powdery mildew. During the trial, disease incidence was quite high. The first infections appeared on berries in the second week of June in untreated plots. On 19 June, 40% of bunches on untreated plants showed symptoms of powdery mildew, with a McKinney’s index of 7% (Table 4). Later, the pathogen’s activity continued and, at the time of the assessments carried out on 2 and 30 July, the percentages of infected bunches were 89% and 97% with McKinney’s index values of 40% and 66%, respectively (Table 4). Under such conditions, all the tested spray programmes allowed a significant control of the disease on berries, without showing any appreciable differences among them. In the presence of the highest levels of disease pressure, the lowest values of infected berries were observed on the theses treated with the alternation of proquinazid and myclobutanil or with quinoxyfen and myclobutanil. Similar results were observed in the plots treated with pyraclostrobin+metiram and metrafenone. On 30 July, rachis infections were also observed on 94% of bunches in untreated plots. No rachis symptoms were observed in the treated plots for the whole duration of the trial.
Table 3. Trial A (2007) - Disease incidence on berries.

<table>
<thead>
<tr>
<th>Protection schedules</th>
<th>Sprays</th>
<th>12 June</th>
<th>27 June</th>
<th>20 July</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>McKinney Index</td>
<td>Infected bunches</td>
<td>Severity</td>
</tr>
<tr>
<td>Untreated check</td>
<td>-</td>
<td>12.8 a A</td>
<td>47.3 a A</td>
<td>1.8 a A</td>
</tr>
<tr>
<td>Kresoxim-methyl+boscalid</td>
<td>x</td>
<td>X x x x x x</td>
<td>0.2 b B</td>
<td>1.2 c B</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x</td>
<td>X x x x x x x</td>
<td>0.4 b B</td>
<td>3.0 bc B</td>
</tr>
<tr>
<td>Kresoxim-methyl+boscalid</td>
<td>x</td>
<td>X x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x</td>
<td>x x x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Pyraclostrobin+metiram</td>
<td>x</td>
<td>x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x</td>
<td>x x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Boscalid</td>
<td>x</td>
<td>x x x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Penconazole</td>
<td>x</td>
<td>x x x</td>
<td>0.1 b B</td>
<td>0.7 c B</td>
</tr>
<tr>
<td>Trifloxystrobin</td>
<td>x</td>
<td>x x</td>
<td>0.7 b B</td>
<td>5.0 b B</td>
</tr>
<tr>
<td>Quinoxyfen</td>
<td>x</td>
<td>x</td>
<td>0.7 b B</td>
<td>5.0 b B</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>x</td>
<td>x x</td>
<td>0.8 b B</td>
<td>4.6 bc B</td>
</tr>
<tr>
<td>Proquinazid</td>
<td>x</td>
<td>x x x</td>
<td>0.8 b B</td>
<td>4.6 bc B</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>x</td>
<td>x</td>
<td>0.8 b B</td>
<td>4.6 bc B</td>
</tr>
<tr>
<td>Kresoxim-methyl+boscalid</td>
<td>x</td>
<td>x x</td>
<td>0.8 b B</td>
<td>4.6 bc B</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x</td>
<td>x x x</td>
<td>0.3 b B</td>
<td>1.4 c B</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>x</td>
<td>x x x</td>
<td>0.3 b B</td>
<td>1.4 c B</td>
</tr>
</tbody>
</table>

Dates of sprays: 1) 23 May (start of blossoming); 2) 31 May (end of blossoming); 3) 11 June; 4) 19 June; 5) 27 June (beginning of berry-touch); 6) 5 July; 7) 13 July.
Table 4. Trial B (2008) - Disease incidence on berries.

<table>
<thead>
<tr>
<th>Protection schedules</th>
<th>Sprays</th>
<th>19 June</th>
<th>2 July</th>
<th>30 July</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>McKinney Index</td>
<td>Infected bunches %</td>
<td>Severity</td>
</tr>
<tr>
<td>Untreated check</td>
<td>- - - - - - - - - -</td>
<td>7.0 a A</td>
<td>40.1 a A</td>
<td>1.2 a A</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x x</td>
<td>0.0 c B</td>
<td>0.0 c B</td>
<td>0.0 b B</td>
</tr>
<tr>
<td>Proquinazid</td>
<td>x x x x</td>
<td>0.0 c B</td>
<td>0.0 c B</td>
<td>0.0 b B</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>x x x x</td>
<td>0.0 c B</td>
<td>0.0 c B</td>
<td>0.0 b B</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x</td>
<td>0.1 bc B</td>
<td>0.5 bc B</td>
<td>0.5 ab AB</td>
</tr>
<tr>
<td>Quinoxyfen</td>
<td>x x x x</td>
<td>0.1 bc B</td>
<td>0.5 bc B</td>
<td>0.5 ab AB</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>x x x x x</td>
<td>0.1 bc B</td>
<td>0.5 bc B</td>
<td>0.5 ab AB</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x</td>
<td>0.2 bc B</td>
<td>1.2 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x x x x</td>
<td>0.2 bc B</td>
<td>1.2 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Kresoxim-methyl+boscalid</td>
<td>x x x</td>
<td>0.2 bc B</td>
<td>1.2 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x</td>
<td>0.2 bc B</td>
<td>1.2 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>x x x</td>
<td>0.5 b B</td>
<td>3.4 b B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Trifloxystrobin</td>
<td>x x x x</td>
<td>0.5 b B</td>
<td>3.4 b B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x</td>
<td>0.2 bc B</td>
<td>1.5 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Metrafenone</td>
<td>x x x x</td>
<td>0.2 bc B</td>
<td>1.5 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Pyraclostrobin+metiram</td>
<td>x x x</td>
<td>0.2 bc B</td>
<td>1.5 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Sulphur</td>
<td>X x x</td>
<td>0.2 bc B</td>
<td>1.5 bc B</td>
<td>0.8 ab AB</td>
</tr>
<tr>
<td>Meptyldinocap+myclobutanil</td>
<td>x x x x</td>
<td>0.1 bc B</td>
<td>0.8 bc B</td>
<td>0.5 ab AB</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>x x x x</td>
<td>0.1 bc B</td>
<td>0.8 bc B</td>
<td>0.5 ab AB</td>
</tr>
<tr>
<td>Quinoxyfen+sulphur</td>
<td>x x x</td>
<td>0.1 bc B</td>
<td>0.8 bc B</td>
<td>0.5 ab AB</td>
</tr>
</tbody>
</table>

Dates of sprays: 1) 5 May; 2) 12 May; 3) 19 May (start of blossoming); 4) 29 May (end of blossoming); 5) 9 June; 6) 19 June; 6) 19 June; 7) 27 June; 8) 7 July; 9) 17 July; 10) 25 July.
Discussion

The protection strategies for table-grape from powdery mildew in Southern Italy must be addressed to prevent the outbreak of the disease, due to its high frequency and damaging effects under the environmental conditions of that area. In fact, beyond direct damages, the disease can also induce the development of the causal agents of bunch rots, settling down on berries through the injuries caused by the pathogen. Therefore, bunches need to be continuously protected for a long period: at least from the start of blossoming until véraison and even later, against infections on the rachis. This results in a high number of sprays needing to be carried out during the season, with the risk of inducing resistance to fungicides in the target pathogen.

During recent years, a number of new active substances have been permitted for use against powdery mildew on grapevine, some of which have been tested in field trials in Southern Italy (e.g. Santomauro et al., 1997, 2003; Faretra et al., 1997, 1998; Giampaolo et al., 2006; Dongiovanni et al., 2008).

The field trials reported in this paper were aimed at evaluating the effectiveness of protection strategies based on the alternation of different fungicides with different modes of action. The results showed that, even under a high level of disease pressure, all the tested spray schedules always caused a significant reduction in disease incidence, as compared to the untreated check. In particular, the best results were obtained when kresoxim-methyl+boscalid, pyraclostrobin+metiram, proquinazid or quinoxyfen were applied during the periods of highest disease pressure.

It seems reasonable to specify that the high incidence of the disease was caused by both the environmental conditions favourable to the pathogen and by the considerable inoculum of *E. necator* coming from the untreated rows surrounding the plots. It is clear that higher levels of efficacy may be expected under normal field conditions with uniform applications on large vineyard surface.

In conclusion, the availability of new fungicides with novel modes of action allows the establishment of more suitable protection strategies, with particular regard to the management of resistance in the target pathogen. On the other hand, the choices to be made by the farmers for the correct positioning of the different fungicides through the season are made more difficult, particularly in consideration of important aspects such as their pre-harvest interval, residual activity etc. Therefore, it becomes increasingly evident that there is a need for qualified technical assistance that should transfer updated information coming from research and experimentation to the farmers.

References

Erickson, E.O., and Wilcox, W.F. 1997: Distribution of sensitivities to three sterol demethylation inhibitor fungicides among populations of *Uncinula necator* sensitive and resistant to triadimefon. Phytopathology, 87, 8: 784–791.


A multiphasic approach to evaluating the effects of biofumigation for management of wilt in strawberries

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Abstract. The use of isothiocyanate-releasing plant materials has been proposed as a method for replacing methyl bromide for reduction of inoculum densities of *Verticillium dahliae*, causal agent of wilt in strawberry. We have documented reductions in numbers of *V. dahliae* propagules in both microcosm tests and in the field. In addition to the benefits of reduced pathogen inoculum, we are evaluating non-target affects of the strategy in the field: plant health and yield; changes in functional and taxonomic community profiles in both bulk and rhizosphere soils; changes in culturable bacterial and fungal populations (both taxonomic and functional); and colonisation by arbuscular mycorrhizal fungi. Preliminary data will be presented for each of these indicators.

Keywords: *Verticillium dahliae*, biofumigation, Brassicaceae, Cruciferae, *Fragaria*, strawberry, non-target effects

Introduction

Verticillium wilt of strawberries can reduce yields by up to 75%. Most commercial varieties are highly susceptible to the disease, which has been managed, until recently, by soil sterilization with methyl bromide. This has encouraged a dependence on soil sterilization, as soils frequently become infested with pathogens as primary colonists, a consequence of the vacuum effect and monocultures. With the banning of methyl bromide, alternative strategies are being sought to alleviate the problem of wilt. Among these strategies is the use of green manure plants that release fungitoxic chemicals upon decay – so-called biofumigation. East Malling Research has taken the leadership role in the technical and scientific aspects of exploring green manure alternatives to chemical soil sterilization, a joint industry-government sponsored HorticultureLINK project.

The multiphasic nature of the project is illustrated in Figure 1. The agronomy of two biofumigant green manures: *Brassica juncea*, brown or Dijon or Indian mustard, and *Sinapis alba*, white mustard, are being evaluated, as well as their impact on the viability of the pathogen in soil and disease expression in the host. In addition, the use of BioFence seed meal, produced from *B. carinata*, Ethiopian mustard, and of lavender-based materials are being investigated. Also being studied are the effects on disease in strawberry and effects on yield and marketability. Chemists from the Natural Resources Institute, Chatham, are elucidating the production and fate of chemicals produced after incorporation of green manures in soil, both in laboratory microcosms and in the field. The pathology of the system is being studied in laboratory and field-based tests on *Verticillium dahliae* viability and in disease expression in strawberry at two sites – a conventionally managed site and one where strawberries are being produced under organic management. The microbiology of the soils after biofumigation is also being studied in order to determine if this mode of disease management has possible non-salient, non-target effects. Finally, the economics of biofumigation are being evaluated in terms of costs of using the technology and value-added use of several materials.
Materials and methods

Design of field experiments
All experiments have been designed as complete randomized blocks. Those using cruciferous biofumigants involved four treatments: untreated control; *Brassica juncea* cv. ISCI 99; *Sinapis alba*; and BioFence seed meal pellets, all supplied by Plant Solutions, UK. Experimental units were either 8.1 or 9 m wide (depending on site) and either 10 m or 50 m long (depending on experiment). Two sites were used: a conventionally managed site in Mereworth, Kent on Marlowe series soil, a typic paleo-argillic brown earth; and an organically managed site in Tuesley, Surrey on Fyfield 2 series soil, a typic argillic brown earth.

Experiments with lavender (*Lavandula angustifolia* cv Minette), lavandin (*L. x intermedia* cv. Grosso) and their wastes were established at East Malling Research, Kent, on Marlow series sandy silt loam. These experiments were performed on raised (ca. 30 cm) beds, with each bed representing a block and five blocks, each block comprised of six treatments: untreated control; BioFence pellets; the fresh lavender and lavandin and post-extraction wastes of lavender and lavandin added at approximately 10% by volume.

Parameters evaluated and methods for their evaluation
Inoculum density of *V. dahliae* is estimated using the wet-sieve method developed by Harris and co-workers (1973). Community properties, both metagenomic (ribosomal DNA intergenic transcribed spacer regions) (Steele and Streit, 2006) and community-level physiological profiles using BIOLOG eco-plates (TechnoPath, IR) (Garland and Mills, 1991) are being evaluated for both bulk soil and strawberry rhizosphere microbial communities.

We are also characterizing the identities (Rademaker and De Bruijn, 1997) and several of the properties of microorganisms recovered from the bulk and rhizosphere soils used in the experiments, including activities associated with biological control of plant pathogens and plant growth promotion; and the infection of roots by mycorrhizal fungi under the various
Results and discussion

To date, we have demonstrated marked reductions in pathogen population density in biofumigated soils, on the order of 50% or more. However, because we performed these experiments in highly infested areas, we have not yet shown a decrease in disease on strawberry. We are continuing to investigate several of the materials under conditions of lower inoculum pressure in order to find out if their is a meaningful reduction in disease incidence when biofumigant materials are applied where the threat exists but is not so great.

We have begun studies to investigate possible non-target or undesirable effects of biofumigation (Figure 2).

Figure 2. The strategy for investigation of non-target effects of biofumigation (the various terms and acronyms are defined in the text)

Mycorrhiza are important in nutrient uptake, particularly P, in water use efficiency and disease tolerance. Strawberries are highly mycorrhizal while Brassica spp. are not. The spores of mycorrhizal fungi are of unknown sensitivity to isothiocyanates, chemicals identified in disease suppression that are derived from members of the Brassica family.

Among the materials examined in our early screening experiments, lavender was the most effective. Later, we discovered that lavandin, a hybrid species, and both lavender and lavandin wastes were as effective in reducing Verticillium inocula as fresh lavender. Incorporation of this waste material into strawberry beds is currently being investigated and achieves added value for what is, essentially, a waste material with no present use. Should lavender and lavandin wastes prove non-viable due to lack of sufficient supply, an abundance of other materials with similar chemistry remain to be evaluated and exploited.

By examining the profiles of soil communities and the properties of the culturable fraction it may be possible to identify complementary methods to biofumigation that either increase the activity of beneficial microbes or result in smaller perturbations of the resident microflora.
Acknowledgements

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References


Armillaria root rot on highbush blueberry in Northern Italy: monitoring, identification and inoculum sources

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¹FEM-IASMA Research Centre, Plant Protection Department, S. Michele all’Adige TN 38010, Italy; ²SafeCrop Centre, S. Michele all’Adige TN 38010, Italy

Abstract: Highbush blueberry plants infected by Armillaria spp. were reported in north-eastern Italy (Province of Trento). After inspection, 13 blueberry orchards were found to be infected in the Valsugana valley. Armillaria sp. samples were collected from blueberry plants, from bark spread on the blueberry rows and from infected trees and stumps in the orchard surroundings. The species determination was performed using a species-specific multiplex PCR approach. Efficacy trials with potential biocontrol agents against Armillaria sp. were carried out on young blueberry plants. The average percentage of stunted plants in the infected fields was 11%, while the percentage of dead plants was generally very low (average of 1.5%). The most frequent species infecting blueberries were A. gallica and A. mellea: in each field one species largely dominated the other. The tested Trichoderma strains, especially T. atroviride SC1, were the most effective biocontrol agents against A. gallica and A. mellea.

Key words: Armillaria mellea, Armillaria gallica, Vaccinium corymbosum, mulching barks.

Introduction

Highbush blueberry plants infected by Armillaria sp. were found in north-eastern Italy (Province of Trento) (Prodorutti et al., 2006a). Three Armillaria species (A. gallica, A. mellea and A. ostoyae) were reported on highbush blueberry (Caruso, 1995; Prodorutti et al., 2006b). These three species are also present in the forests of the Province of Trento (La Porta et al., 2006). Disease symptoms observed on blueberry roots were white mycelium between the bark and the hardwood, and rhizomorphs developing inside and around the rotted wood. In this region blueberries are cultivated in an area of 70 ha, mainly located in Valsugana valley. Blueberry plants are usually mulched on rows with a layer of coniferous bark collected by the growers in the forest, where the logs are peeled.

Armillaria spp. can survive for a long time in the soil, on wood and root debris, even in the absence of any living host (Fox, 2000). Mycelium and rhizomorphs assignable to Armillaria sp. were found in the bark heaps and in the bark spread in the orchard rows, as well as in stumps and old trees near the orchards. Therefore infected root residues of forest and old fruit trees and infected mulching barks may represent a possible source of inoculum for blueberries planted on the same site.

The aims of this study were: i) the determination of the Armillaria species infecting blueberry orchards and the spreading of the disease in Trentino region; ii) the assessment of potential inoculum sources in and around blueberry orchards; iii) the determination of the efficacy of some microbial biocontrol agents (BCAs).

Material and methods

Highbush blueberry orchards with aerial symptoms of Armillaria infections (poor shoot...
growth, premature reddening of leaves and dieback), initially identified according to notifications from growers and advisors, were accurately investigated. The percentage of stunted and dead plants was recorded in each orchard. In addition, two infected orchards were monitored from 2003 to 2006 to assess disease increase over time.

Armillaria spp. samples were collected from infected blueberry plants, from bark spread along the blueberry rows and from old infected trees/stumps and bark heaps in the orchard vicinity. The determination of Armillaria spp. was performed using a species-specific multiplex PCR reaction that allowed the discrimination among *A. mellea*, *A. gallica* and *A. ostoyae* (Prodorutti et al., 2009).

The efficacy of several BCAs against *Armillaria* spp. was evaluated on one-year-old potted blueberry plants, under greenhouse controlled conditions. The BCAs tested in this study were: *Trichoderma harzianum* T22 (Koppert, The Netherlands), *T. harzianum* T39 (Y. Elad, Israel), *T. atroviride* SC1 (FEM, Italy), *Phlebiopsis gigantea* CBS 935.70 (Centraalbureau voor Schimmelcultures, The Netherlands), *Bacillus subtilis* F77 (FEM), *Gliocladium catenulatum* (Verdera, Finland). Trials were carried out by inserting wood pieces artificially inoculated with *A. gallica* and *A. mellea* between blueberry roots and treating the soil at the same time with the BCAs grown in Potato Dextrose Broth (Oxoid, UK). The percentage of infected blueberry plants was assessed one year after inoculation.

**Results and discussion**

Thirteen out of 350 Valsugana orchards were found to be infected by *Armillaria* spp. The average percentage of stunted plants in these fields was 11%, reaching a maximum of 20% in two orchards. The percentage of dead plants was generally very low (0.2-2.5%), with an average of 1.5% and values higher than 5% only in two orchards.

In both the two fields monitored annually, the percentage of stunted plants increased from 10 to 20% during the four-year survey. Dead plants increased from 1.5 to 2.5 % in the first orchard and from 4.5 to 5.5 % in the second one. The disease usually started from individual blueberry plants placed inside and/or at the borders of the orchards and developed in patches.

The dominant species were *A. gallica* and *A. mellea*. In each orchard a single species largely dominated over the other: *A. gallica* and *A. mellea* were the most widely distributed species in eight and five orchards, respectively. *A. gallica* was identified on blueberry plants, on bark spread on rows, on bark heaps and on fruit/forest trees at the field margins. *A. mellea* was found on blueberries and on fruit trees in the orchard surroundings but not on bark spread on the rows. No *A. ostoyae* was identified in infected fields; therefore in the biocontrol efficacy trials only *A. gallica* and *A. mellea* were used.

Experiments carried out on potted blueberry plants showed that *T. atroviride* SC1 was the most effective BCA, both against *A. gallica* and *A. mellea*. One year after inoculation, it significantly reduced the percentage of infected plants from 60-80% (untreated controls) to 20% (SC1 treatments). *T. harzianum* T39 was also effective against *A. mellea* (Figure 1).

This study demonstrates that highbush blueberry is susceptible to Armillaria root rot and that infected mulching barks as well as infected roots of old trees can act as a dangerous inoculum source. *T. atroviride* SC1 was effective on blueberry plants against *A. gallica* and *A. mellea* and could be applied directly on blueberry roots and/or on mulching barks to prevent Armillaria infections in the new orchards. Agronomical practices and sanitation procedures before and after planting (i.e. removal of infected roots and rhizomorphs, inspection and heat treatment of barks) are also important in order to reduce the risk of infection to young blueberry plants.
Figure 1. Percentage of infected blueberry plants after treatment with some biocontrol agents and artificial inoculation carried out by inserting *Armillaria mellea* or *A. gallica* infected wood pieces between roots. The assessments were done one year after inoculation. The experiments were carried out on potted plants, under greenhouse controlled conditions. Different letters indicate significant differences ($P \leq 0.05$) according to the Kruskal-Wallis test.

**Acknowledgements**

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blueberry (*Vaccinium corymbosum* L.) in North-eastern Italy (Trentino region). IOBC/WPRS Bull. 29 (9): 75-80.

The Working Group „Integrated Protection of Fruit Crops“ is celebrating its 50th Anniversary

Historic Review by: Ernst F. Boller¹, Albert K. Minks², Jerry V. Cross³, Joop C. van Lenteren⁴ & Theo Wildbolz⁵

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Abstract: The Working Group looks back at 50 years of successful work. The fruit entomologists are the pioneers within WPRS with respect to the development of integrated plant protection (IPP) and integrated production (IP) and their introduction into practice.

Developments occurring during the early 1970s brought a change in the general approach reflected in the change of name in 1974 from “Integrated control in orchards” to the broader term “Integrated plant protection in orchards”. A further milestone was the establishment of the holistic concept of Integrated Production as has been described in the “Message of Ovronnaz” which should be considered as a historic landmark for IOBC as a whole.

The publications of the WG reflect the broad range of its activities and its important function as scientific platform for information exchange and joint programs: 13 proceedings of International Symposia on Integrated Plant Protection and Production in orchards, 14 technical handbooks (brochures) and 41 WPRS Bulletins covering specific topics of the various subgroups. The first international symposium organised by the working group took place in Wageningen in 1961 with 36 participants from 9 countries, the most recent symposium was held in Avignon in 2008 with 250 participants and celebrating the 50th anniversary. Hundreds of experts have participated in the WG’s activities over the past 50 years. The impact of these activities on the development and application of IPP and IP in practise was and still is significant. Concepts and tools developed by the WG became not only general WPRS standards but have influenced significantly the international standards for Integrated Plant Protection. The WG has generated the approach and practical implementation of Integrated Production in the major crops of the WPRS region.

A summary of important events is given in the following table. The full text of this historic review is published on the IOBC/WPRS homepage www.iobc-wprs.org

<table>
<thead>
<tr>
<th>Year</th>
<th>Name of Working Group (Convenor)</th>
<th>Important events</th>
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</thead>
<tbody>
<tr>
<td>1959</td>
<td>Integrated control in orchards</td>
<td>February: Establishment of WG “Lutte intégrée dans les vergers” in Wageningen/NL by representatives of The Netherlands, Germany, Switzerland and France.</td>
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<tr>
<td>1961</td>
<td>(H.J. de Fluiter/NL)</td>
<td>First meeting and colloquium on integrated control in orchards at Wageningen, (NL), 5-9 September.</td>
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<td>1964</td>
<td></td>
<td>Week of applied research in Saxon (CH) on faunistic monitoring</td>
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<td>1965</td>
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<td>3rd Symposium at Montreux (CH), 13-15 September.</td>
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<tr>
<td>1968</td>
<td>(H. Steiner/D)</td>
<td>Establisment of WG on “Genetic Control of Carpocapsa &amp; Adoxophyes” (Th. Wildbolz/CH J. de Wilde/NL becomes chairman of Commission “Integrated Control”.</td>
</tr>
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<table>
<thead>
<tr>
<th>Year</th>
<th>Event Description</th>
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<tr>
<td>1969</td>
<td>4th Symposium at Avignon (F), 9 - 12 September.</td>
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<tr>
<td>1973</td>
<td>25 May–1 June. Meeting in Lana (I) with decisions to split WG into SG pome fruits and SG stone fruits. June: Joint EPPO/IOBC/FAO meeting on IPP concepts.</td>
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<tr>
<td>1976</td>
<td>Important meetings on potential IC labels for fruit. Message of Ovronnaz (birth of the IP concept) Establishment of ad hoc Commission on “IP endorsement” (M. Baggiolini/CH) as subunit of WG. WG on “Genetic Control of Carpocapsa &amp; Adoxophyes” merged with orchard group.</td>
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<tr>
<td>1979</td>
<td>6th Symposium at Vienna, 8-12 October in the frame of the 25th anniversary celebrations.</td>
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<tr>
<td>1981</td>
<td>Meeting at Colmar: proposal to produce a list of selective pesticides for orchards. General Assembly at Antibes: The IP Commission becomes independent of the WG (J.P. Bassino/F &amp; A. Stäubli/CH).</td>
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<tr>
<td>1984</td>
<td>New Subgroup “Pear” (T. X. Nguyen/F).</td>
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<tr>
<td>1986</td>
<td>New Subgroup “Package-apple” (L. Blommers/NL) recommending choice of pesticides for IPP.</td>
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<tr>
<td>1988</td>
<td>New Subgroup “Peach” (H. Audemard/F).</td>
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<tr>
<td>1990</td>
<td>8th International Symposium on Integrated Plant Protection in Orchards at Gödöllő (H), 31 July 31 - 5 August.</td>
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<tr>
<td>1996</td>
<td>New Subgroups “Arthropod Pests” (M. Solomon/UK), and “Soft Fruits” (D. Gajek/PL).</td>
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<tr>
<td>1998</td>
<td>4th International Conference on Integrated Fruit Production (10th Symposium on Integrated Plant Protection in Orchards) at Leuven (B), 27 July -1 August. (Joint IOBC-ISHS International Conference).</td>
</tr>
<tr>
<td>2001</td>
<td>(J. Cross/UK).</td>
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<tr>
<td>2003</td>
<td>WG “Pome fruits” (P. Cravedi/I) merged with orchard group.</td>
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<tr>
<td>2004</td>
<td>Integrated protection of fruit crops 6th International Conference on Integrated Fruit Production at Baselga di Piné (I), 26 - 30 September.</td>
</tr>
<tr>
<td>2008</td>
<td>(C. Ioriatti /I) 7th International Conference on Integrated Fruit Production at Avignon (F), 26 - 30 October.</td>
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Peach orchard management strategies: aphid communities as a case study

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¹ Ecodéveloppement, INRA, Site Agroparc Cedex 9, 84914 Avignon, France, ² Plantes et Systèmes de culture Horticoles, INRA, Site Agroparc Cedex 9, 84914 Avignon, France

Abstract: Because of the various negative side effects of intensive chemical pest control practices, there is a shift in horticulture towards the adoption of alternative approaches for crop protection. In order to characterise and evaluate management strategies being used, we carried out comprehensive interviews to obtain details of the peach orchard protection schedules of 20 organic and conventional fruit farms in south-eastern France. It appeared that besides the regular use of direct control, farmers also used cultural and/or alternative methods and indicators to optimize their orchard management. Combining the latter methods with IOBC’s technical guidelines for plant protection, four strategies have been identified. Their efficacy on aphid communities was then evaluated through visual monitoring of aphids and of beneficial populations at plot level. Brachycaudus persicae and Myzus varians were the most frequent species. The two most efficient strategies were dominated by chemical treatments, whereas the two others, less detrimental to aphid antagonists, were predominantly used by organic farmers and in agreement with IOBC’s guidelines. Variations in aphid communities could be explained by: (i) the use of efficient and therefore toxic products, correlated with low infestations and low abundance and diversity of antagonists; (ii) the link between pre-blooming treatments, cultural and alternative methods (as weed strips management and manual pruning of infested branches) and high populations of aphid communities. Against all expectations, such communities were neither related with kaolin applications, nor with management of vigour and nearby environment. According to the literature, the strategies identified can be interpreted as steps towards a redesign of orchards’ protection.

Aphid, community, Antagonist, Peach orchard, Integrated pest management, Crop protection, Organic production
Adapting to New Management Strategies for Cherry Fruit Flies in British Columbia, Canada

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Abstract: The western and black cherry fruit flies (CFF), *Rhagoletis indifferens* and *R. fausta*, are serious risks to production of sweet cherries *Prunus avium* in British Columbia and Canada, particularly to late-season or high-value export crops with zero tolerance for pest infestation. The availability of new reduced risk chemicals and of “soft” formulations, such as GF-120® NF Naturalyte® Fruit Fly Bait, has led to adaptations and changes in several aspects of crop protection. At the same time, there is interest in the development of an area-wide program using “soft” techniques. Information is being gathered from commercial and organic orchards, private gardens, and abandoned sites within the mixed urban-rural landscapes that are common in Canada. We present some recent results from experiments and experiences of fruit-growers. These include the compatibility of reduced risk pesticides with the key predatory mites of fruit-growing in western and eastern Canada, of the use of GF120 Fruit Fly Bait, the importance of alternate host plants, and new knowledge of CFF flight and movement.

Key words: IPM, cherry fruit fly, insecticide, control strategy, bait, phytotoxicity, movement, non-target

Introduction

The movement of western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae) and black cherry fruit fly, *R. fausta* (Osten Sacken) (Diptera: Tephritidae) from native hosts onto cherries occurred recently in British Columbia (B.C.): changes in host range and species distribution were observed in the 1960s. Control by organo-phosphate insecticides, primarily dimethoate, was implemented successfully. However, by 1999, crop protection from cherry fruit flies was the major challenge of cherry-growers. In 2001, packing houses and agencies reported record levels of damage at harvest. Management of a cherry fruit fly complex (*Rhagoletis* species) was by then a challenge world-wide, owing in large part to the withdrawal of most organophosphate and carbamate insecticides. Also, major changes in cherry-growing have occurred in western Canada including an emphasis on high-value export crops, aided by the introduction of varieties with late season harvest dates, and causing a shift in land use from apple or pear.

In response to similar changes, governments across North America and Europe restarted research on cherry fruit flies from approximately 2001. At that time, the control techniques were for (a) organic growers to avoid the insect by having very early cultivars and pick them before the insects become apparent, sometimes combined with mass trapping with yellow sticky traps; or (b) conventional growers to apply “old-generation” insecticides against adults or larvae. Western Canadian growers, in particular, are export-oriented and because of quarantine restrictions, there is a critical need for management techniques which are acceptable to the international marketplace. The success of the B.C. industry is founded upon the development of increasingly late-maturing cultivars, so early picking is impossible; average picking dates of new varieties have increased from early July to mid-August.
Insecticide use is thereby complicated by the need for frequent entry into small orchards for picking. Also, the existence of an area-wide management program (Thistlewood & Judd 2003) for the key pest of pome fruit, *Cydia pomonella*, has aroused interest for fruit flies.

Since 2002, we conducted research to enable area-wide management of these flies in many habitats, not only commercial orchards. The studies have covered key aspects of use of novel “reduced-risk” insecticides and GF-120 Naturalyte bait spray, or biology and flight of fruit flies. As significant differences now exist between European and North American industries, we describe below some of our results and the grower experience to date.

**Related Studies**

*Compatibility of new insecticides with mite predator*

In Canada, information about the effect on beneficial species of pesticides is not required for registration and is often absent. The western predatory mite *Galendromus occidentalis* (Nesbitt) is the main predator of spider mites in dry parts of B.C. Since 1968, crop protection has been based on the selective use of pesticides that are relatively harmless to this predator. We are conducting laboratory studies to examine the toxicity of new compounds and report on seven to date: imidacloprid (Admire 240), thiacloprid (Calypso 480SC), acetamiprid (Assail 70WP), methoxyfenozide (Intrepid 240F), spinosad (Tracer 44.2%), thiamethoxam (Actara 25WG) and the acaricide spirodiclofen (Envidor 240SC). We compared different types of toxic effects using various bioassays (Bostanian et al. 2009).

Of four neonicotinoids evaluated, acetamiprid and imidacloprid were rated as extremely toxic to adult predators. The label rate of acetamiprid was 5.6-fold the LC₅₀, the estimated concentration that killed 50% of the population. Imidacloprid was even more toxic with a label rate 10.3-fold the estimated LC₅₀ value. By contrast, two other neonicotinoids, thiamethoxam and thiacloprid, as well as spirodiclofen, methoxyfenozide, and spinosad, were rated as harmless to adults. When we examined the effect on reproduction and egg-laying of female predators, we noted again that acetamiprid and imidacloprid were toxic, whereas no effect on egg-laying was obvious within 3 days after treatment with the other five pesticides. None of the seven new pesticides were toxic to freshly laid eggs within 6 days after treatment. An important aspect of modern pesticides can be their repellency, causing predators to avoid treated surfaces. We found imidacloprid, acetamiprid and thiacloprid to be highly repellent, thiamethoxam and spinosad slightly repellent, and spirodiclofen and methoxyfenozide to be non-repellent (Bostanian et al. 2009).

*Compatibility of new fungicides with mite predator*

We also examined in the laboratory (Bostanian et al. 2008) the impact of seven fungicides on *G. occidentalis*: microscopic sulphur 92WP, fenbuconazole (Indar 75WSP), bosalid (Lance 70WDG), fenhexamid (Elevate 50WDG), pyraclostrobin (Cabrio F500), propiconazole (Topas 250E) and myclobutanil (Nova 40W). Using recommended label rates of each product and a water control, we examined their effects on adults and on immature stages, on female egg-laying, and on the number of treated eggs that hatch successfully. We used the “worst possible” conditions and applied each material to leaves containing all stages of the predatory mite and its prey, spider mites (Bostanian et al. 2008).

The synthetic fungicides fenbuconazole, propiconazole, and myclobutanil, are triazoles that work on a biochemical pathway that is not common in the animal kingdom. The mortality of eggs, larvae, and adult stages of predators within six days after treatment with them was no greater than with distilled water (1-7% by stage). The other three synthetic fungicides all act differently on fungi but were similar in causing no greater mortality of eggs, larvae, and adult stages of predators than distilled water. By contrast, microscopic sulphur (92%WP) was
relatively harmless to eggs and adults, but killed 72.4% of the young larvae within six days, which would cause plant-feeding mites to increase very significantly. When we examined the effect on egg-laying of female predators, we found that none of the products significantly increased or reduced the numbers of eggs laid for three days after treatment, compared with a distilled water spray (Bostanian et al. 2008).

**Observations on bait spray use and improvements**

GF-120 Naturalyte bait spray was developed originally for tropical flies (e.g., Moreno & Mangan 2002), and the combination of a bait matrix and insecticide is unusual for temperate fruit orchards. It was tested on cherry in western Canada by growers and researchers in 2005 and permitted for commercial use since 2006.

In 2005, it was noted in every study that the first bait application must be earlier than expected. Generally, some growers had difficulty in adjusting to reaplication of the material after each rain-fall, which is critical. However, the application of bait sprays for fruit fly control has become widespread. Application is usually by a low-pressure modified herbicide sprayer mounted on an all-terrain vehicle, emitting two streams of 4-6 mm droplets of a 4:1 mixture of water to bait concentrate, at 1-2.5 L per hectare (described by Smith 2009). It is fast, often 20 minutes per hectare, or seven-fold that of a typical orchard sprayer. Grower reports and research studies have shown control to be near 100% in formerly “clean” orchards, and typically 95% or more in gardens or single trees when applied by hand.

As expected, the movement of flies from extra-orchard hosts into fruit trees is important. By 2008, growers reported concern with the importance of sources of flies outside orchards and some damage that was being observed at orchard edges, where fertile flies were active and lay eggs. By contrast, a prediction of increased injury from other insects has not yet been noticed, despite the relatively selective nature of the bait spray, and although likely pests (e.g. Grapholitha packardi) are found adjacent to some orchards.

Our research has examined the best placement of a small amount of material within trees relative to fly activity, and phytotoxicity and leaf-feeding that was observed at the bait droplets. DeLury et al. (2009) tested six sweet cherry cultivars with GF-120 containing spinosad (0.2 g L$^{-1}$ spinosad bait) or without it (blank bait). Spinosad bait and blank bait did not differ significantly with respect to damage observed. Leaf damage was found almost exclusively on the undersides of leaves at the doses (0, 17, 20, 25 or 40%) and cultivars tested. The effects of the bait on lower leaf surfaces increased from 24 to 168 h, and with dose, in terms of the proportion of droplets (0, 0.4, 0.5, 0.75 or 0.94) and area (0, 18.7, 23.5, 40.5 or 91.6 mm) burned. In addition, chlorophyll level was reduced with increasing dose on underside of leaves, but not on upper surfaces. The chlorophyll level in undamaged leaves (upper surfaces) differed by cultivar. Cherry leaves were less damaged by a 20% bait application in June (0.26) than in July (0.46) and August (0.50). Incidental insect leaf feeding at bait locations occurred at a low rate and was highest on lower leaf surfaces.

**Host plants**

Many unmanaged trees occur in fruit-growing areas of the B.C. interior. Surveys enabled us to evaluate the importance of wild hosts, abandoned orchards, and unmanaged trees. We visited 30 sites from May to September of 2002 and 2003, and an additional 40 sites in more remote locations in 2004. In all years, yellow sticky card traps (Pherotech Inc., Delta, B.C., Canada) with ammonium carbonate lures were hung by hand, as high as possible from the ground or using a two-step ladder, in suitable host trees. Beginning in 2003, some sites also received a matching Rebell trap (Andermatt Biocontrol, 6146 Grossdietwil, CH) with ammonium carbonate lure. Larvae were collected from fruit or berries, permitted to pupate into sand, and pupae reared in subsequent years for identification of species and any parasitoids.
Both *R. indifferens* and *R. fausta* were caught on traps around pin cherry *Prunus pensylvanica* and bitter cherry *Prunus emarginata*. Pin Cherry was always associated with *R. fausta*, and in fruit collections was host to proportionally more *R. fausta* than was Bitter Cherry, but both were very suitable reproductive hosts for *R. indifferens*. About half of the Mahaleb cherry, *Prunus mahaleb*, sites were reproductive hosts of *R. indifferens*. By contrast, numerous *R. indifferens* were found on traps inside the plant canopies of Red and Black Chokecherry, *Prunus virginiana*, but these were rarely (1 of >20 samples) reproductive hosts.

**Movement of flies**

Flight is the main dispersal method in *R. indifferens*, but the movement of these flies between hosts is difficult to quantify in the field. Two of us (Thistlewood, Senger, unpublished) have measured the movement and dispersal of *R. indifferens* in and around fruit orchards. One series of studies (Thistlewood, unpublished) used a grid of 28 yellow sticky traps placed across an area of mixed land use from 2002-2004 and monitored weekly each growing season. It revealed that *R. indifferens* flies moved regularly but at relatively low numbers over distances of approx. 200m. The flies moved more readily through contiguous orchard blocks of different fruits than across open spaces. In apple blocks to the South of an infested cherry orchard, about 26% of flies were found 10-20m away, and 3% about 75 m away, compared with 0.9% the same distance away from the cherry orchard but across an open area. However, a 5m roadway was no barrier to significant fly movement. Simultaneous mark-release-recapture (Thistlewood, unpublished) of flies occurred from two release sites placed in an open area and in a young cherry block. We found that flies (15% of those released) were recaptured in cherry orchards within two days at up to 150m from both of the release sites. In other orchards, *R. indifferens* was captured on yellow sticky traps that were within the canopy of apple and peach trees located 140m from the nearest cherry trees, but not captured on traps placed above and below the canopy of those trees.

A tethered flight mill system was used in the laboratory to examine the flight behaviour of sexually mature flies exposed to different levels of conspecific contact and resource availability (Senger et al. 2007). A 2×2×3 factorial experiment compared the relative influence of the factors ‘context’ (crowded, isolated), ‘sex’ (female, male), and ‘resources’ (low = food only; medium = food + leaf; high = food + leaf + cherries) on flight performance including distance flown, net trial time, and stopping patterns. Of 160 flies tested, 86.9% flew <500 m on the flight mill. Individuals from both sexes were capable of maximum flights of ca. 3 km. Distance flown was significantly influenced by ‘context’ such that crowded individuals flew >1.5-fold farther than isolated individuals. Sex influenced the frequency and duration of stops made, with females stopping more often and longer than males. Although females and males in high resource treatments had the shortest net trial times, the factor ‘resources’ did not produce any highly significant main effects, but did generate significant interaction terms with the factors ‘context’ and ‘sex’, suggesting that past experience with ‘resources’ modifies individual flight behaviour. Senger et al. (2007) showed using a flight mill that *R. indifferens* flight behaviour is context dependent and sensitive to adult crowding. The implications for dispersal are discussed further in Senger et al. 2007. In a related study, Senger et al. (2008), showed how *R. indifferens* females can develop their egg load in response to host availability. Egg counts varied significantly with ‘crowded’ females and those in the ‘cherry’ resource treatments producing the most mature eggs. Although the average mature egg count for females from the ‘leaf’ and ‘food’ resource treatments was similar, these two groups differed in the proportion of females that produced no mature eggs at all. They concluded that the effect of social interactions and resources on the maturation of eggs is additive in *R. indifferens*, and that egg load may trade off with dispersal ability in *R. indifferens*.
Discussion

The removal of most organophosphate and carbamate insecticides from the market, and changes in cherry-growing practices, are leading to the rapid adoption of new materials and methods in western Canada. Novel pesticides are undergoing some local evaluations in laboratory and field trials. The insecticide study by Bostanian et al. (2009) suggests that spinosad and methoxyfenozide may be used in IPM programs without concern. Thiacloprid, thiamethoxam and spiropidiclofen should be evaluated by growers and advisors in the field for their effects before being used generally in an IPM program. They did not recommend the use of imidacloprid or acetamiprid. Unfortunately, results from the limited field evaluations of the latter have been contradictory to date, under our conditions.

The fungicide study of Bostanian et al. (2008) concluded that, from a grower perspective, none of the seven fungicides were harmful to the adult stage of predators, nor affected significantly their egg production, nor egg hatch. Although the larvae (young stages) were unaffected by the six new synthetic products, microscopic sulphur was lethal. Bostanian et al. concluded that use of sulphur should be avoided in favour of less toxic alternatives in order to conserve predatory mites. This finding agrees with several studies, e.g. Prischmann et al. (2005) which recommended that sulphur be replaced by less toxic fungicides in wine grapes and other perennial crops so as to avoid mite outbreaks. Bostanian et al. (2008) concluded that, apart from microscopic sulphur, the other six materials (fenbuconazole, boscalid, fenhexamid, pyraclostrobin, propiconazole and myclobutanil) appear to be a good fit for IPM programs where the western predatory mite is an important predator of leaf-feeding mites. The studies were conducted with a predator population from the interior of B.C., and experience shows that the location of the orchard and historical pest management practices can have an effect on the predatory mite population and the outcome of any control program. Therefore, these reports are more of a guide when developing pest management strategies rather than a recommendation for all Canadian regions.

The widespread and generally successful introduction of the Naturalyte GF120 bait formulation of spinosad was enabled by earlier work conducted in the USA. Thistlewood (unpub.) more commonly trapped and observed flies in the upper parts of the canopy than elsewhere within, above, or below, the canopy. One conclusion is to apply the relatively small amounts of bait materials as high up the tree as possible. DeLury et al. (2009) concluded that application to the upper leaf surface, or at doses of $\leq 20\%$, will minimize leaf phytotoxicity.

Unmanaged cherry trees are common in the B.C. interior, and a problem for area-wide management programs because of emigration of fertile flies each year. However, growers are familiar with these trees and they can be located, mapped, and removed or treated as required. Surveys revealed the relative importance of other species of concern. Mahaleb cherry, *P. mahaleb*, is a reproductive host of *R. indifferent*, but in the arid interior of B.C. this bush is often un-noticed by fruit-growers. By contrast, although numerous *R. indifferent* are found on bushes of Red and Black Chokecherry, *P. virginiana*, the latter were rarely a reproductive host.

Research is continuing into the flight and movement of *Rhagoletis* species attacking cherry in B.C. Better knowledge of fly dispersal and movement leads to understanding of the risk posed by urban or abandoned trees in the mixed land use areas found in fruit-growing areas. From trap catches, it appears that relatively small numbers of flies moved long distances every year out of the cherry trees and throughout the entire area of mixed land use. When released in an open area (centre of alfalfa field) with no immediate fruit trees nearby, as might happen the year following removal of a cherry tree, flies moved quickly into the main cherry block or smaller groups of cherry trees. Small numbers of *R. indifferent* can be expected to fly at least 150m in any given season and there is no evidence that a line of flight distance of 250m would be a barrier to establishment of small populations. Even such a short
distance causes difficulty in B.C., requiring treatment of many cherry trees and abandoned sites. In other fruit trees, flies were captured only within the canopy. In general, the flies appeared to move more easily through contiguous fruit tree canopies than across open spaces.

Acknowledgements

We thank Gaétan Racette for technical assistance, Heidrun Vogt for many discussions in our Germany-Canada collaboration, and the Pesticide Risk Reduction and Minor Use Programme, Pest Management Centre of Agriculture and Agri-Food Canada, for additional funding.

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Plant protection in organic apple production of two North Spanish regions

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Abstract. Researchers of two Spanish research institutes, IRTA in Catalonia (North-East region with Mediterranean climate) and SERIDA in Asturias (North-West region with Atlantic climate) have been working in collaboration on projects involving organic apple production since 2002, with the aim of finding optimum production methods. In this paper, trials for the control of apple scab (Venturia inaequalis (Cke.) Wint.), rosy apple aphid (Dysaphis plantaginea Pass.) (Homoptera: Aphididae)) and codling moth (Cydia pomonella L.) (Lepidoptera: Tortricidae) are described. These common pest species were successfully controlled by products and methods allowed by the European organic rules (EC 834/2007).

Key-words: organic apple, plant protection, apple scab, rosy apple aphid, codling moth.

Introduction

Between 1995 and 2007 there was a significant increase in the size and number of operators engaged in organic farming throughout the European Union (Willer and Jussefi, 2007). At present, Spain is the second country of the European Union in organic acreage (988,923 hectares), but organic fruit growing occurred on a very small scale mainly due to the scarcity and dispersion of information (Descombres et al., 2006) and technical difficulties such as: knowledge of resistant cultivars; thinning; fertilization; weed control, and, pest and disease management. Since 2002, two Spanish research institutes, IRTA (Catalonia) and SERIDA (Asturias), have joined forces and combined their expertise to provide more effective research aimed at furthering the development of organic apple farming.

Materials and methods

The potentially harmful agents most likely to affect apple production include the pathogenic micro-organism Venturia inaequalis (Cke.) Wint., which causes apple scab, and two insect pests: the rosy apple aphid (Dysaphis plantaginea (Passerini)) and the codling moth (Cydia pomonella (L.)) which both cause direct damage to the fruit. Several experiments took place between 2001 and 2008 and were located in either Catalonia (IRTA-Mas Badia en Girona; or IRTA-EE Lleida en Lleida) or Asturias.
**Apple scab**
In 2004, trials were conducted in Asturias, using the cultivars ‘Reineta Encarnada’ (RE) and ‘Reineta Blanca de Canada’ (RBC) to explore alternatives to copper for controlling apple scab: (i) lime sulphur (Luqsa S.A.) at 2%, the clays (ii) Mycosan (Andermatt Biocontrol) at 0.8% with the pine oil Nufilm-17 (Agrichem) at 0.1 %, (iii) Mycosin (Andermatt Biocontrol) at 0.5% together with wettable sulphur (Bayer 80% pp, at 0.4%) and Nufilm-17 (0.1%) and (iv) B-Ulmasub (0.5%) with wettable sulphur (0.4%) and Nufilm-17 (0.1%). In 2007-08, trials were carried out in microplots at the IRTA-Mas Badia (Girona) on the cultivar Brookfield Gala with a copper salt Kdos (DuPont) at 2 kg / ha and lime sulphur (Sulfoluq (Luqsa)) at a dose of 10 l/ha.

**Rosy apple aphid**
At SERIDA, the effectiveness and optimal application time of various neem-based botanical insecticides were evaluated. At IRTA-EE Lleida, trials were aimed preventing the autumn colonization with two strategies: (i) alteration of the recognition of the host plant (defoliation, garlic extract and kaolin sprayings) and (ii) reducing or eliminating oviparous females (potassium soap and pyrethrum sprayings).

**Codling moth**
Trials aimed at reducing infestation and consisted of applying bioinsecticides such as the codling moth granulosis virus (CpGv) or products allowed in Organic Farming, alone or in combination with mating disruption methods.

**Results and discussion**

**Apple scab**
In Asturias, damage in the untreated plots reached the 30.7% of the leaves in RE and 11.2% in RBC. Damage was drastically reduced by the application of Mycosan (1.1%), lime sulphur (2.2%), Mycosin (3.3%) and Ulmasub-B (5.6%). In IRTA-Mas Badia (2007), the lowest incidence of pre-harvest lesions was obtained using copper hydroxide (6.5% affected leaves and no lesions to the fruits) and lime sulphur (13.5% affected leaves and 0.3% of the fruits) while in the control, 20% of leaves and 2.1% of the fruit presented symptoms. Similar results were obtained in the 2008 trial.
application in H and another in I).

**Rosy apple aphid**

Of the seven products tested in Asturias, only NeemAzal-T/S (Trifolio-M GmbH) was effective. In our study, it proved very effective when applied in preflowering (phenological stages D1 and E2), whereas efficacy was reduced significantly when applied after flowering (Figure 1). In some trials, there was good aphid control with a single application.

With regard to treatments designed to alter the insects’ recognition of the apple trees, only defoliation prevented the colonization of the host plant in the fall and the resulting infestation during the following spring (Figure 2). As for treatments aimed at reducing colonization, potassium soap application in no case controlled spring infestations. In contrast, the pyrethrum significantly reduced colonization in autumn in each of the three years studied.

![Figure 2](image_url)

**Figure 2.** Population density of the rosy apple aphid (% of apple tree leaves occupied by oviparous (autumn 2005) and viviparous (spring 2006)). \( D^{(1)} \) indicates the mean separation after the analysis of repeated measurements in time. Treatments followed by the same letter are not significantly different according to Duncan's Multiple Range Test \( (p< 0.05) \). In the top line of the autumn graph, \( O \) indicates when kaolin and garlic extract was applied, while \( \bullet \) indicates when potassium soap and pyrethrum were sprayed. T: Control group, A: garlic extract, C: kaolin, S: potassium soap Q: pyrethrum, D: defoliation.

**Codling moth**

In Asturias, with six to eight applications of two trademarked granulovirus, Madex (Andermatt Biocontrol) and Carpovirusine (Arysta, formerly Calliope) succeeded in lowering the percentage of infested fruit from 25% to less than 2%. The method of mating disruption as the basic defence, coupled with initial applications of granulovirus, gave similar results. In Girona, efficacy trials were carried out in microplots with granulovirus (Madex) and Spinosad (Spintor 48, Dow AgroSciences); at the end of the first generation Spinosad had significantly greater capacity to control codling moth compared to CpGV and the control.

In terms of plant health protection in apple orchards, ecological management is achieving a satisfactory degree of control of diseases and pests for production of commercial varieties. After several years of tests, we can conclude that preventive applications of lime sulphur, clay and copper derivatives, either in sequence or in repetition in each case, has proved effective in controlling apple scab in susceptible varieties (bearing in mind the incompatibility of lime sulphur with mineral oils and the negative side effects of applying copper salts in those varieties susceptible to russetting, a physiological disorder). Preflowering applications of
NeemAzal-T/S were effective in controlling rosy apple aphids. For the control of the codling moth, mating disruption is considered sufficient when the pressure of infestation is low, but should be reinforced with aptly timed treatment by CpGV or, in areas where infestation pressure is high, by Spinosad. Using only CpGv was also, on occasions, effective in controlling codling moth infestation.

Acknowledgements

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References

Field efficacy of slaked lime against European fruit tree canker and introduction into practice

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Abstract: Fruit tree canker, caused by Nectria galligena, is an increasing problem in fruit growing areas with wet periods during the leaf fall period. Several effective fungicides against the disease, such as benzimidazoles, will be banned in future in Europe. There is an urgent need for environmentally friendly solutions for this disease. Several field experiments were done to determine the efficacy of slaked lime (calcium hydroxide) against European fruit tree canker. Pieces of wood with sporulating canker were suspended in the top of trees during leaf fall to secure a high inoculum pressure. Infection was through natural wounds like leaf scars and no artificial wounds were made. Newly formed cankers were counted in the following spring. Three spray applications of 100 kg/ha slaked lime at 10, 50 and 90 % leaf fall reduced the number of newly formed cankers by 57 % compared to untreated plots. The number of newly formed cankers was reduced by 60 % when 50 kg/ha of slaked lime was applied in a comparable experiment in the following year. A comparison between 25, 50 and 100 kg/ha of slaked lime resulted in a reduction of 34, 53, 37 % of newly formed cankers. Slaked lime was applied through the overhead sprinkler system in experiments at commercial growers’ sites. The average efficacy was 60 and 62 % in two years respectively. Further demonstrations resulted in the regular use of slaked lime by commercial growers.

Calcium hydroxide, Fruit tree canker, Nectria galligena, Slaked lime
Relation of duration of wet period and number of Nectria cankers for leaf scars and pruning wounds during the summer

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Abstract: Fruit Tree Canker (Nectria galligena Bres.) is an important fungal disease in apple (Malus X domestica Borkh) in the Netherlands. The fungus causes cankers on the shoots, main branches and trunks of apple trees. It takes a lot of effort to control the disease and when infection takes place whole trees can be lost especially when they are young. This makes the pathogen a problem not only for fruit growers but also for fruit tree nurseries. Some of the most effective fungicides no longer permitted in the Netherlands. Therefore, interest from fruit tree growers is increasing for a warning system to optimize the use of the remaining less effective fungicides. This model should be used during the whole year because on several occasions wounds are made. To build this model data about the infection conditions are needed. Detailed information of these conditions during the summer is lacking. Therefore an experiment was done with potted trees in the summer. To investigate a possible difference in susceptibility, two types of wounds were made, a pruning wound and a leaf scar. Trees received different length of wet periods at 20°C after inoculation with N. galligena spores. It was found that no wet period was needed to get a successful infection in the summer. Also no relation between the duration of the wet period and the amount of canker formation was found. Finally, it was found that pruning wounds were more susceptible than leaf scars in summer.

Warning system, Nectria galligena, Apple, Fruit
Detection of latent infections of fruit tree canker (Nectria galligena) in planting material of apple

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Abstract: Fruit tree canker (Nectria galligena) is a serious problem in (organic) apple production. Infections cause direct loss of yield by damage to productive shoots and branches, often leading to tree death. Control measures are applied to protect infection sites, notably leaf scars from external inocula. Young apple trees can be infected symptomlessly during propagation (latent infections). A test was developed for screening young apple trees from tree nurseries for latent infection by fruit tree canker caused by Nectria galligena, prior to planting in the orchard. Under specific conditions (high temperature and relative humidity) it was possible to induce symptoms in infected planting material within 8 weeks. Tests were performed with artificial inoculations to determine the sensitivity of the test. Screening of commercial planting lots with the newly developed method revealed infection incidences that were higher than recorded after planting in the orchard. The developed method is suitable for screening apple planting material for fruit tree canker infections before planting. The method also detects infections that initially stay latent under field conditions. The method seems valid to screen organically and conventional apple trees. However, the method is destructive; therefore an adequate sampling strategy needs to be developed.

Apple canker, Propagation material, Disease control, Screening method
Development of semiochemical attractants, lures and traps for raspberry beetle, *Byturus tomentosus* at SCRI; from fundamental chemical ecology to testing IPM tools with growers

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**Abstract:** Raspberry beetle adults are attracted to flowers of their hosts primarily by colour and odour (floral volatiles). SCRI scientists have investigated this chemical ecology interaction for several years, using a multi-disciplinary approach involving phytochemistry, insect behaviour, and GC-EAG electrophysiology. We will present a historical overview, explaining how these techniques have allowed us to identify the key flower attractants from a complex mixture of volatiles emitted by raspberry flowers. We will then go on to explain how recent (EU-CRAFT, Horticulture Development Council) and current (Defra HortLINK) work has progressed the optimization of raspberry beetle traps for U.K. growers needing IPM solutions due to demands for zero pesticide residue levels on fruit. We will explain how we are developing and testing slow release lures and different trap designs, together with collaborators at East Malling Research, Natural Resources Institute, AgriSense Ltd and also with Norwegian scientists, testing prototype traps on organic soft fruit farms.

**Key words:** raspberry beetle, trapping, host attractant, semiochemicals, IPM

**Introduction**

The raspberry beetle, *Byturus tomentosus* (Degeer) causes severe damage to raspberries by both the adults (which feed on buds and flowers) and by larvae (which feed inside the developing fruit). The threshold for damage is very low in fresh raspberries because the presence of only a few larvae can mean that the whole consignment is rejected by the fruit marketing agents (Gordon *et al.*, 1997). Due to ongoing reductions in allowable pesticide residues on fresh raspberries to a point that is effectively zero in the U.K. and a large increase in protected raspberry production (under plastic tunnels) there is a strong demand for alternative means to control this pest using semiochemical-enhanced trapping methodologies. At SCRI, we have developed a novel trap, based on the key visual and olfactory characteristics of the raspberry flower. This trap is now being tested on commercial farms in Scotland and England (conventional/IPM), and on organic smallholdings in Norway (see Trandem *et al.*, this volume). Previous research at SCRI successfully identified the most active floral attractant volatiles from raspberry flowers using a combination of GC-EAG (gas chromatogram linked to electro-antennogram) and behavioural bioassays using linear track olfactometers and a wind tunnel (Woodford *et al.*, 2003; Birch *et al.*, 2004; Mitchell *et al.*, 2004).

**Materials and methods**

**Sites and duration of on-farm experiments in Scotland**  
The trials were conducted at two farms in Eastern Scotland from 1 May – 11 July 2007. Two commercial Scottish plantations were chosen. Each were growing cv. Glen Ample under
Spanish type tunnels covered with polyethylene sheeting from flowering to after harvest and each plantation was approximately 1 hectare in size. These sites were chosen as they were thought to have low to moderate populations of raspberry beetle due to previous use of insecticides.

### Site 1 details
**Wester Essendy, Blairgowrie, Perthshire, Scotland**

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</tr>
<tr>
<td>E2</td>
<td>NO 135 435</td>
<td>Glen Ample</td>
<td>5</td>
</tr>
<tr>
<td>E3</td>
<td>NO 135 435</td>
<td>Glen Ample</td>
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### Site 2 details
**Blairgowrie, Perthshire, Scotland**

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</tr>
<tr>
<td>J2</td>
<td>NO 216 462</td>
<td>Glen Ample</td>
<td>8</td>
</tr>
<tr>
<td>J3</td>
<td>NO 216 462</td>
<td>Glen Ample</td>
<td>8</td>
</tr>
</tbody>
</table>

### Treatments
Devices were modified AgriSense bucket traps with white Correx cross-vanes and with a polythene vial dispenser containing initially 2.5 ml of attractant coded ‘B’. The funnel traps contained 3 cm of 25% antifreeze (ethylene glycol) in water with a drop of detergent (Teepol®) to reduce surface tension. The trap treatments were applied at least two weeks before flowering commenced in each plantation. Three trap deployment treatments were used: perimeter, lattice and control. Perimeter trapping consisted of the traps being suspended from the top wire at the outer most position in the plantation at 8 m spacing around the entire perimeter of the plantation (50 traps / hectare around the edge of each 1 ha plantation). The traps in the lattice trapping treatment were positioned regularly throughout the plantation, suspended from the top wire, at a density of 50 traps per hectare. The control treatment contained no buckets traps but did contain a small number of sticky traps for monitoring local raspberry beetle numbers in untreated areas.

### Assessments
Eight bucket traps per treatment were checked weekly and the number of beetles recorded. Sampling of all the traps for adult raspberry beetles was undertaken at flowering and at the end of the trial. The number of non-target insects was also recorded. Standard white sticky traps were positioned in all three treatments to allow weekly monitoring of the raspberry beetle populations in control areas without traps.

### Results and discussion
Up to the green fruit stage the lattice treatment (within crop) was x 6 more effective for trapping raspberry beetles than the perimeter layout at one site, but not different at the other site (due to a lower raspberry beetle population following previous seasons’ pesticide
applications). After the green fruit stage, both spatial layouts worked equally well in terms of catching adult raspberry beetles, but catch numbers were lower than in the pre-green fruit stage. In control plots (without bucket traps) numbers of raspberry beetles were variable between sites but more were caught around the edges than in the middle. The numbers of raspberry beetle eggs found on sampled flowers was very low in all trap treatments, which was also reflected in the very low incidence of larvae and damage to berries. Although some non-target insects were caught (mainly bees, wasps, flies and other beetle species), the numbers were low and had no obvious effect on pollination or biocontrol within the crop. These first year results indicate that the enhanced bucket traps are effective in trapping (for monitoring pest numbers and temporal activity) and possibly also for controlling damage caused by raspberry beetle in protected raspberry plantations with low-moderate populations. However, because pest numbers were low due to previous crop management history (pesticide usage), climate and local habitat, trials will be continued at sites in the UK, Norway, Switzerland and France with higher pest populations. The aim is to investigate alternative usage of the enhanced traps and lures for control of the pest and fruit damage. The most effective time to use the traps is pre-flowering of the raspberry crop, when there is no competition from raspberry flowers, although the traps continue to trap raspberry beetles during the flowering period and may help to reduce populations in subsequent years. Further trials will be undertaken to fine tune the spatial deployment under different environmental conditions. Collaborative trials in France and Switzerland, to complement the ongoing trials in Norway, are providing a wider range of pest pressures (organic and conventional producers) and growing conditions to optimise trap deployment strategies for monitoring and/or pest control via a ‘lure and kill’ approach.

Acknowledgements

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References

Prospect for crop protection in Europe: vision from the ENDURE Network

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Abstract: The IOBC has been a pioneer in defining and promoting the Integrated Pest Management and Integrated Production concepts. Despite a wide theoretical recognition of these concepts, the extent to which they are translated into practice at the field level is quite variable, so that European agriculture is still largely relying on pesticide use. Under increasing pressure from public concern on the consequences on human health and on the environment, a more stringent policy is being elaborated at the EU level that will reduce the range of available pesticides and impose a rapid shift towards IPM. In this context, research and extension have to engage even more than before in elaborating and implementing innovative solutions. As practical solutions are generally devised at national or local levels, there is an immediate benefit in comparing them, considering their transferability between countries, identifying their performance and shortcomings, exploring their potential for combination and detecting the gaps and needs for additional knowledge. ENDURE (www.endure-network.eu) - a Network of Excellence gathering 18 institutions from 10 European countries – takes advantage of its multinational point of view to perform such analyses. It also explores new technologies such as precision spraying and early detection of pests and pathogens which have not been much developed yet to assess their potential for reducing pesticide use. In the mid-term, however, introducing technologies for mitigating pesticide impacts and some alternative methods may not suffice to meet the expectation of a sustained crop protection reconciling low impacts and high productivity. With the objective of reducing the vulnerability of crops to pests, pathogens and weeds altogether, changes in the farming system must be considered, as well as the role of the whole food chain from input providers to retailers and consumers. Thanks to the large range of disciplines gathered in this Network, ENDURE is in a unique position to adopt this holistic approach and to take into account the interactions between crop protection, agronomy, ecological and landscape factors as well as the socio-economic framework in which innovative crop protection strategies need to be implemented. Work is in progress on some agricultural systems most representative of European agriculture. As a typical perennial cropping system subject to multiple pest and disease constraints, pomefruit orchards are one of them. Current results on this system will be emphasised.

IPM
State of the Art of Control Strategies of Codling Moth, Apple Scab and Brown Spot in Europe

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Abstract: ENDURE (www.endure-network.eu) is a European Network of Excellence which aims to the reduction of insecticide use in European agriculture, and the identification of gaps of knowledge in pest control science. Among the diverse actions of this network, a survey of the state of the art of control strategies of codling moth, apple scab and brown spot in Europe was conducted. These are 3 key pests of pome fruit production all over Europe, and they are responsible for most of the phytosanitary treatments applied in these crops. The survey was conducted at least in 5 European regions, Rhône Valley (France), The Netherlands, Emilia Romagna (Italy), Lake Constance (Switzerland and Germany), and Lleida (Spain); and in some cases additional regions were surveyed. The survey was carried out by means of a questionnaire for each pest that was filled in by regional experts with close relationship with growers. Questionnaires requested information on monitoring, decision support systems, sanitation practices, use of environmentally friendly products, pesticide resistance management, cultural methods, emerging secondary pests, functional biodiversity, and bottlenecks; all considered basic elements to define a pest control strategy. The results of the survey are shown and discussed regarding specially durability of the strategy, major actual control tools, important bottlenecks, and discrepancy and heterogeneity among regions, for the control of the different pests.

IPM, codling moth, apple scab, brown spot, pomefruit, control strategy
Investigations on the bark beetle species (Coleoptera: Scolytidae) in cherry and peaches in the East Mediterranean Region of Türkiye

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Abstract. This two-year long study was carried out in cherry and peach orchards in Adana, Mersin, Osmaniye and Kahramanmaraş provinces in the East Mediterranean Region of Türkiye in 2004-2005. In this study the species of bark beetles -Scolytidae spp.-, the distribution and the infection rates of this pest were determined.

As a result of this study, five species of the Scolytidae family, which are one of the major pests of cherry and peaches in the region, were found. These species were Scolytus rugulosus Müller, Scolytus amygdali Guerin, Xyloborus dispar Fabricius, Taphrorynchus villifrons Dufour and Scolytus pygmaeus Fabricius. S. rugulosus was found to be the most common species followed by S. amygdali.

It was determined that the first adults appeared in the beginning of May (3rd-5th of May) and survived until mid-September. It was found that all provinces in the study area were infected by the pest at different rates. The infection rates of bark beetle species in Mersin, Adana, Osmaniye and Kahramanmaraş were determined to be 4.3, 5.6, 7.0 and 7.4 % respectively. The area where the survey was conducted was found to be infected at an average of 5.8 %.

Key words: cherry, peach, pest, bark beetle species

Introduction

In 2007, Türkiye had 398,141 tons of crop obtained from 12,048,104 cherry–bearing trees. The East Mediterranean Region of Türkiye produces 5.2 % of the total cherry production of the country. In this region, the provinces of Mersin, Adana, Kahramanmaraş and Osmaniye produce 9,637; 5,991; 2,375 and 1,940 tons respectively (Anonymous, 2007).

Peach production, another export product which is important for the economy of Türkiye, is concentrated in the Marmara, Aegean, Black Sea and Mediterranean regions. In 2007, the peach production was 539,435 tons in the country (Anonymous, 2007). The East Mediterranean Region produces 16.5 % of the total peach production of the country.

There are many pests causing problems to stone fruits in Türkiye. Among these pests, Scolytidae species is one of the major pests causing economic losses in cherry and peach trees (Naredran et al., 1995; Tezcan and Civelek, 1996; Ben-Yahuda et al., 2002; Çınar et al., 2004). Most of these Scolytidae species are considered as “secondary” insects because they require a weakened or stressed host for successful colonization and development. In spring, the emerging adults bore an entrance hole in the bark to excavate a brood gallery in the inner bark or phloem. The adults feed and reproduce inside of the gallery. As a result of their feeding inside of the phloem tissue, lack of development in the leaf and flower buds occur which, in turn, causes crop losses. If the measures to prevent the build up of the pest aren’t taken, starting from the thin branches, the whole tree may eventually die (Nizamlioğlu, 1961; Malavolta et al., 1995; Kaplan and Yücel, 2000).

In this study, it was determined which bark beetle species caused damage in cherry and peach trees. The distribution and infection rates of these species in cherry trees in the East
Mediterranean Region were also determined.

**Materials and methods**

The main materials of this study were bark beetle species, cherry and peach trees, Steiner funnel, aspirator, pruning scissors, killing jar, and culture boxes.

*The determination of bark beetle (Scolytid spp.) species*

This study was conducted mainly in cherry orchards in the East Mediterranean Region provinces of Adana, Mersin, Osmaniye and Kahramanmaraş. The provinces were separated into districts [Adana: (Pozantı, Saimbeyli, Feke); Mersin: (Arslanköy, Güzelyayla, Fatih); Kahramanmaraş: (Andırın); Osmaniye: (Hasanbeyli, Bahçe)] based upon the production amount of cherries. Some of the peach orchards adjacent to cherries were also investigated and bark beetle samples were also taken from those orchards. The studies in the orchards were done non-periodically between April and September in 2004 and 2005. Monitoring and strike methods were used to collect the adult bark beetles.

*The spreading and the infection status of the bark beetle species*

In this study, samplings were done in at least three and at the most nine orchards which were selected randomly and could represent the region. Each district was visited non-periodically two times in the first year and once in the second year. The numbers of trees monitored were decided according to Lazarov and Grigorov’s (1961) method. The trees which showed infection symptoms were checked according to the method to see if they contained bark beetle adult or larva. The trees which had beetles (adult or larva) under the bark were accepted as infected. The spread and the infection status of the pest in cherry orchards were determined by the number of infected trees.

**Results and discussion**

*The determination of bark beetle (Scolytid spp.) species.*

At the end of this study, the names of the species determined, the location where they were found, and the name of the plant which they were found on are given in Table 1.

Table 1. The bark beetle species determined in cherry and peach orchards in the region.

<table>
<thead>
<tr>
<th>Class</th>
<th>Family</th>
<th>Species</th>
<th>Locations</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coleoptera</td>
<td>Scolytidae</td>
<td><em>Scolytus rugulosus</em> Müller</td>
<td>Pozantı Tarsus</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bahçe Andırın (Çokak)</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mersin (Arslanköy)</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pozantı (Ömerli)</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Andırın Hasanbeyli</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saimbeyli Bahçe (Y.Karderesi)</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Seyhan</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Scolytus amygdali</em> Guerin</td>
<td>Tarsus (Yenice)</td>
<td>Peach</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Xyloborus dispar</em> Fabricius</td>
<td>Kozan</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Taphrotrypochus villifrons</em> Dufour</td>
<td>Kozan</td>
<td>Cherry</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Scolytus pygmaeus</em> Fabricius</td>
<td>Andırın (Çokak)</td>
<td>Cherry</td>
</tr>
</tbody>
</table>
It can be seen in Table 1 that a total of five *Scolytus* species belong to *Scolytidae* family of Coleoptera order were found in cherry and peach orchards. It was discovered that the adult population of the pest was low in quantity in the beginning of the vegetation but increased over time (July-August). It was also found that overwintering larva was affected negatively by the hard winter conditions and the cultural upkeep processes applied in autumn and spring. It also seems that pesticide applications done in early growing season reduces the beginning population of the pest (Tezcan and Civelek, 1996).

As a result of this study, *S. rugulosus* was found the most abundant species and was followed by *S. amygdali*. It was noted that *S. rugulosus* was a significant and abundant species in the cherry orchards in Elazığ - Mardin (Çınar et.al., 2004) and in Niğde - Ulukışla (Ulusoy et.al., 1999). Kaplan and Yücel (2000) declared that *S. rugulosus* prefers cherry, plum, apricot and peach in sequence among other stone fruits. Mustaga (1991) suggested that *S. rugulosus* prefers primarily cherry, peach and plum species. In some studies it was determined that *S. amygdali* causes serious damage on stone fruits in Mediterranean and South Europe countries by adult feeding on the buds and the larva feeding under the bark (Anonymous, 2007a). In another study, it was noted that *Xyloborus dispar* species caused serious damage on *Populus* spp., *Acer* spp., *Betula* spp., *Fagus* spp., *Quercus* spp., *Salix* spp., *Castanea sativa*, *Malus domestica*, *Prunus communis*, *Prunus armeniaca*, *Prunus cerasus*, *Prunus domestic*, and *Prunus persicae* (Anonymous, 2007b).

The spreading and the infection status of the bark beetle species

This study was conducted simultaneously with the study to determine the species. The infection percentages of bark beetles in the cherry orchards in the East Mediterranean Region were calculated and the results are given in Table 2. The first adults appeared in the beginning of May (3rd-5th of May) in the orchards of Pozantı (Adana). After the beginning of May, bark beetles were found during every orchard examination.

Table 2. The infection rates of bark beetles in the cherry orchards in region (%)

<table>
<thead>
<tr>
<th>Provinces</th>
<th>Districts</th>
<th>Checking dates (dd.mm.yyyy)</th>
<th>No. of trees checked</th>
<th>No. of infected trees</th>
<th>Infection rate (%)</th>
<th>Average infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adana</td>
<td>Pozantı</td>
<td>03.05.2004</td>
<td>103</td>
<td>6</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pozantı</td>
<td>12.08.2004</td>
<td>91</td>
<td>6</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pozantı</td>
<td>04.05.2005</td>
<td>97</td>
<td>6</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saimbeyli-Feke</td>
<td>20.05.2004</td>
<td>141</td>
<td>5</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saimbeyli-Feke</td>
<td>08.09.2004</td>
<td>130</td>
<td>9</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saimbeyli-Feke</td>
<td>19.07.2005</td>
<td>88</td>
<td>4</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Mersin</td>
<td>Güzelyayla-Arslanköy-Fatih</td>
<td>05.05.2004</td>
<td>110</td>
<td>3</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Güzelyayla-Arslanköy-Fatih</td>
<td>05.08.2004</td>
<td>134</td>
<td>8</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Güzelyayla-Arslanköy-Fatih</td>
<td>08.06.2005</td>
<td>145</td>
<td>6</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>Osmaniye</td>
<td>Bahçe</td>
<td>31.05.2004</td>
<td>22</td>
<td>2</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hasanbeyli</td>
<td>21.06.2004</td>
<td>73</td>
<td>4</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hasanbeyli-Bahçe</td>
<td>12.09.2004</td>
<td>106</td>
<td>7</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bahçe-Hasanbeyli</td>
<td>18.05.2005</td>
<td>122</td>
<td>8</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Kahraman maraş</td>
<td>Andırın</td>
<td>05.07.2004</td>
<td>117</td>
<td>10</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Andırın</td>
<td>03.09.2004</td>
<td>109</td>
<td>8</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Andırın</td>
<td>30.06.2005</td>
<td>113</td>
<td>7</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1701</td>
<td>99</td>
<td>5.8</td>
<td></td>
</tr>
</tbody>
</table>
When Table 2 is checked it is seen that the rate of infection is varied between 2.7 and 9.1% with an average of 5.8%. The infection rates of Mersin, Adana, Osmaniye and Kahramanmaraş were 4.3, 5.6, 7.0 and 7.4% respectively. According to the table, the most extensive infection rates were seen in the orchards in Bahçe (9.1%) and the lowest rates were seen in Güzelyayla-Arslanköy-Fatih (2.7%). In a study conducted by Kaplan and Yücel (2000), in the East and Southeast Anatolia region it was determined that the infection rates of S. rugulosus in Diyarbakır, Elazığ, Adıyaman, Mardin, and Malatya were 9.1, 6.7, 4.7, 4.4 and 4.1% respectively and it was also determined that the infection rate of the region was 5.8%.

As a result of this study, five species of bark beetles were determined with S. rugulosus being found the most widespread one. Although the infection rate of bark beetles in the region was found to be 5.8%, it was determined that this rate wasn’t high enough to necessitate pesticide application. Pesticide usage during unsuitable periods must be avoided because the pest has many predator and parasitoids. Healthy, vigorous trees that are well cared for are less subject to attack. Keeping the trees healthy and removing the infected trees and debris (such as the cut branches) will be the best measures to prevent the pest to build up.

**Acknowledgements**

The authors are grateful to the expert Prof. Dr. Erdal SELMİ (İstanbul University, Forestry Faculty, Bahçeköy/Istanbul) who identified the specimens of bark beetles.

**References**

zararlıları, bölüm II. Ankara, s: 68 – 69.
The incidence and control of cranberry tipworm, *Dasineura vaccinii* S., in cranberry plantations in Latvia

Ilze Apenite  
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**Abstract:** The commercial cultivation of American large-fruited cranberry (*Oxycoccus macrocarpus* (Ait.) Pers.) began in Latvia in the last decade of 20th century, because the area of natural cranberry (*O. palustris* Pers. and *O. microcarpus* Pers.) had decreased. The spread, development and progress of the most harmful pests were regularly monitored in a field trial located in the Aluksne region in the north-eastern part of Latvia. Mainly the cranberry variety ‘Stevens’ was observed. One of the most important reasons for cranberry yield loss is insect damage. After three years (2004-2006) it was concluded that the most widespread and harmful pest of this crop in Latvia is cranberry tipworm *D. vaccinii*. At the beginning of the experiment it was established in north-eastern part of Latvia (2004, 2005) but in 2006 the cranberry tipworm appeared also in other regions. In North America cranberry, tipworm is controlled with flooding, sanding and chemical control (insecticide treatments). In Latvia in many cranberry plantations it is difficult to perform flooding and sanding treatments (intensive growth of weeds- neutral soil). Therefore it was necessary to carry out experiments to test the effects of insecticide treatments. Currently no insecticide is registered for cranberry in Latvia. One of the tasks was to test the efficacy of the insecticide Fastac, 10% EC (a.i. -cypermetriner) for control of cranberry tipworm at different dosages and treatment times and to compare the efficacy with an untreated control. The experiment was carried out from 2005 to 2006. Higher efficacy was obtained with two treatment times with the highest dosage of Fastac applied.

*Insects, Development, Weeds, Insecticide, Treatments*
Preliminary trials for a continuous rearing of *Bactrocera oleae* (Rossi) on its natural host *Olea europaea* L. in the laboratory and future perspectives

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**Abstract:** A simple and affordable small-scale rearing technique to supply olive fruit fly (*Bactrocera oleae* Rossi) instars continuously throughout the year, even when fresh fruits are not available naturally to oviposit, is essential to optimize biological studies. Olive fruits came from a typical olive grove of northern Lazio (Cura di Vetralla, VT, central Italy), organically managed. Cages, feeders and instruments were specially designed. The fruits were kept fresh for more than 1 year in special “muffs” of straw and tulle, assembled directly on fruiting branches. The rearing started in 2005, as soon as emergences occurred. Adults were collected from the field and moved to the rearing cages with a bunch of sound and fresh fruits, to allow egg laying. Thereafter, every time a new lab generation started emerging, a bundle of fresh fruits was moved from the field into the cages to let new ovidepositions occur. Temperature and RH were maintained at standard lab conditions, 20°C ± 2°C, 60% ± 5% RH, and natural photoperiod. From 11 October 2005 to 22 January 2007, the fly gave 13 continuous reproductive cycles in the lab, 1 generation every 40 days on average. An exception was the 9th generation (27 August - 27 September) which lasted 31 days because of accidental high temperatures (26-27°C). This is the first method which has succeeded in obtaining olive fly generations continuously on its natural host. Fine tuning this technique will make it suitable for every other study (i.e. physiological, biological and behavioural studies, parasitoid rearing and release, sterile insect technique, etc.).

**Key words:** olive, olive fruit fly, life-cycles, biocontrol

**Introduction**

Several attempts have been made in the past in order to rear olive fly *ex-situ* with the aim of studying different bioecological and behavioural aspects. Most of the studies carried out under laboratory conditions referred to only one generation (Economopoulos *et al*., 1976; Remund *et al*., 1977; Pucci *et al*., 1982; Tzanakakis & Koveos, 1986; Koveos & Tzanakakis, 1990; Raspi *et al*., 1997). Other investigations on multiple generations relied on artificial diets (Tsitsipis, 1977).

It seems that genetic changes occur when olive flies are reared on artificial diet causing adverse effects on their performance and fitness if compared to wild individuals (Tsakas & Zouros, 1980; Kostantopoulou *et al*., 1986). For this reason, researchers are now focussing on obtaining laboratory colonies and maintaining them on their natural host (Genç & Nation, 2008).

The aim of this work was to design a simple and affordable small-scale rearing technique to supply olive fly instars continuously throughout the year, even when fresh fruits are not available for oviposition. This is essential to optimize biological studies as well as for mass rearing in sterile insect techniques.
Material and methods

Olive fruits came from a typical olive grove of northern Lazio (Cura di Vetralla, VT, central Italy), organically managed. All trees are of Canino cv, and 40 years old. To provide fresh fruits year-round, some fruiting branches were wrapped in special “muffs” of straw and tulle, assembled directly on fruiting branches starting from late July-early August (as soon as the fruits reach the right size and before the pit’s hardening). Securing both the olives’ soundness and slow ripening, this method usually provides fresh, healthy fruits through 13 months and over. Every 10 days some branches were picked from the ‘muffs’, deprived of leaves to prevent a quick dehydration of fruits, then placed into the cages.

Cages, feeders and other tools were specially designed. Rearing cages are cylindrical (diameter = 30cm, h = 40cm), made of plexiglass with a removable bottom and a little side-window. The cage top is closed with a 0.5 mm-mesh tulle (Figure 1). Feeders are made from a cylindrical glass container (diameter=3cm and h=10cm) with a plastic screw top. As soon as the container is filled, the nutritional solution pours, through a bottom connection, into a little tray (diameter=4cm, h=1cm) (Figure 2). The tray is plugged up with a porous material to let solution surface slowly and allow adults to feed comfortably. To avoid overflowing, a pipe (not capillary) must traverse the container’s cap, with a free end outside the container (see Figure 2). Feeders were washed once a week using sodium hypochlorite, then soaked in water for 3 hours, and again washed in plenty of water. Cages were cleaned when needed, using a common detergent. A proper displacement of the adults from a rearing cage to others (shifting cages) allowed us to separately maintain individuals of each generation. At least 3 cages are necessary to maintain population in every generation.

The rearing started in 2005, from wild emergences, collecting and moving adults from the field to the cages with a bunch of sound and fresh fruits, to allow egg laying. Thereafter, every time a new lab generation emerged, a bundle of fresh fruits was moved into the cages to let new ovidepositions occur. Temperature and RH were maintained at standard lab conditions, 20°C ± 2°C, 60% ± 5% RH, and natural photoperiod.

Results and discussion

From 11 October 2005 to 22 January 2007, we succeeded in maintaining 13 continuous fly reproductive cycles in the lab, 1 generation every 40 days on average. An exception was the 9th generation (27 August - 27 September) lasted 31 days because of accidental high temperatures (26-27°C).

Figure 3 shows part of the lab generation emergence series. Emergences are reported as percentage of flies per sex per generation versus date. Net mortality is also displayed (bar charts) as % of dead individuals per sex per generation versus date.

The tested method appears to be both practical and cost-effective, for the first time enabling the continuous lab rearing of *B. oleae* on its natural host without interruptions, thanks to the year-round availability of olive fruits. Several possible improvements could be made to fine tune the method, such as for example administering a refined diet to adults or providing a more comfortable media to pupate (we tested successfully moistened paper towels as well as layers of moistened fine sand).

The rearing technique reported succeeded for the first time in obtaining olive fly generations continuously on its natural host and, after standardization, it appears to be very promising for optimizing further studies (i.e. physiological, biological and behavioural studies, parasitoid rearing and release, sterile insect technique, etc.).
Figure 1 Scheme of the rearing cage (different components and flies are not in scale)

Figure 2 Scheme of the feeder (olive fly not in scale)
Figure 3 Emergence and mortality of generations from IV to VIII
References


The current issue of codling moth control in Croatian apple orchards

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Abstract: In recent times, high populations of codling moth (Cydia pomonella) have been observed in Croatian apple orchards. The appearance of large populations of this pest is in accordance with data from other parts of Europe and the world. In the last ten years in orchards in which monitoring of codling moth by pheromone traps is conducted increasing daily moth catches and earlier appearance of the pest have been observed. More than ten years ago codling moth was caught to the end of July. Today adult flight lasts until the end of September. The number of treatments against codling moth has increased seven times. Reasons for the increased number of treatment are complex; global warming, resistant strains of codling moth, a third generation of the pests. Monitoring the appearance of the first generation of adults and efficient temperature sums in field conditions indicate the emergence of pests with a lower temperature requirement. The extended flight of adults to September and the dynamics of adult catches on pheromone traps indicate the presence of a third generation of the pest. Integrated protection measures against codling moth are encountering a series of problems. Environmentally more favourable measures of protection against codling moth, like the mating disruption technique, which is applied in Western Europe has not shown satisfactory results in Croatia because of the small size of orchards. Biological products such as those based on the virus are not available on the Croatian market. The number of insecticides registered for codling moth control is small, with only a few active substances, which will lead to a greater number of applications per year and increase the rate of development of resistant strains of the pest.

IPM, Codling moth, Apple, Resistant types
Loquat and Pomegranate Thrips in the Eastern Mediterranean Region of Turkey

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Abstract: A thrips survey was conducted during 2006-2007 in pomegranate and loquat trees in the Eastern Mediterranean Region of Turkey which includes Adana, Mersin, Hatay and Osmaniye provinces. For the extraction of thrips in the laboratory, new shoots with terminal buds and flowers were collected and 400 pomegranate and 1000 loquat fruits were randomly checked visually for any damage. A total of 511 adult thrips were collected. Seven species of thrips were identified: Frankliniella occidentalis Pergande (loquat, pomegranate), Thrips tabaci Lindeman (loquat, pomegranate), Thrips major Uzel (loquat, pomegranate), Pezothrips kellyanus Bagnall (pomegranate), Frankliniella intonsa Trybom (pomegranate), Thrips meridionalis Priesner (loquat), Melanthrips fuscus Sulzer (loquat). Among these species, T. major was the most widely distributed species (90.6%), occurring throughout all loquat growing districts in the Eastern Mediterranean Region followed by T. meridionalis (3.5%) in both years. However, F. occidentalis was the most widely distributed species (94%), occurring throughout all pomegranate-growing districts in the Eastern Mediterranean Region followed by T. tabaci (3%) in both years. Thrips are presently of little economic importance as pests of pomegranate (little damage) and loquat (damage rate % 17) in the region.

Key words: Loquat, pomegranate, thrips, pests

Introduction

Loquat, Japanese plum (Eriobotrya japonica), a small or medium sized tree is native to China and Japan. It was introduced into Japan and became naturalized there in very early times. It has been cultivated in Japan for over 1,000 years. It has also become naturalized in India and many other areas. The loquat is adapted to a subtropical to mild-temperature climate. Well established trees can tolerate a low temperature of -11°C (Anonymous, 2007a). It is estimated that loquat came to Turkey about 150-200 years ago. China (200,000 tons production and 42,000 ha) is the leading producer of loquats, followed by Pakistan (28,800 tons production and 11,000 ha), Spain (41,487 tons production and 2,914 ha) and Japan (10,245 tons production and 2,420 ha) (FAO, 2003). Turkey is an important world producer of loquat. According to 2003 data; production of loquat 13,000 tons. Turkish loquat production of 97.5% is ensured from Mediterranean region of Turkey (Anonymous, 2007b). In Turkey, the Mediterranean region has the most suitable ecological conditions for growing loquat. Within the Eastern Mediterranean region, Mersin, Hatay and Adana provinces are the major producers.

Pomegranate (Punicae granatum L.), can be grown in almost every regions of Turkey. This plant is resistant to dry climate conditions and can easily adapt to various soil types. Turkey is accepted as the homeland of pomegranate and takes the first place in growth quantity among the other grower countries (Özgüven and Yılmaz, 2000). Recently many pomegranate orchards were planted, especially in Mediterranean, Aegean and southeast Anatolia regions of Turkey, owing to increasing export demands. As a result of this increase in
growing areas, in addition to cultivation problems, various plant protection problems that cause economical loses in pomegranate occur every year (Öztürk and Ulusoy, 2006). Total production of pomegranate 106,560 tons (t) in Turkey. The Eastern Mediterranean region is one of the most important areas of pomegranate production (Hatay (4090t), Adana (2962t), Osmaniye (1217t), Mersin (8705)) and has increased in recent years (Anonymous, 2007b).

After the economic value of loquat was realized, demand for commercial production rapidly increased. Further future expansion of the loquat and pomegranate growing areas is expected. Parallel to this increase the pests incidences have also increased including thrips. The aim of this study is to identify thrips species and their damage to loquat and pomegranate in the eastern Mediterranean region.

**Material and methods**

Sampling was done in loquat orchards in the eastern Mediterranean region. Loquat flowers and shoots were collected from three sites; Adana, Mersin, Hatay. Flowers and shoots were also collected from pomegranate from different sites; Adana (Seyhan, Yüreğir, Karataş), Mersin (Erdemli, Silifke, Mut), Hatay (Erzin, Dorytol, Samandağ, Arsuz), Osmaniye (Merkez), during 2006-2007. In the laboratory, all other pests on leaves and flowers were discarded. To determine the damage caused by thrips, pomegranate leaves and 400 pomegranate fruits were assessed. To determine damage by thrips on loquat, 1000 fruits were checked. All the thrips species were identified by Prof. Dr. İrfan Tunç (Akdeniz University, Agricultural Faculty, Plant Protection Department, Antalya, Turkey).

**Results and discussion**

**Loquat thrips**

During 2006-2007, five Thysanoptera species were identified (Table 1). *Thrips major* was the most dominant species (91%) and was the main thrips species on found on loquat flowers from December to February. Fewer *T. major* (231 individuals - highest number) were collected from loquat leaves and fruits were few. (Table 1). Cravedi and Molinari (1984) indicated that the greatest damage is caused by the feeding of young nymphs of *T. major* on the flowers. Later eggs are laid on the developing fruits (nectarine), after which subsequent generations migrate to other flowering plants.

Although *Frankliniella* species are major pests of many plants *T. major* was the major pest in Loquat, in the eastern Mediterranean region, Turkey. Three *T. major* (1.3%) and one *M. fuscus* (25%) male thrips were found, but no males were found of *T. tabaci, T. meridionalis, F. occidentalis* (Table 1).

In this study, injury caused by thrips on loquat was 17 %, enough to cause an economic loss. *Orius* spp. effectively reduces thrips populations under certain situations (Ananthakrishnan 1993). There are very few studies determining natural enemies and pests on loquat is few in Turkey.
Table 1. Number of male and female Thysanoptera on loquat trees in the Eastern Mediterranean Region of Turkey during 2006-2007.

<table>
<thead>
<tr>
<th>Thysanoptera species</th>
<th>Total thrips number</th>
<th>Relative abundance of total number (%)</th>
<th>Female thrips number</th>
<th>Male thrips number</th>
<th>Female/Male thrips ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thrips major</em></td>
<td>231</td>
<td>90.6</td>
<td>228</td>
<td>3</td>
<td>1.3</td>
</tr>
<tr>
<td><em>Thrips meridionalis</em></td>
<td>9</td>
<td>3.5</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Thrips tabaci</em></td>
<td>6</td>
<td>2.3</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Melanthrips fuscus</em></td>
<td>5</td>
<td>1.9</td>
<td>4</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td><em>Frankliniella occidentalis</em></td>
<td>4</td>
<td>1.5</td>
<td>4</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>255</td>
<td>100</td>
<td>251</td>
<td>4</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Pomegranate thrips

Five Thysanoptera species were identified (Table 2). *F. occidentalis* and *T. tabaci* were found in every province (Adana, Mersin, Hatay and Osmaniye) and *T. major* (Adana), *P. kellyanus* (Hatay), *F. intonsa* (Adana) were found in these regions. *F. occidentalis* was the most dominant species (up to 241 individuals) (Table 2). This is hypothesized to be because the pest has a high number of hosts and is well suited to the climate. *F. occidentalis* was first found on vegetables and ornamentals in the Mediterranean region in 1993 (Tunç and Göçmen, 1994).

Table 2. Number of male and female Thysanoptera on pomegranate trees in the Eastern Mediterranean Region of Turkey during 2006-2007.

<table>
<thead>
<tr>
<th>Thysanoptera species</th>
<th>Total thrips number</th>
<th>Relative abundance of total number (%)</th>
<th>Female thrips number</th>
<th>Male thrips number</th>
<th>Female/Male thrips ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Frankliniella occidentalis</em></td>
<td>241</td>
<td>94.1</td>
<td>225</td>
<td>16</td>
<td>7.1</td>
</tr>
<tr>
<td><em>Thrips tabaci</em></td>
<td>8</td>
<td>3.1</td>
<td>8</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Thrips major</em></td>
<td>3</td>
<td>1.1</td>
<td>3</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Frankliniella intonsa</em></td>
<td>2</td>
<td>0.7</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Pezothrips kellyanus</em></td>
<td>2</td>
<td>0.7</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>100</td>
<td>240</td>
<td>16</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Lublinkhof and Foster (1977) indicated that the highest reproductive rate of *F. occidentalis* (95.5 eggs/female) was obtained at 20°C in laboratory conditions. Another reason for the high number of *F. occidentalis* is that some pomegranate orchards are planted with or around other fruit orchards in the region. *P. kellyanus* is found on citrus in Turkey and was recorded on pomegranate trees in this study. Teksam and Tunç (2007) suggested that based on the experience in other countries and the increase of invasion and abundance in 2007 this pest species should be monitored. *F. occidentalis* populations consisted of 7% male thrips, *T. tabaci*, *T. major*, *P. kellyanus*, *F. intonsa* had no observed male thrips (Table 2). In this study, injury of thrips on pomegranate is only minor. *Orius* spp. effectively reduces thrips population under
certain situations (Ananthakrishnan, 1993). Studies on determining natural enemies on pomegranate are very recent, other crop studies have been carried out during the past 15 years, in Turkey (Mart and Altun, 1992; Öztürk et al., 2005).

In summary, seven Thysanoptera species were identified during 2006–2007 on loquat and pomegranate grown in the east Mediterranean region of Turkey. *F. occidentalis* was the species in both years in pomegranate orchards. Loquat orchards harboured *T. major* more than other thrips species. Some Thysanoptera species carry plant viruses. For this reason, more attention should be given to the knowledge of the biology and ecology of pomegranate and loquat thrips species. Research is needed into the control of these pests as part of an integrated pest management in pomegranate and loquat.

**Acknowledgements**

The authors are very grateful to Prof. Dr. İrfan Tunç, Akdeniz University, Agricultural Faculty, Plant Protection Department, Antalya, Turkey, for the identification of thrips species and other help with the research.

**References**

Cravedi, P., Molinari, F., 1984: Thysanoptera injurious to nectarines. Informatore Fitopatologico. 34: 12–16.
Two Spotted Mite, *Tetranychus urticae*, a new pest in persimmon orchards; approaches to reduce its density

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Abstract: Oriental persimmon, *Diospyros kaki* Thunb., endemic to East Asia, is one of the major fruit crops in Korea. After several decades two spotted mite (TSM) finally emerged recently as one of the key pest in the orchards. To solve the mite problem we have undertaken faunal surveys and defined the dominant species. We are identifying and conserving predators, assessing the status of the mite as a pest in orchards, developing effective miticides against TSM, and attempting to analyze the fluctuations of populations. The faunal survey of mites in 2006 in Korea showed that most of the collected tetranychid mites belonged to the genus *Tetranychus*, and additional collections of tetranychids made in 2007 were identified as *Tetranychus urticae* Koch. Among phytoseiid species collected, *Amblyseius eharai* was the most abundant. Most *A. eharai* were found on the branches in pedicels. In early spring, *A. eharai* was abundant before the extension of persimmon leaves, so was considered to be overwintering on the trees. Seventeen populations of TSM from farmer’s orchards were monitored. Among these orchards, only 2 were properly managed, 5 farms should have applied control measures but the farmers had little information on the mite and its damage, and 10 orchards were not in danger of mite damage. For control of TSM in fields, applications of spiromesifen 20SC and acequinocyl 15SC showed more than 90% control activity. Fluctuations of TSM populations may have been caused by pesticide activity and spray, density of predacious mites, rainfall, and weeds in the persimmon orchards.

*Persimmon, Fauna, Tetranychus urticae, Amblyseius eharai, Miticides, Control strategy*
Autumn control of aphid pests of tree and bush fruit crops

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Abstract: The aphid species that are significant pests of tree and bush fruit crops in Europe are almost all host-alternating. They spend the autumn, spring and early summer on their winter woody tree/bush fruit host but migrate to a herbaceous host in summer. In the autumn, there is a return migration to the winter woody host by males and pre-sexual females (gynoparae), the latter producing sexual females (oviparae) which mate with the males and lay overwintering eggs on the bark. The normal strategy to control aphid pests is to apply an aphicide in spring shortly after the eggs have hatched to avoid the subsequent development of damaging colonies, which cause severe curling of leaves on shoots and stunting. Work on apple, raspberry and blackcurrant is reported, which has shown that good control of all the important aphid pests of these crops can be achieved by autumn application of an aphicide timed to kill the returning winged forms before egg-laying occurs. The advantages of autumn application are that the aphids are vulnerable to direct interception by sprays and that pesticide residues on fruit due to aphicide application do not occur. Possible methods for gauging the size and timing of the autumn migrations to rationalise the use of autumn aphicide sprays, including suction and sex pheromone trapping and surveying the incidence of gynoparae and oviparae on trees in the autumn, are discussed.

Keywords: Dysaphis plantaginea, Amphorophora idaei, Hyperomyzus lactucae, pirimicarb

Introduction

The aphid species that are significant pests of tree and bush fruit crops in Europe are mostly host-alternating. They spend the autumn, spring and early summer on their winter woody tree/bush fruit host but migrate to a herbaceous host in summer. In the autumn, there is a return migration to the winter woody host by males and pre-sexual females (gynoparae), the latter producing sexual females (oviparae) which mate with the males and lay overwintering eggs on the bark (Figure 1). Other important species, e.g. the large raspberry aphid Amphorophora idaei, also have a migration period in summer or autumn but do not host alternate. The normal strategy to control aphid pests is to make one or more aphicide applications in spring shortly after the eggs have hatched to avoid the subsequent development of damaging colonies, which cause severe curling of leaves on shoots and stunting.

The objective of this work was to investigate whether as good control of the most important aphid pests of tree and bush fruit crops could be achieved by autumn application of an aphicide timed to kill the returning winged forms before egg-laying occurs.
Figure 1. Host alternating life cycle of the rosy apple aphid, *Dysaphis plantaginea*

**Materials and Methods**

A data base of over 40 years of weekly records of the numbers of several important tree and bush fruit aphid pests including rosy apple aphid, apple-grass aphid (*Rhopalosiphum insertum*) and currant-sowthistle aphid (*Hyperomyzus lactucae*) were available from the network of Rothamsted Insect Survey 12.2m suction traps. The data were used to examine the timings of the autumn migrations of the different species. It was assumed that the best time to spray would be towards the end of the autumn migrations of the gynoparae but at the start of the male migrations so that sprays were applied before egg laying.

Large scale replicated field experiments were conducted in commercial apple, blackcurrant and raspberry plantations in Kent, SE England examining the efficacy of different timings of aphicide sprays in the autumn.

**Results**

Suction trap records indicated that numbers autumn migrants vary greatly from year to year but for most species the bulk of the autumn migration of gynoparae generally occurs before week 41 (1st week October) with males migrating mainly after this time (Figure 2).

Sprays of aphicides applied in the trials at different times in the autumn greatly reduced number of aphids that developed the following spring on apple, blackcurrant and raspberry. For some aphid species, e.g. the blackcurrant aphid (Figure 3), time of spray application made little difference to the degree of control though two sprays were better than one, but for other species e.g. the currant-sowthistle (Figure 3) and large raspberry aphids (Figure 3) the degree of control depended on the date of application with best control being achieved by sprays in early October.
Fig. 2 Suction trap records for the currant-sowthistle aphid (*Hyperomyzus lactucae*) at Wye in Kent in 2004

**Discussion**

Autumn application of aphicides gave good control of all the most important aphid pests of tree and bush fruit crops. Where timing was critical, the best time of application was in early October, coinciding with the end of the migration of gynoparae and the start of the migration of males. The advantages of autumn application are that the aphids are vulnerable to direct interception by sprays and that pesticide residues on fruit due to aphicide application do not occur. Possible methods for gauging the size and timing of the autumn migrations to rationalise the use of autumn aphicide sprays include suction and sex pheromone trapping and surveying the incidence of gynoparae and oviparae on trees in the autumn.

**Acknowledgements**

This work was funded by the UK Horticultural Development Council (apple), The GlaxoSmithKline Growers’ Research Fund (blackcurrant) and through a UK industry and Defra funded HortLINK project (raspberry).

**Reference**

Figure 3. Numbers of aphids that developed after treatment with aphicides at various times in the autumn.

Blackcurrant aphid (*Cryptomyzus galeopsidis*)

Sprays of pirimicarb

**Currant sowthistle aphid (*Hyperomyzus lactucae*)**

Sprays of pirimicarb

**Large raspberry aphid (*Amphorophora idaei*)**

Thiacloprid spray
New infestation outbreaks of *Panonychus ulmi* Koch (Acari: Tetranychidae) in apple orchards of North-West Italy

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Abstract. The fruit tree red spider mite, *Panonychus ulmi*, has been a major pest in almost all fruit growing regions of the world, due to the negative effects of chemical sprays on natural enemies, until integrated pest management became widespread. Indeed the reduction of insecticide applications allowed the biocoenosis of antagonists, to control the red spider mite. In cases of use of certain insecticides this mite again became a local problem. In 2003 and 2005 in North-West of Italy inexplicable spread of infestations of this mite both on apple and peach orchards has been recorded. Our studies conducted in 2006, 2007 and 2008 assessed that *Panonychus ulmi* Koch (Acari: Tetranychidae) is still the main species in the orchards of north-west Italy and *Amblyseius andersoni* (Acari: Phytoseiidae) is its main antagonist. Hypothesis of an involvement of grass chemical control in infestation outbreaks of red spider mite was not confirmed and, it seems that it can be excluded as a cause of red spider mite infestation outbreaks.

Keywords: *Panonychus ulmi*, apple

Introduction

The fruit tree red spider mite, *Panonychus ulmi* Koch (Acari: Tetranychidae), has been a major pest in almost all fruit growing regions of the world, due to the negative effects of chemical sprays on natural enemies, until integrated pest management became widespread. Indeed the reduction of insecticide applications allowed the complex of antagonists (mainly the coccinellid *Stethorus punctillum*, anthocorid *Orius* sp. and mites as *Amblyseius andersoni* (Acari: Phytoseiidae), the main predator of tetranychids in North-West Italy) to control the red spider mite. Only in cases of use of certain insecticides this mite became a local problem again. In 2003 and 2005 in North-West of Italy infestations of this mite, both on apple and peach orchards, have been recorded. Our studies conducted in 2006, 2007 and 2008 aimed at understanding the reasons for these new outbreaks.

Materials and methods

In 2006, two apple orchards and two peach orchards located in a fruit growing area on the plain and two apple orchards and two peach orchards located in a hilly fruit growing area were monitored regularly for the presence of the red spider mite and its antagonists. In 2007, 20 farms were monitored for infestation outbreaks. Infestation data for these orchards for the two years before (2005 and 2006) and intervention information for 2007 have been collected. The orchards were checked regularly for red spider mite presence and, when present, a sample was collected.

Twenty-four leaves from each side of six plants per orchard were taken monthly. At the
same time, samples of prevalent grass species were taken. Tetranychids and their antagonists were identified in the laboratory on fresh leaves or on material collected by Berlese funnel and stored at 4°C in 1/3 alcohol, 2/3 water and a drop of glycerine.

In order to test the influence of herbicide sprays on red spider mite outbreaks, randomized blocks were established, apart from chemical control sprays (Glufosinate ammonium, 4-7 l/ha) and monitored for red spider mite infestation after 1-2 weeks. Samples were taken, as described above, both on trees and grass.

Results

Infestation outbreaks
In 2003 and 2005, red spider mite infestation outbreaks were recorded in many apple and peach orchards, regardless of spray interventions that are standardized in the fruit growing area of North-western Italy. The aim of experimentation was to understand the origin of those outbreaks by monitoring many orchards from the beginning of the season. In 2006 two apple and two peach orchards were monitored as well as almost 20 orchards in 2007, but no infestation was recorded regardless of past infestations, spray interventions and presence of red spider mite eggs during the winter.

Red spider mite and antagonists
The species collected during the surveys was identified: the red spider mite was *Panonychus ulmi* Koch (Acari: Tetranychidae), although no large populations were recorded (Table 1). The main antagonist found out was *Amblyseius andersoni* Chant, both on trees and grasses, although they were less numerous on the latter (Table 1). In both 2006 and 2007, the populations of this phytoseiid were relevant. Less represented were Tideid species found only in hilly apple orchards in 2006.

<table>
<thead>
<tr>
<th>Date</th>
<th>Species</th>
<th>A. andersoni</th>
<th>P. ulmi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>3 May</td>
<td><em>Epilobium</em> sp.</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td>Taraxacum officinale</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Peach</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8 May</td>
<td><em>Senecio vulgaris</em></td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Stellaria sp.</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14 May</td>
<td>Apple</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>30 May</td>
<td>Apple</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>13 June</td>
<td>Apple</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>5 July</td>
<td>Apple</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td>Apple</td>
<td>0.1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>23 Aug</td>
<td><em>Echinochloa crus-galli</em></td>
<td>3</td>
<td>0.2</td>
</tr>
<tr>
<td>Poa pratensis</td>
<td>9.6</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>25 Set</td>
<td>Apple</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>Apple</td>
<td>0.7</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
**Role of grass chemical control in infestation outbreaks**

Grass chemical control was hypothesized to play a role in red spider mite infestation outbreaks. A technician of the extension service suggested that chemical control of grass made under intense sun irradiation condition or just before these conditions could induce outbreaks of red mite. The hypothesis was that chemicals under the sun evaporates affecting antagonists of the red spider mite. To assess the validity of this hypothesis randomized plots in two apple and two peach orchards were sprayed with herbicide just before very warm days in July and August. Other plots were left untreated. Infestation was recorded in each plot.

Table 2. Red spider mite (*P. ulmi*) and its antagonists *A. andersoni* mean per apple leaf in treated and untreated plots a week after spray.

<table>
<thead>
<tr>
<th></th>
<th><em>A. andersoni</em></th>
<th><em>P. ulmi</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical control</td>
<td>0.4a</td>
<td>0.4a</td>
</tr>
<tr>
<td>Un-treated</td>
<td>0.1a</td>
<td>0.2a</td>
</tr>
</tbody>
</table>

**Discussion**

Unluckily, our studies conducted in 2006 and 2007 did not encounter an infestation outbreak of red spider mite and this fact confirms the unpredictability of the phenomenon, at least with current information.

In any case, it was possible to confirm that *Panonychus ulmi* Koch (Acari: Tetranychidae) is still the main species in the orchards of north-west Italy and *Amblyseius andersoni* is its main antagonist.

Hypothesis of an involvement of grass chemical control in infestation outbreaks of red spider mite was not confirmed and, from experience, it seems that it can be excluded as a cause of red spider mite infestation outbreaks.

**Acknowledgements**

We thank all the farmers and the field technicians involved in the present study. This research was supported by Regione Piemonte (Italy) within the program: “Programma di ricerca, sperimentazione e dimostrazione agricola in frutticoltura e orticoltura 2007 and 2008”.

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Ostrinia nubilalis Hübner (Lepidoptera, Pyralidae) as a threat to apple

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Abstract. Over the last few years, damage to fruits due to the European Corn Borer, Ostrinia nubilalis Hübner, has been recorded in apple orchards of Piedmont (North-West Italy). Investigations carried out in 2006 and 2007 aimed to understand the phenomenon, evaluate a better way to monitor the insect and perhaps modify the pests management strategy to control O. nubilalis. Field surveys confirmed that the main damage occurs in orchards close to corn fields or, in a few cases, in orchards with the grass Echinochloa crus-galli. Researches demonstrated that pheromone-baited mesh cone traps are more efficient than delta sticky traps for monitoring the flight of European corn borer and that in the Piedmont area the E strain is prevalent. Field surveys confirmed that ECB generally lives and reproduces on corn, and migrates onto apple trees when the main host plants is harvested. In Piedmont this happens at the beginning of September, even if damage sometimes appear earlier in August when high populations of ECB are present. Information collected allowed the extension services to monitor the pest and modify the pest management strategy.

Keywords : European Corn Borer, Ostrinia nubilalis, apple.

Introduction

Over the last few years, damage to fruits due to the European Corn Borer, Ostrinia nubilalis Hübner, has been recorded in apple orchards of Piedmont (North-West Italy). Investigations carried out in 2006 and 2007 aimed at understanding the phenomenon, evaluating a better way to monitor the insect and possibly modify the apple pest control strategy keeping into account O. nubilalis.

Materials and methods

Apple orchards are located in the fruit growing area of North-West Italy, which frequently are proximity with corn fields. Data have been collected in 2006 and 2007 in 5 orchards (Golden Delicious, Fuji, Red Delicious). During July and August, 50 corn plants have been monitored weekly for the presence of eggs and larvae of O. nubilalis. TrapTest (Isagro, Milan, Italy) and Heliothis Trap (Scentry, Billings, Montana, USA) have been placed in both corn fields and apple orchards to monitor adult flight. Each trap was lured with both Phenyl-AcetAldehyde (PAA) and E or Z pheromone. PAA was replaced at 15 days interval and the pheromone lure at 4 week intervals. Two of each combination trap/lure were placed in each field/orchard. Traps were checked weekly from the second generation in 2006 and from the first in 2007, until the end of September.
Results

Location of the pest
At the end of August 100% of corn plants in field close to orchards were visited at least once by European Corn Borer (ECB) (Fig. 1). These data suggest that populations are similar to those that can be found in corn production areas. It must be noted that this percentage is the sum of first and second generations. Since a third generation can develop, late ripening apple varieties are the most susceptible. No orchard far from corn fields showed ECB damage, except for orchards with fully developed *Echinochloa crus-galli*, a grass that allows ECB to develop quite high populations, that can move to apple trees and cause relevant damage.

![Graph showing percentage of infested corn plants and mean number of ECB larvae per corn plant.](image)

Figure 1. Per cent of infested corn plants and mean number of ECB larvae per corn plant.

Monitoring European Corn Borer
In order to assess whether the E or Z strain was prevalent, TrapTest traps were lured with Phenyl-Acetaldehyde and alternately with the two pheromones. As shown in fig. 2 the prevalent strain is E as in other corn production areas of Northern Italy.

![Graph showing mean number of European Corn Borer adults caught in apple orchards in 2006 with two different lures in TrapTest traps.](image)

Figure 2. Mean number of European Corn Borer adults caught in apple orchards in 2006 with two different lures in TrapTest traps.
TrapTest and Heliothis traps were placed in both corn fields and apple orchards to understand the best way to monitor *O. nubilalis* population. The second gave a better response: higher number of adults and a well defined generational succession as shown in Figure 3.

![Figure 3](image1.png)

Figure 3. Mean number of European Corn Borer adult catches in apple orchards in 2007, with two different models of traps.

**When the pest goes to apple**

Since corn dries out starting from the end of August, this period is the mostly likely to induce ECB to cross to apple. Indeed damage often arises in August with a peak at the end of August and the beginning of September (Fig. 4).

![Figure 4](image2.png)

Figure 4. Mean number of European Corn Borer adult catches in apple orchards with Heliothis net traps. The arrow indicates the time of ECB crossing from corn to apple.
Discussion

In conclusion we can say that:

- Populations of the European Corn Borer (ECB) in North-West of Italy reach similar levels to those that can be found in the main corn production areas.
- In the fruit-growing area of Cuneo province the E-strain is prevalent and the Z-strain is very rare. The best way to monitor the pest are Heliiothis net traps baited with both E-pheromone and Phenyl-Acetaldehyde.
- The problem of damage by ECB on apples can arise when corn fields are close to apple orchards. Rarely, damage due to ECB has been reported in orchards not close to corn fields as found at the beginning of the research. It is often confused with codling moth (*Cydia pomonella* L.) damage. Sometimes, however, in the presence of fully developed *Echinochloa crus-galli* grass ECB can easily reproduce and then move to apple.
- The end of August is the time of highest risk of ECB crossing from corn (or *E. crus-galli*) to apple. Indeed, at that time, higher population levels are reported and corn is harvested. Those are the better condition for migration. As to defence, it seems that the active ingredients usually sprayed for controlling codling moth are also effective on ECB.

Acknowledgements

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Investigations on the occurrence of the quarantine fruit fly species *Rhagoletis cingulata* and *Rhagoletis indifferens* on *Prunus avium* and *Prunus cerasus* in Austria

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Abstract: During the growing seasons 2007 and 2008 the occurrence of the two quarantine fruit flies *Rhagoletis cingulata* (Loew) and *R. indifferens* (Curran) was monitored in Austria. *R. cingulata* originates from the eastern and *R. indifferens* from the western part of North America. Both species are important pests of cherries in North America and potentially in European cherry orchards, causing severe quality problems after fruit infestation. While *R. cingulata* mainly infests various *Prunus* species, *R. indifferens* also occurs on *Crataegus* sp. and on *Rhamnus* sp. After the first findings of American cherry fruit flies in Europe in 1983 in Switzerland, *R. cingulata* was also detected during surveys in other European countries, such as the Netherlands, Germany, Hungary, Slovenia and Croatia. Recent findings of *R. cingulata* were located near the south-eastern border of Austria while no findings of *R. indifferens* were reported from this region until now. The survey in Austria was carried out in the main cherry production areas and in those areas where high invasion potential was most probable. Sampling sites were located in variable orchards in the eastern part of Austria, along the border to Hungary and Slovenia. Fruit flies were baited and caught with yellow panels of the type Pherocon® AM. Traps were placed in cherry trees to catch adult flies, which emerged under or near the sampling trees. In 2007, two traps were installed and replaced weekly at each of the seven sampling sites from May 2nd until 2 weeks after the last seasonal occurrence of the fruit flies. In 2008, the survey was carried out on 6 cherry production sites including 4 new monitoring sites compared to 2007. Traps were replaced in fortnight intervals from the end of May in 2008 until 2 weeks after the last seasonal occurrence of the fruit flies. Identification of the caught individuals was carried out morphologically.

In both years, a high number of European cherry fruit flies (*R. cerasi* Linné), which is considered an important cherry pest in Austria, was caught in many traps. In 2007, at each of two of the sampling sites, 1 individual of *R. cingulata* was found. No further non-native fruit flies were caught. We assumed that the captured specimens were separately introduced specimens and that there were no established populations in the monitoring area during the seasons 2007 and 2008.

Keywords: quarantine pest, *Rhagoletis cingulata*, *R. indifferens*, cherry, distribution, Austria
Population evolution of Ceratitis capitata (Wied.) in the NE of Spain and its implications for the establishment of control methods.

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Abstract: The Mediterranean fruit fly Ceratitis capitata (Wied.) is a worldwide pest that has increased its populations in the last 10 years in Girona province (NE of Spain, 42º North latitude). The adult population has been carefully monitored, using dry food based attractants containing three components, in peaches (2005-2007) and apples (2007) in the two main fruit growing areas of Girona. One trap per orchard was installed and the captures were registered using SIG technology; interactive distribution maps were drawn on a weekly basis using two software programs jointly, Hesperides® and Google map®. An area-wide control project was applied using mass trapping in both areas hanging 50 traps/ha in each fruit orchard, bated with dry food based attractant of three components. The project acreage started on 300 ha in 2005 and grew to 774 ha in 2007. Damage level and chemical treatments were recorded and sanitation methods were applied as a compulsory requirement. Results showed a seasonal population evolution, with maximum catches at the end of September or early October in both fruit species studied. The highest population was found in the Northern part of the two Girona fruit growing areas. SIG technology has enabled us to determine the zones with the highest population in each area and to choose the control strategy in each orchard. Mass trapping as a control method on an area-wide basis gave good protection of fruits and in only a few cases it was necessary to apply reinforcement with chemical spraying. Sanitation measures have proved to be necessary to complete mass trapping as a control method of the Mediterranean fruit fly. All these results will be discussed in order to improve the control of Medfly in the Girona fruit area.

Medfly, Ceratitis capitata, Population evolution, Monitoring, Mass trapping, Area-wide control, SIG
Preliminary studies about the effect of ‘Candidatus Phytoplasma mali’ on the psyllid Cacopsylla melanoneura (Homoptera: Psyllidae)

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Abstract: Cacopsylla melanoneura Föster (Homoptera: Psyllidae), an univoltine psyllid, is a vector of ‘Candidatus Phytoplasma mali’, the etiological agent of apple proliferation disease (AP), which is a severe problem in Italian apple orchards. Preliminary studies were conducted about the influence of ‘Ca. Phytoplasma mali’ on the fitness of C. melanoneura. Couples of overwintering adults of the psyllid collected in the field were exposed to the phytoplasma by feeding on infected and non-infected apple (Malus domestica L.) (Rosaceae) shoots. The effect of the exposure to the phytoplasma with the diet was determined by measuring some of the life history traits correlated to the fitness of the individuals such as longevity of the females, number of eggs laid, egg hatching and development of larval instars. The longevity of AP-exposed adult females was not significantly different to that of psyllids fed on healthy apple shoots. However, the AP-exposed females laid significantly less eggs than unexposed ones, and the eggs produced by AP-exposed females were significantly delayed in hatching. Moreover, the progeny of AP-exposed females (number of nymphs emerging from eggs laid on apple shoots) was significantly less numerous than the progeny of unexposed females, while there were no significant differences in their development to adulthood. Further studies are necessary to establish whether such differences are due to the presence of AP phytoplasma in the body of the psyllid or in the plant.

Apple proliferation, Psyllid, Apple, Phytoplasma
New insights into management of the white grub, Polyphylla olivieri in fruit orchards of Iran

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Abstract: Polyphylla olivieri (Col., Melolonthidae) is the most destructive white grub in the Iran. This Scarabaeid has a wide host range including different fruit trees in most part of Iran. Chemical pesticides is the common for controlling this pest. Considering side effects of this method, application of biocontrol agent has been considered in management programmes. Among the natural pathogens, several isolates of entomopathogenic nematodes from both genus of Steinernema and Heterorhabditis were isolated from third and second larval stages of this pest in Iran. This isolates belonged to Heterorhabditis bacteriophora, Steinernema carpocapsae and Steinernema glaseri. Laboratory assay showed that the last species, S. glaseri had the highest mortality potential. The prevalent pathogen of this melolonthid in Tehran province was Metarhizium anisopliae and after this Beauveria bassiana. Compatability studies on application of entomopathogenic nematodes and fungi indicated that application of entomopathogenic nematodes and M. anisopliae can reduce population of this white grub considerably. In addition to natural pathogens as natural biocontrol agents, some isolates of nematodes were isolated from soil habitats of this pest. Among this, Steinernema feltiae and Heterorhabditis megidis had the highest virulence compared with other species. A survey for characterization and introduction of isolates with high virulence can provid a good alternative in integrated management of Polyphylla olivieri in future.
First evidence of the walnut husk fly (*Rhagoletis completa*) in Austria

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**Abstract:** The walnut husk fly *Rhagoletis completa* (Tephritidae, Diptera), originating from North-America, is listed as a quarantine pest on the Annex I/Al (directive 2000/29/EC). As the main host plants of *R. completa* are various species of *Juglans* spp., infestations could become a problem for walnut production because larval feeding in the mesocarp (nutshell) could also damage the pericarp and the nut itself. Under certain conditions peaches (*Prunus persica*) may also be attacked. In international trade, the major means of dispersal is the transport of infected fruits (containing live larvae).

In Europe (Switzerland) some specimens were collected in the late 1980s for the first time. During the last years *R. completa* also occurred in Slovenia, Italy and Germany, and recently in France (2007). Due to the fact that there are still no individuals of the walnut husk fly documented for Austria a monitoring program was started by the Institute of Plant Health (AGES) in 2008. The monitoring took place in Tyrol, near Innsbruck in private gardens following up on information of the Tyrolean Plant Protection Service. Sticky yellow traps were used to catch the fruit flies and were set up and recorded from the end of June at 14-day intervals.

In the first half of July the first individuals were caught and the first presence of *R. completa* was demonstrated for Austria. In autumn, fruits that were infested by the walnut husk fly were found in other regions of Austria (Vienna, Styria, Carinthia), too. Monitoring in other parts of Austria will be continued in 2009.

**Key words:** quarantine pest, *Rhagoletis completa*, walnut, distribution, Austria
The occurrence of leaf rollers in Polish apple orchards and possibilities of their integrated control

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Abstract: Leaf rollers constitute the major pests of fruit crops - particularly apple and pear in many regions with temperate climate. Among a dozen or so species occurring in fruit orchards in Poland only four are important or even (depending on year) very important. They are: dark fruit-tree tortrix (Pandemis heparana), summer fruit tortricid (Adoxophyes orana), apple bud moth (Spilonota ocellana) and european leaf roller (Archips rosanus). The harmfulness of these pests during warm seasons is particularly serious. Since 2002 an increasing significance of Adoxophyes orana has been observed which is probably connected with warming of the climate. This complicates the control of the above leaf roller species as its larvae are present at a different time to those of the others, and especially the summer generation of larvae of Adoxophyes orana. Several monitoring techniques can be used to evaluate the occurrence and abundance of Adoxophyes orana, tree inspections used along with the use of sex-pheromone traps seem to be the most effective ones. Despite of wide host range Adoxophyes orana prefers to feed on apples, so together with other tortrix species (see above) it is able to cause serious problems in many orchards. Several pesticides are registered in Poland for chemical control of these pests in orchards along with IFP programs. These are thiacloprid (Calypso 480 SC), acetamiprid (Mospilan 20 SP), indoxacarb (Steward 30 WG), metoxyfenozid (Runner 240 SC) and spinosad (Spintor 480 or 240 SC). The insecticide indoxacarb, metoxyfenozid and spinosad are used mainly in the summer because they reduce the codling moth population as well. In the case when other pests (e.g. aphids) occur along with tortrix species, neonicotinoids (thiacloprid and acetamiprid) are recommended. Since 2006 a few experiments with the new active ingredient rynaxypyr have been conducted and very promising results for leaf roller control were obtained.

IPM, Leaf rollers, Pomefruit, Control Strategy
The control of the *Cacopsylla pyri* L. (Sternorrhyncha, Psyllidae) in a pear orchard in the Czech Republic.

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**Abstract:** A field trial was conducted in 2008 in the Czech Republic to test the efficacy of kaolin (aluminosilicate mineral) against over wintered adults of *Cacopsylla pyri*. It aimed to prevent the females laying their eggs. Ekol (90% coleseed oil) was also tested in order to suffocate adults and eggs. In addition, the insecticides Samnite 20 WP (pyridaben), Insegar 25 WP (fenoxycarb) and Calypso 480 SC (thiacloprid) were applied to reduce nymphs. These treatments were repeated on the first and the second generation. Efficacy was compared with an untreated control. Beating tray samples were taken in both plots (control, treatment) to monitor the density of adults. Egg-laying and nymph infestation were visually monitored. The *Cacopsylla pyri* population was not reduced under a damaging level. This observation might be explained by a high initial infestation level and the immigration of pear suckers from the untreated control plot. However, during the vegetation period it was observed that there were lower number of adults, nymphs and eggs on treated trees compared to the untreated control. The population density was significantly decreased, but not under the economic threshold (10 eggs or nymphs / 100 leaves). Yield was not decreased and no honeydew and sooty moulds were observed on the fruits.

**Key words:** integrated pest management, *Cacopsylla pyri*, pears, kaolin, oil, insecticides

**Introduction**

The psylla, *Cacopsylla pyri* is one of the most significant pests of pear orchards in the Czech Republic. Increasing populations were caused by emergence of resistant populations due to intensive use of insecticides and by the eradication of natural enemies (*Anthocoridae*, *Chrysopidae*, *Coccinellidae*) of the pest, caused by using non-selective preparations (Falta 2008). In order to renew the ecological equilibrium in orchards and increase the efficacy of insecticides, it is necessary to apply the rules and principles of integrated pest management.

As a sap-feeding insect, *C. pyri* produces large amounts of honeydew, causing the growth of sooty mould and black russet on fruits. Pear psylla also serves as a vector for the phytoplasma organism responsible for Pear decline. In case of heavy and prolonged infestations, the toxins injected by a pear suckers when they feed can lead to the exhaustion or even death of the tree (“Psylla shock”) (Michelleti *et al.* 2005).

The European pear sucker appears in 3-6 generations every year. In February/March the over wintering adults start to feed and lay eggs. Each female can lay up to 400 eggs over 4-5 weeks. After egg hatch, pear psylla nymphs pass through five development instars.

Control of this pest is very difficult. The most important control is to start with protection at the beginning of vegetation growth, when there are fewer life stages present. During the vegetation period all stages are often present and it is difficult to select a suitable product for control and to predict optimum timing for its application (Michelleti *et al.* 2005).

Tested product kaolin is an aluminosilicate mineral. Kaolin particles create on plants coating, which causes visual disorientation of adults, pests insects struggle with moving over treated surface, and particles fill up suitable places for eggs which leads to reduce feeding and oviposition (Kocourek and Stará 2007, Pultar 2007). Kaolin does not kill insects but acts as a...
repellent or barrier against adults (Daniel and Wyss 2004). Ekol (90% coleseed oil) was also tested in order to suffocate adults and eggs.

**Material and methods**

The study was conducted in 2008. The research site was in Holovousy village, Czech Republic. Orchards with main pear varieties “Konference” and “Lukasova” were established in 1993. The initial infestation level was high. In several preceding years improper control methods were practised, the population density increased and crops were significantly damaged by honeydew and, as a result, yields decreased. The research area was divided into two uniform parts – treated and untreated. Size of experimental area was 1 ha.

Kaolin was tested against over-wintered adults of pear sucker to prevent egg-laying on the branches and shoots. The first application was done before egg laying (BBCH 51-53). Altogether there were three treatments of kaolin at intervals 10-14 days. During the snapping of browses (BBCH 55) followed up two treatments of oil Ekol (coleseed oil) at intervals 12 days. After the leaf flush (BBCH 57), three types of insecticides were applied - Sanmite 20 WP (pyridaben), Insegar 25 WP (fenoxycarb) and Calypso 480 SC (thiacloprid). These treatments were repeated at the same concentrations against the second generation. Chemical control ended 25.6.2008 (Table 1).

Table 1. System of treatments against pear suckers in 2008.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Active ingredient</th>
<th>Dose /ha</th>
<th>Water /ha</th>
<th>Date of application</th>
</tr>
</thead>
<tbody>
<tr>
<td>kaolin</td>
<td>Al₄Si₄O₁₀(OH)₈</td>
<td>50 kg</td>
<td>1000 l/ha</td>
<td>8.2.2008</td>
</tr>
<tr>
<td>kaolin</td>
<td>Al₄Si₄O₁₀(OH)₈</td>
<td>25 kg</td>
<td>1000 l/ha</td>
<td>25.2.2008</td>
</tr>
<tr>
<td>kaolin</td>
<td>Al₄Si₄O₁₀(OH)₈</td>
<td>25 kg</td>
<td>1000 l/ha</td>
<td>5.3.2008</td>
</tr>
<tr>
<td>Ekol</td>
<td>90% coleseed oil</td>
<td>9,0 l</td>
<td>1000 l/ha</td>
<td>4.4.2008</td>
</tr>
<tr>
<td>Ekol</td>
<td>90% coleseed oil</td>
<td>9,0 l</td>
<td>1000 l/ha</td>
<td>16.4.2008</td>
</tr>
<tr>
<td>Sanmite 20 WP</td>
<td>pyridaben</td>
<td>0,75 kg</td>
<td>400 l/ha</td>
<td>23.4.2008</td>
</tr>
<tr>
<td>Insegar 25 WP</td>
<td>fenoxycarb</td>
<td>0,75 kg</td>
<td>400 l/ha</td>
<td>1.5.2008</td>
</tr>
<tr>
<td>Calypso 480 SC</td>
<td>thiacloprid</td>
<td>0,20 kg</td>
<td>400 l/ha</td>
<td>9.5.2008</td>
</tr>
<tr>
<td>Sanmite 20 WP</td>
<td>pyridaben</td>
<td>0,75 kg</td>
<td>400 l/ha</td>
<td>29.5.2008</td>
</tr>
<tr>
<td>Insegar 25 WP</td>
<td>fenoxycarb</td>
<td>0,75 kg</td>
<td>400 l/ha</td>
<td>10.6.2008</td>
</tr>
<tr>
<td>Calypso 480 SC</td>
<td>thiacloprid</td>
<td>0,20 kg</td>
<td>400 l/ha</td>
<td>25.6.2008</td>
</tr>
</tbody>
</table>

For establishment of long-term effect of control the monitoring of all developmental stages continued in both plots. Efficacy was compared with an untreated control. A tractor mounted sprayer, Tifone Vanguard 1075, was used in the trial.

Beating tray samples were taken to monitor the density and the activity of *C. pyri* adults. One sample comprised 25 beatings on 25 branches over a 0.25m² tray. Eggs-laying and nymph infestation were visually monitored on 20 randomly chosen clusters of blossoms or young shoots. These parts of the tree were cut off and observed in the laboratory under a microscope. Three samples for each evaluation were taken in both plots (control, treatment) at 6 or 7 days after application.
Results and discussion

Adults
The kaolin particle film does not kill adults, but acts as repellent or barrier and reduces feeding and oviposition. Hence, the application of kaolin did not reduce numbers of adults, it only prevented females laying their eggs. Ekol was ineffective. The best results of adult psylla control being Calypso 480 SC (biological efficacy was 100% and 81, 25%, first and second generation respectively). The population reduction of pear sucker when applying Insegar 25 WP was about 60% compared to the control. Sanmite 20 WP was effective only on the second generation (biological efficacy was 81, 43%). At the end of the growing season the difference between number of adults in the treated and non-treated plots was 83, 33%.

Eggs
The first kaolin treatment was applied before egg-laying. This precaution put behind and narrowed period of mass egg-laying. Until the first half of March the number of eggs in treated plot was low. Air temperature was low (about 5°C) and there was much precipitation during the second half of March, washing off the kaolin shortly after the application. Because no more applications were possible the numbers of pear sucker eggs on both plots had risen by the end of the month. Following two treatments of coleseed oil, the amount off eggs decreased again. Sanmite 20 WP was not effective against eggs of the first generation, but very good control on the first and second generations was achieved with Insegar 25 WP with ovicidal activity (biological efficacy was 97, 4% and 98, 54%) and Calypso 480 SC (biological efficacy was 73, 83% and 89, 84%). At the end of growing season the difference between number of eggs in the treated and non-treated plots was 88, 89%.

Nymphs
The highest efficacy was achieved by Calypso 480 SC (82, 29% and 91, 43%). Efficacy of Insegar differed between generations (83, 15% – 31, 40%). Sanmite 20 WP achieved good control in the first pear sucker generation (78, 79%). At the end of the growing season the difference between number of nymphs in treated and non-treated plots was 38, 1%.

During the vegetation period there were lower numbers of adults, nymphs and eggs of Cacopsylla pyri on the treated trees compared to the untreated control. However, the C. pyri population did not remain under a damaging level and controls had to be applied on the second generation as well. This observation might be explained by the high initial infestation level and the immigration of pear suckers from the untreated control plot.

In general, the population density was significantly decreased, yield was not decreased, and no honeydew or sooty moulds were observed on the fruits in the treated area. In the beating tray samples made in the end of growing season there were green lacewings (Chrysoperla carnea), seven spotted lady beetles (Coccinella septempunctata), bugs from Anthocoridae family and many species of spiders on all plots.

These treatments will be repeated in a second year. Reducing pear sucker populations at the end of growing season and presence of beneficial organisms and predators in the pear orchard are good indicators for the efficacy of the treatments.

In accordance with the results of Daniel and Wyss (2004) there was a high efficacy of kaolin in the trials conducted in Switzerland. Triple applications of kaolin (30 kg/1000 l) against over wintered adults before blossom reduced amount of adults as well as amount of nymphs consequently. Kaolin acts as repellent. Since it is not toxic to beneficial organisms this product is suitable for organic farming (Daniel and Wyss 2004). Similar results with kaolin treatments against Cacopsylla pyri are described by Pasqualiny et al. (2002), who
achieved a 99 to 100% reduction of eggs and nymphs by two applications in February / March. Glenn et al. (1999) and Coupard (2001) also showed a significant reduction of eggs and nymphs. According to Glenn et al. (1999) the adults get heavily coated with kaolin particles within 24 hours and appeared preoccupied by attempts to remove these particles from their body, unable to feed or to oviposit.

The coleseed oil Ekol is more effective in use with insecticides. In combination with spinosad it was recommended for registration against pear suckers in pear orchards (Daniel and Wyss 2004). The insecticides tested here were able to reduce pear sucker, but there are very few products for growers to choose from, in the Czech Republic, against psyllids. Neonicotinoides (Calypso 480 SC) may have a negative influence on beneficial organisms. In addition the repeated use of products with the same active ingredients or same mode of action may result in resistant psyllid populations (Falta 2008).

We tested new active ingredients; not only chemical (abamectin, spinosad), but botanical (azadirachtin) and physically acting products as well. It is advised to insert mechanical as well as biological (e.g. Anthocoris nemoralis introduction) methods of control to the integrated pest management against pear suckers.

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Geographical distribution and population dynamics of the European cherry fruit fly, Rhagoletis cerasi (Diptera: Tephritidae) in Greece

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Abstract: Although the European cherry fruit fly Rhagoletis cerasi L (Diptera: Tephritidae) poses a major threat to cherry production in Greece, there are only a few studies on its bioecology. Following extensive fruit sampling (during 2004 – 2008) we studied the geographical distribution of R. cerasi in several areas all over Greece. Infested fruit samples were collected in the areas of Macedonia (Thessaloniki, Katerini, Kozani, Halkidiki, Kavala), Thessaly (Trikala, Magnisia, Larissa, Karditsa), Peloponnesus (Ilea, Achaia), Thrace (Komotini), Crete island (Chania), North Aegean sea (Lesvos island). In addition to sweet cherries, R. cerasi pupae have been recovered from sour cherries (Thessaloniki), wild growing cherries (Prunus spp.) (Kozani, Trikala, Magnisia) and Prunus mahaleb (Trikala). Infestation levels varied greatly among sampling years, areas, and fruit species. Adults obtained from pupae collected from samples, from all the above areas except Crete, were examined for infections by the intracellular bacterium Wolbachia, which is known to exist in many European populations of R. cerasi. All populations were found to be singly infected by the same Wolbachia strain (wCer1). Pupal diapause termination and adult flight have been studied in a lowland – coastal (Kalà Nera Magnisias) and a highland area (Dafni Kozanis). Considerable differences exist both in diapause intensity and adult flying period between the two populations. The above data together with earlier data, collected in our laboratory, were used to construct population models for both areas.

Geographical distribution, Infestation levels, Wolbachia, Diapause termination, Adult flight
Population Dynamics and Damage Analysis of *Cetonia aurata* / *Potosia cuprea* in Croatian peach orchards

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Abstract: During some of the last fifteen years in the coastal part of Croatia it was observed that scarab beetles from the subfamily Cetoniinae caused damage to ripening peaches. With further analysis it was shown that these species were *Cetonia aurata* and *Potosia cuprea*. In 2007 we monitored their appearance and population dynamics, and we calculated the damage they caused. This was done in two locations in Zadar, Ravni kotari region. The Csalomon® VARb3k traps with baits consisting of 100 µl phenethyl alcohol + 100 µl methyl eugenol + 100 µl trans anethol were used. The total number of trapped beetles from the two locations was 569 *Cetonia aurata* and 200 *Potosia cuprea*. The damage percentage ranged from 0 % up to 7 %.

Key words: *Cetonia aurata*, *Potosia cuprea*, peaches, damages, population dynamics, attractant traps.

Introduction

During some of the last fifteen years in the coastal part of Croatia it was observed that scarab beetles from the subfamily Cetoniinae (Coleoptera, Scarabaeoidea, Cetoniidae) caused damage to ripening peaches. Several damage reports were received from the farmers. These beetles have started to make damage not only during flowering, but also during fruit ripening. It was noticed that they cause damage by biting the fruits and making lesions that way. Attacked fruits were not acceptable to the market, especially if mould diseases appeared on the damaged parts, which happened quite often.

The literature data about the damage they cause in Croatia are quite poor. They have been known as beetles that are attracted to flowers and to ripe and over ripe fruits (Endrödi, 1956; Miksic, 1965). They have been reported as a fig pest, but almost without any importance (Bakaric et al., 1989), and as a peach flower and fruit pest, but with a remark that the fruit wound had been caused by a bird or some other pest (Maceljski, 2002). Very recently they have been reported as a severe peach fruit pest (Baric et al., 2006).

These beetles cannot be efficiently suppressed with insecticides because they are very resistant. Most insecticides cannot be applied during flowering without affecting honeybees, bumblebees or other beneficials. Also, almost no insecticide can be applied during the ripening period because of the waiting period of the insecticide.

Materials and methods

Locations

The experiments were conducted during 2007 at two locations in Zadar, Ravni kotari area. The first location was a peach orchard near Skabrnja village, total area 1.5 ha, with the following peach and nectarine cultivars: Flavorcrest, Glohaven, Elegant Lady, Fayette, Stark
Redgold and Caldesi 2000. The rootstock is GF 677. The other location was also a peach orchard near Prkos village, total area 0.25 ha, some 5 km away, with the following cultivars: May Crest, Caldesi 2000, Glohaven, Suncrest and Maria Aurelia. The rootstock is also GF 677.

**Traps**

We used the Csalomon® VARb3k funnel traps, which are light blue, the optimal visual attractant cue for Cetonia, and contain a floral attractant bait for Cetonia/Potosia. These baits are composed of 100 µl phenethyl alcohol + 100 µl methyl eugenol + 100 µl trans anethol (Schmera et al., 2004; Tóth et al., 2005; Vuts et al., 2007). Photos of the trap can be viewed at www.julia-nki.hu/traps. The traps were set at a sunny place on the peach tree, at about 1.2 – 1.5 m height. We set four traps in Prkos and two in Skabrnja. The traps were set 15 m from each other, on different rows, on 8 May, and they were taken off on 30 August, which gave a monitoring period of 114 days. The baits were set on the trap setting date, and changed on 8 June and 6 July.

**Monitoring**

Traps were inspected twice weekly, when captured insects were collected and identified.

**Damage Analysis**

The damage percentage was calculated in the following way: we marked 8 trees of each cultivar, and checked 25 fruits per tree, 8 fruits from the first branch floor, 9 fruits from the second branch floor and again 8 fruits from the third branch floor, at the time that each cultivar ripened. Every fruit was visually inspected, and if we noticed damage typical of Cetonia / Potosia, we considered this as one damaged fruit. All together, we checked 8 trees and 200 fruits of each cultivar. The assessment dates were as follows: In Prkos on 26 May, 8 June, 2, 11 and 20 July, and 8 August, and in Skabrnja 26 May, 8 and 19 June, 2, 11, 20 and 31 July, and 8 August. We counted the damaged fruits and divided that number with the total number of inspected fruits to calculate the damage percentage.

**Results and Discussion**

**Population Dynamics**

In Prkos *Cetonia aurata* and *Potosia cuprea* were found on the first inspection date (11 May). The peak was reached on the 12 June, but it can be noticed that there are actually 3 peaks which follow the bait change. It can be seen that the bait attractance diminishes after 30 days. The last samples were trapped on 20 July (*C. aurata*) and 3 August (*P. cuprea*). All together we trapped 523 *C. aurata* and 189 *P. cuprea*. It seems that *C. aurata* ended its flight earlier than *P. cuprea*.

In Skabrnja *C. aurata* and *P. cuprea* appeared on the second inspection date, on 14 May. Here we can barely recognize three peaks of *C. aurata*, and almost no peaks of *P. cuprea*.. The catches were small compared to Prkos, but only 2 traps were used. The last samples were trapped on 11 July of both species. All together we trapped 46 *C. aurata* and 11 *P. cuprea*. If we took into the consideration that with 4 traps we caught 523 pcs. of *C. aurata* and 189 pcs. of *P. cuprea* in Prkos, and with 2 traps we caught 46 pcs. of *C. aurata* and 11 pcs of *P. cuprea* in Skabrnja, we could say that in Skabrnja there were less *Cetonia* and *Potosia* that in Prkos. The total number of trapped beetles from both locations was 569 *C. aurata* and 200 *P. cuprea*.
**Damage Analysis**

There was less damage in Prkos than in Skabrnja. One part of this result is due to only 2 traps in Skabrnja, compared to 4 traps in Prkos. Also, the orchard in Skabrnja is much bigger (1.5 ha) than the orchard in Prkos (0.25 ha). The results show the cultivar damage differences in each orchard. In Prkos, the earliest cultivar, May Crest was attacked the most (3%). Suncrest was a little bit less damaged (2%). There was no damage at all on nectarine Caldesi 2000 and peaches Glohaven and Maria Aurelia. The date of the damage assessment on May Crest and Suncrest when there was damage seen actually follows the catch peaks in the traps, so we can say that there obviously is a connection between the number and the dynamics of *Cetonia* and
In Skabrnja, the biggest damage was on Glohaven peach (7%), little bit less on Flavorcrest (5%), 4% on nectarines Caldesi 2000 and Stark Redgold and peach Elegant Lady, and 3% on Fayette. In this orchard there was damage on all cultivars. Here we cannot draw the parallel between the beetle dynamics and damage. When the highest peak was recorded (26 May), there was no damage observed. At the time of the second peak (22 June), it could be said that there is a parallel, since at that time we observed the 4% damage on Caldesi 2000 (19 June) and 5% damage on Flavorcrest (2 July). The most attacked cultivar was Glohaven. The nectarines were less attacked than some peaches.

In small orchards such as the one in Prkos (0.25 ha), with 4 traps we could control the damage. This is the reason why there was more damage in Skabrnja. Thus, in an orchard of 1 ha we would need about 15 traps to control the damage below the economical threshold.

Acknowledgements

We thank the County of Zadar, The Department of Agriculture, who financially supported the research and this article.

References

Spatial patterns and Sampling of predatory mites (Acari: Phytoseiidae) on apple orchards

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Abstract: The spatial distribution of the predatory mites (Acari phytoseiidae), was studied by applying Taylor’s power law and Iwao’s regression models. Studies were carried out during two consecutive growing seasons (2003 and 2004) in a two apple orchards (Cvs: Royal Gala and Golden Smoothee) of Northwest Portugal. The species present, there was a complex dominated by the generalist predators Euseius stipulatus (Athias-Henriot) and Kampimodromus aberrans (Oudemans) in Ponte de Lima and by Amblyseius andersoni (Chant) and E. stipulatus in Braga. The relationship between mean and variance was studied by Taylor’s power law and Iwao’s regression models. Both models showed good fit to the data (Taylor R²=97.3%, Iwao R² = 90.3%, p<0.001), concluding that the phytoseiid species has an aggregated distribution on vineyard fields. The spatial distribution of phytoseiids was aggregated, according the Taylor (b = 1.195 ± 0.021; t1,987 = 8.921; d.f. = 88; p <0.001) and Iwao (b = 1.652 ± 0.058; t1,987 = 11.292; d.f. = 88; p <0.001) coefficients. The Taylor’s regression coefficients were commons for both places and cultivars, which justifies a common sampling program for the complex species presents. The optimal sample size (leaves) for phytoseiids populations with fixed precision levels of 0.15, 0.20 and 0.25 where estimated with Taylor’s regression coefficients. The results showed that a smaller number of leaves are required for the detection of high phytoseiids densities and the required sample sizes, increased considerably with increased levels of precision. A binomial sampling procedure has been developed through the relationship between the proportion of leaves occupied and the men number of phytoseiids per leaf. The strong significant relationship between the estimated and observed proportion of occupied leaves (R² = 87.5%; d.f. = 89; F = 614.48; p <0.001), makes it possible to use a binomial or presence-absence sampling approach. Presence-absence sampling is an efficient method for crop management purposes because less time is needed to process the samples compared with a method where all phytoseiids are counted.

Phytoseiidae, Biocontrol, Sampling methods, Taylor power law, Integrated pest management
An inventory of tortricids (*Lepidoptera, Tortricidae*) in Swedish apple orchards as a basis for future management strategies

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**Abstract:** Over the last couple of years, growers, researchers, advisors and plant protection companies have noticed increasing problems with tortricids in Swedish apple orchards. Since the insecticide Gusathion (azinphosmethyl) has been banned (end of 2008; KemI 2008), a further increase of tortricid populations can be expected. In order to get a picture of species composition and population densities among the tortricids, an inventory of seven species, *Adoxophyes orana*, *Archips podana*, *Archips rosana*, *Cydia pomonella*, *Hedya nubiferana*, *Pandemis heparana* and *Spilonota ocellana* was made in 11 orchards in southern Sweden (Skåne) in 2008. Population densities were estimated by bud sampling (April 20-25), pheromone trapping (May 5-September 22) and assessment of fruit damage (September 9-12). In all orchards *A. podana* was the dominating species followed by *A. rosana* and *P. heparana*. Generally trap catches of *C. pomonella* were low, but flight activity was recorded over a longer period of time. Similar flight curves were observed for *P. heparana* and *S. ocellana*. Trap catches of *H. nubiferana* were very low at all sites. *A. orana* only occurred in one of the orchards and exhibited two peaks in flight activity, indicating that there were two generations. Infestation levels of tortricid larvae were low in bud samples, possibly due to sampling being done too early in the season. Average fruit damage was 5%, varying from 1.6 to 21%. The inventory will be the basis for development of future management strategies and forecasting tools.

**Key words:** Tortricids, pheromone traps, apple orchard, inventory, bud samples
Spread of European stone fruit yellows in Piedmont (northwestern Italy) and presence of Cacopsylla pruni Scopoli in plum and apricot orchards

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Abstract: In recent years, high percentages of declining plants showing symptoms ascribable to the European stone fruit yellows (ESFY) disease were recorded in plum and apricot orchards in Piedmont, north western Italy. Since 2006, visual inspections were carried out in dozens of orchards to assess the incidence of symptomatic plants in early spring (premature budbreaks) and late summer (yellowing and leafroll). Surveys with yellow sticky traps and beating tray were carried out from the beginning of March until the beginning of June to monitor the presence of Cacopsylla pruni and other possible vectors in the orchards and in the surroundings on wild Prunus species. The presence of “Candidatus Phytoplasma prunorum” in plum and apricot trees as well as in the insects was ascertained by PCR and RFLP analyses. The very low C. pruni population density recorded and the presence of “Ca. Phytoplasma prunorum” in recently planted orchards (1 year old) suggest an early infection possibly occurring in the nurseries.

Key words: European stone fruit yellows, Cacopsylla pruni, plum, apricot.

Introduction

European stone fruit yellows (ESFY) is one of the most important fruit tree diseases inducing serious damage in cultivated Prunus species in Europe. It is caused by a phytoplasma, “Candidatus Phytoplasma prunorum” belonging to the 16S rX phylogenetic group (Seemüller and Schneider, 2004). The typical symptoms are early foliation at the end of the winter, yellows and leaf roll in summer, dieback and a more or less rapid decline. Its prevalence has increased in all Europe in recent years especially after the introduction of Japanese plums. Carraro et al. (1998; 2001) identified the psyllid Cacopsylla pruni Scopoli as the vector of this phytoplasma in Italy, while Jarausch et al. (2001), Laviña et al. (2004) and Fialova et al. (2004) confirmed the vectoring ability of this species in France, Spain and Czech Republic respectively. Also in Piedmont, northwestern Italy, high percentages of declining plants showing symptoms ascribable to the ESFY disease were recorded in Japanese plum and apricot orchards in the last years. For this reason, since 2006, preliminary surveys to assess the incidence of the disease through the region and the presence of the insect vectors have been carried out.

Materials and methods

Field surveys
The surveys were carried out since 2006 in differently aged stone fruit orchards located in the province of Cuneo, Piedmont, north western Italy. Visual inspections were performed in
Phytoplasma detection and identification

Plant DNA was isolated from phloem tissue from field-collected branch samples using the Pure Link TM Plant Total DNA Purification kit (Invitrogen, Carlsbad, USA). Approximately 1 g of fresh plant material was used for each plant. Insect DNA was extracted following a protocol adapted from Marzachi et al. (1998) and already applied for psyllids (Tedeschi et al., 2002). Insect and plant DNAs were amplified with a nested polymerase chain reaction (PCR) firstly with phytoplasma universal primer pair P1/P7 (Schneider et al., 1995) and then with the AP-group specific primer pair fO1/rO1 (Lorenz et al., 1995) after a 1:40 dilution. Reaction and cycling conditions were as described in the original papers. PCR amplification products were analysed by 1% agarose gel electrophoresis, stained with ethidium bromide, and visualised on a U.V. transilluminator. Moreover, the amplicons were restricted with the endonuclease RsaI which allows the specific identification of the “Ca. Phytoplasma prunorum” in RFLP analysis. Seven microlitres of the amplicon were digested with 5 U of RsaI at 37°C overnight.

Results

Spread of the disease

Different symptoms were observed on plum and apricot plants. We distinguished between suspected symptoms such as chlorosis without leaf roll or non-chlorotic leaf roll and specific symptoms such as early foliation in the late winter period and chlorotic leaf roll in the summer. Ninety percent of the orchards investigated had at least one symptomatic plant (Tab. 1), and 33% of the tested plants were positive to phytoplasmas belonging to the 16SrX group. RFLP analyses confirmed the presence of “Ca. Phytoplasma prunorum”.

A high correlation was observed between specific symptoms and phytoplasma detection by PCR, but also, 8.6% of plum tested plants and 55.5% of apricot tested plants with suspected symptoms were infected. In plum orchards the presence of suspected symptoms is normally more common in young plants while the incidence of the disease evaluated by means of specific symptoms remains quite constant during the years. Particularly interesting is the presence of typical symptoms in 1-year-old plants, supported also by phytoplasma detection by PCR.
Table 1. Presence of suspected (e.g. chlorosis without leaf roll or non chlorotic leaf roll) and specific symptoms (e.g. early foliation and chlorotic leaf roll) in differently aged plum and apricot orchards.

<table>
<thead>
<tr>
<th>Plant age (years)</th>
<th>Monitored orchards n°.</th>
<th>Monitored plants n°.</th>
<th>Suspected n°.</th>
<th>%</th>
<th>Specific n°.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plum</td>
<td>1</td>
<td>8</td>
<td>469</td>
<td>111</td>
<td>23.7</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2-3</td>
<td>18</td>
<td>4779</td>
<td>218</td>
<td>4.6</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>4-5</td>
<td>4</td>
<td>184</td>
<td>69</td>
<td>28.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>6-7</td>
<td>5</td>
<td>1771</td>
<td>104</td>
<td>5.9</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>&gt;8</td>
<td>9</td>
<td>2881</td>
<td>204</td>
<td>7.1</td>
<td>198</td>
</tr>
<tr>
<td>Apricot</td>
<td>4-5</td>
<td>1</td>
<td>473</td>
<td>1</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6-7</td>
<td>3</td>
<td>1984</td>
<td>4</td>
<td>0.2</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>&gt;8</td>
<td>3</td>
<td>1772</td>
<td>49</td>
<td>2.8</td>
<td>76</td>
</tr>
</tbody>
</table>

Insect vectors

During the three years, the number of *C. pruni* collected with yellow sticky traps on apricot and plum was always very low (Fig. 1). Five *C. pruni* specimens collected with yellow sticky traps and two specimens collected with the beating tray method on cultivated plum plants and analysed by PCR were negative for “*Ca. Phytoplasma prunorum*”. Other psyllid species, *C. melanoneura* (Förster), *C. pulchella* (Löw) and *Trioza alacris* Flor were collected in the orchards with higher densities, but they always resulted negative for the phytoplasma. Surveys on wild *Prunus* species enabled the collection of 57 specimens of *C. pruni* on *P. spinosa* L. and “*Ca. Phytoplasma prunorum*” was detected in 3 of them.

Discussion

The present, preliminary research pointed out the widespread presence of “*Ca. Phytoplasma prunorum*” in Japanese plum and apricot orchards in Piedmont, a region where the plum cultivation is consistently increasing. After a long tradition of only European cultivars, new Japanese cultivars were introduced in the nineties and some of them, e.g. Angeleno, had a great success because of the belated harvest period and an extremely good keeping quality. Our results demonstrated that the spread of the disease is not, at this moment, attributable to the known vector *C. pruni*, because of its very low population density recorded. Thus specific control strategies against this psyllid are not necessary now.
Moreover, the detection of “Ca. Phytoplasma prunorum” in very young plants suggests the role of propagation material in the diffusion of ESFY, and more attention on this aspect is required in the future. But the psyllid C. pruni should not be underestimated: the spread of plum cultivation may stimulate the population density of C. pruni, that might became worrying considering also the relevant inoculum source of the phytoplasma in the region. At the same time the possibility of other insect vectors should not be excluded. Further attention should be focused on P. spinosa, which proved to be the favourite host plant (Carraro et al., 2002), considering also the location of it’s hedges that are often in the surroundings of cultivated stone fruit orchards.

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References

Observations of *Rhagoletis cingulata*, an invasive species from North America, on cherry in Germany

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Abstract. Since 2003, the Eastern cherry fruit fly, *Rhagoletis cingulata* (Loew), an introduced Tephritid fly from North America, has been observed in Germany in increasing abundance. We present an overview of the increase in distribution and discuss the consequences for management programs for sour cherry (*Prunus cerasus*). Following the identification of a single female in a malaise trap in Rhineland-Palatinate (central Rhine region) in 1999, a trapping program was conducted near the original host site and in several cherry growing regions from 2002 onward. In 2003, a few specimens of *R. cingulata* were reported on yellow traps in cherry orchards in the Rhineland-Palatinate area. Since 2004, the number of individuals found in Rhineland-Palatinate cherry growing regions increased considerably and the species was also found in other Federal states. At the present time, the species has been collected from nearly all cherry-growing regions of Germany. In Germany, *R. cingulata* is emerging 3-4 weeks later than does the European cherry fruit fly, *R. cerasi*, and mainly attacks sour cherries. In some years and locations, the Eastern cherry fruit fly has caused more than 20 % damage in sour cherries, whereas infestation due to *R. cerasi* in sour cherries usually is of low importance. The species status has been confirmed by Dr. Allen Norrbom, Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture, USA.

Keywords: *Rhagoletis cingulata*, *Rhagoletis cerasi*, phenology, infestation, host plants

Introduction

The Eastern cherry fruit fly *Rhagoletis cingulata* (Loew) is a severe pest of cherries, originating from North America. After the record of a single female in 1999 in the middle Rhine valley (Merz & Niehus 2001), a monitoring program was conducted with yellow traps and fruit samples. Since 2003 the species has been observed in Germany in increasing abundance (Lampe & Krauthausen 2005, Lampe et al. 2006, Vogt 2007a & b, Maring &Kirchner 2008) and thus is a further threat for cherry growing beside the native species, the European cherry fruit fly *R. cerasi*.

Material & Methods

Monitoring was done with yellow traps, type Rebell® in all cherry growing regions of Germany. Fruit samples were taken in Rhineland-Palatinate in an extensive production area of sour and sweet cherries between Mainz and Bingen in the Rhine valley. Cherries were put in boxes with a grid bottom, so that larvae could leave them for pupation. Pupae were collected and stored for diapause at 3 to 5° C for 5 to 7 months. For post-diapause development, pupae were brought to a climate chamber (25 ± 0.5°C / 18 ± 0.5°C, RH 65 ± 5 %, photoperiod light:dark 16:8 h, 4 to 6 klx). The same procedure was followed for fruit samples from *Prunus mahaleb* and *Prunus serotina*. Emerged adults were determined to species (Carroll et al. 2002). We also checked the wing pattern as it is known to be variable. Characteristics used for
the discrimination between *R. cerasi* and *R. cingulata* larvae and pupae were the mandibles of the cephalopharyngeal skeleton (Carroll et al. 2004) and the color of the puparium. The puparium of *R. cingulata* is brown, that of *R. cerasi* is beige. Individuals of *R. cingulata* were sent to the Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture, USA, for species confirmation.

Results

**Monitoring with yellow traps**

*R. cingulata* has been detected in the main cherry growing regions of Germany: in Baden-Württemberg, Brandenburg, Bavaria, Hesse, Hamburg, Lower Saxony, North Rhine-Westphalia, Rhineland-Palatinate, Saxony, Saxony-Anhalt and Thuringia, attaining high abundances in some of these regions. It occurs mainly in sour cherry (*Prunus cerasus*) orchards, and in areas where *Prunus mahaleb* and *Prunus serotina*, its main native host, are present.

The flight peak of the species occurs 3–4 weeks later than that of the European cherry fruit fly, *R. cerasi* (Fig. 1). In cherry orchards, late varieties, especially sour cherries like the economic important variety “Schattenmorellen” are most threatened.

![Graph showing flight activity of cherry fruit flies in 2007 in a sour cherry orchard without insecticide treatment (Heidesheim F4)](image_url)

Fig. 1. Flight activity of cherry fruit flies in 2007 in a sour cherry orchard without insecticide treatment (Heidesheim F4)

**Fruit samples**

2004 was the first year in which we provided evidence for *R. cingulata* infestation in sour cherries in the Mainz-Bingen area. More than 150 collected pupae had the typical brown colour and only *R. cingulata* emerged from these pupae in the following year after diapause. In 2005 the infestation level in sour cherries from four localities in Rhineland-Palatinate without insecticide treatment was between 2 and 11 % with a proportion of 7.4 % *R. cerasi* and 92.6 % *R. cingulata* on average (Table 1). In 2006, fruit samples of tart cherries from four untreated cherry orchards revealed infestation levels due to *R. cingulata* up to 21 %. One treatment with dimethoate (which is no longer registered for cherry fruit fly control in Germany) reduced the infestation level from 21 to 2.4 %. Fruit samples from *P. mahaleb* in
the Mainz-Bingen area from 2008 were infested by both fruit fly species to a level of around 10 % (82.5 % *R. cerasi*, 17.5 % *R. cingulata*) in 2008. Up to 6 % of *P. serotina* fruits from forests in the Upper Rhine Valley near Mannheim were infested with *R. cingulata* in 2008. A low *R. cingulata* attack of 0.6 % was detected in sweet cherries, *Prunus avium* (untreated single tree at a field path) in 2008.

Table 1. Infestation of sour cherries by *R. cerasi* and *R. cingulata* at four localities in the Mainz-Bingen area, 2005

<table>
<thead>
<tr>
<th>Locality</th>
<th>Infestation %</th>
<th>No. of pupae</th>
<th>Proportion %</th>
<th>R. Cerasi</th>
<th>R. cingulata</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Heidesheim, Heuweg</td>
<td>3.5</td>
<td>115</td>
<td>12.2</td>
<td>87.8</td>
<td></td>
</tr>
<tr>
<td>2 Wackernheim, Rabenkof</td>
<td>1.7</td>
<td>66</td>
<td>12.1</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td>3 Heidesheim, Sandgrube</td>
<td>11.4</td>
<td>211</td>
<td>5.2</td>
<td>94.8</td>
<td></td>
</tr>
<tr>
<td>4 Wackernheim</td>
<td>2.2</td>
<td>42</td>
<td>0.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>434</td>
<td>Mean 7.4</td>
<td>Mean 92.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. *R. cingulata* wing patterns; left: type A (example from Dossenheim, 2004); right: type B (example from Rheinhessen 2006) (Photos: JKI Dossenheim)

Table 2. Proportion of *R. cingulata* adults found in Germany with wing patterns type A or B (see text for explanation)

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dossenheim 2007-2008</td>
<td>8</td>
</tr>
<tr>
<td>Dossenheim 2004-2006</td>
<td>15</td>
</tr>
<tr>
<td>Rheinhessen 2004-2006</td>
<td>119</td>
</tr>
<tr>
<td>Bayern 2006</td>
<td>1</td>
</tr>
<tr>
<td>BW, Zavelstein 2006</td>
<td>0</td>
</tr>
<tr>
<td>Thüringen 2005</td>
<td>9</td>
</tr>
<tr>
<td>Brandenburg 2006</td>
<td>21</td>
</tr>
<tr>
<td>sum</td>
<td>173</td>
</tr>
<tr>
<td>percentage</td>
<td>74.2</td>
</tr>
</tbody>
</table>

**Wing pattern**

In 74% of the collected individuals of *R. cingulata* from Germany the anterior arm of the apical band forms an isolated spot (=type A) and in 26% the anterior arm of the apical band is more or less complete and connected to the posterior apical band (= type B) (Fig. 2 & Table
2). Both wing patterns occurred in all regions.

Discussion

*R. cingulata* is established in Germany. Date and method of introduction are not known. The native host plant, *P. serotina*, introduced to Europe since the 17th century and now widely distributed in hedges, windbreaks and forests, is probably involved, as *R. cingulata* has been detected in *P. serotina* fruits from a forest by the first author and by Holz from windbreak plants in Brandenburg (personal communication 2007). Genetic studies might help to elucidate these questions.

As a consequence, cherry fruit fly control is now necessary in the economic important tart cherry variety „Schattenmorellen“, which was not the case before. Late sweet cherry varieties are also threatened. The rise in abundance is probably correlated with changes in insecticide control of cherry fruit fly (no highly effective organophosphates are allowed anymore) and with the increase of abandoned tart cherry orchards, which serve as reservoir. Furthermore, *P. mahaleb*, which is used as rootstock for sour cherries, often imbrutes in these areas and thus is serving as further host plant.

With regard to the control of cherry fruit fly, there are severe problems, as in Germany, no insecticide is registered for chemical control of cherry fruit fly. Only exceptional permits for the use of Mospilan (a.i. acetamiprid) have been given. The application dates have to be carefully adapted as *R. cingulata* prolongs the infestation period. Actually, no alternatives exist to chemical CFF control, though research is ongoing e.g. bait sprays.

Acknowledgements

We thank colleagues from the plant protection service for their cooperation in the trapping program: Dr. K. Geipel (Freising, Bavaria), U. Holz (Frankfurt/Oder, Brandenburg), E. Maring (Erfurt, Thuringia), D. Mohr & Dr. G. Palm (Jork, Lower Saxony), G. Sartorius (Friedberg, Hesse), Dr. H. Saucke (Wittenhausen, Hesse), Dr. A. Trapp (Dresden, Saxony). We thank Dr. Allen Norrbom, US Department of Agriculture, USA, for the determination of the species.

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Vogt H. 2007a: Short information about an invasive *Rhagoletis* species in Germany. Editors:
Selectivity of phytosanitary products used on citrus orchards to *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae)

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Abstract: The effect of some phytosanitary products used on citrus orchards on *Chrysoperla externa* was evaluated. The maximum dosages of thiametoxan, imidacloprid, milbemectin, pyriproxyfen and spirodiclofen were sprayed on eggs of this chrysopid in a Potter tower, with toxicity evaluations on this and subsequent development phases. The experiment was conducted at 25±2°C, 70±10% RH and 14-hour photophase in a complete randomized design with six treatments and thirty replicates. Survivorship of contaminated eggs, larvae, pupae and adults originating from contaminated eggs was evaluated. Additionally, number and viability of F1 generation eggs were evaluated. Imidacloprid and spirodiclofen were statistically different from control with egg viability of 76.7% for both products, with 96.7% for control. As for thiametoxan, milbemectin and pyriproxyfen, they did not influence egg survival rate, with 93.3%, 80.0% e 80.0%, respectively. Spirodiclofen received the slightly noxious (class 2) classification.

Key words: lacewing, insecticide, toxicity.

Introduction

Brazilian citriculture significantly contributes to socio-economic growth, promoting the generation of indirect and direct jobs, besides its importance as an export product. In this context, this activity generates annually US$ 1.0 billion in foreign currency and approximately US$ 5.0 billion of GNP (Abecitrus, 2008). Citrus orchards are distributed over approximately 700,000 hectares, which favours the incidence of numerous arthropod pests, which need to be controlled chemically. Neves et al. (2002) reported that a considerable amount of money is spent with phytosanitary products in this crop, which surpasses expense for several crops like coffee, corn, soybean and sugarcane.

Large-scale use of insecticides can reach not only the target pests but beneficial organisms as well, e.g. chrysopids, which are susceptible to several products used in pest control. Chrysopids are important predators in larval stages and are found in several crops of economic importance, including cotton, citrus, corn, soybean, alfalfa, tobacco, grape, apple, rubber and others. They can feed on neonate caterpillars, aphids, scales, mites and several small arthropods with easily perforated teguments (Souza & Carvalho, 2002). Thus, several phytosanitary products used on citrus orchards were evaluated for their activity on eggs and subsequent stages of *Chrysoperla externa*.

Material and Methods

The experiment was run according to IOBC’s methodology (Hassan et al. 1994; Hassan & Degrande 1996; IOBC/WPRS 1992). The evaluated products with dosages expressed in g or mL of a.i. L⁻¹ of water were: Actara 250WG (thiametoxan – 0.05), Confidor 700WG (imidacloprid – 0.07), MilbekNock (milbemectin – 0.008), Tiger 100CE (pyriproxyfen –
Twenty-four d old eggs obtained from an F3 generation of laboratory reared *C. externa* were used. The eggs were placed in Petri dishes and sprayed using a Potter tower calibrated to deliver 1.5±0.5 µL/cm² at 15 lib/pol² (unfamiliar unit!). The dishes were left under shade for two hours for evaporation of excess water on eggs, and individually placed in glass tubes for a total of 30 eggs per treatment. Eggs were kept in climatic chambers at 25±2ºC, 70±10% RH and 12-hour photophase. Evaluations started two days after the application of the products, from larval emergence, through pupal formation to adult emergence. Five chrysopid pairs were formed after emergence on each treatment and kept in PVC cylindrical cages internally covered by filter paper and closed on the top with plastic film. Larvae were fed *ad libitum* with *Anagasta kuehniella* eggs and adults with brewer’s yeast and honey (1:1). Eggs were collected from the F1 generation every other day and counted for four consecutive weeks. Fertility was determined in 96 eggs per treatment, placed individually in plastic trays, covered by plastic film and kept under the same climatic conditions. The experiment was performed in a completely randomized design with six treatments and thirty replicates with fertility evaluations and survivorship of larvae and pupae. Data obtained from fertility evaluations were submitted to the Z test at 5% probability (Triola, 2005).

For the larval period, data were subjected to the Kaplan-Meier (Kaplan & Meier, 1958) estimator for the analysis of survivorship. Fertility data were subjected to analysis of variance and means compared by the Tukey’s test ($P \geq 0.05$).

**Results and Discussion**

A survival rate of 76.7% for both imidacloprid and spirodiclofen was observed compared to 96.7% for the control. Rocha (2008) reported fertility of 77.5% in *C. externa* eggs treated with these same compounds, as compared to 92.5% in controls, indicating their toxicity for chrysopid eggs. Some compounds can show ovicide action, interfering with embryonic respiration (Riedl *et al.*, 1995). There were no significant differences among thiametoxan, milbemectin and pyriproxyfen as compared to control, with fertility reaching 93.3%; 80.0% and 80.0%, respectively. There was evidence of some tolerance of *C. externa* eggs to these products, which was also reported by Carvalho *et al.* (2002), Godoy *et al.* (2004), Bueno & Freitas (2004) and Ferreira *et al.* (2006). The lower toxicity of the tested compounds is possibly associated with the corion which may prevent penetration of some products, thereby avoiding contamination of the developing embryo.

In relation to the survivorship of subsequent development phases to this insect a significant difference of the tested products on third instar larvae and pupae was detected, but not on first and second instar larvae, which had similar tegument alterations for all tested products and control, resulting in no interference on insect biology and 100% probability of survivorship (Table 1). No interference of phytossanitary products on subsequent stages after the egg stage is perhaps associated with their low residue level on corions at hatching time, reducing contamination of neonate larvae, but Godoy *et al.* (2004) found deltametrin caused 50% mortality of neonate larvae and interference in subsequent stages, with 62% mortality on the first instar.

Significant differences were detected in survivorship of third-instar larvae of *C. externa* originating from treated eggs, ranging from 68.2 to 95.0 %. Significantly higher survivorship was observed in treatments with thiametoxan and spirodiclofen, relative to all others. Only spirodiclofen lasted 4 days for median time change of instar, with all other ones with not more than 3 days (Table 1). Duration of these stages originated from treated eggs suggest that there was no “latent effect” as reported by Croft (1990) as the effect expressed in subsequent stages to the one when the organism was exposed to a chemical product.
There was no significant difference in median pupal longevity, corresponding to 12 days, but Rocha (2008) reported significant differences in pupal stage duration in organisms originated from previously treated stages. These differences may be possibly associated to other variables like products of distinct chemical group, mode of action, geographic origin of the insect population under study, dosage, application methods, etc.

Table 1. Median time (MT) in days, and survivorship probability (S) in %, of *Chrysoperla externa* first, second and third instar larvae, and pupae.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1st MT</th>
<th>1st S</th>
<th>2nd MT</th>
<th>2nd S</th>
<th>3rd MT</th>
<th>3rd S</th>
<th>Pupa MT</th>
<th>Pupa S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiametoxan</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>92.31</td>
<td>12</td>
<td>91.30</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>68.20</td>
<td>12</td>
<td>68.20</td>
</tr>
<tr>
<td>Milbemectin</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>68.20</td>
<td>12</td>
<td>72.73</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>4</td>
<td>95.00</td>
<td>12</td>
<td>56.25</td>
</tr>
<tr>
<td>Spirodiclofen</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>4</td>
<td>95.00</td>
<td>12</td>
<td>56.25</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>100.0</td>
<td>3</td>
<td>82.50</td>
<td>12</td>
<td>83.90</td>
</tr>
<tr>
<td>Log – Rank (P-value)</td>
<td>0.9996</td>
<td>0.9999</td>
<td>0.0017</td>
<td>0.2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although no differences have been detected among treatments in the pupal stage, spirodiclofen, pyriproxyfen and imidacloprid showed low probability of survival (56.25%; 68.18% and 68.20%, respectively, Table 1).

The influence of the compounds on female fecundity of the F1 generation revealed a significant difference for spirodiclofen, 34.69 eggs/female as compared to 49.11 eggs/female for control. The remaining products showed from 40.94 to 52.59 eggs/female on average, during the four weeks of evaluations. Reduction in number of eggs layed by females originating from eggs treated with spirodiclofen may be associated with the mode of action. This compound is part of the cetoenol group, a molecule derived from tetronic and tetromic acids which inhibit lipid synthesis with consequent interference in egg production and, in some cases, on their oviposition capacity. For egg survival rate, no significant difference was found between spirodiclofen and control (Table 2).

Table 2. Mean1 number (± SE) and F1 *Chrysoperla externa* fertility (%).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of eggs</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiametoxan</td>
<td>40.94 ± 3.09 ab</td>
<td>89.50 ± 0.88 c</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>46.83 ± 2.86 ab</td>
<td>94.33 ± 0.88 b</td>
</tr>
<tr>
<td>Milbemectin</td>
<td>51.65 ± 2.52 a</td>
<td>94.42 ± 0.88 b</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>52.59 ± 2.67 a</td>
<td>93.33 ± 0.88 b</td>
</tr>
<tr>
<td>Spirodiclofen</td>
<td>34.69 ± 3.78 b</td>
<td>95.92 ± 0.88 ab</td>
</tr>
<tr>
<td>Testemunha</td>
<td>49.11 ± 2.86 a</td>
<td>99.42 ± 0.88 a</td>
</tr>
<tr>
<td>CV (%)</td>
<td>55.32</td>
<td>10.24</td>
</tr>
</tbody>
</table>

1Means followed by the same letter are not statistically different by Tukey’s test at 5%.

With the results of *C. externa* mortality during its development plus fecundity and fertility data obtained, the total effect (E) of the tested pesticides for the egg stage of this predator was calculated. Following the IOBC methodology, only spirodiclofen was included in toxicological class 2 (= slightly noxious) in relation to the predator; all other products were
included in toxicological class 1 (= non toxic) (Table 3).

Table 3. Percent of *Chrysoperla externa* egg mortality when treated with insecticides, number of eggs/day/female, fertility (%), total effect (E).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Infertile eggs</th>
<th>Dead neonate larvae</th>
<th>Dead larvae</th>
<th>Dead pupae</th>
<th>M%¹</th>
<th>Mc%²</th>
<th>R1³</th>
<th>R2%⁴</th>
<th>E%⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiametoxan</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>23.33</td>
<td>0.0</td>
<td>17.55</td>
<td>89.50</td>
<td>24.4</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>26.67</td>
<td>4.36</td>
<td>20.07</td>
<td>94.33</td>
<td>12.8</td>
</tr>
<tr>
<td>Milbemectin</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>30.00</td>
<td>8.70</td>
<td>22.13</td>
<td>94.42</td>
<td>8.1</td>
</tr>
<tr>
<td>Pyriproxyfen</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>26.67</td>
<td>4.36</td>
<td>22.54</td>
<td>93.33</td>
<td>2.9</td>
</tr>
<tr>
<td>Spirodiclofen</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>46.67</td>
<td>3044</td>
<td>15.26</td>
<td>95.92</td>
<td>51.3</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>23.33</td>
<td>-</td>
<td>20.85</td>
<td>99.42</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Cumulative mortality (%).
²Mortality (%) corrected by Abbott (1925).
³Number of eggs/day/female.
⁴Egg fertility (%).
⁵Total effect (E) (%) of treatments.
*IOBC Class of Toxicity, class 1 non toxic (E%< 30%) and class 2 = slight noxious (30 ≤ E% ≤ 79%) To what does this refer?

**Conclusion**

Imidacloprid and spirodiclofen were the most toxic to *C. externa* eggs. Thiametoxan, imidacloprid, milbemectin, pyriproxyfen and spirodiclofen did not influence the subsequent stages after the application to eggs. Spirodiclofen caused fecundity reduction in adults of the F1 generation. Thiametoxan, imidacloprid, milbemectin and pyriproxyfen reduced fertility in the F1 female generation.

**Acknowledgements**

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First record of the parasitoid *Copidosoma varicorne* (Nees) (Hymenoptera: Encyrtidae) in Greece

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Extended abstract

Biological control has been a fundamental element of the Integrated Pest Management (IPM) concept since its initial definition more than 30 years ago. Region specific and naturally occurring biological control agents play a significant role in agro-ecosystems. The objective of this study was to record and observe the impact of native beneficial species on the overwintering population of the peach twig borer *Anarsia lineatella* Zeller (Lepidoptera: Gelechiidae) in IPM orchards. In previous studies we reported the presence of numerous beneficial arthropods, belonging to different families observed in overwintering sites (hibernacula) of *A. lineatella* (Damos and Savopoulou-Soultani 2008a). Substantial mortality because of parasitic wasps belonging to parasitoids of the family Braconidae was also reported (Damos and Savopoulou-Soultani 2008b). In this study, we report the mortality of *A. lineatella* overwintering larvae caused by the endoparasitoid *Copidosoma varicorne* (Hymenoptera: Encyrtidae).

The faunistic survey was conducted in two important regions in central Macedonia of northern Greece (Veria 40.32°N-0.22.18°E and Velvendo 40.16°N-0.22.04°E). Hibernacula of overwintering larvae were collected randomly from conventional and IPM peach orchards. All collected material was transferred to the Laboratory of Applied Zoology and Parasitology of the Aristotle University of Thessaloniki. Overwintering larvae of *A. lineatella* were placed at constant laboratory conditions for development (20°C, 16:8h L:D and 65±5%RH). Individuals of *C. varicorne* were reared from overwintering *A. lineatella* larvae during 2005, 2006 and 2007. A number of dry samples were sent to the Natural History Museum of London for a confirmative taxonomic identification. This is the first record of *C. varicorne* in Greece.

Hymenoptera is one of the richest in species groups in the Palaearctic region (Werner 2001, Gahari et al. 2006), while Copidosomatine encyrtids are well known for their unique polyembryonic development and larval soldier caste in which a single zygote generates multiple embryos by clonal proliferation (Zhurov et al. 2004). Comprehensive classification of Encyrtidae (Triapitzin, 1989) placed *Copidosoma* in the tribe Copidosomatini, subtribe Copidosomatina, together with *Paralitomastix* Mercet 1921 and *Copidosomopsis* Girault 1915 (Guerrieri and Noyes 2005).

Species of the genus *Copidosoma* Ratzeburg 1844 have been recorded as parasites on more than 20 Lepidoptera families, including members of the Gelechiidae and Tortricidae (Daane et al. 1993, Guerrieri and Noyes 2005). Encyrtidae species are considered as important parasitoids and are generally widespread, including species that have the potential to be used as biocontrol agents of lepidopteran pests (Guerrieri and Noyes 2005). In addition, species recorded as *Paralitomastix varicornis* (Mercet 1921) or *Encyrtus varicornis* Nees by original designation are synonyms with *Copidosoma* (Kazmi and Hayat 1998, Guerrieri and Noyes 2005), while on a recent detailed revision of the European species of Encyrtidae by Guerrieri and Noyes (2005), the species *C. varicornis* is also synonymous with *C. varicorne*.
which is the valid name. The species has also been recorded as *P. varicornis* in Northern Italy as a cause of mortality in overwintering *A. lineatella* larvae (Molinari et al. 2005), while in the USA the Encyrtidae *Paralitomastix pyralidis* (Ashmead) has been recorded as parasitizing overwintering larvae of *A. lineatella* (Daane et al. 1993).

Overwintering larval parasitization was especially high during the winter of the year 2006-2007. Moreover, although mortality of *A. lineatella* larvae due to *C. varicorne* parasitization was relatively low (5-15%), the species was present on samples collected from all different IPM peach orchards in northern Greece. This fact indicates a stable presence of *C. varicorne* in peach orchards of northern Greece during the last years. The identification and evaluation of local natural beneficial species under field conditions should be of special interest, as part of an overall IPM program.

**Key words:** *Copidosoma varicorne*, *Paralitomastix varicornis*, *Anarsia lineatella*, *Biological control*, *IPM*

**References**


Behaviour and biological control of two-spotted spider mite (*Tetranychus urticae*) in floricane red raspberry plantations

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**Abstract:** The biology, behaviour and reciprocal relationships of *Tetranychus urticae*, *Neotetranychus rubi* and the phytoseiid mite *Amblyseius andersoni* were investigated from 1999 to 2007 on floricane red raspberry in Trentino, Northern Italy. From 2005 to 2007, in a plantation in Mocheni’s Valley the efficiency against two-spotted spider mite of an *A. andersoni* local strain and the commercially available predators *Amblyseius californicus* and *Phytoseiulus persimilis* was also evaluated. Two introduction rates (26 and 52 individuals/m, equivalent to 10.4 and 20.8 individuals/m²) at different times of release were compared for these two last predators. *A. californicus* releases, applied before the middle of June, were more effective than late releases in every year, in comparison with check plots (no release). The best control result was recorded where the highest dose was introduced. However, *A. californicus* didn’t perform as well as *A. andersoni* in the reintroduction plot. In our trials, *P. persimilis* established in the crop with very small populations, probably indicating important ecological requirements (prey density, release rate, climate under polyethylene rain covers, etc.) for its establishment. The information we collected was used to produce a two-spotted spider mite management recommendation scheme for Trentino’s raspberry growers.

**Key words:** *Tetranychus urticae*, *Neotetranychus rubi*, floricane red raspberry, behaviour, biological control.

**Introduction**

Tetranychid mites, *Neotetranychus rubi* (raspberry mite) and particularly *Tetranychus urticae* (two spotted spider mite, TSSM) represent key pests on floricane red raspberry cultivated in Trentino, Northern Italy. Their natural control relies on many indigenous predators, among which the phytoseiid mite *Amblyseius andersoni* is the most important and effective.

A deeper knowledge of how this mite system works in our raspberry growing conditions is crucial to correctly manage TSSM, integrating as well as possible the available preventative and curative control measures, to recommend to the growers a more sustainable approach. With this aim, we carried out investigations in Trentino from 1999 to 2007.

**Material and methods**

Investigations were conducted in two plantations, both cultivated with floricane fruiting red raspberry cv.Tulameen, and located at Canzolino (500 m a.s.l) and S.Orsola – Mocheni’s Valley (1150 m. a.s.l).

The efficiency against TSSM of the predatory phytoseiids *A. californicus* and *P. persimilis* (supplied by Koppert Italia S.r.l) and of a local strain of *A. andersoni*, was evaluated from 2005 to 2007 in non-replicated blocks, 8-16 m long, set up in the S.Orsola plantation. We compared two introduction rates, 26 and 52 individuals/m of row, equivalent to 10.4 and 20.8 individuals/m² of the commercially available predators. *Amblyseius andersoni* was introduced only in 2005 spring (about 12 individuals/m²) by means of occupied cv. Heritage
primocanes collected from another plantation in Mocheni’s Valley and hung on the fruiting canes of the selected block. Pest and predatory mite populations were recorded both on fruiting and on vegetative canes by means of visual inspection (under binocular microscope) of 1 terminal leaflet/2 m of row, at approximately 7-15 d intervals. The density of the TSSM population was determined by counting the number of motile stages, or estimated according to a ranking system (Guignard, 1968 unpub.), while the phytoseiids were determined by counting the number of mites per leaf. The percentage of leaves occupied by the mites was calculated. Cumulative mite-days/leaf index was also calculated to express the load of TSSM in the whole season.

The overwintering behaviour of the mites was investigated in both sites inspecting by visual control or floatation system, samples of 30-40 cm basal portion of fruiting canes and old dead leaves collected in winter. In S.Osola plantation, we also inspected (by visual control with a 10x hand magnifier) spontaneous weeds collected from the alley-rows, to assess TSSM overwintering females population.

**Results and discussion**

**Overwintering and crop colonization**

TSSM overwinters in Trentino’s red raspberry plantations as adult females mainly on various spontaneous weeds. *Neotetranychus rubi* (Nr), also widespread on raspberry, often in association with TSSM, and the indigenous phytoseiid mite *Amblyseius andersoni* (Aa), prefer to spend the winter directly on the canes and on old dead leaves on the soil. At the resumption of growth in spring, *N.rubi* and *A.andersoni* can rapidly reach the new leaves on laterals of the fruiting canes (FC). TSSM normally occurs later. Nr and pollen are probably an alternative food for Aa at the beginning of the season (Figure 1). Since it overwinters on weeds, TSSM easily infests the new small vegetative canes (VC) that are emerging from the soil. The new VC act as a “rearing box” and as a real “lift” to allow the pest to reach the laterals that develop above soil level on FC later in the season (May/June) (Figures 1 and 2). A well established and large Aa population is already on leaves when TSSM reaches the FC, so the pest is easily and rapidly subjected to control (Figure 1).

**Demographic rise, summer development of the population and stimulating factors**

Starting approximately from the second half of May (pre-bloom or bloom), TSSM usually begins a progressive demographic rise, that leads the population to peak during harvest (Figures 1 and 2). The leading factor that induces and regulates this demographic rise is the progressive increase in temperature. The climate becomes hotter and drier, ideal for the pest, which speeds up its lifecycle, increases its eggs production and its dispersion on the leaves.

Some agronomic interventions in this fragile moment of the balance, may contribute to further destabilize it and shifting it to favour TSSM. Nylon rain cover, even if not integral, modifies the microclimate, increasing the maximum air temperature. Some chemicals (e.g. etofenprox) cause a disproportionate increase of the pest population (Figure 2 – 2006 year), that may be due to a probable direct stimulation of reproductive abilities of the mite, and to a rather persistent toxic action on its indigenous predators.

**More and less stable systems**

The reaction of the system to this critical moment of the balance is different according to whether or not the indigenous phytoseiids (*A.andersoni*) are present. In Table 1 we can observe that, after its re-introduction, Aa steadily and permanently colonised the crop. The pest developed every year a smaller population than in the check (no release) plots.
The use of commercially available phytoseiids

Are the commercially available predators able to guarantee to the system the same stability in raspberry fields not endowed with an indigenous Aa population? Summary data concerning the trial we carried out in S.Orsola site from 2005 to 2007 reported in Table 2, indicate that *A.californicus* early releases (before the middle of June) were more effective every year than late releases in reducing TSSM on FC in comparison with the control plots. The high rate of introduction (52 ind./m, or 20.8 ind./m²) was the most effective in 2005. *A.californicus* developed a smaller population than *A.andersoni* on leaves every year. Except for the early release of a high dose in 2005, results with it were worse than with *A.a* every year in the re-introduction block. Concerning *P.persimilis*, we recorded a weak and late establishment only in 2006 (0.77 motile stages/leaf on FC at the end of August).

TSSM management recommendations

With the information we obtained, a scheme of recommendations for TSSM management on raspberry has been produced. The presence/absence of indigenous *A.andersoni* (Aa) is considered as the main discriminating factor for the control actions to undertake. A provisional natural control threshold (NCT) is suggested (on terminal leaflets of fruiting canes, TSSM/Aa motile stages ratio must be ≤ 1). Short periods of moderate NCT exceeding (up to 3-4 TSSM:1 Aa) are tolerated without interventions. High risks situations for the prey/predator balance (e.g sudden, continuous and noticeable NCT increases, marked Aa population failures, TSSM eggs production increase and occurrence of stimulating factors, etc.) require a release of *A.californicus* by the middle of June (low or high dose depending on the risk evaluation) and/or *P.persimilis* (> 50 individuals/m) if close to harvest. If no or very few indigenous Aa are detected in the plantation, a massive re-introduction in spring is suggested. If it is not possible to find an Aa source, the actions described above for a high risk situation should be undertaken. A prudent threshold of 1 TSSM motile stage/leaflet on fruiting canes before bloom, is suggested for an acaricide application.

Figure 1. Development of TSSM, *N.rubi* and *A.andersoni* at Canzolino site in 1999.
Figure 2. Development of TSSM at S.Orsola site.

Table 1. Development of TSSM and *A. andersoni* in re-introduction plot at S.Orsola site.

<table>
<thead>
<tr>
<th>S.Orsola site - fruiting canes</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSSM</td>
<td>24.94</td>
<td>55.62</td>
<td>3.29</td>
</tr>
<tr>
<td>mean n° of mobile stages/leaf</td>
<td>0.04</td>
<td>0.33</td>
<td>0.46</td>
</tr>
<tr>
<td>mean % of occupied leaves</td>
<td>2.08</td>
<td>8.33</td>
<td>22.91</td>
</tr>
<tr>
<td>CMD/leaf</td>
<td>107.93</td>
<td>64.67</td>
<td>9.87</td>
</tr>
<tr>
<td>mean n° of mobile stages/leaf</td>
<td>0.25</td>
<td>0.29</td>
<td>1.87</td>
</tr>
<tr>
<td>mean % of occupied leaves</td>
<td>14.66</td>
<td>18.77</td>
<td>52.11</td>
</tr>
<tr>
<td>CMD/leaf</td>
<td>39.03</td>
<td>1112</td>
<td>15.79</td>
</tr>
<tr>
<td>mean n° of mobile stages/leaf</td>
<td>0.01</td>
<td>0.23</td>
<td>0.98</td>
</tr>
<tr>
<td>mean % of occupied leaves</td>
<td>1.52</td>
<td>11.55</td>
<td>31.63</td>
</tr>
</tbody>
</table>

Note: Aa releases; 2, 11, 20/5 etofenprox spray; 01/7 hexatiazox spray; 10/4

Table 2. Summary data of the trials with TSSM predatory mites at S.Orsola site.

<table>
<thead>
<tr>
<th>check blocks (no releases)</th>
<th><em>A. andersoni</em> reintroduction block</th>
<th><em>A. californicus</em> release blocks</th>
</tr>
</thead>
<tbody>
<tr>
<td>early releases (before the middle of June)</td>
<td>late releases (after the middle of June)</td>
<td>26 individuals/m</td>
</tr>
<tr>
<td></td>
<td>26 individuals/m</td>
<td>52 individuals/m</td>
</tr>
<tr>
<td>year</td>
<td>cane</td>
<td>period</td>
</tr>
<tr>
<td>2005</td>
<td>VC</td>
<td>115-6/8</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>36</td>
</tr>
<tr>
<td>2006</td>
<td>VC</td>
<td>235-218</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>1030</td>
</tr>
<tr>
<td>2007</td>
<td>VC</td>
<td>194-267</td>
</tr>
<tr>
<td></td>
<td>FC</td>
<td>194-167</td>
</tr>
</tbody>
</table>
Natural regulation of the rosy apple aphid (Dysaphis plantaginea) in organic apple orchards

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Abstract: Rosy apple aphid, Dysaphis plantaginea (Passerini) (Hemiptera: Aphididae), is the most detrimental aphid species in European organic apple orchards. This study aimed to evaluate the natural regulation of D. plantaginea and the effect of installing hail nets on this regulation. The study was carried out during spring 2008 in one experimental apple orchard without pesticide and four organic commercial apple orchards located in southern France. The density and the diversity of natural enemies observed in the experimental orchard were higher than those in the organic orchards. The colonies of D. plantaginea were exploited by a multispecific guild of natural enemies. Hoverflies, lady beetles and earwigs were the most abundant groups. Hoverflies tended to arrive first, followed by lady beetles and earwigs. A high level of aphid infestation was observed in two organic orchards, presumably related to a low level of natural enemies and to a high level of ants. Regarding the effect of hail nets, the study revealed a positive influence of the hail nets on regulation by earwigs but a negative influence on the presence of other natural enemies especially lady beetles. To sum up, this field study indicated that the population dynamic of D. plantaginea was strongly affected by natural enemies, but not sufficiently to maintain it under the tolerance threshold. So, new management practices aiming at enhancing this natural regulation need to be found.

Keywords: rosy apple aphid, Dysaphis plantaginea, natural regulation, natural enemy, organic apple orchard

Introduction

The holocyclic, dioecious rosy apple aphid (RAA), Dysaphis plantaginea (Passerini) (Hemiptera: Aphididae), is undoubtedly the most injurious aphid species in European organic apple orchards (Blommers et al., 2004; Cross et al., 2007; Delorme et al., 1997). If left uncontrolled, this aphid causes a severe leaf deformation and fruit stunting and may reduce return-bloom (Blommers et al., 2004).

Currently, it appears that the control of RAA is difficult because of the very low treatment threshold (usually if the pest is detected) which leads to routine treatment (Cross et al., 2007; Miñarro et al., 2005). New control strategies, which respect organic production principles, have been developed (e.g. Miñarro and Dapena, 2008) to avoid the negative effects of pesticides and the development of resistance in aphid populations (Delorme et al., 1997).

Although contradictory and often inconsistent results are reported (Brown and Mathews, 2007; Miñarro et al., 2005; Wyss et al., 1999), biological control, and factors that enhance this control, remains one of the best solutions to minimise pesticide use (Miñarro et al., 2005).

Thus, this study aimed to determine, to which point, the population dynamic of RAA is affected by naturally occurring enemies, and to identify predators of importance for controlling its population. In addition, because the use of hail nets increases in Provence (southern France), we wanted to study the possible effects of these nets on this regulation, namely the RAA populations and the abundance and diversity of their natural enemies.
Materials and Methods

Study apple orchards
The studies were carried out in four commercial orchards (n°51, 145, 125, and 126), 3.4 ha in total, managed following organic production rules and located close to Avignon (southern France). They were planted with three cultivars: Golden Delicious (n°51 and 145), Akane (n°125), and Royal Gala (n°126). In addition, a 0.23 ha experimental orchard not treated with insecticides and located in the INRA experimental site in Avignon, was used for this study. It was planted in 2001 with Granny Smith and Royal Gala cultivars and presented a 5-row per 24 trees design for each cultivar. Four rows of each cultivar were covered with white hail nets (mesh size: 3×7.4mm) on 17 April 2008, i.e. when RAA and some natural enemies, e.g. hoverflies, were already present.

Entomological assessments
In each orchard, 50 shoots, among those infested with RAA, were randomly selected and marked with coloured ribbons. In the experimental orchard, 50 more were marked in the eight covered rows. The presence of RAA, its natural enemies, and ants were assessed on these shoots by weekly visual observations, depending on orchard, from 7 April (infestation beginning) to 3 July 2008 (aphid migration to the secondary host plant: Plantago spp. was then complete). Aphid infestation was recorded using six classes (A = no aphid; B = 1 to 5 aphids; C = 6 to 25; D = 26 to 50; E = 51 to 125; F > 125). In the same way, the presence of ants was recorded using four classes (I = 1 to 5 ants; II = 6 to 25; III = 26 to 50; IV > 50). Aphid and ant numbers that were analysed were the mean number of the recorded classes. In contrast, presence of the predaceous stages of natural enemies was recorded in exact numbers.

Results and discussion

Existence of natural regulation of RAA
The beginning and the intensity of RAA infestation differed according to orchard (Figure 1). These differences may be due to differences in site characteristics, surrounding habitat, cultivar, cultural practices and/or ant and natural enemy presence (Brown and Mathews, 2007; Miñarro and Dapena, 2008; Stewart-Jones et al., 2008). The abundance and diversity of natural enemies observed in the experimental orchard were higher than those in the organic orchards (Figure 1, Table 1). The RAA colonies were exploited by a guild of natural enemies. Hoverflies (Syrphidae), lady beetles (Coccinellidae), and earwigs (Forficulidae) were the most abundant groups. Hoverflies arrived first, followed by lady beetles and earwigs. This sequential arrival may be explained by their lower developmental thresholds: 4, 10, and 6°C for hoverflies, lady beetles (Dixon et al., 2005), and earwigs (Helsen et al., 1998), respectively. The latter appears naturally late because of the parental care for eggs and young nymphs in nests (Helsen et al., 1998). These thresholds could give the hoverflies and earwigs some advantages over most aphid enemies for their use in augmentation biological control programs in early spring.

Natural enemy abundance increased progressively from the beginning of the infestation to peak population phase and reached their maximum at (INRA orchard) or after (organic orchards) the infestation peak. Thereafter, their abundance decreased following the decline of RAA population. Hence, the population development of RAA may thus be affected by their natural enemies -and this would indicate a potential for these enemies to negatively impact RAA population growth- and/or by the alatae migration, which began at the peak phase of RAA in our study.
Figure 1. Temporal development (Mean ± SE) of RAA, natural enemy (NE), and ant populations in each orchard: (A) INRA experimental orchard “without nets”; (B) INRA experimental orchard “with nets”; (C) organic orchard n°145 *: similar results found in orchard n°51; (D) organic orchard n°125 **: similar results found in orchard n°126”.

Table 1. Total number of predaceous stages of natural enemies assessed on 50 infested shoots randomly selected in each studied orchard (pooled numbers of weekly assessments from 7 April to 3 July 2008). (Δ): the number is calculated after net installation.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>INRA Uncovered</th>
<th>CoveredΔ</th>
<th>n°51</th>
<th>n°145</th>
<th>n°125</th>
<th>n°126</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Orchard</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n°51</td>
<td>n°145</td>
<td>n°125</td>
<td>n°126</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Coccinellidae</td>
<td>170</td>
<td>11</td>
<td>123</td>
<td>136</td>
<td>36</td>
<td>47</td>
<td>523</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>Dermaptera</td>
<td>Forficulidae</td>
<td>84</td>
<td>142</td>
<td>130</td>
<td>82</td>
<td>218</td>
<td>128</td>
<td>784</td>
</tr>
<tr>
<td></td>
<td>Cecidomyiidae</td>
<td>2</td>
<td>-</td>
<td>11</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>32</td>
</tr>
<tr>
<td>Diptera</td>
<td>Syrphidae</td>
<td>280</td>
<td>194</td>
<td>105</td>
<td>162</td>
<td>45</td>
<td>44</td>
<td>826</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Heteroptera</td>
<td></td>
<td>56</td>
<td>21</td>
<td>8</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>Braconidae</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>Neuroptera</td>
<td>Chrysopidae</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>Spiders</td>
<td></td>
<td>47</td>
<td>22</td>
<td>14</td>
<td>26</td>
<td>14</td>
<td>19</td>
<td>142</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>705</td>
<td>403</td>
<td>406</td>
<td>443</td>
<td>314</td>
<td>240</td>
<td>2511</td>
</tr>
</tbody>
</table>

| Number of assessments | 10 | 8 | 11 | 11 | 10 | 9 |

Effect of installing hail nets
This study shows that the RAA population in the net-covered rows was lower than in the
uncovered ones. Furthermore, the infestation in the net-covered rows disappeared about 10 days prior to that observed in the uncovered ones. Since the caging conditions (Wyss et al., 1999) and hail nets (Tasin et al., 2008) have negligible effects on the temperature and relative humidity within the canopy and thus for RAA development, we can explain our results by the positive influence of nets on RAA regulation by its natural enemies, especially earwigs (Table 1), despite its late arrival in RAA colonies. Nevertheless, this study revealed a negative influence of nets on the presence of some natural enemy groups, especially lady beetles. These nets may form a physical barrier to the insects’ entry (Graf et al., 1999). Hence, only small-sized insects such as Scymnus spp. lady beetles (personal observation) can pass through this barrier.

In conclusion, this field study indicates that the population dynamic of RAA was affected by natural enemies, but not sufficiently to maintain it under the tolerance threshold. Therefore, new management practices aiming at enhancing this regulation are required.

References


Pest management practices and environment factors affect natural regulation of the codling moth

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Abstract: Numerous arthropod predators and parasitoids species attack codling moth eggs and larvae, but these antagonists do not efficiently control the pest in commercial orchards. Parasitism of diapausing larvae was assessed in 79 apple and pear orchards from South-eastern France (2007-2008). The predation and parasitism of egg masses was investigated on a sub-sample of 13 orchards in 2008. Diapausing larvae were observed to be parasitize in only 21.0% and 16.4% of orchards in 2007 and 2008, respectively. The mean parasitism rate over the two years was 3.7 %, 2.3% and 0.8% in the organic and conventional with or without mating disruption orchards, respectively. It was higher in apple than in pear orchards, for high than low densities of windbreak hedgerows and for low than for high densities of orchards surrounding the analysed fields. Six parasitoid species were identified, among which Ascogaster quadridentata, Pristomerus vulnerator and Perilampus tristis were the most frequent whatever the management practices. The composition of the parasitoid community was explained by both local (27%) and landscape factors (16%). On average 12.5% and 54.1% of egg masses exposed to natural antagonists were consumed by predators in July and August 2008, respectively. The highest predation rates were also recorded in the organic orchards and close to hedgerows. Egg parasitism was negligible (0.1%). It appears from this analysis that of egg and larval parasitism, the most frequently described in the literature, has lower impact on codling moth populations than the predation of eggs. Comparing with previous analyses in the same area, it appeared that larval or egg parasitism was much more affected by the protection practices than egg predation.

Key-words: Cydia pomonella, predation, parasitism, apple orchard

Introduction

The codling moth, Cydia pomonella L. is a major pest in apple and pear orchards, damaging a large part of the world production. It has been controlled by synthesis insecticides that select resistant populations in conventional and organic production systems as well. New control techniques have been recently adopted, such as mating disruption, but success is subjected to biological and environmental factors. Studies on natural regulation of the codling moth by parasitoids and predators are therefore of economic and ecological interest. This regulation may be affected by the production or protection practices, and also by the diversity of the plant species that make up the grass cover of the orchard and the surrounding hedges.
We here assessed how the production system affects the parasitism of diapausing larvae and the predation and parasitism of eggs of the codling moth in a small agricultural landscape of the low valley of Durance, in Southern France.
Material and Methods

Parasitism of diapausing larvae was observed in 79 apple and pear orchards of a 70 km² area near St Rémy de Provence, in southeastern France. Corrugated cardboard traps were placed on trunks of geo-referenced trees in August 2006 and 2007, and the larvae were harvested in October of the same year. The larvae were weighed, then placed in individual plastic cups (2 x 2 x 2 cm) and kept in a shelter under natural conditions of temperature, humidity and photoperiod. The adults of codling moth and parasitoids started to emerge in April 2007 and 2008.

The predation and the parasitism of codling moth eggs were assessed in a sub-sample 13 orchards in this area. Codling moth were allowed to lay eggs on paper sheets that were cut in 2x1 cm pieces containing on average fifteen 12 hour-old eggs. The cards were fixed on leaves in the orchards (30 cards per orchard) and exposed to the natural enemies for three days. The numbers of parasitized and predated eggs were assessed seven days after exposing them in the orchards, at the start of July and August. The orchards were assigned according to production system, organic or conventional. In the conventional system, the orchards in which the codling moth was controlled exclusively with chemical insecticides (conventional) were also differentiated from those in which mating disruption was implemented, alone or with reduced pesticides (integrated).

Results

The parasitism of diapausing larvae occurred in 21% and 16.4% of the orchards sampled in 2006 and 2007, respectively. The mean rate of parasitism in these two years differed significantly between the production systems (P=0.00051), reaching 3.7%, 2.3% and 0.8% in the biological, integrated and conventional system, respectively (Figure 1). When considering only the orchards where larval parasitism occurred, the production systems did not differ significantly (Figure 2). Pristomerus vulnerator was the most frequent species in 2006, followed by Ascocaster quadridentata and the hyperparasitoid Perilampus tristis (Figure 3). Ascogaster quadridentata was far the most prevalent species in 2007, followed by P. tristis. The kinetics of emergence of P. vulnerator and P. tristis were closely related to those of C. pomonella, while A. quadridentata emerged earlier (Figure 4).

The predation of C. pomonella eggs was significantly higher in organic orchards than in conventional ones (Figure 5). In August, the mean percentage of cards with predated eggs reached 64% in the organic orchards and 48% and 42% in the integrated and conventional orchards, respectively. The predation rate was significantly higher in August than in July. It was confirmed that Trichogramma cacoeciae was present in this area as a parasite of C. pomonella eggs, but this occurred only in August in a single egg sheet of a single orchard under conventional system (ie 3.8% parasitism in this orchard).

Discussion

The presence of several species of parasitoids was detected on C. pomonella diapausing larvae during the two years of experimentation. The low rate of parasitism indicated that the regulation C. pomonella was not strongly under the dependence of these organisms. The larval parasitism occurred more frequently in the organic than in the conventional or integrated orchards. However, the rate of parasitism no longer differed between the tree production systems when considering only the ten orchards where diapausing larvae were parasitized.

In contrast to the low rate of larval or egg parasitism, the rate of egg predation was
relatively high in the three production systems, as observed in the previous study on untreated pear and apple orchards of the same area, and was the highest in the organic system. This observation raised the question of egg predators as a way of regulation of codling moth populations under organic or integrated pest management strategies.

Furthermore, both predation and parasitism rates were significantly lower in the orchards surrounded by large surfaces of conventional orchards in the 100 m buffers. This suggests that the protection practices in the surroundings may affect the potential of immigration of the natural enemies towards the orchards.

![Figure 1. Mean parasitism (%) of diapausing larvae of *C. pomonella* in apple and pear orchards of Southern France](image1)

![Figure 2. Mean parasitism (%) of diapausing larvae of *C. pomonella* in parasitized orchards of Southern France](image2)
Figure 3. Nature of *Cydia pomonella* parasitoids in orchards of Southeastern France.

Figure 4. Kinetic of emergence of *Cydia pomonella* and its parasites, in day degrees

Figure 5. Mean rate of predation (%) of sentinel eggs of *Cydia pomonella* in 13 orchards
A geostatistical approach to evaluate the side effects on non target species using a non repeated plot.

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Abstract: A geostatistical approach to evaluate the side effects of insecticides on non target species using a non replicated treatments. Field research was carried out, in pear orchards in the Emilia-Romagna region (I), to study the side effects on populations of Anthocoris nemoralis F. of three different strategies to control the first generation of Cydia pomonella L. The strategies were: i) soft = application of CpGV, ii) OP = application of phosmet and chlorpyrific and iii) reduced risk = thiacloprid and methoxyfenozide. These strategies were applied on large single block plots and the responses on Cacopsylla pyri L. and A. nemoralis F. populations were analysed by means of geostatistical approach. The A. nemoralis data was collected by means of a grid sampling plan based on referenced points while C. pyri were sampled as average of mobile instars per each plot. The population density of C. pyri and A. nemoralis were higher in OP and soft strategies than in the reduced risk strategy, but the prey/predator population ratio was similar for the three strategies. The geostatistical monitoring method could be adapted to measure the effects of different products on some target and non target species populations, also on non replicated large plots or wide areas.

Anthocoris nemoralis, Cacopsylla pyri, Side effects, Geostatistical monitoring
The effect of rosy apple aphid and beneficial insect dynamics in an orchard

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Abstract: The natural control of the rosy apple aphid Dysaphis plantaginea, a major pest in European apple orchards, was studied from 2006 to 2008 in three organic apple orchards planted with Smoothee 2832T®, Ariane and Melrose cultivars. The development of D. plantaginea and the beneficial complex associated with aphid colonies were visually assessed in the spring, and the effect of the interaction within the orchard (edge, inner and intermediate areas) was studied. Infestation of orchard edge trees by D. plantaginea was higher. Beneficial numbers and the predator/prey ratio were also higher in edge trees in 2007, and 2008. Predatory arthropods that were assessed within infested shoots mainly comprised Syrphidae, Cecidomyiidae, ladybirds and earwigs, but their proportion differed between cultivars. It also differed between areas of the orchard: Cecidomyiidae were assessed earlier and also prevailed in edge areas, whereas Syrphidae prevailed in the inner parts of the orchards. However, even the most favourable situations did not permit the natural control of D. plantaginea. These results suggest that the cultivar affects both D. plantaginea and associated predatory arthropods, and that the management of edge effects through orchard redesign and/or cultural practices deserves to be considered for the management of the rosy apple aphid in IPM orchards.

Key-words: aphid, Dysaphis plantaginea, predatory arthropod, edge effect, apple orchard

Introduction

The dioecic rosy apple aphid, Dysaphis plantaginea (Passerini) (Hemiptera: Aphididae), is a major pest in European apple orchards. Due to serious detrimental effects and a high multiplication rate, the treatment threshold is very low (ca. 1% infested shoots or presence in French orchards). As early pesticide applications can affect natural enemy populations that begin to settle and/or to increase in orchards, such applications are not compatible with IPM strategies. Besides, alternative methods are not available in orchards, despite promising studies on biological and conservation biological control (Wyss, 1996; Wyss et al., 1999); pheromones (Fitzgerald et al., 2005); and autumn kaolin applications (Bürgel et al., 2005). Studies on the natural control of D. plantaginea in European orchards also indicate that the beneficial complex of natural enemies is generally not able to provide control of this aphid (Blommers, 1999; Miñarro & Dapena, 2001; Miñarro et al., 2005).

A better understanding of the relationships between the rosy aphid and the beneficial complex within the orchard is thus necessary for an alternative control of this foliar pest. The aim of this study is to describe the functional relationships between D. plantaginea and its natural enemies, for different situations within the orchard.

Materials and methods

Experimental design
The study was carried out from 2006 to 2008 at the INRA experimental site in Saint-Marcel-
lès-Valence (South-Eastern France). Three organic orchards, planted with three cultivars (Smoothee 2832T®, Ariane (scab Vf-resistant) and Melrose) were studied. Orchards were planted in 2005 at a density of 1000 trees ha⁻¹; they were lined by mixed hedgerows and fallow fields. Each orchard had an 8-row per 46 trees design (0.37 ha). No post-bloom aphicide treatment was applied during the experiment. For assessments of aphid infestation and natural enemy numbers, each orchard was divided into three areas: edges (end line trees), intermediate (5 trees adjacent to edge trees within the line) and centre (other trees).

**Aphid and arthropod assessments**

Visual assessments of tree infestation in each orchard were made at two dates (first symptoms, infestation peak) (30s per tree) of all the trees in the 4 central rows and of all edge trees. Tree infestation was recorded using 3 classes: 0 no symptoms; 1 ≤ 10% shoots with symptoms; and 2 >10% shoots with symptoms. The three cultivars were pooled for analyses.

Visual assessments of 80 (2007) to 125 (2008) marked infested shoots per orchard (Ariane and Melrose cultivars only), were carried out every ten days after bloom and until aphid migration to its secondary host plant *Plantago* sp.. Aphid infestation was recorded using six classes: A no aphid; B 1-5 aphids; C 6-25 aphids; D₁ 26-50 aphids; D₂ 51-125 aphids; E > 125 aphids per shoot, and the presence of predatory stages of natural enemies was recorded in exact numbers on each shoot.

**Results and discussion**

**Tree infestation within the orchard**

At peak infestation, the distribution of trees within symptom classes was different (CHI² test, \( P<0.05 \)) between edge and centre areas for the 3 study years. Edge and intermediate areas were different in 2007 only. Infestation was higher in edge trees compared to the centre of the orchard; intermediate areas tended to be similar to edges (Table 1).

Table 1. Infestation according to orchard area (% infested trees, i.e. % (cl1+cl2) trees).

<table>
<thead>
<tr>
<th>Orchard area</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12-may</td>
<td>23-may</td>
<td>3-may</td>
</tr>
<tr>
<td>Edge</td>
<td>10.4</td>
<td>22.9</td>
<td>58.3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7.4</td>
<td>20.4</td>
<td>44.4</td>
</tr>
<tr>
<td>Center</td>
<td>6.0</td>
<td>10.9</td>
<td>25.4</td>
</tr>
</tbody>
</table>

**Potential for natural control**

Hoverflies (Syrphidae) prevailed until mid-May among predatory arthropods observed within *D. plantaginea* colonies. At the end of the infestation period (end of May), the complex of beneficial arthropods was more diversified with aphid predators (Coccinellidae, predatory Cecidomyiidae) and generalist or other predators (earwigs, lacewings, predatory bugs).
Differences in the proportion of beneficial taxa (cumulative numbers across the season) were displayed whatever the cultivar between edge and centre areas (CHI2 test, \( P<0.05 \)). Cecidomyiidae were always observed earlier and in higher numbers in edge areas, whereas, Syrphidae prevailed in the orchard centre. The proportion of beneficial taxa also differed between cultivars (Figure 1).

![Figure 1](image1.jpg)

Figure 1. Complex of beneficial arthropods observed in *D. plantaginea* colonies in 2008: (a) Ariane centre area; (b) Ariane edge area; (c) Melrose centre area.

![Figure 2](image2.jpg)

Figure 2. Mean beneficial and aphid numbers for Melrose cultivar in 2008: (a) centre area; (b) edge area.

Both aphid and beneficial numbers increased at first (Figure 2), as did the predator/prey ratio. In 2007 and 2008, in the centre areas, the decrease in aphid numbers only occurred at the migration of *D. plantaginea* towards its secondary host. In 2008, in the edge areas (Figure 2b), aphid numbers remained high despite a slight decrease observed before aphid migration. The predator/prey ratio was similar in all three areas in 2008 and higher at the plot edges in 2007. No efficient predatory effect on aphids was observed in either year or cultivar.

**Towards integrated management of *D. plantaginea***
The situation within the orchard has an effect on both aphid infestation and the beneficial
guild: infestation was higher at the edges of the orchards and qualitative differences in the predatory complex were observed between the orchard areas. However, as already stated by other authors (Blommers, 1999; Miñarro & Dapena, 2001), natural control of *D. plantaginea* by natural enemies is unreliable, despite non-disruptive pest management and a diversified plant environment. The most aphid favourable habitat is observed at orchard edges, which may support the proposal of redesigning orchards by plant management to enhance pest control.

Lastly, as the aphid population level that can be tolerated in orchards is very low, the combining of all existing control methods, including these beneficial augmentative measures and cultivar susceptibility (Simon *et al.*, 2008), should be used in the integrated management of *D. plantaginea*.

**Acknowledgements**

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**References**


Susceptibility of codling moth populations originating from the Czech Republic to *Cydia pomonella* granulovirus (CpGV)

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Abstract: Baculoviruses are very important agents of organic and integrated crop production due to their favorable ecotoxicological qualities, high selectivity and efficacy. Whereas many European countries and the USA have been using *Cydia pomonella* granulovirus (CpGV) products to control codling moth for many years, registration of CpGV in the Czech Republic is still in progress. However, in the last six years, populations of the codling moth resistant to CpGV-M isolate were locally found in some European countries. With regard to this experience, the object of this research is to evaluate the susceptibility of various codling moth populations in the Czech Republic to CpGV-M and also to propose a suitable anti-resistance strategy for the Czech Republic. In 2008, the first monitoring of wild codling moth populations’ susceptibility to CpGV-M was evaluated by laboratory bioassays. Three wild populations (Prague-Ruzyne, Bulhary and Velke Bilovice) and a reference (sensitive) laboratory strain were assessed. LC50 in the 7th and 14th day after the infection of the first larval instar was determined by probit analysis. No decreased sensitivity to the CpGV-M was demonstrated.

Key words: codling moth, *Cydia pomonella* granulovirus, resistance, LC50

Introduction

Baculoviruses generally are the major group of arthropod viruses studied for their biological potential to control pests in agriculture and forestry. Considering their high environmental stability, virulence, host specificity and lack of any known negative influence on the environment, non target organisms or human health, baculoviruses are used as efficient bioinsecticides (Asser-Kaiser et al., 2007). The host range of individual baculoviruses is usually limited to a few closely related species especially from the insect orders Lepidoptera, Hymenoptera and Diptera. *Cydia pomonella* granulovirus (CpGV) (genera Granulovirus) is highly pathogenic for codling moth (*Cydia pomonella*, (L.)) and its infection results in high mortality of early instar larvae. Infected larvae succumb within 5-10 days.

In the late 1980s, the first CpGV biopesticide was registered for commercialization and use in some European countries. CpGV biopesticides are now commercially available in most European countries, in North America, Argentina, Australia, New Zealand, and South Africa. In the Czech Republic, the CpGV based biopesticides called Madex (Andermatt Biocontrol AG, Switzerland) and Carpovirusine (Natural Plant Protection, France) are just being registered. While in the Czech Republic the registration process is still not finished, Germany, France, Switzerland, Italy and Netherlands have already reported the occurrence of several populations of codling moth resistant to CpGV (Jehle, 2008). The selection of resistant populations is related to applications of only one isolate of CpGV, discovered in 1963 in Mexico, called CpGV-M. Foreign research institutes and the preparations’ producers are currently searching for the main reasons for the development of resistance as well as possible solutions to this situation. Andermatt Biocontrol AG laboratories managed to obtain a virus
strain efficient against the resistant codling moth population by subsequently passaging CpGV-M through the resistant population. The commercial name of this new biopesticide is Madex Plus. Its efficiency was evaluated not only in the laboratory, but also in field experiments in selected orchards (Jehle, 2008). The aim of our work was to compare the sensitivity of selected Czech codling moth populations to CpGV-M in bioassays.

Material and methods

Laboratory strain of codling moth
The neonate larvae of codling moth used to establish 50% lethality concentration (LC50) values of the CpGV-M were taken from a laboratory strain reared on an artificial diet under standard conditions of 26°C and a photoperiod of 16:8 h (L:D). The Crimean laboratory strain of codling moth (Vsesajuznyj Institut Zascity Rastenij, Saint Petersburg, Russia) was used as susceptible reference.

Field populations of codling moth
The susceptibility of three codling moth field populations to CpGV was tested in laboratory conditions. For these experiments, the overwintering larvae of codling moth were collected from July to November 2007 in apple orchard in Velke Bilovice and apple alleys in Bulhary and Prague-Ruzyne. The Velke Bilovice orchard is located in South Moravia (approximately 153 miles from Prague) in an intensive apple growing farm, following integrated pest management strategies since 1992. The tested codling moth population came from a 80-ha plot at a distance of 200m from the plot treated with CpGV. In this orchard, resistance to insecticides was proved (Stará and Kocourek, 2007). The Bulhary alley is located in South Moravia along the road near to the village of Bulhary and has never been treated with chemical pesticides or CpGV. The Prague-Ruzyne alley is located in Prague and belongs to the Crop Research Institute. This alley is at a distance of 400m from the experimental orchard treated for several years with CpGV. The overwintering larvae were collected in apples using paper belt traps and stored for 3-4 months in a climatic chamber at 6°C temperature. At the beginning of February 2008, the temperature in the climatic chamber was gradually increased to 20°C. Adult codling moth emerged during April and they were transported into an ovipositing box situated in the glasshouse with natural light conditions. The branches of apple trees were placed round the boxes to simulate natural conditions and support the oviposition.

Bioassay
The methodology of bioassays and rearing of adults of wild codling moth populations was carried out according to Fritsch et al. (2005) and Asser-Kaiser et al. (2007). Neonate larvae (L1) were placed individually on the surface of the diet prepared from instant premix (Manduca Premix-Heliothis Premix, Stonefly Industries; USA). Purified and quantified CpGV-M isolate was mix into the diet in concentrations from 10^{2} to 3x10^{4} occlusion body (OB)/ml. Twenty to fifty L1 larvae were exposed to each tested concentration of CpGV-M. Larvae of the laboratory strain were exposed to six concentrations of CpGV-M, larvae of field populations were exposed to two – four concentrations of CpGV-M depending on the number of larvae available for the test. One day post infection the dead larvae were removed to avoid assessing the larvae injured during manipulation from the bioassay. Mortality of larvae in the bioassay was evaluated symptomatically 7 and 14 days post infection.

Statistical analysis
Corrected mortalities (Abbott, 1925) of the larvae from the laboratory strain of codling moth were analyzed by probit analysis ( STATISTICA 6.0, StatSoft Inc. 2005). LC50 values, 95%
Results and discussion

Laboratory strain of codling moth
The average mortality of codling moth larvae from the laboratory strain one day post infection (mortality due to manipulation) was 1.25%. In untreated controls, the mortality of larvae 7 and 14 days post infection was 0%. Seven days post infection, CpGV-M had intermediate efficacy against larvae from the laboratory strain with an LC$_{50}$ of 5.42x10$^3$ OB/ml of diet (95% confidence limit 3.55 – 8.96 x10$^3$ OB/ml, slope 0.95), while 14 days post infection the LC$_{50}$ was 3.47x10$^2$ OB/ml of diet (95% confidence limit 1.73 – 5.69 x10$^2$ OB/ml, slope 1.86). These results correspond to results published by Fritsch et al. (2005) and confirm the sensitivity of the codling moth laboratory strain to CpGV-M. Asser-Kaiser et al. (2007) stated LC$_{50}$ values of ~170 to ~970 OB/ml of the susceptible laboratory and field populations 14 days post infection. These data correspond with our results too.

Field populations of codling moth
The average mortality of codling moth larvae from wild populations from Bulhary, Prague-Ruzyně and Velké Bílovice one day post infection was 7.05%, 7.35% and 4.75%, respectively. The mortality in controls without CpGV-M 7 and 14 days post infection was 0%. Mortality of larvae from wild populations expressed in probit in dependence on log of CpGV-M concentrations is presented in Figure 1. The low number of codling moth larvae available for the bioassay did not enable the determination of LC$_{50}$ values of CpGV-M against wild populations. However, according to the position of points corresponding to mortality of larvae from the tested wild populations in Figure 1 with respect to the confidence intervals of concentration-mortality response curve of the laboratory strain, the sensitivity of wild populations did not differ significantly from the laboratory strain. In most cases, mortality rate of wild populations lie inside the confidence interval of the concentration-mortality response curve of the laboratory strain. According to Schmitt et al. (2008), codling moth larvae surviving infection by CpGV in concentrations of 10$^4$ OB/ml (evaluated 14 days post infection) can be considered to be resistant. In our experiments, mortality of larvae from the tested wild populations 14 days post infection by CpGV-M in concentrations 10$^4$ OB/ml (log c 4.00) and 3x10$^4$ OB/ml (log c 4.48) reached always 100%. Hence, any tested wild population did not exhibit decreased sensitivity to CpGV-M. In addition to this, codling moth populations collected from localities with different management of pests control did not differ significantly in their sensitivity to CpGV-M. Recently, for the population of codling moth from the commercial apple orchard in Velké Bílovice, cross resistance to fenoxy carb, teflubenzuron and phosalone has been detected (Stara and Kocourek, 2007). According to our results, CpGV-M is efficient against this population and no cross-resistance to CpGV-M and fenoxy carb, teflubenzuron and phosalone was indicated. Similarly, Schmitt et al. (2008) did not prove cross-resistance between CpGV and insecticides. Hence, after registration of biopesticides based on CpGV-M, we can expect high efficacy of these biopesticides against codling moth populations from the Czech Republic. These biopesticides will be suitable for use in organic apple orchards as well as in IPM orchards and will serve as a good alternative to chemical insecticides in orchards with incidence of codling moth populations resistant to insecticides.
Figure 1. Comparison of efficacy of CpGV-M tested against sensitive laboratory strain and against the wild populations from Bulhary, Prague-Ruzyne and Velke Bilovice 14 days post infection.

Acknowledgements

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References


Indicators to assess the environmental impact of protection practices in apple orchards

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Abstract: Apple fruit production requires the application of numerous pesticides, mostly targeted against scab, codling moth and aphids. A Principal Component Analysis of the protection practices in 54 randomized apple orchards of a small production area near Avignon, in south-eastern France, produced 4 groups of growers relying on the protection strategy against *Cydia pomonella*: organic production, exclusive use of mating disruption (MD) against *C. pomonella*, intensive use of chemical insecticides (intensive), and a fourth group with both MD and chemicals (intermediate). The environmental impacts of these management strategies were assessed using two different indicators i) the environmental impact quotient (EIQ) accumulating the impacts on farmers, consumers and non human biota ii) I-PHYARBO, a fuzzy expert system focusing on the environmental impact of pesticides. The outputs of these two indicators strongly differed from each other, the highest environmental impact being attributed to the organic orchards by EIQ while according to I-PHYARBO organic farming had the safest protection program. The three other protection systems did not differ strongly from each other whatever the indicator. This range discrepancy, which is conserved when considering only the beneficial organisms, is mainly due to the structure of the models. Unlike I-PHYARBO, EIQ assumes dose proportionality and a strict additivity of the effects of successive treatments, thus attributing high adverse effects to the organic programs that involve frequent applications of mineral fungicides. Attention has to be paid to the significance of these indicators, which may become useful tools to establish the consistency of pest-control strategies and recommendations.

Keywords: Pest management, agri-environmental indicator, EIQ, I-PHYARBO, mating disruption, organic orchard

Introduction

Apple fruit production requires the application of numerous pesticides, mostly targeted against scab, codling moth and aphids. Forecasting models, population survey and alternative control methods are increasingly implemented against these pests, tending to reduce the number of chemical treatments and the environmental impact of protection practices. There is at the same time an increasing concern regarding the impact of these practices on the environment, strongly depending on the toxicity of each pesticide, the dose and technique of application, the time the active ingredients remain in the soil, and surface and groundwater contamination. The first approach to assess impacts is the direct measurement on communities, and vast scientific literature is dealing with the impact of production strategies on the biotic environment (Hole *et al.* 2005). However these analyses are highly time consuming, and the relations between agricultural practices and the environment are not always demonstrated. Different abiotic environmental indicators were thus developed to assess the risk associated with different protection scenarios (*Reus et al.*, 2002, *Devilliers et al.*, 2005). Two of them, implemented at the plot level and initially designed for fruit crops, were used in this study to compare the management strategies of apple growers in south-eastern France. The first one is the environmental impact quotient (EIQ) accumulating the impacts on farmers, consumers and non human biota (*Kovach *et al.*, 1992). The second one, I-PHYARBO, is a
fuzzy expert system focusing on the environmental impact of pesticides (Devilliers et al., 2005).

**Material and methods**

*Indicators.* Both EIQ and I-PHY\textsubscript{ARBO} indicators score the potential risk for a treatment program based on the toxicity of each applied pesticide and measures of potential exposure based on the half-life, runoff or leaching potential, and pattern of use.

The overall EIQ for each pesticide is the average of three general risk categories, the farm worker, the consumer, and the ecological component including aquatic organisms, bees, birds, and beneficial arthropods. The EIQ value of a pesticide application is the product of the active ingredient index by the rate of application; the EIQ for a crop protection program is the sum of the values obtained for each pesticide application.

I-PHY\textsubscript{ARBO} is an expert system adapted for fruit crops from the indicator “Ipest” (Van der Werf and Zimmer, 1998), initially designed for field crops. The four modules of Ipest reflect the presence of the pesticide and the risk for ground water, surface water and air compartments, their input variables being pesticide properties and conditions of application. A fifth module on beneficial arthropods was included in I-PHY\textsubscript{ARBO}. The value of each module is calculated according to the degree of membership of the input variables to the fuzzy subsets favourable or unfavourable. The five modules can be considered individually, or aggregated according to membership to fuzzy subsets and a set of decision rules. Unlike the EIQ, a high value of I-PHY\textsubscript{ARBO} means a low environmental impact.

*Orchards.* The protection practices including the treatment recordings were collected and described by the way of a direct inquiry in 54 randomized apple orchards of a small production area near Avignon, and subjected to a Principal Component Analysis (PCA) to establish a typology of grower strategies.

**Results**

The PCA designed four groups of orchards relying on the protection strategy against *Cydia pomonella* and on the intensity of chemical applications (Figure 1): a first group of seven orchards under organic fruit production guidelines (Organic), a group of six orchards under conventional farming with use of mating disruption against *C. pomonella* and a few chemical insecticides against other pests (MD), a group of 12 orchards with exclusive use of chemical insecticides (Intensive), and a last group of 22 orchards using both MD and chemical treatments against *C. pomonella* (Intermediate).

Both indicators were able to discriminate the protection programs, despite a high variability of practices and of indicator values within each of the groups (Figure 2). The outputs of the two analyzed indicators strongly differed from each other. According to EIQ, the highest environmental impact was attributed to the organic orchards whereas the three other protection systems did not differ significantly from each other. According to I-PHY\textsubscript{ARBO} organic farming had the safest protection program, and the introduction of MD technique also significantly minimized the environmental impacts when compared to the most intensive system.
Figure 1. Protection strategies of apple growers in the study area designed using a PCA analysis considering the nature and number of treatments or the alternative protection strategies against the codling moth.

Conclusions

I-PHY\textsubscript{ARBO} fits better than EIQ with the observations of biological communities of the same area, where Organic is the safest system on soil macrofauna (Denoyelle \textit{et al} 2007), arthropods (Simon \textit{et al} 2007) and birds (Bouvier \textit{et al} 2005). Direct observations on biological communities seem a good way to validate such indicators, but only a few studies considered simultaneously the outputs of abiotic indicators and selected field related organisms, which was the case for carabids, weeds, earthworm, microbial soil activity in Heyer \textit{et al} (2003). The range discrepancy observed between the two indicators, which is conserved when considering only the beneficial organisms, is mainly due to the structure of the models. Unlike I-PHY\textsubscript{ARBO}, EIQ assumes dose proportionality and a strict additivity of the effects of successive treatments, thus attributing high adverse effects to the organic programs that involve frequent applications of mineral fungicides. Particular attention has to thus be paid to the significance of these indicators, which may become useful tools to establish the consistency of pest-control strategies and recommendations in fruit production.
Figure 2. Values of the EIQ (high value = high impact) and of the I-PHY_{ARBO} (high value = low impact) indicators according to protection strategy in 2006 in apple orchards.

References


Potential new storage rot problems in UK Cox apples

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Abstract: Recent surveys of rotting in Cox apples in the UK have identified new fungal rots due to Botryosphaeria obtusa, Basidiomycete fungi and Phomopsis mali causing losses in the orchard and in store. Only studies on B. obtusa are reported here. B. obtusa causes a brown rot of fruit in the orchard and in store a purple rot, usually at the stalk end, and with a distinct medicinal smell. The fungus can invade fruit directly or via wounds. All apple varieties tested were susceptible to B. obtusa, but Cox was most susceptible. The rot also occurs on pears but at a much lower incidence. Studies on B. obtusa invasion of wood showed that the fungus did not form cankers on trees or invade wounds, but rapidly colonised dead 1-3 year-old branches on the tree. Similarly prunings on the ground were also rapidly colonised by the fungus. Dead apple twigs in orchards are therefore the main source of inoculum. Botryosphaeria infected apple twigs were present in all orchards examined. B. obtusa was rarely found on alder or Chamaecyparis twigs and windbreaks do therefore not appear to be a source of the fungus for apple trees. Monitoring fungal activity on infected twigs showed that conidia were produced all year round. Studies on B. obtusa rot in store on Cox, Gala and Fiesta showed that rot development was very slow and secondary spread to healthy fruit unlikely to be significant. Losses in store will therefore depend on the level of fruit infection in the orchard. Changes in orchard management practices relating to pulverisation of prunings in the orchard rather than removal and burning have probably contributed to the increase in incidence of Botryosphaeria.

Botryosphaeria obtusa, Phomopsis mali, Inoculum,
Is it possible to predict the aerial concentrations of *Venturia inaequalis* ascospores in apple orchards?

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**Abstract:** Daily aerial concentrations of ascospores of *Venturia inaequalis*, the infectious agent responsible for apple scab, were observed over four years in apple orchards in the Drôme and Maine-et-Loire departments in France. These concentrations were recorded throughout the entire primary ejection period with Burkard 7-day volumetric spore traps, placed directly on the ground at the inter-row level of the orchard. During days with particularly high ejections, *i.e.*, greater than 5% of the total quantity of ascospores trapped for the year, concentrations of more than 400 ascospores/m\(^3\) of sampled air could be observed in the two regions. Using meteorological data recorded by the weather stations located near the orchards studied, it was possible to model daily ascospore ejections with two types of decision support software used on a regular basis in France for agricultural warning systems. However, these models did not correctly estimate a significant number of large ejections for some of the years. It would therefore be unrealistic to recommend the use of these modelled values of daily ascospore ejections for pest control strategies requiring precise details about these quantities, without taking excessive risks. On the other hand, it seems possible to use these two models to determine the period (from 1 to 2 months, depending on the year) during which the aerial concentrations of ascospores are the highest.

**Key-words:** apple scab, *Venturia inaequalis*, ascospores, aerial concentrations, modelling

**Introduction**

Apple scab, caused by *Venturia inaequalis* (Cooke) G. Wint., is one of the most serious diseases of apple worldwide. Ascospores released from pseudothecia on overwintered infected leaves in the leaf litter are the main source of scab primary inoculum (Holb *et al*., 2004). In France, the most commonly used disease management strategy to control apple scab relies on a chemical protection applied during the primary period of ascospore ejections for any “Mills contamination risk” level, including the “Angers risk” (Olivier, 1986). A total of 15 to 20 fungicide treatments per year may thus be necessary to control the disease, according to years and regions.

However, it is possible to reduce the chemical protection during this primary period (Brun *et al*., 2007). In the case of low-susceptibility cultivars, the integrated control scheme proposed by Olivier (1986) takes the inoculum present in autumn in the orchard and the intensity of the ejection of ascospores into account. In the case of *Vf*-resistant cultivars, it is recommended to protect high contamination risk periods (only the main period of ascospore ejections associated with some levels of Mill’s risks have to be protected).

To be able to apply such strategy, it is necessary to determine accurately the main period of ascospore ejections (from 5 to 95 % of the ascospore stock ejected).
Materials & Methods

The aerial concentration of ascospores was assessed with Burkard 7-day volumetric spore traps (Burkard Manufacturing Co. Ltd., Rickmansworth Hertfordshire, England) installed on the orchard ground. Monitoring was made for 4 years in organic orchards of the Valence region (Drôme, France) and in experimental orchards not sprayed against scab in Maine-et-Loire (France).

The modelling of ascospore ejections was based on data from weather stations located near the orchards computed by the 2 softwares used in France by extension advertising networks:
*Pulsowin®* (Pulsonic, Orsay, France), decision aid software intended for use by fruit growers, and Melchior®, software used by the French Regional Plant Protection Services, 2 similar softwares based on maturation curves of Lagarde (1988);
*RIMpro®* (version 2.0.5; Bio Fruit Advies, Zoelmond, the Netherlands) based on maturation curve known as the New Hampshire curve (Giraud & Trapman, 2006).

Results & Discussion

In this study, the aerial ascospore traps were placed directly *in situ* in the orchards during the entire primary season. Working directly *in situ* and not on artificial apple leaf litters outside of the orchard allowed us to take the actual maturation conditions of the perithecia in the orchard into consideration. By measuring the aerial concentration of ascospores at 45 cm above the ground, we were able to accurately estimate the inoculum ejected from the leaf litter that was then deposited on the foliage (Carisse *et al.*, 2007). During days with particularly high ejections, *i.e.*, greater than 5% of the total quantity of ascospores trapped for the year, concentrations of more than 400 ascospores/m³ of sampled air could be observed in the two regions.

Table 1. Differences in day numbers for the 5% and 95% ascospore ejection points between models and trap measures in Maine-et-Loire en 2004, 2005, 2006 and 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Total of ejected ascospores</th>
<th>5% of the stock</th>
<th>95% of the stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>RIMpro® 6289</td>
<td>1 day B</td>
<td>1 day B</td>
</tr>
<tr>
<td></td>
<td>Pulsowin® 100%</td>
<td>3 days A</td>
<td>26 days A</td>
</tr>
<tr>
<td>2005</td>
<td>RIMpro® 6476</td>
<td>4 days B</td>
<td>8 days B</td>
</tr>
<tr>
<td></td>
<td>Pulsowin® 100%</td>
<td>17 days B</td>
<td>2 days A</td>
</tr>
<tr>
<td>2006</td>
<td>RIMpro® 4753</td>
<td>The same day</td>
<td>21 days B</td>
</tr>
<tr>
<td></td>
<td>Pulsowin® 100%</td>
<td>3 days B</td>
<td>7 days B</td>
</tr>
<tr>
<td>2007</td>
<td>RIMpro® 6070</td>
<td>4 days B</td>
<td>17 days A</td>
</tr>
<tr>
<td></td>
<td>Pulsowin® 100%</td>
<td>22 days A</td>
<td>36 days A</td>
</tr>
</tbody>
</table>

A: after spore trap; B: before spore trap

Applying specific strategies of treatment in the period between 5 to 95% of the ejections will only be possible if the discrepancy between the prediction of this period by the model(s) and biological facts is not too large. Pulsowin® predict 5% of the stock ejected too late 1 year/4 in Maine-et-Loire, and Melchior® and RIMpro® 2 years/4 in the Drôme department (Table 1 and 2; Figure 1). Pulsowin® and RIMpro® predict the end of important ejections (95% yet ejected) too early 1 and 2 years/4 respectively in Maine-et-Loire, and RIMpro® 1 year/4 in Drôme (Table 1 and 2; Figure 2).
For the Maine-et-Loire Department, the announcement of 5% of the ascospore stock ejected according to RIMpro® could therefore be used to identify the beginning of the period of dense ejections. In contrast, the end of the dense ejection period could not be identified with the models at no risk.

For the Drôme Department, it is therefore not possible to predict the beginning of the dense ascospore ejection period with these two models. We thus propose, by default, to consider the ejections as potentially dense as soon as the apple trees have reached the stage of susceptibility to apple scab (C-C3 Fleckinger stage). On the other hand, it seems that the announcement of 95% of the ascospore stock ejected according to Melchior® can be used to detect the end of the dense ejection period in the Drôme at no risk.

Table 2. Differences in day numbers for the 5% and 95% ascospore ejection points between models and trap measures in Drôme in 2004, 2005, 2006 and 2008.

<table>
<thead>
<tr>
<th></th>
<th>Total of ejected ascospores</th>
<th>5% of the stock</th>
<th>95% of the stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loriol 2004</td>
<td>5314</td>
<td><strong>5 days A</strong></td>
<td>2/3 days A</td>
</tr>
<tr>
<td>Melchior®</td>
<td>100%</td>
<td>The same day</td>
<td>21/22 days A</td>
</tr>
<tr>
<td>Pulsowin®</td>
<td>100%</td>
<td>The same day</td>
<td>21/22 days A</td>
</tr>
<tr>
<td>Gotheron 2005</td>
<td>5675</td>
<td>1 day A</td>
<td>3 days B</td>
</tr>
<tr>
<td>Melchior®</td>
<td>100%</td>
<td>9 days B</td>
<td>3 days B</td>
</tr>
<tr>
<td>RIMpro®</td>
<td>6097</td>
<td><strong>6 days A</strong></td>
<td>2 days A</td>
</tr>
<tr>
<td>Melchior®</td>
<td>100%</td>
<td><strong>8 days A</strong></td>
<td>4 days A</td>
</tr>
<tr>
<td>RIMpro®</td>
<td>5590</td>
<td>6/10 days B</td>
<td><strong>19 days B</strong></td>
</tr>
<tr>
<td>Melchior®</td>
<td>100%</td>
<td><strong>2/6 days A</strong></td>
<td>3 days B</td>
</tr>
</tbody>
</table>

A: after spore trap(s); B: before spore trap(s)

Figure 1: Ascospore ejections according to trap information and models in La Rétuzière (Maine-et-Loire) in 2007.
Figure 2: Ascospore ejections according to trap information and models in Gotheron (Drôme) in 2008.

Acknowledgements

The authors acknowledge Claudine Foubert, Christophe Brouard, Sylvain Hanteville, the students Estelle Dumont, Benjamin Langendorf and Maël Baudin for their contribution to this work.

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Efficiency of association of scab control methods on resistance durability of apple: the case study of cultivar Ariane

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Abstract: The major resistance gene \textit{Vf} has been deployed in several commercial cultivars. This resulted in the emergence of virulent isolates of \textit{Venturia inaequalis} in Europe. In France, isolates virulent to \textit{Vf} developed since 1995, mainly in North-West region. To increase the durability of resistance of \textit{Vf} cultivars in regions where virulent isolates are not yet present (or present at a low frequency), it was recommended that leaf litter be destroyed in winter and to apply fungicides at times of the highest scab risks. These recommendations, however, had not been evaluated experimentally previously. In 2004, we initiated a project to test these recommendations on cultivar ‘Ariane’, which has been deployed in France since 2002. Our objective was to evaluate the efficiency of association of scab control methods to delay the breakdown of \textit{Vf}. In an experimental orchard planted with ‘Ariane’, we compared scab development in 3 untreated and 3 treated plots. In untreated plots, scab increased quickly to 98% of scabbed trees and 35% of scabbed fruits in 2008, showing the high susceptibility of ‘Ariane’ in case of breakdown of its resistance. In treated plots, destruction of leaf litter was performed each year, and 5 to 9 fungicides were sprayed each spring to cover medium and high risks of scab development following Mill’s curves. For comparison, about twice the fungicide sprayings were applied in the same region on susceptible cultivars. In 2008 on the treated plots, 4% of the trees presented a very low severity of disease, and 0.2% of scabbed fruits were observed. This study shows the efficiency to associate sanitation and reduced number of fungicide sprayings to complement \textit{Vf} resistance and delay its breakdown.

Key words: major resistance, apple scab, \textit{Venturia inaequalis}, durability of resistance, fungicide, sanitation

Introduction

Most of the apple cultivars grown in orchards are susceptible to scab, and numerous fungicide sprays are necessary to control the disease. The use of scab resistant cultivars constitutes one of the alternatives to fungicide treatments, but presents the disadvantage that breakdown of resistance can occur, at least in case of major genes. The major resistance gene \textit{Vf} has been deployed in several commercial apple cultivars. This resulted in the selection of virulent isolates of \textit{Venturia inaequalis} in Europe (Parisi \textit{et al.}, 1993; Parisi \textit{et al.}, 2004). In France, \textit{Vf} has been used mainly in the northwest of the country (in regions: Nord, Normandie and Bretagne). The first virulent isolates on \textit{Vf} were observed in 1995 on cultivar ‘Judeline’ (Parisi \textit{et al.}, 2000), and developed in the 2000s (Guerin and Le Cam, 2004), resulting in the susceptibility to scab of ‘Judeline’ in this region. Other \textit{Vf} cultivars have been planted in France, but represented a very low acreage. Since 2002, the percentage of acreage planted with \textit{Vf} cultivars has increased with the planting of cultivar ‘Ariane’ (Laurens \textit{et al.}, 2005) in the different areas of apple production in France (with 500 ha in 2007).
To increase the durability of resistance of \( V_f \) cultivars in regions where virulent isolates are not yet present (or present at a low frequency), it was recommended that leaf litter be destroyed in winter (Gomez et al., 2007) and to apply fungicides when the the highest scab risks were found (Olivier, 1986). These recommendations, however, had not been evaluated experimentally. The aim of this study was to evaluate the efficiency of association of control methods to increase the durability of \( V_f \) in the case of ‘Ariane’, in a region where the frequency of isolates virulent to \( V_f \) was initially low.

**Materials and methods**

The experimental orchard was planted in 1999 in Loire Valley (Champigné, France), and comprised 6 plots of ‘Ariane’ (78 trees per plot) surrounded by non-host hedges (for details, see Didelot et al., same volume).

‘Ariane’ carries the major gene \( V_g \) in addition to \( V_f \), but \( V_g \) does not increase the efficiency of resistance, because of the existence of race 6 overcoming \( V_f + V_g \) (Parisi and Lespinasse, 1996). In this orchard, the first symptoms of scab were observed on ‘Ariane’ in 2004 in one plot adjacent to the present experiment.

Since 2005, 3 plots were maintained without any protection against scab and 3 plots were protected against scab by the association of leaf litter removal and applications of fungicides in case of moderate and severe Mill’s risks (for details, see Didelot et al., same volume).

From 2005 to 2008, scab was assessed on each tree on a 1 to 9 scale (adapted from Latour et al., 1998) according to the % of scabbed leaves in June and September. At harvest, fruits were sampled randomly on the 4 central rows of each plot and the percentage of scabbed fruits was evaluated.

**Results**

Five to 9 fungicide treatments against scab were applied each spring from 2005 to 2008 (Table 1), which covered all periods of medium and high risk. No fungicide was applied after the end of the discharge of ascospores.

<table>
<thead>
<tr>
<th>Year</th>
<th>N° of scab risks* (Mill's curves)</th>
<th>N° of fungicide treatments against scab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Angers/Low Medium/High</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>7 5</td>
<td>6</td>
</tr>
<tr>
<td>2006</td>
<td>5 4</td>
<td>5</td>
</tr>
<tr>
<td>2007</td>
<td>11 6</td>
<td>6</td>
</tr>
<tr>
<td>2008</td>
<td>9 10</td>
<td>9</td>
</tr>
</tbody>
</table>

* Number of risks between susceptible stage of apple and end of projection of ascospores

In untreated plots, a large increase of scab was observed between 2007 and 2008 on leaves and on fruits (Figure 1), leading to 98% of scabbed trees and 35% of scabbed fruits in 2008. This increase resulted from climatic conditions very favorable to scab. In treated plots, the increase of scab was slow with 4% of scabbed trees at the end of June 2008 and 0.2% of scabbed fruits at harvest 2008. On these scabbed trees, less than 5% of leaves were infected, whereas on untreated plots the mean scab severity corresponded to about 15% of scabbed
leaves, with the presence of trees with more than 50% of scabbed leaves (Figure 2).

**Figure 1.** Increase in scab incidence on ‘Ariane’ from 2005 to 2008 for treated (leaf removal + fungicides) and untreated plots. A: scab on leaves in June; B: scab on fruits at harvest

**Figure 2.** Comparison of the distribution of scab severity in June 2008 on ‘Ariane’ for treated (leaf removal + fungicides) and untreated plots; 1: no scab, 5: 25% of scabbed leaves, 7: 50% of scabbed leaves, 9: >90% of scabbed leaves

**Discussion**

This study shows that scab quickly increased in untreated plots, showing the high susceptibility of ‘Ariane’ when virulent isolates of *V. inaequalis* develop. In case of breakdown of resistance, ‘Ariane’ should be treated as a susceptible cultivar (e.g. ‘Gala’). In the 3 untreated plots of our experiment, leaf removal will be performed and fungicide treatments will be applied in 2009 for all scab risks. This result confirms the importance of resistance management of *Vf* apple scab resistant cultivars (cf Trapman, this volume) in order to delay as much as possible the increase in frequency of virulent isolates.

By contrast, the level of disease on the treated plots remained low, although climatic conditions were very favourable to scab development. These results show the efficiency of combining sanitation with reduced number of fungicide sprays to complement *Vf* resistance and delay its breakdown. This experiment was carried out in severe conditions for scab, with a higher amount of primary inoculum than usually found in commercial orchards. The combination of control methods resulted in the maintainence of good efficiency of the *Vf*
resistance, and the number of treatments against scab was reduced to about half the number of fungicide treatments applied in the same region on susceptible cultivars. This experiment will continue in the future, in order to evaluate the efficiency of these practices on the control of disease and resistance durability precisely.

Acknowledgements

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References

Control of oriental fruit moth, *Cydia molesta* (Busck), by Isomate OFM rosso dispensers in peach orchards in Bulgaria – preliminary results

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Abstract. Peach is the major fruit in South-East Bulgaria. Its main pest is the oriental fruit moth (OFM), *Cydia molesta* Busck. For a long time pest management in stone fruit production in Bulgaria relied on organophosphate and pyrethroid insecticides. Although originally quite effective, recently their effectiveness decreased, apparently due to the resistance developed in many pests. Hence, alternative means of control are urgently needed. The most common environmentally friendly methods are those related to sex pheromones. Until recently, their use has been limited mainly to monitoring, aiming at precise timing and reduction of chemical treatments. Mating disruption (MD) presents a more promising solution, however. The trials on mating disruption in the present study were carried out with Isomate OFM rosso dispensers (Shin-Etsu, Japan) in an isolated 10-ha peach orchard in 2007 and 2008. Pheromone trap catches were completely inhibited in the MD block whereas they were numerous in the reference, i.e. conventionally treated orchard. The Isomate OFM rosso dispensers, installed before the first flight of OFM at the rate of 500 units per ha, efficiently reduced fruit damage – down to 0.1-0.2% at harvest. In the reference orchard, with 5-6 insecticide treatments against OFM, damage still reached 5-6%. The results indicate that mating disruption for control of oriental fruit moth may be effective in Bulgaria. Its use will be helpful in meeting the requirements of EU for residues free fruit production.

Keywords: IPM, peach, oriental fruit moth, mating disruption, Isomate OFM rosso dispensers

Introduction

Peach is one of the major fruit species in the south-eastern part of Bulgaria. It provides rapid recovery of investments due to early bearing and relatively small phytosanitary problems (Kolev and Jivondov 2000). Its main pest is the oriental fruit moth (OFM), *Cydia molesta* Busck. The larvae of early OFM generations damage current season shoot tips; then they feed in the developing fruitlets and fruits. For a long time pest management in stone fruit orchards in Bulgaria relied on organophosphate and pyrethroid insecticides. Albeit originally effective, they caused environmental problems and increasing consumer concerns. Recently their effectiveness decreased, apparently due to resistance developed in many pests. Therefore, alternative means of control are urgently needed. The most frequently applied environmentally friendly methods are those related to sex pheromones. Their use has been first limited to monitoring, aiming at precise timing and reduction of chemical treatments. Mating disruption (MD) presents a more promising solution, however though.

Positive results of mating disruption of *Cydia (Grapholitha) molestata*, have been reported from South Africa by Barnes & Blomefield (1997), from Italy by Trematerra *et al.* (2000) and from Australia by Sexton & Il’ichev (2000). According to the studies of Molinari *et al.* (2000), efficacy of synthetic pheromones applied for mating disruption of *C. molestata* and *A. lineatella* was very high. In 74 tests in 1998 and in 119 tests in 1999 damage was below 1%.
In the recent investigation of Molinari (2007), confusion methods, involving 300-1000 pheromone dispensers per ha, reduced OFM reproduction in peach orchards provided that the dispensers had been installed before the start of the first flight. They remained effective through the second and third generations of the pest. MD proved also to be effective in control of OFM in peach orchards of Slovenia (Rot & Blazič 2005) and New Zealand (Lo & Cole 2007).

Materials and methods

**Trial orchard**
The well-isolated, 10-ha commercial orchard near village Chokoba, Sliven region was established in spring 2005. In the years 2007-2008 a trial on mating disruption (MD) of the oriental fruit moth (*Cydia molesta* Busck.) was carried out there with Isomate OFM rosso dispensers and aimed at the assessment of this method applied for the first time in Bulgaria.

The Isomate OFM rosso dispensers were installed in the trial orchard at the beginning of April, about the start of OFM flights. The dispensers were hung in the upper third of tree canopies at a density of 500 pieces per ha. According to the manufacturer, each dispenser is loaded with a minimum of 240 mg of the OFM pheromone mixture. Against other pests occurring in the trial plot, only one aphicide treatment was applied each in 2007 and 2008.

**Reference orchard**
Another orchard of 2-ha in area served as a reference and was treated conventionally. It was located near the city of Sliven and established in 2003. Twelve treatments (14 active ingredients) were applied there during each season, to control OFM and other pests. Six of the treatment applications were timed against oriental fruit moth. The fruit damage by OFM in this orchard in the years 2004-2006 was about 5%. The economic threshold in Bulgaria is 4-6% damaged fruits at harvest time.

**Indices studied**
Monitoring of OFM flights was carried out by sex trapping in both seasons. Three triangular traps were installed in the trial orchard – they were baited with a standard capsule (Csalomon) containing orfamone. The traps were installed in the centre and at the edge of the trial orchard before OFM flights started. For comparison, 2 standard traps were installed in the reference, conventionally treated orchard. All pheromone traps were checked twice a week.

Early in the season sampling of damaged shoots was carried out on 50 trees, randomly chosen in the trial plot and in the reference orchard. During the season, sampling for fruit damage was carried out in the trial and reference plot on 1000 fruits at each sampling. At harvest, 3000 fruits were sampled in both orchards, to evaluate the final damage rate.

**Evaluation of data**
Data on catches of male moths in the pheromone traps were considered as totals for each date of control and presented in a graphical form. The rate of fruit damage by OFM was expressed as percentage of damaged fruits. Significance of differences in damage rate between the trial and reference orchard was estimated by use of the Chi-square test.

**Results**

**OFM flight dynamics**
In the reference commercial orchard with conventional treatments the first flight of oriental fruit moth in 2007 began on April 2. In the trial plot two moths were recorded in the
pheromone traps on April 5, when the Isomate OFM rosso dispensers were installed. The flights of the overwintering generation OFM reached their maximum by the third decade of April and continued with varying intensity, till the end of May (Fig. 1). The flight of the first summer generation, which overlapped the overwintering one, started at the beginning of June, reached its maximum in the third decade of June and continued till the end of July. The second generation began to fly at the end of July and finished at the end of August. The third generation began at the end of August and finished on October 8. The traps installed in the reference orchard caught in total 442 moths. In the trial plot, after installation of Isomate OFM rosso dispensers, no moths were caught in the pheromone traps.

In 2008, the first flight of OFM in the reference orchard began on April 4. In the trial plot no moths were recorded in the pheromone traps till April 10, when the Isomate OFM rosso dispensers were installed. The flights of the overwintering OFM generation reached its maximum in the second decade of April and continued till the end of May (Fig. 2). The flights of the first summer generation, that overlapped the overwintering one, began at the beginning of June, reached its maximum in the third decade of June and continued till the end of July. The second generation began at the end of July and finished at the end of August. The flights of a partial third summer generation began at the end of August and finished on September 19. The traps installed in the reference orchard caught in total 559 moths. In the trial plot, no moths were caught in the pheromone traps during the whole season.
Evolution of shoot and fruit damage by OFM during the season

In the reference, conventionally treated orchard, severe shoot damage caused by OFM was noted at the end of May in both years of study (Table 1). At the same time, no shoots were damaged in the trial plot, where Isomate OFM rosso dispensers were installed. Damage rates on shoots were significantly different between the treated plots and the reference orchard on 25 May of both years (Chi-square test, $P < 0.01$).

First signs of fruit damage were noted in the reference orchard at the end of June of each year. Starting from the end of June, through August and September, fruit damage rate steadily increased, reaching 6.2% in 2007 and 6.4% in 2008 at harvest. In the trial plot few damaged fruits were noted at the end of the season; at harvest it was also negligible: 0.1% in 2007 and 0.2% in 2008. Damage rates were significantly different between the treated plots and the reference orchard already on 29 June and 29 July 2007, 21 June and 18 July 2008 (Chi-square test, $P < 0.01$) and thereafter until harvest in both years of the study (Chi-square tests, $P < 0.001$).

Table 1. Evolution of shoot and fruit damage by OFM in the Isomate OFM rosso trial plot and in the conventionally treated reference orchard in two successive years of study

<table>
<thead>
<tr>
<th>Index</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>Trial plot</td>
</tr>
<tr>
<td>Shoot damage</td>
<td>2007</td>
<td>2008</td>
</tr>
<tr>
<td>[%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>May 13</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>May 25</td>
<td>0.0</td>
<td>30.0</td>
</tr>
<tr>
<td>June 9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>June 29</td>
<td>0.0</td>
<td>1.5</td>
</tr>
<tr>
<td>July 5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>July 29</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>August 12</td>
<td>0.0</td>
<td>4.8</td>
</tr>
<tr>
<td>September 6</td>
<td>0.1</td>
<td>6.2</td>
</tr>
<tr>
<td>preharvest</td>
<td>0.03</td>
<td>5.3</td>
</tr>
<tr>
<td>at harvest</td>
<td>0.1</td>
<td>6.2</td>
</tr>
</tbody>
</table>

| Fruit damage  | 2007 | 2008       |
| [%]           | 2007 | 2008       |
| preharvest    | 0.03 | 5.3        |
| at harvest    | 0.1  | 6.2        |

Discussion

In the reference orchard, damage caused by OFM was considerable, in spite of 6 conventional treatments applied against OFM. It is suspected that the population of OFM in this orchard has developed resistance to some of the insecticides used. Resistance of OFM to organophosphate, pyrethroid and carbamate insecticides was detected in Canada by Pree et al. (1998) and Kanga et al. (1990) and was considered as the main cause of failure of conventional plant protection. Apparently a similar situation may have occurred in Bulgarian peach orchards.

Application of mating disruption with use of Isomate OFM rosso dispensers significantly reduced OFM incidence and damage caused by this pest. This is in line with the reports from other countries (Barnes & Blomefield 1997; Trematerra et al. 2000; Molinari et al. 2000; Sexton & Il’ichev 2000; Rot & Blazič 2005; Molinari 2007; Lo & Cole 2007) and indicates that the MD method may be successfully introduced in Bulgarian peach orchards. This new technology may serve as an alternative means for control of OFM. Its use will be helpful in
meeting the requirements of EU for residue free fruit production.

Acknowledgements

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References


An integrated approach for reducing fungicide sprays against scab in organic apple orchards

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Abstract: The aim of this study was to evaluate scab control efficacy in integrated approaches of i) three sanitation treatments (fallen leaf removal combined with winter pruning and non-sanitized control), ii) three onsets of first fungicide sprays (dormant bud, early tight cluster and pink bud stage), and iii) three final dates for finishing fungicide programs (mid-July, mid-August and mid-September) in an organic apple orchard on a moderately scab susceptible cultivar, Jonathan. A delay in the onset of first spray until pink bud stage resulted in higher scab incidences on both leaves (16-21 %) and fruits (13-15 %) compared with the non-delayed spray treatments (5-8 % and 6-9 %, respectively). Final leaf and fruit scab incidences increased significantly when sprays were omitted after mid-July compared to spray treatments finished at mid-August or mid-September. A combination of leaf removal with pruning resulted in lower scab incidence (5-12 %) compared with the non-sanitized plots (7-15 %) when spray treatments were finished at mid-August or mid-September. Results on cv. Jonathan suggested that scab sprays could only be omitted before early tight cluster and after mid-August if leaf removal and pruning was applied.

Key words: organic production, apple scab, apple, sanitation, pruning, spray omission

Introduction

MacHardy et al. (1993) developed a ‘potential ascospore dose (PAD)’ action threshold in New Hampshire for delaying the onset of scab fungicide programs in the early spring. With this action threshold, one to four sprays can be saved at the beginning of the year (MacHardy et al., 1993; MacHardy, 1996). Action thresholds were also developed for timing of final fungicide application on the harvest scab threshold (1% fruit incidence) (Scheer, 1992; Holb et al., 2003). Using this strategy, another two to four sprays are likely to be saved at the end of the growing season (Scheer, 1992; Holb et al., 2003). Action thresholds were developed in integrated orchards where PAD dose of V. inaequalis was low. These were not tested in organic orchards.

Winter pruning and leaf removal of fallen leaves for reducing primary scab inoculum sources were extensively studied in order to decrease pesticide use in apple orchards (Holb, 2006; Holb et al., 2005). It might be possible that a broad-scale integrated approach including leaf removal, winter pruning and savings of sprays at both the beginning and the end of the growing seasons might provide reductions of fungicide use in organic orchards.

The aim of this study was to evaluate scab control efficacy in integrated approaches of i) three sanitation treatments including fallen leaf removal and its combination with winter pruning, ii) three onsets of first fungicide sprays, and iii) three final dates for finishing fungicide programs.

Material and methods

A two-year study (2005 and 2006) was carried out in a 15-ha organic apple orchard in
Eperjeske, eastern Hungary. Trees were grafted on M26 rootstock and pruned to spindle shape. Orchard soil type was brown forest soil. Organic production guidelines (Anon., 1998) have been applied since the orchard was established in 1996. Leaf wetness duration (hr) was detected from 10 March until 30 September in both years and apple scab infection periods were calculated.

The experimental design was a split-plot with the two years as blocks, three sanitation treatments combined with three onsets of first fungicide spray as main plots and three final spray dates as sub-plots. Nine equally sized treatments of orchard sanitation practices combined with first spray application timing were applied as follows: 1) sprays from dormant bud stage without sanitation; 2) sprays from early tight cluster without sanitation; 3) sprays from pink bud stage without sanitation; 4) sprays from dormant bud stage with leaf removal; 5) sprays from early tight cluster stage with leaf removal; 6) sprays from pink bud stage with leaf removal; 7) sprays from dormant bud stage with leaf removal combined with pruning; 8) sprays from early tight cluster stage with leaf removal combined with pruning; 9) sprays from pink bud stage with leaf removal combined with pruning. In treatments 4-9, fallen leaves were collected according to Holb (2006). In treatments 7-9, the upper third of all shoots of a tree was pruned according to Holb et al. (2005). In treatments 1, 4 and 7, the first spray was applied at dormant bud stage; in treatments 2, 5 and 8, at early tight cluster stages; in treatments 3, 6 and 9, at pink bud stages according to the ‘PAD’ action threshold criterion of MacHardy et al. (1993). Each treatment was replicated three times and each replicate consisted of a minimum of 330 trees (0.33 ha). Within each main plot, three final spray date treatments were applied. Final spray date was 1) in mid-September, which followed the final spray date in the general orchard spray schedule, 2) in mid-August following the ‘foliar scab’ action threshold criterion of Holb et al. (2003) and 3) in mid-July, which was based on the fact that fruit susceptibility to scab greatly decreases after this date (Schwabe et al., 1984). Each sub-plot consisted of a minimum of 110 trees.

In spring, the potential ascospore dose (PAD), was calculated in each sub-sub-plot treatment according to Gadoury and MacHardy (1986). PAD was calculated as the average of 10 replicates for each year and treatment. Disease assessments were made on leaves and fruits of cv. Jonathan at mid-July, mid-August, and mid-September. Observations were made in five randomly selected trees. For leaf assessment, disease was assessed on 50 leaves of five selected twigs. For fruit assessment, 25 typical fruits for the given phenological stage were observed.

Disease incidences were analyzed by split-split-plot analysis of variance (Statistical Analysis System v. 8.1; SAS Institute Inc., Cary, NC). Then, the nine treatments were partitioned into pre-planned contrasts including: i) 1, 2 and 3 vs. 4, 5 and 6; ii) 1, 2 and 3 vs. 7, 8 and 9; iii) 1, 4 and 7 vs. 2, 5 and 8; iv) 1, 4 and 7 vs. 3, 6 and 9; v) 1, vs. 2 and 3; vi) 4, vs. 5 and 6; and vii) 7, vs. 8 and 9. Means were separated by Least Significance Difference (LSD)-test using LSD0.05 values.

**Results and Discussion**

The Mills infection periods from mid-March until mid-October were severe on 19 and 16, moderate on 8 and 6, and low on 7 and 7 occasions (in total 34 and 29) in 2005 and 2006, respectively. Estimated mean PAD ranged between 5977.1 and 8345.2 ascospores m\(^{-2}\) in the non-sanitized plots and between 235.7 and 579.0 ascospores m\(^{-2}\) in the sanitized plots.

Analysis of variance of final scab incidence indicated significant differences ($P<0.05$) between the two cultivars, among nine treatments, three final dates of spray applications, and two years (data not shown). For leaf incidences, when the spray schedule finished in mid-July, there were no significant differences among sanitation treatments combined with onset of first
except one case in 2005 when non-sanitation treatments were compared between sprays started at dormant bud stage and pink bud stage (Table 1). At final spray dates of mid-August or mid-September, leaf scab incidence at harvest was significantly lower ($P<0.05$) in all treatments sprayed from dormant bud stage (1, 4, and 7) compared to all treatments sprayed after pink bud stage (3, 6, and 9) (Table 1). For fruit incidences, at final spray date of mid-July, there were no significant differences in either sanitation treatments or various onsets of first sprays (Table 2). At final spray dates of mid-August and mid-September, fruit scab incidence was significantly higher ($P<0.05$) in all non-sanitized treatments compared with corresponding treatments of leaf removal combined with pruning, as well as in the sanitized plots combined with spray programs started after pink bud stage (6 and 9) compared with those started at dormant bud stage (4 and 7).

Table 1. Leaf scab incidence (%) of cultivar Jonathan at three final spray dates in nine treatments of sanitation practices combined with onset of first spray applications in an organic apple orchard at Eperjeske (Hungary, 2005-2006)

<table>
<thead>
<tr>
<th>Treatments/Final spray date</th>
<th>mid-July</th>
<th>mid-August</th>
<th>mid-Sept.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NonsanitDorm (1)</td>
<td>14.5</td>
<td>8.3</td>
<td>6.7</td>
</tr>
<tr>
<td>NonsanitCluster (2)</td>
<td>16.1</td>
<td>11.4</td>
<td>9.5</td>
</tr>
<tr>
<td>NonsanitPink (3)</td>
<td>20.6</td>
<td>14.7</td>
<td>13.5</td>
</tr>
<tr>
<td>RemovalDorm (4)</td>
<td>14.2</td>
<td>7.8</td>
<td>6.2</td>
</tr>
<tr>
<td>RemovalCluster (5)</td>
<td>14.3</td>
<td>10.4</td>
<td>9.0</td>
</tr>
<tr>
<td>RemovalPink (6)</td>
<td>17.1</td>
<td>13.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Removal+PruningDorm (7)</td>
<td>13.8</td>
<td>6.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Removal+PruningCluster (8)</td>
<td>13.1</td>
<td>9.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Removal+PruningPink (9)</td>
<td>16.9</td>
<td>12.9</td>
<td>11.8</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>5.93</td>
<td>5.06</td>
<td>4.16</td>
</tr>
</tbody>
</table>

Significance of contrasts

| Nons vs. Rem               | 0.212$^c$ | 0.435 | 0.567 | 0.802 | 0.391 | 0.545 |
| Nons vs. Rem+Prun          | 0.106     | 0.246 | 0.259 | 0.332 | 0.096 | 0.367 |
| Dorm vs. Cluster           | 0.893     | 0.071 | 0.051 | 0.474 | 0.120 | 0.450 |
| Dorm vs. Pink              | 0.058     | 0.001 | <0.001 | 0.602 | 0.001 | 0.001 |
| NonsDorm vs. NonsClus-Pink | 0.049 | 0.038 | 0.011 | 0.144 | 0.026 | 0.037 |
| Rem+PruningDorm vs. Rem+PruningPink | 0.592 | 0.049 | 0.021 | 0.180 | 0.044 | 0.047 |

$^a$ Non-sanitized Dorm, from dorm bud stage without sanitation; Non-sanitized Cluster, from early tight cluster without sanitation; Non-sanitized Pink, from pink bud stage without sanitation; Non-sanitized Dorm+Pruning, from dorm bud stage with leaf removal; Non-sanitized Cluster+Pruning, from early tight cluster stage with leaf removal; Non-sanitized Pink+Pruning, from pink bud stage with leaf removal; Non-sanitized Dorm+PruningDorm, from dorm bud stage with leaf removal combined with pruning; Non-sanitized Dorm+PruningCluster, from early tight cluster stage with leaf removal combined with pruning; Non-sanitized Dorm+PruningPink, from pink bud stage with leaf removal combined with pruning.

$^b$ For details of the treatment comparisons using contrasts see Table 1, footnote b.

$^c$ Probability values from analyses of variance. Values in bold represent significance level at $P<0.05$.

Table 2. Fruit scab incidence (%) of cultivar Jonathan at three final spray dates in nine treatments of sanitation practices combined with onset of first spray applications in an organic apple orchard at Eperjeske (Hungary, 2005-2006)

<table>
<thead>
<tr>
<th>Treatments/Final spray date</th>
<th>mid-July</th>
<th>mid-August</th>
<th>mid-Sept.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NonsanitDorm (1)</td>
<td>12.4</td>
<td>9.9</td>
<td>9.4</td>
</tr>
<tr>
<td>NonsanitCluster (2)</td>
<td>13.3</td>
<td>10.3</td>
<td>9.9</td>
</tr>
<tr>
<td>NonsanitPink (3)</td>
<td>15.3</td>
<td>12.9</td>
<td>12.7</td>
</tr>
<tr>
<td>RemovalDorm (4)</td>
<td>11.8</td>
<td>8.5</td>
<td>7.7</td>
</tr>
<tr>
<td>RemovalCluster (5)</td>
<td>12.8</td>
<td>9.1</td>
<td>8.1</td>
</tr>
<tr>
<td>RemovalPink (6)</td>
<td>14.3</td>
<td>12.1</td>
<td>11.7</td>
</tr>
<tr>
<td>Removal+PruningDorm (7)</td>
<td>11.5</td>
<td>8.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Removal+PruningCluster (8)</td>
<td>12.9</td>
<td>8.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Removal+PruningPink (9)</td>
<td>13.7</td>
<td>12.0</td>
<td>11.8</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>6.536</td>
<td>2.49</td>
<td>2.44</td>
</tr>
</tbody>
</table>

Significance of contrasts

| Nons vs. Rem               | 0.627$^c$ | 0.067 | 0.053 | 0.846 | 0.215 | 0.226 |
| Nons vs. Rem+Prun          | 0.585     | 0.047 | 0.034 | 0.897 | 0.036 | 0.049 |
| Dorm vs. Cluster           | 0.585     | 0.428 | 0.402 | 0.698 | 0.302 | 0.504 |
| Dorm vs. Pink              | 0.190     | 0.004 | 0.002 | 0.307 | 0.003 | 0.002 |
| Non-sanitized Pink         | 0.544     | 0.120 | 0.116 | 0.404 | 0.127 | 0.127 |
| Rem+PruningDorm vs. Rem+PruningPink | 0.505 | 0.049 | 0.046 | 0.948 | 0.047 | 0.025 |

$^a$ For details of treatments see Table 1, footnote a.

$^b$ For details of the treatment comparisons using contrasts see Table 1, footnote b.

$^c$ Probability values from analyses of variance. Values in bold represent significance level at $P<0.05$. 

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Potential ascospore dose was reduced below 600 ascospores m\(^{-2}\) orchard floor in all sanitation treatments and a combination of leaf removal and winter pruning significantly reduced scab incidence compared with non-sanitized plots. Under such conditions, the first spray may be delayed until pink bud stage (MacHardy et al., 1993). Despite the low amount of spring inoculum (PAD < 600 ascospores m\(^{-2}\) orchard floor), spray applications could only be omitted before early tight cluster due to the low efficacy of scab fungicides in organic apple production. This resulted in omitting two copper sprays at dormant bud and green tip stages.

Final leaf scab incidence significantly increased when sprays were omitted after mid-July. This may also result in a rapid increase of *V. inaequalis* ascospores on fallen leaves and of conidia inside bud scales (MacHardy, 1996; Holb et al., 2005). Earlier completion of the fungicide spray program was also previously shown as the cause of increased storage scab (MacHardy, 1996). However, scab incidences were similar in spray programs completed in mid-August or mid-September if a combination of leaf removal with pruning was applied. This result indicated that one to three sprays may be omitted at the end of the growing season if sanitation of scab inoculum sources is applied and cultivars are similarly susceptible to scab as Jonathan. Previous studies, in well-managed integrated orchards, also recommended omission of two to four sprays at the end of the season (Scheer, 1992; Holb et al., 2003); however, these studies used highly scab susceptible cultivars without using sanitation treatments to reduce scab inoculum.

In conclusion, our results indicated that sprays against scab could only be omitted under Eastern European conditions before early tight cluster stage and after mid-August if removal of fallen leaves combined with pruning was applied and final leaf scab incidence was <15%.

References


Searching inoculum sources of brown spot of pear

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Abstract: *Stemphylium vesicarium* causes brown spot disease on pear and leaf blights in asparagus and onion. Multiple fungicide applications for disease control are common in infested pear orchards. The fungus is also able to colonise plant debris saprophytically. The objectives of our study were (1) to determine the pathogenicity on pear of *S. vesicarium* isolates from different origins, (2) to develop a molecular tool for discrimination between isolates pathogenic or non-pathogenic on pear and (3) to quantify pear-pathogenic populations of *S. vesicarium*. *S. vesicarium* was isolated from infected pear fruits and necrotic leaves of pear, orchard lawn grasses, onion and asparagus. The pathogenicity of 116 *S. vesicarium* isolates was assessed on detached pear fruits and on leaves. Disease incidence was similar for isolates from fruits or leaves of pear or from necrotic orchards lawn grasses. Isolates from asparagus or onion caused no symptoms on pear. AFLP patterns of isolates showed clustering of isolates originating from pear orchards (either from diseased fruits or from orchard lawns), whereas onion and asparagus isolates clusters into separate groups. AFLP bands unique for pear-pathogenic *S. vesicarium* isolates were sequenced and a quantitative detection was developed based on one of these unique AFLP bands. The specific quantification of pear-pathogenic populations of *S. vesicarium* by TaqMan-PCR is currently used in studies on population dynamics in orchards. Results will be used for the development of efficient sanitation measures which will reduce the risks of brown spot epidemics.

*Stemphylium vesicarium, Pathogenicity, Detection*
The initiative “Monitoring of Venturia inaequalis virulences”

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Since the breakdown of the Vf gene in the central-north part of Europe (Parisi et al. 2004) breeders have been looking for new resistance sources to introduce in their breeding programs. Alternative major genes to Vf are available (e.g. Va, Vh, Vbj, Vm, Vr, Vh2, Vh4, Vd, Vg, Vr2,...) but, with few exceptions (e.g. Vm in the cultivars ‘Murray’ and ‘Rouville’, Crosby et al., 1992; Vh4 in the cultivar ‘Regia’, Boudichevskaia et al., 2006), they are not exploited as sources of resistance. For nearly all these R-genes molecular markers suitable for marker assisted selection (MAS) are available (reviewed in Gessler et al., 2006) and a few advanced selections that could be readily used in breeding programs are also available (M. Kellerhals ACW pers. communication).

One of the strategies that can be used to develop apple cultivars with durable apple scab resistance is the pyramiding of major genes. But which genes are the best suited to produce such new cultivars? The most interesting genes are those which have not been overcome by the pathogen. Nevertheless, genes that have been overcome, but with a limited spread of virulent races, may be used in breeding. To find information on the emergence and geographic distribution of isolates virulent to specific resistance genes is difficult and time consuming. Moreover most of the reports found are not up-dated and the correctness of the data are difficult to verify. In addition comparison of the results from different sources is also difficult as often different differential hosts have been used in different studies. To improve this situation the initiative “monitoring of V. inaequalis virulences” has been proposed. The project foresees: 1) the establishment of a large network of orchards of selected differential hosts; 2) the yearly scoring of apple scab incidence by the partners participating in the network; 3) the submission of the data to the curator of the project; and 4) regular publication of collected data through the homepage of the project.

In the first phase of the project 16 differential hosts possibly carrying a single known major resistance gene have been selected (Table 1). To coordinate the studies of the V. inaequalis-Malus pathosystem in the field with those in the laboratory, as far as plant material was available, the same genotypes proposed by Bus et al. (2009) were chosen. All the plant material has been centralized at Agroscope Changins-Wädenswil and molecularly tested. The tests confirmed that all the plant material is “true to type”. Multiplication of the plant material is ongoing.

Partners currently interested in planting a set of differential hosts are in Germany, Italy, Belgium, Czech Republic, Austria, Poland, Chile, Canada and New Zealand. However additional partners are needed. Interested partners are invited to announce their expression of interest through the homepage of the project (www.vinquest.ch) where details as well as the status of the project can also be found. Partners participating in the network should note that this is a long term project and therefore the differential orchards should be maintained as long as possible. On the other hand the time that yearly has to be dedicated to the project is quite limited. In fact it will be requested that the partners assess incidence and severity of scab of each plant once a year at the end of the primary season. Currently a differential host orchard is composed of 80 plants (16 differential hosts, 5 replications each). After the assessment, the data will be submitted to the curator of the project by e-mail. First appearance of virulent isolates will be carefully verified (pictures, verification of the “true to typeness” of the infected...
tree and preparation of single spore cultures). Upon validation of the data, the curator will summarize the collected information and will publish it through the homepage of the initiative. The proposed procedure will provide breeders, advisors, farmers and researchers with up-dated, comparable and validated information on the current geographic distribution of apple scab virulences.

Table 1: New set of differential hosts (adapted from Bus et al. 2008)

<table>
<thead>
<tr>
<th>Host</th>
<th>Accession</th>
<th>R-gene(s) (known)</th>
</tr>
</thead>
<tbody>
<tr>
<td>h0</td>
<td>‘Gala’</td>
<td>none known</td>
</tr>
<tr>
<td>h1</td>
<td>‘Golden Delicious’</td>
<td>Vg alias Rvi1¹</td>
</tr>
<tr>
<td>h2</td>
<td>TSR34T15</td>
<td>Vh2 alias Rvi2</td>
</tr>
<tr>
<td>h3</td>
<td>Q71 (‘Geneva’ x ‘Braeburn’)</td>
<td>Vh3.1 alias Rvi3</td>
</tr>
<tr>
<td>h4</td>
<td>TSR33T239</td>
<td>Vh4 alias Rvi4</td>
</tr>
<tr>
<td>h5</td>
<td>9-AR2T196</td>
<td>Vm alias Rvi5</td>
</tr>
<tr>
<td>h6</td>
<td>‘Priscilla’</td>
<td>Vf alias Rvi6</td>
</tr>
<tr>
<td>h7</td>
<td>M. floribunda 821²</td>
<td>Vf and Vfh alias Rvi6 and 7</td>
</tr>
<tr>
<td>h8</td>
<td>B45 (‘Pacific Beauty’ x M. sieversii GMAL4302-X8)</td>
<td>Vh8 alias Rvi8</td>
</tr>
<tr>
<td>h9</td>
<td>J34 ‘Gala’ x ‘Dolgo’</td>
<td>Vfj alias Rvi9</td>
</tr>
<tr>
<td>h10</td>
<td>A 723-6 (‘Worcester Pearmain’ x PI172623)</td>
<td>Va alias Rvi10</td>
</tr>
<tr>
<td>h11</td>
<td>M. baccata jackii</td>
<td>Vbj alias Rvi11</td>
</tr>
<tr>
<td>h12</td>
<td>Hansen’s baccata #2</td>
<td>Vb alias Rvi12</td>
</tr>
<tr>
<td>h13</td>
<td>‘Durello di Forlì’</td>
<td>Vd alias Rvi13</td>
</tr>
<tr>
<td>h14</td>
<td>‘Dülmener Rosenapfel’</td>
<td>Rvi14</td>
</tr>
<tr>
<td>h15</td>
<td>GMAL 2473</td>
<td>Vf2 alias Rvi15</td>
</tr>
</tbody>
</table>

¹ Nomenclature according to Bus et al. 2009.
² Currently F1 of M. floribunda 821 carrying only Vfh (alias Rvi7) is not available, as h7 has been temporarily choose M. floribunda 821.

References


Late winter climatic conditions influence ascospore production and release in Venturia inaequalis.

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Abstract: Most fungicide applications targeting apple scab aim to control primary infections caused by ascospores and spraying is thereby linked to ascospore availability. We investigated the effect of pre bud break climatic conditions on seasonal patterns of ascospore release. Apple leaves bearing pseudothecia of Venturia inaequalis were overwintered at orchard sites in 8 countries for up to 3 years. Leaf samples were collected 2 to 5 weeks before bud break and again at bud break, air dried, and sent via airmail to Norway. The samples were stored at -18 °C upon arrival until tested. Disks cut from each replicate leaf sample were incubated moist at 20 °C to allow ascospore maturation but prevent discharge. Matured ascospores were induced to discharge twice a week and enumerated until the supply was exhausted. The proportion of ascospores ejected was fitted against degree-day accumulation using logistic regression. The regression intercept (onset maturation), slope (maturation rate), as well as the absolute number of spores counted differed significantly (P < 0.001, P = 0.05, P < 0.001 respectively) among sites and sampling dates. There was a significant interaction between site and sampling date, indicating that climatic conditions prior to bud break differentially impacted the subsequent ascospore availability. Observed differences could perhaps be used to further refine previously described models of ascospore maturity.

Apple scab, Maturation model, Spray timing
Abstract: IOBC guidelines for integrated fruit production prescribe use of forecasting systems in direct plant protection. In Latvia, LPPRC, model RIMpro for apple scab *Venturia inaequalis* control was tested from 2003. Following to FRAC guidelines to reduce the risk of fungus resistance developing, from 2007 efficacy of fungicides mixtures (Chorus, a.i. cyprodinil + Dithane NT, a.i. mancozeb; Effector, a.i. dithianon + Candit, a.i kresoxim-methyl) and alternately curative or strobilurine – protective fungicides use was tested. In all cases the first protective application before scab ascospores discharge was carried out with Cu product Champion 50. In case of emergency Effector was used during the secondary scab infection period. Fungicides registered in Latvia for apple scab control were effective with a mixture of protective/curative or strobilurine products being alternately used, the exception being the strobilurine Candit (Qo inhibitor) which was used separately, until fungal resistance appeared in the 3rd season of Candit use. The efficacy of Candit/Effector mixture was on a level with other treatments and that of the curative product Chorus wasn’t lost after 6 seasons of use when applied no more than 3 times per season. Nevertheless, further strategy of resistance preclusion has to be considered and what request minimal at-risk products to use separately. In all cases fungicides applications, even Chorus/Dithane mixture, were more effective if used before infection and as weather forecasting was not always the number of necessary applications had to increase. Under Latvia conditions frequently there are three severe scab infection periods during the total primary infection period, subsequently 3 or 4 fungicides applications being necessary in addition to the first Champion treatment.
Use of the A-scab model for rational control of apple scab

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Abstract: A-scab is a dynamic model for Venturia inaequalis primary infections. It simulates ascospore maturation, ejection, deposition, and infection during the season based on hourly data of air temperature, rainfall, relative humidity, and wetness duration. A-scab produces a risk index for each infection period and predicts the time of disease onset. Since the validation works showed that the model produces accurate and robust predictions, a 3-year (2006 to 2008) experiment was carried out in order to determine the possibility of using A-scab for scheduling fungicide sprays. Trials were performed in northern Italy (at Ravenna and Bologna) by comparing: i) untreated control, ii) farming practice, iii) A-scab recommendations. The disease incidence on both leaves and fruits in the plots sprayed according to A-scab predictions did not change significantly relative to the farm practice. The use of A-scab led to a general reduction in the number of fungicide applications.

Keywords: disease modelling, Venturia inaequalis, field trials, apple scab control, fungicide reduction

Introduction

Apple scab, caused by Venturia inaequalis (Cooke) Wint., is the most important fungal disease of apple worldwide. It causes repeated infections on leaves and fruits during the season and it can cause severe yield losses when fungicides are not applied efficiently. Strategies for applying fungicides have changed greatly in recent decades, from spraying to a calendar to having a rational schedule based on actual infection risk. Mathematical models represent a valuable tool for estimating infection risk based on meteorological conditions and host information.

A-scab is a dynamic simulation model including all the stages of the infection cycle based on the principles of ‘systems analysis’ (Leffelaar, 1993). It simulates development of pseudothecia, ascospore maturation, discharge, deposition, and infection during the season based on hourly data of air temperature, rainfall, relative humidity, and leaf wetness. A-scab produces a risk index for each infection period and predicts the probable periods of symptoms appearance (Rossi et al., 2007). In a previous work, A-scab was validated under different epidemiological conditions with satisfactory results: neither corrections nor calibrations were necessary to adapt the model to different apple-growing areas (Rossi et al., 2007).

To evaluate model performances for disease control in practice, field trials were planned in two locations in Emilia-Romagna (North Italy) for three years (2006 to 2008), for comparing treatments scheduled according to the farming practice, A-scab suggestions, and an untreated control. In this paper, these field experiments and results are described and
Materials and Methods

The A-scab model

Model parts (Giosuè et al., 2000; Rossi et al., 2000, 2001, 2003a and b, 2006a and b, 2007) and the general structure with algorithms were described in details in Rossi et al. (2007). The model follows all the stages of the infection cycle and links the progression from one stage to another to the influential meteorological conditions. The model starts from overwintering pseudothecia and accounts for ascospore maturation and dispersal during the primary inoculum season. A part of these ascospores is deposited on susceptible apple leaves and cause infection. Finally, symptoms become visible at the end of the incubation period (Fig. 1).

Figure 1 - Conceptual structure of the A-scab model predicting primary infections of Venturia inaequalis; the infection severity is expressed, for each infection period during the primary inoculum season, by an index called Risk$_{inf}$.

The model produces a daily (i) risk index cumulated over each infection period, calculated as: $Risk_{inf} = \sum_{i=1}^{n} SRAdis \cdot IE_{inf} \cdot HOST_{inf} \cdot 100$

where: SRAdis is the ascospore dose ejected during any discharge event; IEinf is the proportion of ascospores that cause infection during the infection period; HOSTinf is an index accounting for host susceptibility.

The model was validated in several Italian apple growing areas by comparing actual data (ascospore releases, disease onset, disease incidence and severity) to model outputs; it never requested calibrations or modifications to be adapted to the different areas (Rossi et al., 2007).

Field trials

Field trials were planned to test the possibility of using A-scab for scheduling fungicide applications compared to the farming practice and an untreated control.
Trials were performed at two locations in the districts of Bologna and Ravenna (Emilia-Romagna region), in the period 2006 to 2008. The trials were performed on ‘Imperatore’ and ‘Pink lady’ trees, 14 and 5-year old, at the two locations, respectively. Orchards were managed according to the common practice, with exception of the fungicide sprays; two treatments with copper were applied on the entire orchard at bud break (4.5 kg/ha) and green tip (3 kg/ha) to prevent Nectria cankers. The experimental design was a randomized complete block design with three replicated plots (7 or 8 plants per plot) for the thesis A-scab and untreated control; the farming practice was applied on the remaining orchard surface.

Hourly meteorological data of air temperature (°C), relative humidity (%), rainfall (mm), and leaf wetness (yes or no) were acquired by the Regional Meteorological Service in the proximity of the experimental orchards. The model was operated each day using the actual weather data until the current day and weather forecasts for the following three days.

Fungicides against scab were applied according to model outputs: when the Risk_{inf} calculated using weather forecasts was ≥0.2, a treatment was applied using a protectant or a curative fungicide, depending on the time of application in relation to the time of the predicted infection; when the Risk_{inf} calculated using actual data was ≥0.4 an additional curative treatment was applied as soon as possible, if a protectant fungicide has been applied before. The protectant fungicides used were Dithianon (1 kg/ha) until blooming and Tryfloxxistrobin (0.22 kg/ha) after this growth stage; the curative fungicide was Difenoconazole (0.15 kg/ha) + Dithianon (1 kg/ha). In the untreated plots no fungicides against scab were applied for the entire primary season.

Disease incidence was assessed on leaves and fruits at the end of the primary inoculum season. These data were angular transformed and analysed using the ANOVA and the Tukey’s test at α=0.05 to test significant differences among averages. Data concerning the farming practice were not statistically analysed.

**Results and discussion**

Disease incidence varied strongly in the two locations and from one year to another (Fig. 2). Scab did not appear at Bologna in 2007 and reached about 60% of affected fruits in the untreated control of Bologna in 2008.

The incidence of affected leaves and fruits was always significantly lower in the plots treated according to A-scab with respect to the untreated control and similar to that obtained using the farming practice (Fig. 2). At Bologna in 2006, the percentage of affected leaves was 16% on the control and it was significantly reduced to 3.8% using A-scab recommendations (3 fungicide applications), data on the farming practice were not available as well as the correspondent number of treatments. Fifty-five percent of scabbed fruits was observed on the untreated control and 6% in the A-scab plots. Similar results were obtained at Ravenna where A-scab suggested 6 treatments which reduced significantly disease incidence with respect to the untreated control on both leaves and fruits (Fig.2); using the farming practice, 1% and 4% of scabbed leaves and fruits was observed, respectively, but the number of applications was not registered.
Figure 2 – Incidence of leaves and fruits affected by scab at Bologna and Ravenna in 2006 to 2008 in plots not sprayed against scab, treated according to the A-scab model, and following the farming practice. Bars with different letters are significantly different at P=0.05 (Tukey’s Test). Numbers in italics indicate the number of fungicide treatments in the different spray programs, na means ‘data not available’.

At Bologna in 2007 the disease did not appear: A-scab suggested only one spray while the farmer performed 8 treatments. At Ravenna the reduction with respect to the control was significant: 37% of scabbed fruits against 19% using A-scab and 18% in the farming practice, in this case both methods suggested 4 treatments.

In 2008, weather conditions were more favourable to disease development at Bologna (58 and 32% of affected fruits and leaves, respectively, on the untreated control) than at Ravenna (8 and 26% of affected fruits and leaves, respectively, on the untreated control). The use of A-scab reduced disease incidence significantly in both orchards (Fig. 2) reaching disease levels similar to those of the farming practice (Fig. 2); the number of treatments was halved at Bologna (5 and 10 with A-scab and the farming practice, respectively) while at Ravenna it was reduced by about 25% (10 against 13 with A-scab and the farming practice, respectively).

In conclusion, the timing of fungicide applications following A-scab, which is able determine accurately when weather and host conditions are favourable for infection, made it possible to save about 50% of fungicide sprays with respect to the farming practice (5 against about 9 on average per primary inoculum season) maintaining the incidence of affected leaves and fruits at comparable levels.

References

Monitoring of Venturia inaequalis strains sensitive to strobilurine fungicides and occurrence of apple scab on resistant cultivars in the Czech Republic

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Abstract: Occurrence of apple scab (Venturia inaequalis) on resistant cultivars was investigated in the Czech Republic however symptoms have never been observed up to the year 2006. Apple scab is currently recorded in six isolated plantings of resistant cultivars in the territory of the Czech Republic. Apple scab was founded only on Vf resistant cultivars (Rubinola, Topaz, Rajka, Otava, Melodie etc.) in all cases indicating that those isolates can be classified as the race 6 or 7. Monosporic isolates of V. inaequalis were prepared for next testing using plant indicators and distinction using PCR methods. At the same time, sensitivity of V. inaequalis to strobilurine fungicides was tested in the orchards, where the chemical treatment against apple scab was ineffective. Leaf samples were collected from 22 commercial orchards, one sample was taken from apple tree solitary growing in natural conditions and one sample was taken in experimental orchard. A germination of spores in aqueous fungicide solutions was assessed. A decrease of strobilurine sensitivity of V. inaequalis was observed in several localities.

Key words: VF gene, apple scab, races, strains, Venturia inaequalis

Introduction

Apple scab caused by Venturia inaequalis (Cooke)Wint. is one of the most important diseases of apples. Scab is controlled mainly by applying chemical preparations at appropriate intervals during growing season. Plant breeding programs in numerous countries have attempted to develop resistant varieties to eliminate the most important disease in apple orchards caused by V. inaequalis. Resistance to apple scab originating from Malus floribunda clone 821 is the most widely form of resistance used in apple breeding programs. According to ability to overcome resistance sources used by plant breeders seven races were defined. Two races virulent to the most-used type of resistance given by Vf gene were founded up recent time - race 6 in Germany in 1993 (Parisi, 1993) and race 7 in England in 1994 (Roberts, 1994). Vf gene was broken in seven locations within Czech Republic up to the year 2008.

Next attempts is creation the functional growing system in IPM and organic regimes. This system will play not only environmental in a landscape view but it should be acceptable from an economic view. The orchard is understood as an agroecosystem with a number of relations among host plants, pests and predators and where not only direct control treatments are important. The strengthening of these relations coupled with the use of environment friendly control methods will result in a higher stability of orchard ecosystem with a lower use of a "hard" chemicals. As a result of all these attributes the production of the safe and top-quality fruit production is excepted. V. inaequalis has developed resistance to some key fungicides including benomyl, dodine, flusilazole and strobilurines (Szkolnik and Gilpatrick, 1969; Smith et al., 1991; Olaya and Köller, 1999; Palm et al., 2004). Rate of pathogen sensitivity to fungicides differs in orchards according to treatment regimes in previous years. For a sustainable fungicide efficacy management, knowledge about changes in the sensitivity of the
target pathogen populations is necessary. In our study, preliminary monitoring of sensitivity of *V. inaequalis* populations to strobilurine fungicides was made in orchards where chemical control was not effective. Evaluation of conidia germination in drops of spray liquids of fungicide Discus was used in our tests.

**Material and methods**

**A) Fungicide sensitivity tests**

*Sampling*

Leaf samples with apple scab lesions were collected from 22 commercial orchards in different regions of the Czech Republic in years 2007 and 2008. A sensitive population of *V. inaequalis* was taken from apple tree solitary growing in natural conditions. This population had never been exposed to any fungicides. One sample was taken in experimental orchard of RBIP Holovousy. Samples were kept in refrigerator and processed within 5 days of collection.

*Sensitivity tests*

The commercial strobilurine fungicide Discus® (kresoxim-methyl; BASF) was used in these laboratory tests. Eighty microlitres of aqueous fungicide solution (concentration – 0.02%) were pipetted into depressions on microscope slides. In one variant, only drinking water without fungicide was used. Conidia of *V. inaequalis* were transferred from infected leaves into drops on slides and incubated at 22± 2 °C for 48 h. After that, the number of germinated spores was counted under a microscope (minimum 500 spores per variant) and the percentage of germination was calculated.

**B) Tests of apple scab virulence on resistant cultivars**

*Sampling*

Samples of leaves were collected from seven orchards with symptoms on resistant cultivars for determination of pathogenicity in greenhouse experiments and distinction by PCR methods.

*Pathogenicity tests*

Evaluation of pathogenicity of apple scab on resistant cultivars was initiated after first occurrence of symptoms in the year 2006. Indicating cultivars were grafted into orchards in main apple growing regions for analyzes of the resistance. The host cultivars range included Gala (susceptible control), Golden Delicious (gene *Vg*), Priscilla, Prima (gene *Vf*), Florina (genes *Vg*, *Vf*). Others cultivars and species were used for indicating apple scab on other forms of resistance (*R 12740-7A, OR 45T132, 9AR2T128*), (Bénaouf, 1997), (Table 1). Samples of leaves were collected from orchards with symptoms on resistant cultivars for determination of pathogenicity in greenhouse experiments and distinction by PCR methods. *V. inaequalis* on culture plates is grown at this time, so results are not available yet.

**Results and discussion**

Obtained results of fungicide sensitivity tests are presented in graphs 1-3. Monitoring of commercial orchards revealed that strobilurine sensitivities of *V. inaequalis* were not uniform throughout plantings. The highest spore germination was recorded in case of population from experimental orchard of RBIP Holovousy. Percentage of germination in drops of spray liquid with kresoxim-methyl was almost as high as percentage of germination in drops of water without fungicide. This result was expected, because the resistant population of *V. inaequalis* was established purposely in this orchard in previous years. In the contrary, the germination of
The wild-type population (sample “Lázně Bělohrad”) was almost suppressed in drops with kresoxim-methyl. The similar results were achieved in case of several commercial orchards. In these localities, occurrence of apple scab might be caused either by bad treatment term or way of fungicide application. Eventually, decrease of sensitivity to other fungicides (for example to DMI fungicides) can not be excluded in these orchards. High percentage of conidia germination (higher than 50% of germination in water) was recorded in case of five populations originated from different commercial orchards. This finding indicates that use of the strobilurine fungicides during following season will lead to significant decrease of fruit quality and strobilurines should be excluded from control in these orchards.

First symptoms on resistant cultivars were recorded in the year 2006 in four locations – Žrnov in Eastern Bohemia, Břasy and Spálené Poříčí in western Bohemia and Buková Lhota in central Bohemia. Two years later in 2008 were recorded symptoms in other three locations – Rohozec in southern Moravia, Branice in southern Bohemia and Zvěstov in central Bohemia. Symptomps of apple scab was founded only on Vf resistant cultivars (Rubinola, Topaz, Rajka, Otava, Melodie etc.) Overcoming of other forms of resistance was not recorded. Consequently those isolates can be classified as the race 6 or 7. Monosporic isolates of V. inaequalis were prepared for distinction of relationship using molecular methods. Izolations of DNA are in the process.

Graphs 1 – 3: Fungicide sensitivity tests
Table 1: Host cultivars and genotypes
<table>
<thead>
<tr>
<th>Cultivar (clone)</th>
<th>Behavior</th>
<th>Resistance gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gala</td>
<td>Susceptible</td>
<td>None</td>
</tr>
<tr>
<td>Golden Delicious</td>
<td>Susceptible</td>
<td>Vg</td>
</tr>
<tr>
<td>Priscilla</td>
<td>Resistant</td>
<td>Vf</td>
</tr>
<tr>
<td>Prima</td>
<td>Resistant</td>
<td>Vf</td>
</tr>
<tr>
<td>Florina</td>
<td>Resistant</td>
<td>Vf, Vg</td>
</tr>
<tr>
<td>R12 740-7A</td>
<td>Resistant</td>
<td>Vr</td>
</tr>
<tr>
<td>OR45T132</td>
<td>Resistant</td>
<td>Vm</td>
</tr>
<tr>
<td>9AR2T128</td>
<td>Resistant</td>
<td>Vm</td>
</tr>
</tbody>
</table>

Map 1: Locations with occurrence of apple scab on scab resistant cultivars

Acknowledgments
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References
Smith, F.D., Parker, D.M., Köller, W. 1991. Sensitivity distribution of Venturia inaequalis to
Apple Proliferation Phytoplasma in South Tyrol – An Integrated Approach

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Abstract: In 2000 and 2001 a severe occurrence of apple proliferation phytoplasma was noticed for the first time in apple orchards in South Tyrol (Italy). At the same time, in 2000 an increased occurrence of Cacopsylla melanoneura and in 2004 for the first time also a second vector, Cacopsylla picta, were detected in the orchards. The, in some cases, rather heavy economic losses caused by these attacks induced all appropriate institutions to look for solutions together with the producers. The phytoplasma had to be controlled in compliance with the principles of integrated fruit production. The complete elimination of all infected trees including the roots in combination with chemical control of the two vectors proved successful and resulted in a considerable reduction in infections in the past two years.

Keywords: apple proliferation, Cacopsylla melanoneura, Cacopsylla picta

Introduction

South Tyrol is with 18,500 ha the greatest contiguous apple growing area in Europe. This area has more than 60 million apple trees. Since 2000 one of the major problems has been the apple proliferation phytoplasma. Phytoplasmas are important phloem-limited, insect-transmitted pathogenic agents. The most important insect vectors transmitting this phytoplasma are the two psyllids Cacopsylla picta and Cacopsylla melanoneura. C. melanoneura is widespread in the orchards throughout the whole region and is found everywhere on apple trees in South Tyrol. It is possible to discover it on the apple trees from the end of January onwards. The behaviour of the second insect vector C. picta is different. It was found in South Tyrol for the first time in 2004. This insect infests the orchard during the blossom period and leaves the trees later in the summer.

The phytoplasma can also be transmitted from one infected tree to another through root contact and it is also possible to receive infected trees from the nurseries; in 2000 and 2001 a severe occurrence of infected trees in the “Burggrafenamt”, in the Vinschgau Valley and in the orchards on the hillsides in the rest of the region was noticed.

The typical and most important symptoms an infected tree shows can be witches’ broom, red coloured leaves on the whole tree, smaller and less coloured fruits with a poor taste and an earlier start in the vegetation period than a healthy tree.

Material and methods

The South Tyrolean Extension Service for Fruit- and Wine-Growing has invested a lot of time in discovering the intensity of the disease in the different parts of the region. One important evaluation method is counting the symptomatic trees (trees with one or more symptoms) in a representative number of apple orchards. This has been done every year since 2005. The orchards in this evaluation were divided into young (second leaf) and older orchards. In this
way it was possible to obtain a large number of data and it was easier to observe the course of
the disease in the selected orchards over the years. In particular, the trees in the second leaf
are good indicators for the control strategy against the vectors, because symptomatic trees
must have been infected the year before. In addition we know that nearly all the trees that
were infected with the phytoplasma in the first leaf show symptoms in the second leaf.

The second evaluation technique was monitoring the two insect vectors *C. picta* and *C.
melanoneura*. For this reason in spring a large number of insects were collected across the
whole region, to establish the population density of the pests and their life cycles in the
orchards.

**Control of the apple proliferation phytoplasma**
So far there are no known direct control measures against *Candidatus phytoplasma mali*. The
first and most important approach to controlling the apple proliferation phytoplasma is to
eliminate the infected trees from the orchards. It is not enough to remove just the part of the
tree above the ground, but also the infected roots have to be eliminated, since the pathogen
can also be transmitted when the roots of two trees grow together. An infected tree produces
small sized and poorly coloured fruits with a poor internal quality and can serve as a source of
inoculum to infect other trees in the orchard. Eradication of all infected trees is a prerequisite
for a successful disease control.

The second approach is trying to eliminate the two insect vectors. This must be done by
using insecticides. The recommended spraying period against *C. melanoneura* is at budbreak
of the apple trees, and a pyrethroid (active agent etophenprox) was used. In order to control
the second psyllid *C. picta* two other sprays are necessary: one before and one immediately
after bloom. In this case the recommended active agent is chlorpyrifos. These two psyllids do
not cause direct damage to the trees, but they transmit the phytoplasma, so the tolerance level
is zero.

**Results**
From 2005 to 2008 the technical staff of the South Tyrolean Extension Service for Fruit- and
Wine-Growing evaluated every year the symptomatic apple trees in more than 150 orchards in
the second leaf. In Table 1 the data from the Gala orchards in the second leaf are shown.

<table>
<thead>
<tr>
<th>District</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burggrafenamt (BGA)</td>
<td>0.7</td>
<td>1.5</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Vinschgau Valley (VI)</td>
<td>0.6</td>
<td>0.7</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td>Etsch Valley (ET)</td>
<td>0.2</td>
<td>0.4</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>Ubertetsch (UE)</td>
<td>0.5</td>
<td>0.1</td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>Unterland (UL)</td>
<td>0.2</td>
<td>0.1</td>
<td>0.09</td>
<td>0</td>
</tr>
<tr>
<td>Leifers (LEI)</td>
<td>0.1</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eisack Valley (EI)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean for South Tyrol</td>
<td>0.5</td>
<td>0.8</td>
<td>0.11</td>
<td>0.02</td>
</tr>
</tbody>
</table>

In 2005 0.43% of the trees showed symptoms, in 2006 0.6%, and in 2007 0.15%. In 2008 we
detected only 0.02% symptomatic trees. The result of this evaluation indicates that the average infestation has decreased significantly over the past four years (Table 1).

*C. picta* prefers trees in the first leaf. We have noticed that the presence of *C. picta* in this type of orchard is much higher than in older orchards. The population density of this vector in orchards in the first leaf decreased significantly in 2007. In the Eisack Valley we have never found *C. picta* in an orchard, which may explain the absence of the disease (Figure 1).

![Figure 1: Percentage of second Leaf Orchards on M9 with (in grey) and without *Cacopsylla picta* in 2006 and 2007](image)

**Conclusion**

By following the two approaches mentioned above we have noticed in a large number of orchards a significant reduction in the population density of the vectors and in the new infections of apple trees. Only these two approaches together have shown an acceptable result in our orchards. Just spraying or cleaning is not enough to reduce the number of infected trees and to protect the healthy ones from an infection.

The target for the future is to reduce the sprays against the insect vectors to a minimum. This may be possible if the controls are carried out carefully and if the infected trees are removed as quickly as possible from the orchards. In two districts of the region this has already been achieved; in the upper Vinschgau Valley and in the Eisack Valley it was possible to do without spraying before bloom.
Development and validation of a rapid method testing of CpGV susceptibility in codling moth populations

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Abstract: In the last five years the phenomenon of emerging resistances of codling moth (Cydia pomonella) against Cydia pomonella granulovirus (CpGV) has been observed in about 30 orchards in different European countries. So far, bioassays with the F1 generation of the diapausing CM larvae have been used for testing CpGV susceptibility. This is labour-intensive and time consuming; results are only available about 9 months after collection of larvae. Therefore, we were seeking for an alternative method by performing a direct test on the younger instars during the season. We developed and validated a more rapid test by optimizing the virus concentration in the bioassay, duration of bioassay and improvement of diet in order to be able to directly test the susceptibility on second to fourth instar larvae extracted from apples. By testing more than 3700 larvae extracted from 12000 infested apples from 20 orchards in Germany, Switzerland, The Netherlands, Austria and Italy we could prove that direct testing is feasible and provide results within 3 weeks after sampling. This new method allows us to make precise predictions about the status quo in resistance of an examined population, even if the orchard was treated with CpGV products, pheromones or chemical insecticides, which, as a matter of course, complicates the identification and determination of a potential resistance.

Codling moth, Cydia pomonella Granulovirus, CpGV, Resistance testing
Effect of a growth enhancer Carbon Kick Booster® on mites and natural mite enemies in apple

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Abstract: The importance of mite pests is increasing in Finnish apple production due to lack of efficient pesticides and the effect of climate change. Integrated pest management has been successful to enhance natural control of mites by indigenous OP-resistant phytoseiid mites but rejection of OP-insecticides will cause increasing problems. Plant derived substances have been successful to restrain pest populations in greenhouses. Tests with a growth enhancer ‘Carbon Kick Booster®’ containing rape seed oil, emulsifiers and triacontanol were conducted in the laboratory and field conditions to evaluate its effect on apple rust mite (Aculus schlechtendali) and fruit tree red spider mite (Panonychus ulmi). In the laboratory 1-2 % solution killed a majority of the pest mites in 1-4 days. In field tests the results were inconsistent but comparable to sulphur treatments. Mites of the families Tarsonemidae and Tydeidae were not affected and in field tests phytoseiid mites survived the enhancer sprayings better than the sulphur sprayings. Predatory cecidomyid larvae were present in the trees and limited both red spider mite and apple rust mite population increases in all treatments.

Key words: Phytoseiidae, apple rust mite, fruit tree red spider mite, Aculus schlechtendali, Panonychus ulmi

Introduction

In the past, the fruit tree red spider mite or the European red mite (ERM) Panonychus ulmi (Koch) was a serious problem in professional apple orchards in Finland. In the 1980’s, OP-resistant predatory mites (Acari, Phytoseiidae) were hardly present in sprayed orchards (Tuovinen and Rokx, 1991) but in the 1990’s, along with the adoption of IPM, natural control of ERM has prevailed in many orchards due to OP-tolerant phytoseiid mites (unpublished data). From 2008 onwards, after the rejection of OP-insecticides in Finland, the success of natural mite control may be threatened if insecticides which are more harmful to predatory mites will replace the OP’s. The expected warming of climate, which has actually already been noticed may lead to better overwintering and even development of one extra generation of ERM. Recently, the introduction of new apple cultivars which are more susceptible to the apple rust mite (ARM) Aculus schlechtendali (Nalepa) has increased the importance of this eriophyid mite in future IPM.

Only few specific acaricides are available for growers and repeated sprayings involve a risk of developing resistance in ERM and ARM populations. As an alternative approach, several plant oil based biorational pesticides have been used especially in organic farming, e.g. in greenhouses (Allen et al., 1993). One product belonging to biorational pesticides, Carbon Kick Booster® (CKB) (Carbon Kick Ltd., Finland) containing turnip rape seed oil (90%), emulsifiers (10%) and a small amount of triacontanol, was tested in the laboratory and open field to evaluate its effect on pest mites and on the natural mite community in apple trees.
Material and methods

Laboratory tests
In the laboratory tests CKB was sprayed as 2.0% and 1.0% solutions, with and without Silwet Gold® (SG) silicone surfactant (0.05 and 0.025%). Tests were performed by spraying pieces of apple leaves in a Potter tower using the amounts similar to 300 and 600 l/ha field sprayings. Leaves were preserved in Petri dishes on moist cotton in a climate room in 25°C and 60% RH. Assessment of the effect on ARM was made 1, 3 and 6 days after treatment. Mites were classified mobile if they moved their legs. Henderson-Tilton formula was used to calculate the effect of sprayings and data were analysed by ANOVA.

Field tests
In 2007, a field test was done at MTT Piikkiö (60°23’N; 22°33’E) by spraying randomly selected trees with a knapsack mist sprayer with 2.0% CKB + 0.05% SG. Additionally the whole block was sprayed by azinphosmethyl (5th July) and malathion (13th July) to control insect pests. In 2008, the similar CKB+SG sprayings were done twice (10th June and 29th July) and, another treatment with 1.0% sulphur (Kumulus®) was added for comparison at the same time. The whole block was sprayed once (10th July) by pyrethrum insecticide to control aphids. Leaf samples were collected and checked before and after sprayings and ARM, ERM and other mites and insects were counted. Statistical analyses were done by ANOVA using log-transformed data.

Results and discussion

Laboratory tests
In the laboratory CKB+SG and CKB had a good effect on ARM, whereas the effect of SG alone was poor when sprayed at lower amount of liquid (Figure 1). After 3 days the results were similar, but after 6 days the number of mobile mites also in untreated went down.

Field tests
In 2007, the effect of 2.0% CKB+0.05% SG was variable between cultivars with a mean effect of 69% on ARM and on ERM 89% compared to the untreated trees (Henderson-Tilton). In 2008, the first spraying of CKB+SG and Kumulus+SG had moderate effects on ARM, 67% and 52% (Henderson-Tilton), respectively (Figure 2). The initial ERM
population was low but variable and therefore the effect of the sprayings remained questionable. The effect of the late July sprayings on both mite species remained insignificant partly because of the emergence of natural mite enemies (Figures 3 and 5).

Figure 2. Numbers of mobile apple rust mites and European red spider mites before treatments and 36 DAT in the field test in 2008. Treatments: CKB 2.0% + SG 0.05%, Kumulus 1.0% + SG 0.05%. Different letters indicate significant differences between treatments (ANOVA, Tukey’s test, P=0.05).

Kumulus+SG sprayings diminished phytoseiid mite and egg numbers in late season compared to the trees sprayed by CKB+SG and the untreated trees (Figure 3). In spite of the pyrethrin spraying (10th July) phytoseiid mite numbers grew fast in July-August except in Kumulus+SG treated trees. The second treatment of CKB+SG in late July may have affected on egg-laying of phytoseiid mites, however, the noticed differences were not significant.

Figure 3. Observations of mobile phytoseiid mites and phytoseiid eggs in the field test 2008. The arrows indicate the treatment dates, see Figure 2.

CKB+SG did not have any notable harmful effect on other non-target mites except Czenspinkia sp. (Acari, Winterschmidtiiidae). Kumulus+SG treatments decreased the number of all non-target mite groups (Figure 4). Besides phytoseiid mites predaceous gall midge larvae, Feltiella spp. and Arthrocnodax spp. (Cecidomyidae) occurred on leaves in moderate numbers and it was obvious that these predators diminished the numbers of ARM and ERM in all treatments (Figure 5).

Oil based biorational pesticides are noteworthy alternatives for conventional or novel synthetic acaricides although their effect cannot be guaranteed in all circumstances (Krawchyk and Hull, 2005). Weather conditions may have a great influence on the effect of CKB, and repeated treatments may be necessary. Variation of the leaf hairiness of cultivars influences spreading of the spraying solution on the leaf surface where ARM lives; this problem is less when a silicon adjuvant is used. CKB sprayings did not cause any unwanted
effects on plants and it was safe to beneficial predatory mites present in the apple trees, which is an advantage compared to the sulphur sprayings.

Figure 4. Numbers of mobile mites of the most common non-target mite families in the field test 2008. Treatments, see Figure 2. Different letters indicate significant differences between treatments (ANOVA, Tukey’s test, P=0.05).

Figure 5. Observations of predatory cecidomyid larvae in the field test in 2008. Treatments, see Figure 2.

References

Biological Efficacy of Botanical Insecticides in Controlling Green Apple Aphid (*Aphis poni* De Geer)

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**Abstract:** The effects of the botanical insecticides pyrethrin (Pyros®), rotenone (Rotenone®) and pyrethrin + rotenone (ShowTop) were monitored in two apple orchards planted with cvs. Granny Smith and Kožara. The trial was set up according to the EPPO PP1/21(2) Protocol. The insecticides were applied in each of four rows. Four leaves from each tree were designated as samples for monitoring the population pressure of *Aphis poni* De Geer. The insecticides were applied in June by spray drift and atomiser. The temperature was 23°C and relative air humidity 63%. The pest population pressure was checked immediately before the treatment and on the 1st, 2nd, 3rd and 7th day after treatment (DAT). The highest efficacious insecticide was Pyros® (83.2%) on 1 DAT followed by ShowTop (82.8%), whereas Rotenone® was the least effective (67.1%) at controlling *A. poni*. Rotenone® was most effective on 2 DAT (72.1%), with subsequent inspections showing a decrease in efficacy (67.3% and 44.7%). For Pyros®, further inspection on 2 and 3 DAT registered a decline in efficacy to 72 – 73%, whereas on 7 DAT it reduced to 55.7%. The inspection on 2 DAT reported the highest efficacy of ShowTop (84%) and a further decline to 76.4% and 69.5% on 3 DAT and 7 DAT, respectively.

**Key words:** apple, pests, *Aphis poni*, botanical insecticides

**Introduction**

Aphids are considered major pests which threaten the quality and yield of fruits. About 250 species of plant aphids harm, both directly and indirectly, cultivated plants worldwide. Indirect damage caused by aphids as virus vectors is an issue of utmost importance (Harris, 1990). Economically important aphids in Serbian apple orchards include green apple aphid *Aphis poni* De Geer (Dimić, 2000; Petrović-Obadović, 2003), followed by *Rhopalosiphum insertim* Walk., *Dysaphis dejecta* Walk., *Dysaphis plantaginea* Pass. (Petrović-Obadović, 2003) and a new pest in Serbian apple orchards - *Aphis spireacola* (Petrović-Obadović et al., 2008). The earlier widespread use of pesticides in controlling aphids in practice was not documented in scientific research and articles (Milenković et al., 2002). Under Serbian conditions, preliminary investigations showed the efficacy of azadirachtin in controlling green apple aphid (Milenković et al., 2001; Milenković et al., 2005). The use of botanical insecticides for controlling *A. poni* in Western Serbia was examined in this study.

**Material and methods**

The effects of the botanical insecticides pyrethrin (Pyros®), rotenone (Rotenone®) and pyrethrin + rotenone (ShowTop) were monitored in two apple orchards planted with cvs. Granny Smith and Kožara. The orchard, located at the 'Vranici' site (Cacak 1), at an altitude of 315m, was planted with cv. Granny Smith in spring 2008. The second site was located at 'Mršinci' (Čačak 2), at an altitude of 235m, and was planted with cv. Kožara in spring 2007.

The trial was set up according to the EPPO PP1/21(2) Protocol. The experimental area
comprised 20 apple trees divided into four treatments, each including five trees (five replicates per treatment). Four leaves were marked on each tree for inspection, i.e. counting the number of mobile specimens for monitoring the population pressure of *A. pomi*. The untreated row (control) was marked likewise.

The insecticides were applied on 6 June 2008, around 19 h, by spray drift (locality 1) and by atomiser (locality 2). The temperature was 23°C and relative air humidity 63%.

The pest population pressure was assessed immediately before the treatment and then on the 1st, 2nd, 3rd and 7th days after the treatment (DAT). The reduction in percentages of aphid population were calculated by Henderson-Tilton formula.

The recommended concentrations of pyrethrum (Pyros® (0.08%)), (rotenone Rotenone® (0.3%)) and pyrethrin+rotenone (Show Top (0.5%)) were applied in order to study initial and residual effects on the green apple aphid. Rothenone and pyrethrum are natural plant-derived substances. Rothenone has a longer residual life than most botanicals and is effective for about a week. In organic production, the use of rotenone as an insecticide is permitted under Regulation EC 2092/91, amended by EC 889/2008.

**Results and discussion**

The first inspection, 24 hours after treatment, revealed that the number of aphids increased to 727 in the control, but decreased to 439 (Rotenone®), 179 (Pyros®) and 76 (Show Top) in all insecticide treatments (Table 1). A further decrease in aphid numbers was observed 2 DAT, 162, 128 and 31, respectively, in the treated, and 316 aphids in the untreated one.

The third and fourth inspections conducted on 3 DAT and 7 DAT, respectively, showed that aphid numbers increased to 204 and 268 in the row treated with Rotenone®. The inspections carried out 3 DAT registered a decline in the number of aphids on trees treated with Pyros® (128 aphids) and Show Top (49 aphids). The assessment conducted at 7 DAT showed an approximately equal number of aphids in both insecticide treatments. The number of aphids in the control, decreased to 341 (3DAT) and 264 (7DAT).

Table 1. The number of *Aphis pomi* on apple trees sprayed with botanical insecticides, site 1 (Čačak 1), cv. Granny Smith

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Aphid No. prior to treatment</th>
<th>Aphid number and percent reduction after treatment initial effects</th>
<th>Residual effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1-day</td>
<td>2-day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N°</td>
<td>R%</td>
</tr>
<tr>
<td>Rotenone® (rotenone)</td>
<td>995</td>
<td>439</td>
<td>67</td>
</tr>
<tr>
<td>Pyros® (pyrethrin)</td>
<td>793</td>
<td>179</td>
<td><strong>83</strong></td>
</tr>
<tr>
<td>Show Top (pyrethrin + rotenone)</td>
<td>331</td>
<td>76</td>
<td><strong>83</strong></td>
</tr>
<tr>
<td>Control</td>
<td>541</td>
<td>727</td>
<td>-</td>
</tr>
</tbody>
</table>

N° - Number of aphids
R% - Reduction % of aphids calculated by Henderson-Tilton formula

The percent reduction of aphids showed that the most effective insecticide at Čačak (site 1) was Pyros® (83.2%), followed by Show Top (82.8%) and Rotenone® (67.1%) (Table 2). Rotenone® showed its highest efficacy at 2 DAT (72.1%) whereupon subsequent inspection
there was a decrease in efficacy (67.3% and 44.7%). On 2 and 3 DAT the efficacy of Pyros® declined (72 – 73%), whereas on DAT 7 it was reduced to 55.7%. The assessment on 2 DAT reported the highest efficacy of ShowTop (84%) and a further decline to 76.4% and 69.5% on 3 DAT and 7 DAT, respectively.

In the first inspection of the autochthonous cv. Kožara (Table2), 24 h after treatment, the number of mobile aphids increased to 168 in the control, but decreased in the treated fields for all insecticides: 16 (Pyros®), 29 (ShowTop) and 38 (Rotenone®). Changes in the aphid number were not registered on 2 DAT. A further decrease to 11 and 8 was recorded on 3 DAT and 7 DAT, respectively, following the Pyros® application. There was an increase in aphid number in the other two insecticides (32 and 36 specimens (ShowTop) i.e. 41 and 50 specimens (Rotenone®), respectively). In the untreated field, the number of mobile aphids increased to 168 (1 DAT), 172 (2 DAT) and 181 (3 DAT). The inspection conducted on 7 DAT registered a decrease in aphid number to 152. The decrease was probably due to an increase in the abundance of predatory entomofauna.

Table 2. The number of Aphis pomi on apple trees sprayed with botanical insecticides, site 2 (Čačak 2), cv. Kožara

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Aphid No. prior to treatments</th>
<th>Aphid number and reduction percentage after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-day</td>
</tr>
<tr>
<td>Rotenone® (rotenone)</td>
<td>157</td>
<td>38</td>
</tr>
<tr>
<td>Pyros® (pyrethrin)</td>
<td>159</td>
<td>16</td>
</tr>
<tr>
<td>ShowTop (pyrethrin + rotenone)</td>
<td>165</td>
<td>29</td>
</tr>
<tr>
<td>Control</td>
<td>165</td>
<td>168</td>
</tr>
</tbody>
</table>

N° – Number of aphids
R% - Reduction % of aphids calculated by Henderson-Tilton formula

The percent reduction of aphids at Čačak 2 site showed that on 1 DAT the most effective insecticide was Pyros® (90%), followed by ShowTop (83%) and Rotenone® (76.3%). The efficacy of Rotenone® was highest on 3 DAT (77%), subsequent inspection showing a decrease (67%). Further inspection on 2 and 3 DAT revealed an increase in the efficacy of Pyros® to 91 – 94%, whereas on 7 DAT it remained unchanged (94%). The inspection on 2 and 3 DAT reported high efficacy of ShowTop (83%) and a further decline to 76% (7 DAT).

A comparison of the reduction percentage between the two localities suggested that the same insecticides showed different efficacy (Fig.1).
Figure 1. Efficacy of two different spray applications of insecticides against *A. pomi*.

The differences in efficacy of the insecticides at the two sites most likely resulted from different methods of application (spray and atomiser). They were manifested through greater efficacy of the atomiser-applied insecticides at the locality 2. The differences were most evident (Fig. 1) with Pyros®.

No symptoms of phytotoxicity (russetting, necrosis) to apple leaves were observed during the trial.

**References**

Evolution of apple surface metabolites throughout the season and codling moth (Cydia pomonella L.) egg-laying behaviour.

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Abstract: Cydia pomonella behaviour is related to plant surface metabolites. Among them soluble carbohydrates (glucose, fructose and sucrose) and sugar alcohols (sorbitol, quebrachitol and myo-inositol) influence plant site acceptance and stimulate egg-laying. It is generally observed in orchards that throughout the season the females shift their egg-laying site whenever a majority of eggs remaining on the leaf surface. On the variety Granny Smith they first lay eggs in majority on the twigs and upper side of corymb leaves and then progressively they lay more eggs on the lower side of corymb leaves and fruits. Our aim is to study the relationship between the chemicals throughout the season and the behaviour shifts. For both varieties Golden Delicious and Granny Smith, we considered different plant organs: twigs, leaves, leaf sides, fruit at several growth stages. Within the six metabolite pattern the concentrations and ratios (ng/cm²) of metabolites vary with the plant organ, leaf side and the season period. Although quantities are different between the varieties, differences remain according to the sites and are rather similar: the upper side of corymb leaves is the richest site throughout the periods. On the twigs, fructose, sorbitol and mannitol increase throughout the periods but quebrachitol decreases dramatically. Apple surface enriches in sorbitol and grow poorer in fructose. On the base of our knowledge on the influence of metabolite blend on egg-laying behaviour we verified a good correlation between them in orchards throughout the season. This study could open new ways of apple tree protection based on the recognition of the host by the insect.

Surface metabolites, Sugars, Sugar alcohols, Cydia pomonella, Egg-laying
Evaluation of integrated management scenarios of the peach tree-Myzus persicae system using a crop-pest model

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Abstract: Integrated Fruit Production (IFP) calls for an adaptation of production processes to improve crop quality and environmental safety. This approach gives priority to alternative methods of pest control. Our study investigates the potential of management scenarios that integrate chemical, biological (inundative release of Harmonia axyridis ladybirds) and cultural (nitrogen fertilization and winter pruning) pest control methods for the peach tree-aphid system. We used a modeling approach to address this question. We defined 108 management scenarios, which were based on theoretical pest control strategies combined with control variables relative to pest control and cultural practices. Then, we performed model simulations of these scenarios and studied the relationships between control variables and model outputs referring to agronomical, economical, sanitary (pest), and sustainability performance. Results showed that ‘agronomical performance’ was largely controlled by ‘agronomical practices’, while ‘pest performance’ was largely controlled by ‘pest control practices’.

Key words: biological control, Harmonia axyridis, chemical control, cultural control, winter pruning, nitrogen fertilization, green peach aphid, population dynamics, foliage growth, fruit quality.

Introduction

Integrated Fruit Production (IFP) aims to develop more sustainable production systems, profiting from natural resources and ecological processes of the system in replacement of agrochemical inputs (Cross et al., 1997). This includes relying on alternative (i.e, natural, cultural or biological) methods of pest control. These methods aim to reduce pest pressure by confining trophic exchanges in the “crop-pest-predator” system within adequate levels. Therefore, IFP calls for an adaptation of agricultural practices that can ensure crop yield while improving crop quality and environmental safety. Our study investigates the potential of crop-pest management scenarios that integrate chemical, biological (inundative release of Harmonia axyridis) and cultural (nitrogen fertilization and winter pruning) pest control methods for the peach tree Prunus persica (L.) Batsch- Myzus persicae (Sulzer) aphid system.

N fertilization and winter pruning are two major cultural practices used in fruit production orchards to improve agronomic performances. On the other hand, by manipulating host plant quality (N content, defensive chemistry) and/or host plant availability (vegetative growth), they were likely to regulate aphid populations. Consequently, they can modify the level of aphid damage on the crop and indirectly affect crop performances. A ladybird release raises the level of predators and insures a top-down control of aphid populations. However cascading effects of variation in plant resources can extend to this third trophic level and affect its performances. All these interactions make comprehension difficult for whole system behaviour in response to management practices. Modeling is a well-adapted tool to analyze a
complex system in a general framework and build up effective Integrated Crop-Pest Management strategies (Getz and Gutierrez, 1982).

**Material and methods**

**Description of the crop-pest simulation model** (Figure 1)

Our deterministic model predicts, for an “average” tree and over a 1-year time horizon, (i) the daily time-course of shoots and fruit dry masses, (ii) the daily time-course of the sizes (in number of individuals) of an “average” aphid population and ladybird cohorts, and (iii) fruit quality at harvest. Sub-models [2] and [3] (Figure 1) were respectively developed by Grechi et al (2008b) and Chen (1997). The global model is fully described in Grechi (2008a).

![Diagram of the “peach-aphid” model and its four sub-models.](image)

**Definition of the 108 integrated management scenarios**

The management scenarios included four theoretical pest control strategies detailed with respect to biological and chemical treatments: no treatment (*strat1*-'no treatment'), only chemical treatments (*strat2*-'conventional' and *strat3*-'organic farming'), and both chemical and biological treatments with high action thresholds for release of ladybirds and application of insecticides (*strat4*-'integrated'). These were combined with (i) control variables relative to insecticide characteristics (maximal mortality rate for insecticides used at bloom, ins1B, or during the season, ins1S) and cultural practices (winter pruning intensity, IP, and leaf N content, NCleaf), and (ii) an uncontrolled variable (aphid inoculum, iniNA).

**Definition of the criteria used to evaluate management scenarios**

The assessment of a management performance relies on a set of criteria that characterize (i) ‘agronomic’ indices of an average fruit (fruit yield, Yield, refractometric index, RI, and fresh mass, MFr), (ii) ‘economic’ indices (selling price, SP, and total number of ladybirds released, TotNLA), (iii) ‘pest’ variables (number of insecticide applications, INS, and total number of aphids per shoot over a year, TotNA), and (iv) ‘sustainability’ properties (number of long

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1 Here ‘organic farming’ is a strategy that is limited to the usage of insecticides authorized according to farming guidelines. Such insecticides are generally less effective against pests than those used in conventional systems.
shoots, \(N_{LS}\), and proportion of long shoots > 30cm, \(pLS_{30}\). They are referred as ‘performance variables’.

**Model simulations and performance analysis of the integrated management scenarios**

Model inputs comprise ‘control variables’, which refer to the management practices. They include ‘agronomic’ (\(I_p\), \(NC_{leaf}\)) and ‘pest’ (\(strat_{i}\in\{1,2,3,4\},\ins1B,\ins1S\)) variables. Each management scenario is characterized by a set of ‘control variables’ and ‘performance variables’ (computed from model outputs). The performances of the simulated management practices were investigated via a principal component analysis with instrumental variables (PCAIV) in correlation plots.

**Results and discussion**

Results of the PCAIV (Figure 2) showed that ‘agronomic performances’ were largely controlled by ‘agronomic practices’ (Figures 2A, 2B). \(N_{LS}\), \(RI\) and \(MF\) were mainly explained by \(I_p\) whereas \(pLS_{30}\) was largely explained by \(NC_{leaf}\), both factors had a positive effect. To a lesser extent, \(Yield\) and \(SP\) were positively correlated to \(NC_{leaf}\). \(Yield\) appeared to be negatively correlated to \(I_p\), while \(SP\) provided no clear correlation with \(I_p\) due to their nonlinear relationship (data not shown). Conversely, ‘pest performances’ were strongly controlled by

![Figure 2. Correlation plots of ‘control variables’ (A, C) and ‘performance variables’ (B, D) for the 1-2 (A, B) and 1-3 (C, D) PCAIV components.](image-url)
‘pest control practices’ (Figure 2C, 2D). \(strat1, strat2, strat3, strat4\) and \(ins1S\) largely explained the two variables \(INS\) and \(TotN_A\). On the contrary, \(ins1B\) and \(iniNA\) explained none of ‘pest performance’ variables. \(INS\) (resp. \(TotN_A\)) was positively (resp. negatively) correlated to \(strat2, strat3\) and \(ins1S\), and negatively (resp. positively) correlated to \(strat1\) and \(strat4\). In spite of peach-aphid interactions, our results did not identify clear interactions between ‘pest’ and ‘agronomic’ variables. Since cultural practices have a partial effect and are generally less effective at regulating pest population than chemical control methods, their effect on aphid dynamics can only be expressed under control strategies without an intensive use of insecticides (e.g., \(strat1\) and \(strat4\)). The low effect of ‘pest control’ practices on ‘agronomic performances’ could be explained by the possible weak effect of aphids on fruit production in some situations (Grechi, 2008a).

Furthermore, among the 11 management scenarios with high \(SP\) values (\(SP > 150\) €/tree) the four pest control strategies were represented. High values of \(SP\) could be reached with different values of insecticide effectiveness but only with the intermediate pruning intensity value (\(IP=0.40\)) and the highest leaf N content value (\(NC_{leaf}=3.5\)). This indicated the importance of \(IP\) and \(NC_{leaf}\) in the determination of \(SP\).

Owing to its ability to investigate multiple criteria performances of management scenarios that integrate several pest control methods, the peach crop-aphid model should be useful for further IFP implementation. This first analysis already revealed that there was not a unique profile of environmentally interesting scenarios, and that environmentally safe pest control strategies could lead to profitable selling prices.

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**References**


Modelling codling moth damage as a function of adult monitoring and crop protection: A survival generalized linear mixed model approach with time varying covariates

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Abstract: The codling moth (Cydia pomonella) is responsible for most insecticide treatments in pear and apple orchards. In a context of reduction in pesticide use, we aim at better understanding factors that affect codling moth damage intensity. We modelled the link between the proportion of damaged fruits and both constant covariables (type of orchard: pear or apple, organic or not, with or without mating disruption) and time-varying covariables (weekly counts of adults and number of insecticide treatments). Observations were collected in 40 orchards in south-eastern France. We found that damage intensity increased with the number of adults trapped. An analysis of the random orchard effect indicated a certain temporal stability in the risk probability of orchards and a lower risk probability in orchards surrounded by numerous pomefruit orchards and windbreak hedgerows.

Keywords: codling moth, damage, trap, mixed model, survival analysis

Introduction

Reduction of pesticide use is an important issue for both human health preservation (Lee et al., 2004) and biodiversity conservation (McLaughlin, 1995). Thus, there is an urgent need to find alternative solutions for crop protection against pests. Codling moth, the major worldwide insect pest in apple and pear orchards, is responsible for most insecticide treatments in European and North American orchards. Despite this intensive insecticide pressure, codling moth can locally have dramatic impact on production. A major reason is probably insecticide resistance that occurs both for chemical pesticides and biological agents (Reyes et al., 2007; Sauphanor, 2006). Codling moth damage is due to fruit perforation by young larvae. Classical insecticide treatments are therefore targeted towards eggs or young larvae. In some cases, mating disruption with pheromones is also associated with insecticides to reduce locally mating and population growth. In this context, two important challenges are (i) to assess to what extent monitoring codling moth adults provide useful information for prediction of fruit damage and (ii) to find potential factors (even with partial effect) that may reduce codling moth damage in orchards.

We addressed these challenges using a statistical approach: we combined survival methods with generalized linear mixed techniques to model the relationships between the risk of fruit injury at the orchard level and several constant or dynamic covariables. The dynamic variables are adult codling moth population densities whose changes a priori parallel damage risk variations and the number of insecticide treatments applied against codling moth. The constant effects are the global orchard pest management strategies (organic or conventional, with or without mating disruption) and the host plant species (apple or pear). To apprehend an unexplained variability among orchards, we also included a random orchard effect. We searched for potential environmental factors affecting risk of damage using a regression
analysis between random orchard effects and some landscape characteristics known to affect codling moth population densities (Ricci et al. in press, 2009).

Material and methods

Sampling sites
In 2006 (respectively 2007), we selected 41 (respectively 40) apple or pear orchards by a stratified random draw of spatial coordinates within an approximately 70 km² pear and apple growing area in south-eastern France. Seven orchards were organic in 2006 (six in 2007). Mating disruption was used in five of these orchards in 2006 and four of them in 2007. In eight orchards in 2006 and ten in 2007, growers used both chemical treatments and mating disruption against codling moth. All other orchards were conventional orchards in which growers used only chemical treatments.

Codling moth trapping, damage observations, and records of insecticide applications
Codling moth population densities were monitored during adult first flight in 2006 and 2007 using delta traps (TRECE® Phérocon® IIB Trap) baited with a mixed codlemone (1mg) and pear ester (1mg) lure (TRECE® CM/DA Combo). We placed one trap per orchard. Traps were checked and adults counted weekly from April 19th to June 21st in 2006 and from April 11th to June 20th in 2007.

Fruit damage intensity (proportion of attacked fruits) caused by the progenies of first flight adults were assessed once each year in each orchard. Observations were made during the week following June 21st in 2006 and June 25th in 2007. Codling moth injuries were recorded by visual inspection of 1000 fruits per orchard distributed over 50 regularly spaced trees (20 fruits per tree, 10 on each side of the row).

Insecticide treatments target eggs and larvae and thus intend to reduce the risk of fruit damage for a given level of adult population. We therefore performed farm surveys in 2006 (2007 not available) to collect records of insecticide applications for each orchard.

Modelling codling moth injury
We modelled damage probability as a temporal function of both fixed effects (the number of adult codling moth in traps and orchards local covariates) and a random orchard effect that cannot be observed. As the records of insecticide applications were not available in 2007, we did not consider both years’ data together, and we thus did not model a ‘year’ effect.

Random effects analysis
To test if random effects had a random spatial distribution we checked if their empirical variogram were within the 98% envelope drawn from 100 permutations of random effects over orchards. To test for temporal stability of random effects over the two years, we checked whether their signs remained the same using a permutation test.

To investigate if some landscape characteristics were associated to estimated random effects, we mapped all pomefruit (pear and apple) orchards and all hedgerows in the study area. We calculated the ratio of hedgerow length over orchard perimeter (hedgerow proportion); the proportion of each 100m wide buffer around focus orchards covered by pomefruit orchards (pomefruit orchards density) and the mean orientation of hedgerows in 150m wide buffers (hedgerow orientation).

We then used a multiple regression to test whether orchard random effects were partly explained by landscape characteristics which were not initially introduced in the model. The four explanatory variables were the three landscape variables defined above together with the orchard area.
Results

Codling moth trapping, damage observations, and insecticide applications recordings
The mean total number of codling moth trapped per orchard was 73.3 (N=41; sd=65.1) during the 10 week period in 2006 and 55.6 (N=40; sd=39.2) during the 11 week period in 2007.

Fruit damage at the end of the first flight period was low: mean percentage of attacked fruits per orchard was 1.07% (N=41; sd=1.43%) in 2006 and 1.22% (N=40; sd=1.79%) in 2007.

In organic orchards, growers mainly used granulosis virus to control the codling moth population with a mean of 14.7 (N=7, sd=2.4) applications. Conventional orchards with mating disruption received a mean of 8.8 (N=8, sd=2.9) insecticide applications. All other orchards (conventional orchards without mating disruption) received a mean of 11.4 (N=26, sd=5.3) applications in 2006.

Parameter estimation and significant covariates
Significant covariates in the full model were the same both years. The risk of fruit damage increased with the number of codling moth in traps. Unexpectedly, neither mating disruption nor the number of insecticide treatments had a significant effect on the risk of fruit damage. On the contrary, damage probability was about 1.4 times higher in organic than non-organic orchards in 2006 and 1.35 times higher in 2007.

Analysis of random effects
Taking into account random orchard effects lead to a large improvement of the model fitting for both years.

Neither the spatial mapping of these random effects over the study region nor their empirical variogram revealed any particular spatial pattern for both years. On the contrary, a certain temporal stability of random effects was evidenced by the permutation test of joint signs (P= 0.050). Orchards with higher or lower than expected risk tended to remain the same from one year to the other.

Multiple regression analyses showed that orchard random effects depended on orchards density and hedgerow orientation but did not depend on the orchard area or on the ratio of hedgerow length over orchard perimeter. The random effects were lower in orchards surrounded by numerous pomefruit orchards and by windbreak hedgerows.

Discussion

The number of adults observed in traps was significantly and positively related to the level of fruit damage both years, indicating that adult trapping provides useful information on the local harmfulness of codling moths. Traditionally, adult counts were performed using pheromone traps but the relevance of these counts for predicting fruit damage has been discussed (Riedl & Croft, 1974). Pheromone traps were more recently discarded because of their low efficiency in orchards under mating disruption. Our results tend to show that traps baited with high loaded combined sex pheromone and pear ester lure do provide information on the local orchard population, either in the presence or not of mating disruption and that they could be used to assess the risk of fruit damage.

On the contrary, the number of insecticide treatments did not explain variation in codling moth damage. This could result from the fact that, despite differences in the number of treatments among orchards, the global insecticide pressure against codling moth is similar. Granulosis virus which was used by organic farmers has a slightly shorter persistence time and, indeed, organic farmers applied pesticides with a higher frequency compared to
conventional growers. This could explain why our models revealed a significant difference between organic and conventional orchards instead of a general effect of the number of treatments.

The large random orchard effects indicate that a great part of variation was not explained by the available fixed effects that we introduced in the model. This precludes the use of the model for prediction of fruit damage only from the knowledge of adults and the orchard pest management strategy. This high variation in the baseline risk between orchards was partially explained by the characteristics of the landscape surrounding the orchards. Landscape harbouring windbreak hedgerows and numerous pomefruit orchards were associated with lower damage. Windbreaks may reduce pesticide drift, therefore increasing insecticide efficiency, or modify plume dispersion and interact with codling moth monitoring with traps. Ricci et al. (2009) found that the presence of surrounding orchards had a negative impact on the density of codling moth diapausing larvae, possibly due to an impact of neighbouring insecticide treatments. The same effect of neighbouring treatments might be at play here.

Acknowledgement

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References


A Comparative Study on Auto-Confusion by Exosex2 GVM-Lb and Mating Disruption by Isonet-L against European Grapevine Moth, *Lobesia botrana* Den.-Schiff. (Lep.: Tortricidae) in Turkey

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**Abstract:** Turkey has more than 300 native grape varieties. Round Seedless (Sultana) is the most important variety. The Aegean Region is the first by possessing 28% of the vineyard surface in Turkey. The production area of Sultana seedless is mostly placed in Manisa Province. Bozcaada Island is in the northwest part of Turkey. The island is very important because of its unique varieties cultivated such as Çavuş and Karasakız. It has 1000 ha of viticulture. The two regions have different agro-ecosystems and ecological conditions. Up to now, synthetic pesticide application has been given priority against European grapevine moth (EGVM), (*Lobesia botrana* Den.-Schiff.) (Lepidoptera: Tortricidae), the key pest of grapes in Turkey. However, negative effects of chemical control on the environment and human health have led up to the necessity of biotechnical methods against the pest. Among them, the mating disruption technique has been tested against EGVM in Turkey. Isonet-L dispensers were proved to be as effective as chemical control against the pest. The objective of this study was to determine in different conditions of Bozcaada Island and Manisa whether the Exosex² GVM-LB auto-confusion system for EGVM, reduces mating and subsequent larval damage to the fruit by comparing with Isonet L, the registered material. By this study, auto-confusion was tested in Turkey for the first time. The auto-confusion (AC) technique by Exosex² dispensers (10 mg pheromone/dispenser) was applied in 17.3 ha and 24.2 ha in Manisa (Aegean Region) whereas it was applied in 12 ha in Bozcaada Island (Marmara Region) in 2007 and 2008, respectively. Only in Manisa, classical mating disruption (MD) technique by Isonet L dispensers (172 mg pheromone/dispenser) was used as a comparative technique in 15 and 6 ha in 2007 and 2008, respectively. Chemical-treated vineyards were also included in the research as comparison (C) vineyard. At the beginning of first flight period, 180 Exosex² dispensers /ha and 600 Isonet L/ha were installed. Exosex² installation was repeated at sixty day-intervals. The need and time of chemical applications was decided by means of Forecasting System against *L. botrana* in C vineyards. In critical periods when the eggs and larvae of first, second, third and fourth generations were expected, and just before harvest; 100 bunches per hectare were controlled in the centre and borders of each AC and MD sampling vineyard, and 100 bunches in each C vineyard separately. Infestation rates were determined. Exosex² dispensers from both locations were analysed by GC. In 2007, the pheromone samples were taken from the first application tablets of both Regions on 31 May 2007. In 2008, the pheromone samples were taken from the second application tablets of Bozcaada on 13 August and third application tablets of Manisa on 09 October. The average infestation rate of all AC vineyards in Manisa was calculated as 6.3 % just before harvest in 2007. In the course of the experiment, 9.6 ha-AC vineyards were treated against *L. botrana* once, whereas 6 ha-AC vineyards were treated two times because of the infestation rate was higher than the threshold of 5%. Fortunately, a 1.7 ha part of AC vineyards did not require any chemical treatment against the pest and auto-confusion has suppressed EGVM in alone. Moreover, the infestation rates were still higher than 5% in 30.6% of the entire AC surface (5,3 ha-9 vineyards) at harvest time. In Manisa, 18.67% part of the entire MD surface had to be sprayed once at least, whereas 13.3% had to be applied twice in 2007. Average infestation rate of all MD vineyards was calculated as 8% at this time.
However, insecticide application has been avoided since the grapes are being harvested. In the last assessment in 2008, average infestation rate of all AC vineyards was calculated as 4.55% at harvest. In the course of the experiment, all AC vineyards in Manisa were totally treated against EGVM twice because of the infestation rates in 3rd generation were higher than the threshold of 5%. In 12% of the entire AC surface, the infestation rates were still higher than 5% at harvest time. They were only 3 vineyards having a surface of 3 ha, totally. In Manisa, 16.67% part of the entire MD surface had to be sprayed once in 2008. Average infestation rate of all MD vineyards was calculated as 4.75% at this time. Only smaller MD vineyard had an infestation rate higher than the threshold at harvest. It can be concluded that small surface of MD caused higher infestation rate. However, insecticide application has been avoided since the grapes are being harvested. In comparison vineyard, broad-spectrum insecticides were applied against L. botrana five times. Infestation rates of the comparison vineyard were always lower than AC plots during the whole season owing to the sprayings of broad-spectrum insecticides. It is also usual to apply chemicals against EGVM in the centre of Manisa Province four or five times per season. No insecticide treatment has been used against any other pest in AC and MD vineyards. The best effectiveness from Auto-confusion has been obtained in Bozcaada against EGVM. No complementary treatment has been applied to suppress the pest. Auto-confusion by Exosex² dispensers was very effective. In comparison vineyard of Bozcaada broad-spectrum insecticides were applied against the pest three times. As occurred in the world, it is possible to have some years and some localities in Turkey, where biotechnical methods are not suitable or successful to control a pest in alone and require complementary insecticide treatment. Mating disruption technique is also registered in Turkey on condition that it should be supported by a biological insecticide treatment preferably to decrease the population density when the infestation rate exceeds 5-6% in the vineyard. By all means, when compared to chemically controlled vineyards, it can be assumed that Exosex² dispensers reduced the number of insecticide applications from 4-5 to 1-2 even in the Aegean Region where population density is higher, flight period is longer and temperatures are higher than Bozcaada Island. Temperature is one of the most efficient factors, which affect the efficacy and stability of pheromone in outer conditions. Average daily temperatures were lower in 2008 when compared with 2007 recorded in Manisa. Results of weekly Isonet-L weights also reflected this phenomenon by consuming their pheromone 3 weeks earlier in 2007 when compared to 2008. According to the results of GC analysis, it was determined that 97.3% of total pheromone amount from Exosex dispensers has been consumed in Bozcaada, whereas only 84% has been released in Manisa in 2007. Despite the higher temperatures in Manisa than Bozcaada in summertime, it can be assumed that the stronger winds might be more effective factor for the emission of pheromone from the dispensers because of lower leaf density in springtime. On the other hand, only 60% of total pheromone amount from Exosex dispensers has been consumed in Bozcaada, whereas 76% has been released in Manisa in summertime in 2008. Therefore, auto-confusion technique can be applied in the vineyards for the control of Lobesia botrana by installing 180 Exosex² dispensers/ha three times per season with 60 days interval. However, it must be combined in the Aegean Region of Turkey with a biological insecticide preferably, if the average infestation rate of the pest exceeds 5-6% once or twice per season.

**Key Words:** Lobesia botrana, auto confusion, mating disruption, grape, Turkey
Identification of the female sex pheromone of the pear midge, *Contarinia pyrivora*

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Abstract: The pear midge, *Contarinia pyrivora* (Riley), is a pest of pear fruitlets and the damage causes severe crop losses. Although it can be controlled by application of insecticide, the timing of application is crucial as *C. pyrivora* is present for only a short period in the year. Identification of the female-produced sex pheromone was undertaken so that it can be used in monitoring and control of the pest. Late larvae of *C. pyrivora* were removed from damaged fruitlets and reared in plastic tubes individually. After sexing, volatiles were collected from both males and females by air entrainment. Collections were analysed by gas chromatography (GC) coupled to electroantennographic (EAG) recording from a male antenna, and by GC coupled to mass spectrometry (MS). Male midges showed EAG responses to two components in collections of volatiles from female. The major and the minor components were identified as 2,7-diacetoxyundecane and 7-acetoxy-2-undecanone respectively. Stereoisomers of the synthetic pheromones were separated by HPLC on a chiral phase and the racemates, individual stereoisomers and binary mixtures were evaluated in field trapping tests. Male *C. pyrivora* were attracted to stereoisomer A of 2,7-diacetoxyundecane and to the first eluting stereoisomer from HPLC fractionation of 7-acetoxy-2-undecanone and these are proposed to be components of the female sex pheromone. However, results were confused by the presence of at least one other midge species in the traps and the experiments will be repeated.

Key words: *Contarinia pyrivora*, pear midge, sex pheromone, electroantennography, traps

Introduction

Pear midge, *Contarinia pyrivora* (Riley) (Diptera: Cecidomyiidae) is a pest of pear fruitlets, causing crop loss in many countries including Europe, USA and Canada and China. In the UK it is reported from every county except Scotland (Barnes, 1948). The overwintering pupae end their pupal stage in mid March and the adults emerge through the soil when flower buds are in pre-blooming stage. After mating, the females lay eggs (10-30) on buds and a few days later the eggs hatch. The larvae emerge and start feeding on the fruit pulp and eventually form a cavity. Attacked fruitlets grow rapidly and get noticeably rounder than normal ones. The growth is arrested after two weeks and the blackened fruitlets crack and fall. Healthy fruitlets in the same cluster get affected owing to immense competition for assimilation of nutrients amongst damaged fruitlets (Barnes, 1948; Alford, 1984). The mature larva leaves the rotted fruitlets and drops to the soil. It burrows a few centimetres into the soil and weaves a cocoon in which it overwinters (Alford, 1984).

The pest has only one generation per annum, emergence occurring over a period of about two weeks in March-April in the UK. *C. pyrivora* is normally controlled by application of insecticide at the white bud flowering stage of pear trees when egg laying commences and when the crop is susceptible to attack. However, different pear varieties reach the critical growth stage at different times and the pest needs to be carefully monitored to determine which crops are at risk. This study was carried out to identify the female sex pheromone of *C. pyrivora*.
*pyrivora* in order to provide a basis for development of improved monitoring and control strategies.

**Materials and methods**

**Insects**
Fruitlets infested with *C. pyrivora* were collected from a pear orchard at Elmston Farm, Preston, in Kent during early June 2006. On emergence, mature larvae were collected and potted individually in tubes containing moistened paper towel. The tubes were stored outdoors under natural conditions. In 2007, tubes containing pupated midges were incubated at 23°-18°C and 16L:8D. Although over 3,000 larvae were collected, only 135 emerged as adults.

**Pheromone collection**
Newly-emerged midges were placed in a glass vessel (5.3 cm i.d. x 13 cm), and charcoal-purified air was drawn in from one end of the glass chamber and out through a Pasteur pipette (4 mm i.d.) containing Porapak Q (200 mg; 80-100 μm; Waters Associates Inc., USA) held between two glass wool plugs. Volatiles trapped on the Porapak filters were extracted with dichloromethane (1.5ml, pesticide grade; Fisher Scientific) and used in the analyses.

**Electrophysiological recording**
Male antennal responses to female volatiles were analysed by gas chromatography (GC) linked to electroantennography (EAG) as described by Cork *et al.*, 1990. The GC used was a HP6890 instrument (Agilent Technologies) with a flame ionisation detector and fused silica capillary columns (30m x 0.32mm x 0.25μm film thickness) coated with polar (Supelcowax-10, Supelco, USA) and non polar (SPB-1, Supelco, USA) phases. The oven temperature was maintained at 50°C for 2 min, then programmed at 10°C/min to 250°C and held for 5 min. Injection was splitless at 220°C and helium was used as carrier gas (2.4 ml/min).

EAG responses were recorded using a portable recording unit (INR-2, Syntech, The Netherlands) comprising integrated electrode holders and amplifier. Glass electrodes were pulled and were filled with electrolyte (0.1 M solution of KCl with 1% polyvinylpyrrolidone). The tips of the electrodes were broken off so that both antennae of the midge could be inserted into the recording electrode and the abdomen inserted into the reference electrode. Signals were amplified and analysed with EZChrom software (Elite v3.0).

**Gas chromatograph linked to mass spectrometry**
For analyses by GC coupled to mass spectrometry (MS) an HP6890 GC (Agilent Technologies) and HP 5973 mass selective detector (Agilent Technologies) were used. Injection was splitless (220°C) with fused capillary columns (30m x 0.25 mm i.d.) coated with polar and non-polar phases as above. GC retention times were converted to Retention Indices (RI) relative to those of acetate esters having even number of carbon atoms from 6-20.

**HPLC separation of stereoisomers of 2,7-diacetoxyundecane and 7-acetoxyundecane-2-one**
HPLC separations were carried out on a Chiralpak AD-H column (150 mm x 4.6 mm i.d.; Daicel Chemical Industries Ltd.) with a pump (Jasco PU-2080 plus) and UV detector (Jasco UV-2075 plus) at 210 nm. A volume of 10μl of a racemic mixture (1 mg/ml in hexane) was separated at a time. The four stereoisomers of synthetic 2,7-diacetoxyundecane (A, B, C, D) were separated using an isocratic solvent system of 0.5% isopropan-2-ol in hexane at 0.3 ml/min. In initial work isomers B and C were not resolved and were collected together (B+C). Later, improvements in column conditioning made it possible to separate all four stereoisomers. The two stereoisomers of synthetic 7-acetoxyundecane-2-one (1 and 2) were separated by eluting with 1% isopropan-2-ol in hexane at 0.5 ml/min. The stereoisomers were collected by hand separately into sample vials and quantified by gas chromatography.
**Field tests**

Field trapping tests were carried out with white delta traps (28 cm long × 20 cm sides; Agrisense, Treforest, UK) **HUNG AT 10 M INTERVALS APPROXIMATELY 1 M ABOVE THE GROUND.** The pheromone was dispensed from rubber septa (Z10,072-2; Sigma Aldrich, Gillingham, UK).

In the first field test, two individual stereoisomers (A and D) and mixed isomers (B+C) of 2,7-diacetoxyundecane and the two stereoisomers of 7-acetoxyundecane-2-one (1 and 2) at 4 μg loadings were tested alongside racemates of the major and minor components (16 μg/dispenser). The test was carried out at Elmstone Court and Mole End Farms near Maidstone, Kent, UK, in early March, 2008. Traps were put in the field at the time when pear buds were green. At each site, three replicates of each treatment were tested in a **RANDOMISED COMPLETE BLOCK DESIGN. TRAPS WERE MONITORED WEEKLY FROM 4-25 MARCH 2008.**

**IN A Second field test the four stereoisomers of 2,7-diacetoxyundecane (A, B, C and D) and the attractive isomer 1 of 7-acetoxyundecane-2-one (4 μg) and binary mixtures of isomers A and D of 2,7-diacetoxyundecane A and D with 1 in 1:10 ratio (4 + 0.4 μg) were tested against unbaited control traps. Four replicates were deployed at Elmstone Court and the traps were monitored from 26 March - 1 April 2008.**

Data were transformed to log (x+1) to fit the assumptions of homogeneity of analysis of variance. The data were subjected to analysis of variance (ANOVA). If significant differences (**P** < 0.05) among treatments were revealed by ANOVA, means were differentiated with the least significant difference (LSD) test.

**Results and discussion**

**Pheromone identification**

![GC-EAG analysis of volatile collections from female *C. pyrivora* with male EAG preparations on non polar GC column. EAG responses are marked with *](image)

Analysis of collections of volatiles from female *C. pyrivora* by GC-EAG showed two responses from male midges (Figure 1). These responses were not observed in analyses of
collections from male midges. Results were consistent when the experiment was repeated with the same or a different insect. On the polar column the responses appeared at RI 1477 and 1435 and on the non-polar column at RI 1311 and 1161. GC-MS traces of male and female volatiles were compared and the active components at the corresponding RI’s were present only in volatile collections from females and these compounds were assumed to be components of the female sex pheromone.

The peaks responsible for the EAG responses were explored using the mass fragmentation pattern and comparison of retention indices with a range of standard compounds available at NRI. They were identified as 2,7-diacetoxynonadecane and 7-acetoxy-2-undecanone as the major and the minor components respectively. These two compounds were synthesised in racemic form and the stereoisomers separated by HPLC on a chiral column.

**Field tests**

In the first field test, significant numbers of males of *C. pyrivora* were caught during the period 13-19 March 2008 with much higher numbers at Mole End (Figure 2).

![Figure 2.](image)

Significantly more males were attracted to traps baited with the first and last eluting stereoisomers of 2,7-diacetoxynonadecane (A, D and B+C), 7-acetoxynonadecane-2-one (1 and 2) and the corresponding racemates (major rac and minor rac) (13-19 March 2008; 3 replicates at each site; means followed by the same letter are not significantly different *(P > 0.05)*).

In the second field test, stereoisomer A of 2,7-diacetoxynonadecane again attracted male...
C. pyrivora (ANOVA after transformation to log(x+1); $F = 42.98$, $df = 7, 21$, $P < 0.001$) (Figure 3). However, in this test isomer C was equally attractive but D was much less attractive. Traps baited with isomer B caught significantly fewer midges than unbaited traps, which possibly explains the unattractiveness of the mixture of B+C in the first test. Enantiomer 1 of the minor component, 7-acetox y-2-undecanone, was significantly attractive, although less attractive than A, as previously. Addition of minor component isomer 1 to major component isomer A did not significantly increase the attractiveness of the latter.

![Figure 3](image.png)

Figure 3. Mean number (± SE) of C. pyrivora males caught at Elmstone Court with HPLC separated stereoisomers of 2,7-diacetoxyundecane (A, B, C and D) and 7-acetox yundecanone-2-one (1) and blends of A+1 and D+1 (26 March – 1 April 2008; 4 replicates; means followed by the same letter are not significantly different ($P>0.05$)).

A third field trial was carried out from 13-17 April 2008 with the stereoisomers of the major component, 2,7-diacetoxyundecane (A-D) and binary blends. All treatments caught similar numbers of midges and closer examination of the catches revealed that at least one other species was present in addition to C. pyrivora. These were identified as a species of Resseliella (Keith Harris, personal communication).

In both field tests stereoisomer A of the major pheromone component, 2,7-diacetoxyundecane (A-D) and binary blends. All treatments caught similar numbers of midges and closer examination of the catches revealed that at least one other species was present in addition to C. pyrivora. These were identified as a species of Resseliella (Keith Harris, personal communication).

In both field tests stereoisomer A of the major pheromone component, 2,7-diacetoxyundecane, attracted significant numbers of male C. pyrivora midges. In the first test stereoisomer D was also attractive, but not in the second test. Only a blend of B+C was available in the first test and this was unattractive. In the second test these isomers were tested separately and C was as attractive as A, while traps baited with B caught significantly fewer than the unbaited trap. Most probably isomer B inhibits attraction and is responsible for the unattractiveness of the blend of B+C and also the racemic mixture of all four isomers of 2,7-diacetoxyundecane. In view of the possibility that at least one other species was being caught in the traps, it is not possible to say whether isomers C and/or D are also attractive to C. pyrivora. Interestingly, stereoisomer 1 of the minor component, 7-acetox y-2-undecanone, was also attractive to male C. pyrivora, although generally not as attractive as A. Moreover, the racemic 7-acetox y-2-undecanone was as attractive as isomer 1 with the other enantiomer 2
being neither attractive nor inhibitory.

2,7-diacetoxyundecane, has a structure similar to those of pheromone components in other species of *Contarinia*, such as 2,12-diacetoxytridecane and 2,11-diacetoxytridecane in the pea midge, *C. pisi* (Hillbur et al., 1999, 2000) and 2,10-diacetoxyundecane and 2,9-diacetoxyundecane in the swede midge, *C. nasturtii* (Hillbur et al., 2005). The acetoxyketone, 7-acetoxy-2-undecanone, is an unusual structure previously only found in pheromones of two other midge species, apple leaf midge, *Dasineura mali* (Hall and Cross, 2005, Cross and Hall, 2009, Cross et al., 2009) and raspberry cane midge, *Resseliella theobaldi* (Hall et al., 2009).

Although it is likely that stereoisomer A of 2,7-diacetoxyundecane and enantiomer 1 of 7-acetoxy-2-undecanone are components of the female sex pheromone of *C. pyrivora*, the results of the field tests were probably confused by the presence of at least one other species of midge in the traps, and the trapping experiments will be repeated with careful identification of the species caught. Furthermore the absolute configurations of the stereoisomers of both components separated by HPLC remain to be determined.

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**References**


Raspberry beetle (*Byturus tomentosus*) flight monitoring and control with semiochemical traps

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**Abstract:** The raspberry beetle, *Byturus tomentosus* is a major pest of Swiss raspberries. In 2008, in the frame of an international cooperation with UK, Norway and France the flight activity of the raspberry beetle has been monitored for the first time in the Swiss Alps with the semiochemical trap (floral attractant) and non-sticky funnel trap developed in Scotland by SCRI. Early results show an irregular attractiveness of the trap. The traps were installed before flowering at the beginning of June and were immediately attractive for 2 weeks. Then the catch of raspberry beetles decreased till end of July. A second important flight activity pattern was observed at the end of July and at the beginning of August. Fruit analysis showed that there was a gradient in the percentage of damaged fruits. Around the traps the damage was about 1% but the average of the whole plot was 5% in one plot and 9% in the second one with semiochemical traps. Neighbouring woods with wild *Rubus* sp. and other wild hosts near the plot could explain high raspberry beetle populations. This monitoring will continue for three years.

**Key words:** *Byturus tomentosus*, controlling, monitoring, raspberry, soft fruits,

**Introduction**

The raspberry beetle, *Byturus tomentosus* (Degeer) is a major pest of Swiss raspberries especially in the higher altitudes (Antonin, 1984). Both adults and larvae cause damage (Gordon & al, 1997, Schmid & al. 2006). The adults feed on raspberry and blackberry, more rarely on *Crataegus*, apple and pear. *Byturus tomentosus* has 1 generation per year. There is a demand for alternative methods to control pests without residues on fresh fruits. The Scottish Crop Institute (SCRI) developed a new type of trap based on the raspberry flower volatiles already tested in Scotland and Norway (Birch & al., 2009; Trandem & al., 2009). The aim of this trial was to test this semiochemical trap in Swiss climatic conditions. The results after one year are presented and discussed hereafter.

**Material and methods**

Flight activity monitoring in 2008 was conducted in two locations: Bruson (1060m alt.) and Nendaz (1300m alt.), with 1 control plot and 2 trial plots. In the control plot in Bruson the raspberry variety Zeva2 was uncovered (open field plantation) and unsprayed, with white sticky traps (10/ha) used to monitor flight activity. In both trial plots the variety was Glen Ample, uncovered and unsprayed. Semiochemical traps and white sticky traps were installed in the trial plots at the stage 53 (flowers in buds) at the beginning of June: 4 traps in Bruson and 11 traps in Nendaz with a concentration of 50 traps/ha for the semiochemical traps and 10/ha for the sticky traps. During the harvest, fruits were scored for presence or absence of larvae. An average of 500
fruits was sampled per plot and per control date. An average of 10 fruits per trap (110 fruits for 11 traps) was extra sampled in the area next to the 11 semiochemical traps. The results around the traps, in the whole plot and in the control plot allowed analysis of the semiochemical trap’s efficiency in monitoring flight activity.

Results and discussion

The semiochemical traps were attractive from the beginning of June during the two weeks before flowering, between stage 53 and 60 (stage 53: flowers in buds; stage 60: first flowers open). Then the catch decreased till end of July. At that time raspberries are fully flowering. Raspberry beetles were more attracted to the flowers (Fig.1). A second important catch period was observed at the end of July and at the beginning of August. During this period, the raspberry flowering is over and the fruits are ripe. The stage 89 (first ripe fruits) was on the 15 of July.

![Figure 1. Number of adult Byturus caught per trap (semiochemical and white sticky trap) in Nendaz in 2008. Average of 11 semiochemical traps and 2 white sticky traps.](image)

The white sticky traps are attractive for a shorter period (7 out of 17 weeks) and to a lesser extent than the semiochemical enhanced traps (Fig.1). The Nendaz plot showed a higher attack of raspberry beetle (total catch/trap: 41) than the Bruson plot (total catch/trap: 24) but both plots showed the same temporal flight activity patterns (Fig.2). In comparison the French results show a different flight activity pattern. The French trial plots were in a warmer and earlier flowering climate than the Swiss ones. The traps were attractive to raspberry beetles only before flowering. There was not a second peak of activity in July and August like in Switzerland (Rivière, 2008).
Figure 2. Cumulated number of adult *Byturus* caught per trap (semiochemical trap) in Nendaz and Bruson in 2008. Average of 11 traps in Nendaz and 4 traps in Bruson.

The average percentage of damaged fruits was 6.8% in the trial plot with semiochemical traps and 9.4% in the control plot (Table 1). However, in the fruits sampled immediately around the semiochemical traps the fruits were less attacked; the average was only 0.9%. The traps efficiency could be improved with a better positioning of the semiochemical traps in the plot. The trial 2009 will investigate this question.

Table 1. Damaged fruits in Bruson in % in the control plot (sticky traps), in the trial plot (semiochemical traps) in the lines between the traps and in each area immediately around the 11 semiochemical traps.

<table>
<thead>
<tr>
<th>Date</th>
<th>Control Bruson (sticky traps)</th>
<th>Trial plot Bruson (between semiochemical traps)</th>
<th>Around traps Bruson (next to semiochemical traps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.07.08</td>
<td>10</td>
<td>5.2</td>
<td>0.5</td>
</tr>
<tr>
<td>28.07.08</td>
<td>11.5</td>
<td>4.2</td>
<td>1.1</td>
</tr>
<tr>
<td>04.08.08</td>
<td>5.2</td>
<td>4.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Conclusions

Semiochemical traps are useful for IPM in raspberries plantations in the mountains. The semiochemical enhanced traps catch earlier, later and more target pests. A considerable reduction of the damages on fruits was observed in Bruson compared to the control plot. The positioning and the concentration (numbers/ha) of the traps must be further studied to reduce further the amount of fruit damage throughout the plots. The aim of the further experiments is to optimize the efficiency of the traps for monitoring and/or population reduction and to define the damage threshold for this new type of trap compared with the standard white sticky trap currently used in Switzerland.
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Control of the Plum Fruit Moth, Grapholita funebrana (Treitsch.) (Lepidoptera, Tortricidae), by false-trail following

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Abstract: Grapholita funebrana (plum fruit moth) is a serious pest in many plum orchards in Italy. Control of the plum fruit moth using the false-trail following technique or ‘sexual disorientation’ is here evaluated in two commercial plum orchards for baby-food production, based on a zero pesticide residue management system. The effectiveness of the false-trail following technique was demonstrated through experimental trials over two seasons in two orchards located in the Ascoli Piceno Province of the Marche Region (central-eastern Italy). Specific, biodegradable, pheromone dispensers, known as Ecodian CF™, were used for each application, with about 2,000/ha. During 2005, three dispenser applications were carried out, with two in 2006. The evaluation of this technique was through monitoring adult males by specific synthetic sex pheromone traps and visual inspections for fruit damage. Anarsia lineatella (peach twig borer), a secondary pest in plum orchards, was also monitored. The efficacy of Ecodian CF™ dispensers was compared with that achieved in commercial plum orchards sprayed with chemical insecticides or managed with mating disruption techniques. Over the two seasons, the control of the plum fruit moth in the experimental orchards was as good as or better than that in the check plots.

Key words: Grapholita funebrana, plum fruit, sex pheromone, false-trail following

Introduction

Grapholita funebrana Treitschke (Lepidoptera: Tortricidae) (Plum Fruit Moth, PFM) is an oligophagous species, feeding on the fruits of several hosts typically within the plant family Rosaceae. PFM is the key pest of plum (Prunus spp.) in most parts of Europe. In Italy, it has three generations per year (Molinari, 1994), while in many areas of central and eastern Europe, it has one or two (Vernon, 1971).

Deregulation of many of the insecticides used in the control of this pest along with public demand for residue-free products, increased the interest for innovative tools in pest management. Behaviour-modifying pheromones can be used for environmentally safe insect management and the technique has become one of the most important instruments for controlling the main pest in the orchards. In the specific situation of plum fruit growing in the Marche region (central eastern Italy) the main factors hindering its spread are the relatively high price as compared to insecticide treatments, and additional pests that are partially controlled by insecticide treatments but not by specific pheromone dispensers. Moreover, in our conditions, most orchards cover areas of less than 2 ha.

Control of the PFM using the false-trail following technique or ‘sexual distraction’ is here evaluated in plum orchards for baby-food production, based on a zero pesticide residue management system. This technique consists of the setting up of several false trails, released by dispensers loaded with low synthetic sex pheromone dosage, able to compete with those of wild females and thus distract males in their search for partners (Maini and Accinelli, 2000).
This diversion of the male’s activities results in either a decrease in the proportion of mated females or a delay in mating.

Material and methods

Ecodian CF™ dispenser release rate. During 2006, at every site, four field-aged dispensers were collected every week and stored at -20°C. Then each dispenser was weighed, dissolved in 20 ml of tetrahydrofurane containing n-hexadecanol as internal standard, and analysed by gas chromatography.

Field tests. Experiments were carried out over a two year period (2005 and 2006), in two commercial plum orchards (Prunus domestica cv Stanley) located in the Ascoli Piceno Province of the Marche Region (central eastern Italy). Plum orchards were located in Capodarco (43°11’27.47”N, 13°45’40.00”E) and Rubbianello (43°3’39.64”N, 13°42’55.44”E) districts. Each experimental orchard was about 1 hectare.

Specific, biodegradable, PFM synthetic sex pheromone dispensers, known as Ecodian CF™, numbering 2000/ha for a total amount of 18 g. a.i./ha of Z8-dodecenyl acetate and dodecyl acetate in 1:1 ratio, were used for each application. Two different application protocols were used: during 2005, three dispenser applications were carried out (March 23-25, May 16 and July 8-15) while only two dispenser applications were made in 2006 (April 20 and June 27) with the purpose of reducing application costs.

The evaluation of the technique efficacy included: (1) monitoring of specific synthetic sex pheromone trap catches; (2) visual inspections on 500 fruits at five different locations per orchards (10 fruits for 10 plum trees) and expressing damage caused by PMF larvae as the proportion of attacked fruits.

The monitoring sex pheromone traps (Traptest® Isagro) were installed in the field on March 18 during 2005 and on April 13 during 2006. Trap captures were checked weekly and sex pheromone dispensers were replaced every 30 days.

The efficacy of Ecodian CF™ dispensers in reducing fruit damage was compared with that achieved in nearby commercial plum orchards sprayed with conventional insecticides (C) (ca. 0.80 ha) or managed with mating disruption techniques (MD) (ca. 1.50 ha).

Anarsia lineatella Zeller (Lepidoptera: Gelechiidae) (Peach Twig Borer, PTB), a secondary pest in plum orchards, was also monitored.

Results and discussion

Ecodian CF™ dispenser release rate. The pheromone emission significantly decreased over the field-ageing period. During the first month of ageing, maybe due to air temperature, the release rate of dispensers placed in the field at the beginning of the summer decreased more quickly than that of dispensers placed in the field in spring. In the climate conditions of the Marche region the life time of the dispensers was at least 75-80 days for the first application (Figure 1).
Field experiments. The field trials confirmed the efficacy of the Ecodian dispensers for PFM control. Over the 2-year study period, in the Ecodian treated plots the percentage of fruit damage recorded was lower than that in the reference plots. Damage caused by PTB larvae was also low in the Ecodian treated plots (Table 1).

In the field trials carried out in 2005 and 2006, the Ecodian treatment strongly inhibited PMF male capture in the sex pheromone traps (Figure 2). The reduction in attraction of sex pheromone traps, in comparison with catches in neighbouring areas, is considered one of the easiest tests to indirectly evaluate the efficacy of pheromone based control methods (Charmillot, 1992).

Three peaks of captures were recorded for *G. funebrana* in the control plots (Figure 2) and two for *A. lineatella* (middle May and end July) in the Capodarco Ecodian treated plot while in the other site the presence of PTB males in the trap was very low (data not shown).

Table 1. Percentage of fruit damaged by PFM and PTB larvae in the different treatments. FTFc=false trail following-Capodarco, MDc=mating disruption-Capodarco, FTFr=false trail following-Rubbianello, CR=conventional insecticides–Rubbianello, - no visual inspection. * On July 25 a hail storm destroyed over 96% of fruit production.

<table>
<thead>
<tr>
<th>Date</th>
<th>FTFc_PFM</th>
<th>FTFc_PTB</th>
<th>MDc_PFM</th>
<th>MDc_PTB</th>
<th>FTFr_PFM</th>
<th>FTFr_PTB</th>
<th>CR_PFM</th>
<th>CR_PTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-06-02</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2005-07-30</td>
<td>0</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0</td>
<td>-*</td>
<td>-*</td>
<td>-*</td>
</tr>
<tr>
<td>2005-08-19</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.2</td>
<td>0</td>
<td>-*</td>
<td>-*</td>
<td>-*</td>
</tr>
<tr>
<td>2006-07-16</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2006-08-20</td>
<td>0</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Our field trials showed that with Ecodian dispensers, PFM can be controlled effectively under the climatic conditions of the Ascoli Piceno Province, confirming the results obtained in other areas (Molinari et al., 2000). Moreover our data suggest that the false trail following technique may be a feasible way for plum fruit moth control in the plum growing areas of the
Marche region and that, in some conditions, it is possible to use only two Ecodian dispenser applications with a large reduction of the technique costs.

The peculiar mode of action of the false-trail following method (low pheromone dosage coupled with high efficacy in small plot size) as well as the possibility to use only two Ecodian dispenser applications for the control of *G. funebrana* open new opportunities for a widespread use of this pheromone based strategy for plum pest control.

**Acknowledgements**

We are greatly indebted to Provino Murri, Giorgio Murri, Umberto Santoni and Carlo Rosati for allowing us to make the observations in their orchards and for assistance in data recording and to G. Caricato, P. Ruggiero and M. Pizzi for their support and help in the work.

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Eight years of practical experience with mating disruption to control grape berry moth, *Lobesia botrana*, in Porto Wine Region

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Abstract: Since 2000 the mating disruption technique has been applied to control *Lobesia botrana* (Den. & Schiff.) in the Porto Wine Region. ISONET-L dispensers have been used in plots whose surface ranged from 3.0 to 25.0 ha. The average percentage of male disorientation for the 8-year experimental period ranged from 80.5 to 100%, being 100% in 55.5% of the 72 sampling periods studied. However, the rate of reduction obtained in larval infestation by the pest, even in favourable conditions (large areas and continuous application), was variable. Some constraints to the technique have been identified, such as the high biotic potential of the species, the high summer temperatures and the local orography (high steepness). In this paper, the results are critically discussed and weak spots are analyzed, as a basis for identifying the real possibilities of the technique in the Porto Wine Region.

Key words: viticulture, pest management, sex pheromone, ISONET-L dispensers

Introduction

*Lobesia botrana* (Den. & Schiff.) is a key pest in the Porto Wine Region (PWR), where considerable losses are being registered, mainly during recent years. In this region, the moth has three flight periods a year, between the middle of March to the middle of October. Public demand for residue-free products, have augmented the interest for innovative tools to control the pest. The mating disruption (MD) technique, which was registered in Portugal for *L. botrana* in 2002, seems to be a good alternative to insecticide applications against this species. In this paper, results obtained with mating disruption to control *L. botrana* during an 8-year period are critically discussed and weak spots are analyzed, as a basis for identifying the real possibilities of its use in the Porto Wine Region.

Material and methods

Vineyards

The experiments were carried out from 2000 to 2007 in commercial vineyards located in PWR (Table 1). Typical vineyards in this region are fragmented into plots of variable size shape, orientation and varieties. The experimental vineyards were grown on slopes ranging from 30-50% (in either small terraces or vertically planted rows), had a surface ranging from 3.0 to 25.0 ha and were planted with several varieties (mainly Touriga Franca, Touriga Nacional, Tinta Barroca and Tinta Roriz).
**Type and density of dispensers**

Pheromone dispensers ISONET-L from SHIN-ETSU Chemical Co., Ltd. with 172 mg (E,Z9-12:Ac) were installed during March, before the beginning of the first flight period at a density of 500-700 ha⁻¹, depending on the experimental site (surface, steepness, winds), with a reinforcement of 10-20% in the 15-20 m of the border and in the top of the hills.

Table 1 – General information about the experimental work carried out

<table>
<thead>
<tr>
<th>Year</th>
<th>Wine-farm</th>
<th>Surface (ha)</th>
<th>Altitude (m)</th>
<th>Dispensers/ ha</th>
<th>Date of installation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Seara d'ordens</td>
<td>4.0</td>
<td>440</td>
<td>500</td>
<td>13-Mar</td>
</tr>
<tr>
<td>2001</td>
<td>Seara d'ordens</td>
<td>4.0</td>
<td>440</td>
<td>500</td>
<td>19-Mar</td>
</tr>
<tr>
<td>2001</td>
<td>S. Luiz</td>
<td>3.0</td>
<td>210</td>
<td>650</td>
<td>12-Mar</td>
</tr>
<tr>
<td>2002</td>
<td>S. Luiz</td>
<td>25.0</td>
<td>210</td>
<td>560</td>
<td>19-Mar</td>
</tr>
<tr>
<td>2003</td>
<td>S. Luiz</td>
<td>15.0</td>
<td>210</td>
<td>600</td>
<td>21-Mar</td>
</tr>
<tr>
<td>2004</td>
<td>S. Luiz</td>
<td>16.2</td>
<td>210</td>
<td>560</td>
<td>12-Mar</td>
</tr>
<tr>
<td>2005</td>
<td>D. Matilde</td>
<td>7.1</td>
<td>200</td>
<td>630</td>
<td>23-Mar</td>
</tr>
<tr>
<td>2005</td>
<td>Vallado</td>
<td>11.8</td>
<td>150</td>
<td>660</td>
<td>23-Mar</td>
</tr>
<tr>
<td>2006</td>
<td>Vallado</td>
<td>11.8</td>
<td>150</td>
<td>700</td>
<td>21-Mar</td>
</tr>
<tr>
<td>2006</td>
<td>D. Matilde</td>
<td>7.1</td>
<td>200</td>
<td>630</td>
<td>22-Mar</td>
</tr>
<tr>
<td>2006</td>
<td>Cidrô</td>
<td>15.2</td>
<td>610</td>
<td>585</td>
<td>20-Mar</td>
</tr>
<tr>
<td>2007</td>
<td>Vallado</td>
<td>19.7</td>
<td>150</td>
<td>560</td>
<td>21-Mar</td>
</tr>
<tr>
<td>2007</td>
<td>Cidrô</td>
<td>16.7</td>
<td>610</td>
<td>600</td>
<td>20-Mar</td>
</tr>
</tbody>
</table>

**Evolution of pheromones in the dispensers**

The active ingredient in the dispensers was calculated by collecting samples of 10 dispensers monthly from March to September, in 2000, 2002 and 2006.

**Evaluation of MD efficacy**

MD efficacy was evaluated by comparing either pheromone-baited trap catches or grape infestation, between treated and untreated plots, at a number of “inspection stations” per plot, depending on plot surface. Pheromone delta traps (AgriSense BCS Ltd.) were installed in the centre of each plot (control and pheromone-treated plot) and, in some years, also in the border zones. Afterwards, the percentage of male disorientation was calculated for each weekly trapping interval. Grape infestation was assessed by inspecting samples of 100 randomly collected inflorescences or grapes, according to the season, during each of the three generations of the insect. In addition a sample of 50 to 100 grapes was collected at harvest and dissected to look for larvae. In the first generation the level of infestation was expressed as the number of nests per 100 inflorescences, while in the second and in the third generations it was expressed as the percentage of grapes with at least one berry infested. The total number of inflorescences plus grapes examined per plot/year was between 1600 and 15,400 depending on the plot size and the year. Results are expressed as the percentage reduction of infestation in the MD plot compared with the control for each generation and also at harvest. Each time the treatment threshold was surpassed a curative insecticide treatment was applied with either an insect growth regulator or *Bacillus thuringiensis*, mainly in the second and the third generations.
Results and discussion

Evolution of pheromones in the dispensers
During the three years, the release of pheromone was almost linear from the period when the dispensers were installed (in the middle of March) until the beginning/middle of August. During this period the dispensers released about 80% of the pheromone, in 2000 and 2006, and as much as 93%, in 2002. These results are in accordance with those reported by Moschos et al. (2004) in Greece, who found that if the temperatures are high during the preceding period, little pheromone is left in the dispensers during the crucial period of the third generation -- August in PWR.

MD efficacy
The average percentage of male disorientation for the 8-year period ranged from 80.5 to 100%, achieving 100% in 55.5% of the 72 sampling periods studied, which suggests that treatment with pheromone almost completely prevented male moths from locating sources of synthetic sex pheromone. As expected, poor results in the reduction of *L. botrana* larval density were obtained in small plots, such as in the Seara d’ordens farm, in both years and in S. Luiz, in 2001 (Table 2). According to Stockel & Chichignoud (1994), the MD area should have a minimal of 10 hectares, to avoid the migration of mated females from the neighbouring vineyards into the MD treated vineyards. In the plots were the technique was applied in more favourable conditions (larger areas and continuous application), namely the S. Luiz and Vallado farms, its efficacy in the reduction of *L. botrana* larval density was variable (Tables 2 and 3), suggesting that other constraints have been involved in this efficacy. The early exhaustion of the pheromone in the dispensers, related to high temperatures registered during summer in the region, could be one of the main reasons of the method’s failure, mainly as the grapevine moth has a long flight period in the region (about 29 weeks, which lasts from middle March to middle October) (Carlos et al., 2008). However, it must be noted that high temperatures, such as those that occurred in 2002, 2005 and 2006, could also have hampered the development of the third generation of the insect, leading to a low infestation level. On the contrary, when weather conditions are favourable, a high biotic potential could be reached, leading to high population densities, which enhances the probability of mating even in a pheromone environment and reduces the effectiveness of MD (Louis & Schirra, 2001; Moschos et al., 2004).

Table 2. Infestation level \(^{(1)}\) by *Lobesia botrana* and infestation reduction (%) at S. Luiz farm

<table>
<thead>
<tr>
<th>Year</th>
<th>/Generation</th>
<th>Control</th>
<th>MD interior</th>
<th>Infestation red. (%)</th>
<th>MD border</th>
<th>Infestation red. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>1(^{st})</td>
<td>28</td>
<td>4</td>
<td>86</td>
<td>4</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>2(^{nd})</td>
<td>9*</td>
<td>1*</td>
<td>89</td>
<td>1</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>3(^{rd})</td>
<td>31*</td>
<td>13</td>
<td>58</td>
<td>13</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>47</td>
<td>55</td>
<td>0</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td>2002</td>
<td>1(^{st})</td>
<td>36</td>
<td>13*</td>
<td>64</td>
<td>18*</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>2(^{nd})</td>
<td>11*</td>
<td>3*</td>
<td>73</td>
<td>10*</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3(^{rd})</td>
<td>6*</td>
<td>2</td>
<td>67</td>
<td>3*</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>5</td>
<td>3</td>
<td>40</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>2003</td>
<td>1(^{st})</td>
<td>31</td>
<td>5</td>
<td>84</td>
<td>9</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>2(^{nd})</td>
<td>13</td>
<td>4</td>
<td>69</td>
<td>4</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>3(^{rd})</td>
<td>1</td>
<td>0</td>
<td>100</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Harvest</td>
<td>52</td>
<td>10</td>
<td>81</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>2004</td>
<td>1(^{st})</td>
<td>71</td>
<td>3</td>
<td>96</td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>2(^{nd})</td>
<td>47</td>
<td>17</td>
<td>64</td>
<td>15</td>
<td>68</td>
</tr>
</tbody>
</table>
The poor results obtained with MD could also be attributable to a deficient distribution of the pheromone cloud in the vineyards, due to either its high gradient or the presence of laneways bordered by olive/almond trees around the plots.

In conclusion, the experience with MD at PWR have demonstrated that to effectively control *L. botrana* in years of high pest pressure, the technique has to be combined with insecticides, at least for the third generation. The solution proposed by Moschos et al. (2004): a second deployment of dispensers just before the beginning of the third flight period, will greatly increase the costs of the technique, which are already high (around 200 €/ha for MD, compared with 75-90 €/ha, for an insecticide treatment).

**Acknowledgements**

Thanks are due to: wine-growers associated to ADVID (Sogevinus Vinhos S.A. Companhia Geral da Agricultura das vinhas do Alto Douro. Quinta do Vallado Soc. Agr. Lda. Quinta D. Matilde - vinhos Lda and Soc. Agrícola Quinta da Seara D’ordens Lda.) who made their vineyards available for the experiments. To Sogevinus Vinhos S.A., CBC (Europe Ltd.) and Shin-Etsu Chemical Co., Ltd., for supplying the pheromone dispensers. To all ADVID colleagues, mainly to Jorge Costa and Branca Teixeira for technical support.

**References**


Use of Sprayable Pheromone Formulations in Europe.

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Abstract: Sprayable formulations of pheromones for the mating disruption control of different moth species offers an innovative alternative to the use of the current dispenser based technologies. While still assuring the same efficacy as the dispenser systems, the sprayables offer greater flexibility and ease of use. Applied using standard spray equipment, the sprayables can be combined with other treatments. The paper will review the technology, efficacy and use strategies of the Checkmate sprayable technology in Europe.

Key words: Pheromone, mating disruption, sprayable, Checkmate.

Introduction
Sprayable formulations of pheromones for the mating disruption control of different moth species offers an innovative alternative to the use of the current dispenser based technologies. While still assuring the same efficacy as the dispenser systems, the Checkmate Flow range offers greater flexibility and ease of use. Applied using standard spray equipment, the sprayables can be combined with other treatments. The large number of small pheromone emission points provides the crop with complete pheromone coverage over the entire crop.

Formulation technology
Encapsulated formulations can contain one or more active pheromones of the insect pest. The pheromone is contained within the central lumen of the capsule and is released through the semi-permeable walls over the required time.

Product Range
Microencapsulation technology has been developed for a wide range of pheromones and pests. Table 1 provides a list of the formulations currently available in Europe.

<table>
<thead>
<tr>
<th>Product</th>
<th>Presentation</th>
<th>Application Rate</th>
<th>Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>CheckMate CM-F * # (Laspeyresia pomonella)</td>
<td>14.3% a.i. 739ml bottle</td>
<td>90ml/ha/15 days</td>
<td>Apples/Pears/Walnuts</td>
</tr>
<tr>
<td>CheckMate OFM-F * (Grapholitha molesta)</td>
<td>23.6% a.i. 390ml bottle</td>
<td>45ml/ha/15 days</td>
<td>Peaches/Nectarines/</td>
</tr>
<tr>
<td>CheckMate PTB-F (Anarsia lineatella)</td>
<td>17.89% a.i. 461ml bottle</td>
<td>145ml/ha/30 days</td>
<td>Peaches/Nectarines/</td>
</tr>
<tr>
<td>CheckMate BAW-F (Spodoptera exigua)</td>
<td>17.14% a.i. 477ml bottle</td>
<td>175ml/ha/30 days</td>
<td>Vegetables</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of sprayable pheromone formulations available in Europe.
Product Application

The formulations are designed for application using conventional spray equipment. They are compatible with most other treatments and are therefore easy to tank mix with other products with a minimum of precautions. This also facilitates their integration with either conventional insecticides or pheromone dispenser products at different times of the season.

The product can offer either season long control, applying treatments at specific intervals, or can be timed with different insect generations to complement other control strategies.

Typical efficacy

The products have been tested over a wide range of conditions over the years. Trials have demonstrated that the sprayable formulations offer the same or better performance as the dispenser based technologies.

Various application strategies have been tested with the products applied at different doses and corresponding intervals. The products can be applied from a low rate at 15 day intervals to a high rate at 28 days without compromising performance.

Rainfastness

Rainfastness was measured by using a specially made UV fluorescing marked variant of the Checkmate CM–F formulation. Orchard trees were treated using a conventional sprayer at higher rates 8-10 times standard field application rate. Leaves were collected and exposed under artificial rain regimes simulating different rain intensities. A photo camera connected to a computer with software able to detect the marked capsules counts them on the treated surface.

The results show that the wash off caused by increasing rain intensity over 60 minutes led to about 15% wash off at 10mm per hour increasing linearly to 60% wash off at a very heavy down pour of 40mm per hour. Measuring wash off over a 30 minute interval confirmed that most of the wash off occurs in the first 30 minutes with. At any given rain intensity, over three quarters of lost material was removed in the first half hour.

As part of the same experiment the distribution of the formulation on the trees was assessed. The data confirm the relative distribution of the pheromone capsules on the different surfaces of the leaves. Using normal spray equipment the study confirmed that the majority of the pheromone capsules are deposited on the lower surface of the leaves. Using a 10x standard application rate an average of 47 capsules were found on the lower leaf surface compared to 12 capsules on the upper leaf surface. The good rainfastness of the product is probably correlated to the deposition pattern of the microcapsules; the capsules landing on the upper surface would be easily removed by rain while those on the lower surface would be protected and more difficult to remove.

CM-F shows good rainfastness under conditions of these trials with circa 50% remaining even under the most severe conditions. There is a clear correlation between rain intensity and fastness. Indications are that most of wash off occurs quickly with increasing duration of rain
having lesser effects. The good persistence is possibly a correlation between persistence and deposition pattern with most of lost capsules likely to be those deposited on top of leaves.

Conclusions

The Checkmate Flow formulations offer an innovative alternative for the control of a range of agricultural pests giving greater flexibility and ease of use compared to other systems. With the same or better efficacy than other pheromone technologies and application with conventional spray equipment, the Flows offer easy and flexible integration with other pest management strategies. Applied at the higher rates, the formulations will last up to 4 weeks but often the simpler approach has been to use lower rates at shorter intervals integrating them with other orchard treatments and at the same time minimizing any adverse effects of rains or bad weather on the performance of the product.

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Cells responding to pheromone components and plant volatiles could affect semiochemical based control strategies of insect pests in agricultural ecosystems

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Abstract: Electrophysiological and behavioural responses by several insect pests have been recently recorded in order to identify plant volatile compounds, and particularly kairomones, involved in the host-finding process and oviposition site selection. Such compounds have been addressed as candidates to be used in semiochemical based control strategies since they are potentially able either to enhance the sex pheromone activity or to monitor female emergence or to interfere on their behaviour. During similar studies, olfactory cells sensitive both to pheromone components and plant volatiles in Cydia pomonella antennae were described. In the present paper we analysed single cell recordings (SCR, surface contact technique) from olfactory neurons of different tortricid moths (C. pomonella, C. splendana, C. fagiglandana, Pammene fasciana, Lobesia botrana) stimulated by the two categories of compounds. Cellular types varying from the specific (relatively to the tested compounds) to the highly generalist ones were identified. The finding of these cells partly supports the observations reported by various authors about the ability of plant compounds to modulate the biological activity of a pheromone component. It seems not inappropriate to hypothesize that these “peripheral interferences” in odour perception could culminate in changeable behavioural responses that should also be of practical importance when pheromone based control strategies are applied in different agricultural environments, where they frequently show a variable efficiency.

Kairomones, Olfactory neurons, SCR, Fruit crops, IPM
Combining pear ester with codlemone improves management of codling moth

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Abstract: Several management approaches utilizing pear ester combined with codlemone have been developed in the first 10 years after the discovery of this ripe pear fruit volatile’s kairomonal activity for larvae and both sexes of codling moth. These include a lure that consistently outperforms other high load pheromone lures within pheromone-treated orchards, and the use of a microencapsulated formulation that can improve both mating disruption and the effectiveness of insecticide sprays. Field studies demonstrating the effectiveness of combining pear ester with codlemone are presented.

Key words: Cydia pomonella, monitoring, mating disruption, apple

Introduction

The adoption of technology utilizing the sex pheromone (codlemone) of codling moth, Cydia pomonella (L.), for mating disruption (MD) has increased dramatically in recent years (Witzgall et al. 2008). These tactics have been effective in allowing growers to significantly reduce their use of insecticides (Knight 2007). However, pest abundance remains a key factor limiting the effectiveness of MD and management programs must still integrate pheromone use with an array of insecticide sprays when populations exceed thresholds.

Pear ester is a potent kairomone attractant for codling moth and action thresholds based on total and female moth catches in pear ester-baited traps have been developed (Knight and Light 2005). However, growers using MD in Washington State have widely adopted a combo lure (Pherocon CM-DA Combo, Trécé Inc., Adair, OK) that is loaded with both pear ester and codlemone. The use of the two attractants together synergizes the catch of male but not female moths (Knight et al. 2005). The emission characteristics and attractiveness of field aged Combo lures have not previously been reported.

A micro-encapsulated (MEC) formulation of pear ester (DA-MEC, Trécé Inc.) has been developed and has been effective in improving the performance of insecticides, such as granulosis virus (Arthurs et al. 2007, Schmidt et al. 2008), and several synthetic insecticides (Light and Bouyssounouse 2006). DA-MEC has also been evaluated when used alone and in combination with a MEC pheromone formulation for MD of codling moth in apple and walnut (Light and Knight 2005). Whether growers can enhance both larval control with insecticides and pheromone-based MD at the same time with the additional use of DA-MEC has not previously been considered. Hand-applied dispensers, by a large margin, are the mostly widely used MD technique (Witzgall et al. 2008). Therefore, seasonal management programs which add DA-MEC to a series of insecticide and other sprays during the season in orchards treated with hand-applied dispensers should first be evaluated with this combined approach.
Material and methods

The attractiveness and emission rate of field-aged Pherocon CM-DA Combo lures
Delta traps were divided into two groups ‘new’ and ‘old’ (n = 10) and baited with Pherocon CM-DA Combo lures pinned to the inner roof of traps on 28 June 2006. Traps were randomly spaced 10 m apart in an unsprayed apple orchard. Each week for 10 wks traps were checked, lures in ‘new’ traps were replaced, and traps were re-randomized. Analysis of variance (ANOVA) with lure type and week as the main factors were conducted with transformed (square-root) data.

Pherocon CM-DA Combo lures were field-aged inside delta-shaped traps in an apple orchard from 30 August – 19 October. Lures (n = 3) were collected on day 0 and after 7, 14, 21, 28, and 50 d. Volatile captures onto a polyurethane foam sorbent were run for 2 h with an airflow of 5L/min using the methods reported by Tomaszewska et al. (2005). Analyses were conducted with GC/MS using an Alltech EC-Wax column. Recovery of the internal standard, myristic acid averaged 106.2%. Lure emission rates (mg) are reported per 24 h.

Adding DA-MEC to pheromone-dispenser and insecticide-treated programs
Quadrants (0.2 – 0.4 ha) were established in two apple orchards in 2008. Four treatments were randomly assigned to each orchard quadrant and included: the use of five insecticide sprays alone (three sprays of acetamiprid [0.25 kg / 935 L ha⁻¹] followed by two sprays of spinetoram [0.49 kg / 935 L ha⁻¹] timed 17 d apart and applied with an air-blast sprayer), the same insecticide sprays but with the addition of 30 ml DA-MEC added per ha, the same insecticides plus the use of Cidetrak CM pheromone dispensers (Trécé Inc.) applied at 1,000 per hectare, and the same insecticides applied with DA-MEC and the use of Cidetrak CM pheromone dispensers. Each plot was monitored with a delta trap baited with a Pherocon CM-DA Combo lure and fruit injury was sampled prior to harvest (1,200 fruits per plot). Transformed data were tested with ANOVA.

Results

The attractiveness and emission rate of field-aged Pherocon CM-DA Combo lures
Field-aged lures caught significantly fewer moths than new Combo lures over the 10-wk study, $F_{1, 280} = 26.78, P < 0.0001$ (Fig. 1). This difference was most pronounced for lures > 2 wks-old. Combo lures released a much higher rate of pear ester than codlemone (Fig. 2).

![Figure 1. Comparison of the attractiveness of new and field-aged Pherocon CM-DA Combo lures.](image-url)
Emission rates of both compounds declined sharply over 14 d and then remained fairly constant for lures aged 21 - 50 d.

**Figure 2.** Emission rate of pear ester and codlemone from Pherocon CM-DA Combo lures aged in the field.

**Adding DA-MEC to pheromone-dispenser and insecticide-treated programs.**
Mean cumulative moth catch per trap and fruit injury both varied significantly among treatments (Table 1). Pheromone treatments had significantly lower moth catch than the insecticide only treatment. The use of DA-MEC had a noticeable but not significant effect on moth catch. The addition of both MD and DA-MEC significantly reduced fruit injury and plots treated with the combination of both had the lowest level of injury.

**Table 1.** Seasonal comparison of moth catch and fruit injury

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (SE) moth catch</th>
<th>Mean (SE) % fruit injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticide</td>
<td>378.5 (2.5)a</td>
<td>10.6 (0.6)a</td>
</tr>
<tr>
<td>Insecticide + DA-MEC</td>
<td>225.5 (72.5)ab</td>
<td>3.5 (0.2)c</td>
</tr>
<tr>
<td>Insecticide + pheromone dispensers</td>
<td>131.5 (0.5)b</td>
<td>6.9 (0.6)b</td>
</tr>
<tr>
<td>Insecticide + DA-MEC + pheromone dispensers</td>
<td>87.0 (10.0)b</td>
<td>1.3 (0.0)d</td>
</tr>
</tbody>
</table>

ANOVA: \(df = 3,7\)

\[ F = 12.44, P < 0.05 \]

\[ F = 141.0, P < 0.001 \]

**Discussion**

Pear ester has proven to be a very attractive plant-derived volatile for codling moth, influencing both larval and adult behaviors (Light et al. 2001, Knight & Light 2001). Pear ester when used in combination with codlemone has helped to create new and more effective monitoring and management tools. Future work is focused on developing new action thresholds using the combo lure and with the use of pear ester in combination with acetic acid (Landolt et al. 2007). The addition of DA-MEC with various insecticides has suggested that pear ester can improve control of codling moth, especially with insecticides that are active via residual contact (Light & Bouyssounouse 2006). Pear ester can also be added to pheromone in combo dispensers to improve MD (Light & Knight 2005). Further evaluations of seasonal...
management programs in which pear ester is added regularly to all insecticide sprays in MD orchards are needed to more fully assess the novel characteristics of this compound.

Acknowledgements

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Using Insect Behavior to Facilitate Precision Agriculture: Odor-Baited Trap Trees For Management of the Plum Curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae)

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Abstract: Management programs for tree fruit have been developed based on an intensively managed perennial monoculture with standardized management practices. This design has had unforeseen consequences for pest management in that horticultural uniformity leads to a homogenous resource distribution requiring protection on a whole-orchard basis. The ecological foundation of insect behavior offers a clear opportunity to replace indiscriminate whole-orchard insecticide treatments with targeted management zones, bringing together the sustainability of IPM and behavioral control with the efficiency of precision agriculture. Behaviorally active stimuli are presented to attract and retain pests within a particular location in the orchard to allow for implementation of precise control strategies, thereby reducing insecticide inputs and increasing sustainability of the cropping system. The plum curculio, *Conotrachelus nenuphar* (Herbst), is one of the most destructive direct tree fruit pests in eastern North America. A novel approach termed the ‘odor-baited trap tree strategy’ (based on the tenets precision agriculture and insect behavior) has been developed to replace standard whole-orchard insecticide treatments. Select apple trees in the perimeter row are baited with a synergistic two-component lure comprised of the synthetic host plant-derived volatile benzaldehyde and the synthetic male-produced aggregation pheromone grandisoic acid in order to aggregate adult activity in specific perimeter row trees. Then by applying insecticides to these select baited trap trees rather than the entire perimeter row or whole orchard after petal fall, substantial reductions in the amount of insecticide applied can be achieved without compromising plum curculio control. Over the course of four years, comparisons of the trap-tree and perimeter-row treatment strategies have revealed that these strategies prevented penetration by immigrating populations of plum curculio and resulted in economically acceptable levels of injury. The trap tree management strategy resulted in a reduction of ~70% total trees being treated with insecticide compared with perimeter row sprays and 93% compared with standard full block sprays. We currently are working to improve this strategy based on deploying even more powerful attractants within tree canopies to increase aggregation activity and reduce the number of required trap trees.
Cage test to assess the mating disruptant activity of different pheromone blends and formulations on Peach Twig Borer (*Anarsia lineatella* Zeller) in the orchards

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(**) CBC Europe Ltd, Nova Milanese, Italy.

Abstract: Mesh cages were used as a method for assessing the disruption of Peach Twig Borer (*Anarsia lineatella* Zeller) and Oriental Fruit Moth (*Grapholita molesta* (Busck)) mating in peach orchards where different blends of synthetic pheromones and different dispenser formulations have been applied. The trials carried out in the seasons 2006-2008 showed that this method is easy to apply for evaluating the effectiveness of MD in the field and gives a reliable feedback allowing fine-tuning of formulations.

**Key words**: Peach Twig Borer, *Anarsia lineatella*, Oriental Fruit Moth, *Grapholita molesta*, cage test, pheromone, peach

**Introduction**

The peach twig borer (PTB), *Anarsia lineatella* Zeller (*Lepidoptera: Gelechiidae*), is a pest of peach, almond and other stone fruits where larvae cause damage by tunnelling into shoots and fruits.

PTB has been increasingly damaging peach cultivation during the last years in Northern Italy. In this new situation, finding effective control measures is particularly important: reliable monitoring and forecasting means, research on the efficacy of different pheromone blends and formulations for mating disruption are required.

In the last few years, the mating disruption method (MD) is increasingly applied especially in organic and integrated pest management orchards; this method can give good results in terms of efficacy and has a very low environmental impact; there are still some problems with the field evaluation of its effectiveness. For this purpose field tests were carried out in special mating cages to assess the effectiveness of the MD method without the problem of evaluating the initial density of the insect population in the orchard.

**Materials and methods**

Field trials assessing the effectiveness of different formulations of synthetic pheromones of *Anarsia lineatella* Zeller (PTB) and *Grapholita molesta* (Busck) (OFM) for mating disruption (MD) were carried out in the province of Forlì-Cesena in 2006 – 2008.

Dispensers containing single blend (Isonet A and OFM rosso, respectively) and the two blends mixed (Isonet A/OFM) were used. Trials were carried out in two organic orchards.

**Mating cages**

Mating cages installed in the orchards consisted of a cubic aluminium structure (side=1.5 m). Two cages were placed in each plot, one for the *Anarsia lineatella* trials and the other for the
*Grapholitha molesta* trials. Two cages, one for each species, were also installed in another orchard and used as a control (Fig. 2).

A pagoda trap, baited with 2 three day old virgin females were placed in a container of wire mesh, which was hung in each cage. Females were free to move and to release their pheromone, reproducing the natural calling conditions (Fig. 3).

In each trial, ten three day old virgin males were put into each cage in order to simulate a high population density of the target insect. Catches were recorded 3 – 4 nights after the introduction of the insects.

![Figure 1. Cage test](image)

![Figure 2. Position of the cages in the orchards](image)
Results

Data were calculated as an average of caught insects/cage. The effectiveness of pheromones was expressed as percent reduction of catches compared to the untreated control for each species, *Anarsia lineatella* and *Cydia molesta*. As shown in Figure 4, the percent reduction was calculated for each formulation (Isonet A, OFM rosso and Isonet A/OFM).

Trials conducted for *Anarsia lineatella* with Isonet A and Isonet A/OFM dispensers, respectively, showed a reduction in the percentage of catches of 77% and 78% compared to the untreated control, showing an average catch of about 1 insect per trap.

Trials conducted for *Cydia molesta* with OFM rosso and Isonet A/OFM dispensers showed a reduction of 80% compared with the control, suggesting an average catch of 1 insect per trap.

![Figure 4. Percentage trap catch of *Anarsia lineatella* and *Cydia molesta*........](image)

Conclusions

Three years of trials confirmed that the “mating cages” are a reliable method for assessing the effectiveness of different products for MD in the field.

The use of cages containing a known number of insects placed in the trial plot allowed us
to overcome the fundamental problem of evaluating the initial density of the insect population in the orchard.

Knowing and evaluating the natural population density in each orchard is almost impossible; this method allows the evaluation of the tested products and the control under the same population density, without any interference by changing the natural population. The major constraint of this method is the unavoidable need of rearing the target insect.

In the trial reported, it has been shown that dispensers containing the two lures of OFM and PTwB together (Isonet A/OFM) give the same control as separate dispensers containing the single blend of the target insect (Isonet A and OFM rosso).

References


Comparison of different pheromone lures to monitor the flight of 
_Cydia pomonella_

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**Abstract:** The control of the codling moth, _Cydia pomonella_, relies on an accurate understanding of its biology and phenology. Pheromone trapping is an effective and timesaving technique to follow the phenology of adults and to estimate the appearance of the different larval instars. In this study we tested three different pheromone lures for monitoring the flight of _C. pomonella_. The Tripheron capsule attracted most males followed by a capsule developed at the University of Neuchâtel and the unattractive PheroNet capsule. In the future, we recommend the use of the Tripheron capsule for monitoring the flight of _C. pomonella_, especially in regions with low population density.

**Key words:** Pomiculture, insect monitoring, delta traps

**Introduction**

The codling moth, _Cydia pomonella_ (L.) (Lepidoptera, Torticidae), is a key pest in European apple orchards and its control is based on insecticide applications and mating disruption (Angeli et al., 2007). Both control strategies strongly rely on a profound knowledge of codling moth population dynamics. The identification of its sex pheromone in 1971 led to the wide use of pheromone traps for monitoring the flight of _C. pomonella_ (Roelofs et al., 1971; Charmillot et al., 2000). Pheromone trapping is an effective and timesaving technique to follow the phenology of adults and to estimate the appearance of the different larval instars. For the last two decades, the PheroNet capsule has been the most common pheromone lure used to catch codling moth in Switzerland. However, the number of males trapped has decreased significantly over the last years as a result of low population densities of _C. pomonella_. Thus, a more attractive pheromone lure would be useful to predict the phenology of the codling moth more accurately. In this paper we compare different pheromone lures with the aim of identifying a more effective monitoring device.

**Material and methods**

Three different types of pheromone capsules were tested. The PheroNet capsule (distributed by Andermatt BioControl) served as a reference. It is charged with 1 mg E8E10-12OH. We also tested a Tripheron® capsule produced by Trifolio-M GmbH loaded with 1 mg E8E10-12OH and 1 mg 12OH as well as a capsule developed by Patrick Guerin at the University of Neuchâtel comprising 1 mg E8E10-12OH. The three different types of pheromone capsule were exposed in delta traps and set up in two untreated apple orchards. Both orchards were situated in the neighbourhood of Nyon (Switzerland) and were about 0.25 ha in size. Pheromone traps were regularly rotated within orchards in order to minimise local side effects. Pheromone traps were checked twice a week between May and September 2007.
Results and discussion

The capsule Tripheron attracted the highest number of codling moths followed by the capsule developed at the University of Neuchâtel (Figure 1a). Both types of capsules were effective over the whole season (Figure 1b). However, PheroNet lures attracted significantly less codling moths than the other two types of lures.

For the future, we recommend the use of the more attractive Tripheron capsule to monitor the flight of C. pomonella males. Particularly in regions with low densities of codling moths this pheromone lure might be a welcome asset for forecasting the phenology of C. pomonella.

Acknowledgments

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References

Effectiveness of mating disruption and granulosis virus against codling moth in central Bulgaria

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Abstract: Due to economical changes, problems of resistance and the parceling of agricultural area, mating disruption (MD) was studied on its own or in combination with granulosis virus (CpGV) against the codling moth (CM), Cydia pomonella L., in the region of Plovdiv (Bulgaria). The effectiveness of MD and CpGV was tested in small orchards with high pest density. Until the 5th of July 2005, the percentage of CM-damaged fruits was at an acceptable level of 5.1% in the 0.5 ha apple orchard treated with Isomate C LR® dispensers. The number of trapped CM males was 11 times lower than in a conventionally treated orchard, which served as a reference. Except for Rhynchites spp. and Stephanitis pyri, fruit damage by other pests was around the economical threshold. In 2007, Isomate C plus® dispensers together with the CpGV as Madex® were applied in a 19 years old orchard of 1.3 ha. Once again, fruit damage by CM was below the economical threshold until the beginning of July. Thereafter, five treatments with chlorpyrifos-ethyl and chlorpyrifos-methyl were made to avoid higher infestation levels. At pre-harvest, only 1.9% of apples had CM larvae, compared to 17.0% in the reference orchard that was treated 11 times with conventional insecticides. The combination of MD and CpGV showed the best results in an 8-year old apple orchard. In this orchard, only 1.5% of apples were infested with CM larvae at pre-harvest and we detected 1.5 diapausing CM larvae per tree. In the accompanying reference orchard, the density of hibernating CM larvae was 23-times higher. Overall, the development of alternative IPM strategies incorporating mating disruption and granulosis viruses seems to be promising.

Key words: codling moth, mating disruption, granulosis viruses, apple orchards, Bulgaria

Introduction

Codling moth (CM), Cydia pomonella L. (Lepidoptera: Tortricidae) is the key pest of the apples all over the world. It causes extremely severe damage on apple fruits ranging from 6% to 65% in south western Bulgaria and from 10% to 87-100% in south central part of the country (Balewsky et al., 1958). In 1980s, integrated pest management programs were developed in which control of CM was based on the application of insect growth inhibitors (IGI). In 1995, we received the first indication about the failure of IGI in Bulgarian fruit protection. Recently, population densities of CM and consequently apple damage increased sharply in many regions of the country. It has been verified that these insects showed a high degree of insecticide resistance (Charmillot et al., 2007). The mating disruption (MD) technique is one of the principal alternatives for controlling resistant CM populations. The aim of this study was to investigate the effectiveness of MD alone and in a combination with codling moth granulosis viruses (CpGV) in apple orchards with different levels of CM infestations. Our results could throw light on the possibilities of “organic” apple production in
Material and methods

It is well known that mating disruption is more effective when it is applied to large areas that are well isolated. However, the agricultural land in Bulgaria was parceled out with the political and economical changes in the 1990s. For this reason, our study has been conducted in the region in conformity with actual conditions.

Location of orchards

In 2005, a MD trial was carried out in a 17 year old experimental organic orchard (EOO) of the Agricultural University of Plovdiv. In the previous year no pesticides were applied in the EOO. The MD treated orchard was compared to an abandoned orchard at Trud, a village near Plovdiv and a conventionally treated orchard in Plovdiv.

In 2007, the second MD trial was conducted in an experimental conventional orchard of the Agricultural University of Plovdiv (CO1) and in a private conventional orchard in Zvanichevo (CO2) (village near the town of Pazarjik in the Plovdiv region). CO1 was planted in 1988. It is more or less isolated spans 1.3 ha and comprises nine different apple varieties. Close to CO1 is a vineyard and then mixed orchard with cherry, plum and a 0.5 ha apple that was used as a treated reference orchard.

CO2 was planted in 1999, is 2.2 ha in size and there are six varieties of apples. CO2 is well isolated and a mixed orchard (plum, apple, cherry and pear) 600 m away was used as treated reference orchard.

Mating disruption

In 2005, pheromone dispensers of the type Isomate C LR® (Shin-Etsu Chemical Company Ltd., Japan) were used. Dispensers were applied on April 21 at the recommended dose of 1000 dispensers per ha. In 2007, Isomate C plus® dispensers from the same company were used. Dispensers were also applied at the recommended dose of 1000 unit per ha and set up on April 16. Dispensers were placed in the tree tops.

Granulosis virus and insecticides

During 2007, granulosis virus (Madex® from Andermatt BioControl, Switzerland) was applied in the two orchards protected with MD in 2007. The application of CpGV at a dose 100ml/ha started with the hatching of eggs of the first CM generation. Thereafter, CpGV treatments were repeated fortnightly at half the initial dose (50ml/ha). The first applications were conducted on April 26 at CO1 and on April 29 in CO2. In the commercial reference orchards, insecticides and acaricides were applied over the whole season in accordance with pest pressure.

Assessment of pest control

Pherocone 1C wing traps were placed in MD treated and reference orchards to follow the dynamic of CM flight. Fruit damage was assessed during the season and at before harvest. Randomly, 25 to 30 apples were counted per tree, resulting in more than 500 sampled apples per date. In the middle of June corrugated cardboard bands were placed in orchards.

Pheromone emission

In 2007, randomly selected dispensers in the CO1 were periodically replaced with new ones and then stored in a refrigerator. In CO2, 20 additional dispensers were set up in the orchard at the date of the exposure of pheromone dispensers. Every week one of them was recovered and stored in a refrigerator. Collected dispensers were analyzed by gas chromatography.

Results and discussion

Mating disruption trial 2005
No insecticide or acaricide treatments were applied during the season. In the EOO, the level of fruit damage by CM exceeded the economical threshold at the beginning of July 2005 (Table 1), around ten days after the first CM males were caught in the pheromone traps placed in the trial orchard. In the insecticide treated reference orchards, fruit damage topped the economical threshold in June and in the untreated control, fruit damage reached 30 % by the end of May. In 2005, the number of overwintering CM larvae was significantly lower in the orchard protected with mating disruption than in the insecticide treated reference orchard (Table 2). The low level of diapausing CM larvae in the corrugated bands in the untreated control orchard can probably be explained by the low yield of the apple trees.

Table 1. Fruit damage (%) in the mating disruption trial 2005.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Isomate C LR Plovdiv</th>
<th>Treated reference orchard Plovdiv</th>
<th>Untreated reference orchard Trud *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of fruits damaged</td>
<td>% of fruits damaged</td>
<td>% of fruits damaged</td>
</tr>
<tr>
<td>24.5.2005</td>
<td>0.71%</td>
<td>0.00%</td>
<td>6.25%</td>
</tr>
<tr>
<td>31.5.2005</td>
<td>1.74%</td>
<td>0.99%</td>
<td>29.26%</td>
</tr>
<tr>
<td>08.6.2005</td>
<td>1.94%</td>
<td>2.05%</td>
<td>~</td>
</tr>
<tr>
<td>21.6.2005</td>
<td>2.16%</td>
<td>2.05%</td>
<td>35.70%</td>
</tr>
<tr>
<td>05.7.2005</td>
<td>5.14%</td>
<td>1.34%</td>
<td>~</td>
</tr>
</tbody>
</table>

* - very low yield. 86.27% damage before harvest

Table 2: Average number of CM caught and the number of diapausing CM larvae per tree in the mating disruption trial 2005.

<table>
<thead>
<tr>
<th>Orchard / year</th>
<th>Isomate C LR Plovdiv</th>
<th>Treated reference orchard Plovdiv</th>
<th>Untreated reference orchard Trud</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004</td>
<td>2005</td>
<td>2004</td>
</tr>
<tr>
<td>Average number CM caught per trap and per week</td>
<td>18.13</td>
<td>2.13</td>
<td>9.13</td>
</tr>
<tr>
<td>Average number of CM larvae per tree in corrugated bands</td>
<td>54.86</td>
<td>23.00</td>
<td>63.68</td>
</tr>
</tbody>
</table>
Mating disruption trial 2007

Overall, Madex® was applied 12 times in CO1 and 10 times in CO2. Aphids were treated twice in CO2, with cypermethrin in the middle of April 2007 and with imidaclorpride at the beginning of July 2007. In CO1, growers decided to start with additional insecticide sprays on July 7 when damage exceeded the economical threshold. Until the end of the season, 5 additional treatments with Reldan and Nurele-Dursband were applied. Only one CM was caught in the pheromone-treated orchard CO1 and none in CO2. In the other orchards, CM flight started at the beginning of April. First individuals of the second flight were caught at the end of July and the flight continued until the end of September.

In CO1, the level of damaged apples was about the same as in the previous year when conventional insecticides were applied, e.g. 27.86% versus 28%. At harvest, only 1.91% of fruits were attacked by alive or fully developed larvae. As a result of granulosis virus, the largest proportion of damaged apples (22.65%) was caused by stopped larvae. In CO2, damage was 14.82%, nearly three times lower than in the insecticide treated reference orchard (37.43%) but much higher than in the previous year (4%). Once again, the largest proportion of damage was caused by stopped larvae (13.37%). The number of overwintering CM larvae was about the same before and after the mating disruption trial, e.g. 14 larvae per band in 2006 and 16 larvae per band in 2007 in CO1. This is in the same order of magnitude (14.93 larvae per band) as in the insecticide treated reference orchard. In CO2, only 1.47 larvae per band were counted, compared to 35.21 larvae per band in the insecticide treated reference orchard.

Pheromone emission

The analysis of pheromone dispensers with gas chromatograph revealed that several dispensers were already empty in the middle of July and nearly all were empty at the end of August. This indicates that in a very hot summer as experienced in 2007 pheromone emission begins to decrease in the middle of the summer and that almost no pheromone is emitted at end of the flight of CM. These results might explain why apple damage increased sharply in August.

Conclusion

This study has shown that a combined control program of mating disruption and granulosis virus can be more effective than classical control schemes. However, a prerequisite is a low to moderate codling moth population. Under high CM pressure, the combined strategy is at least as effective as classical control. Pheromone-mediated mating disruption is most successful when pest insects are suppressed to a low level year after year. Future investigations on the effectiveness of combining MD and CpGV should investigate the preconditions that are required for these environmentally friendly control techniques to keep the level of codling moths under the economical threshold.

Acknowledgements

We would like to thank Pierre-Joseph Charmillot for his introduction to mating disruption and for his helpful comments and Patrik Kehrli for critical review and correction of the manuscript. We also thank Vanq Bojilova and Vasil Mihaylov from Regional Service of Plant Protection-Plovdiv, and Panko Keranov and Ilia Manoilov for placing their orchards at our disposal. Part of the investigation was funded by 6-th Framework of the EC project PlantProCENTRE contr. № 017367.
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Control of codling moth (*Cydia pomonella*) by the means of active mating disruption, different application systems and varieties

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**Abstract:** Alongside standard systems of mating disruption, the activity of Exosex CM and Ecodian® under different application systems and on several varieties were tested in 2007 and 2008 at the research station of the Federal College and Institute for Viticulture and Pomology, Klosterneuburg. Standard mating disruption techniques usually rely on the introduction of amounts of pheromone similar to those emitted by natural populations of pest species into the atmosphere. Exosex CM significantly reduces deployment time and labour costs in the orchards, additionally the flexibility of integrating this technique with IPM programmes was tested. Ecodian® dispensers were distributed at a rate of 2000 dispensers/ha. The tube dispensers of pheromone (Exosex CM) were placed in a three hectare orchard, which was split into three trial fields: one left untreated, one where the first generation was treated and one in which all generations of codling moth (*Cydia pomonella*) were treated. Additionally, in 2008 different application systems were used. Ecodian® was tested on one hectare and compared with untreated areas. The assessments to quantify efficacy were made visually on windfall fruits, fruits on the tree and on all fruits at harvest and statistically evaluated. In 2007, among the fruits sprayed within the IPM system there was an infestation rate by the first generation on the variety Idared of 0.8%. The second generation treated with Exosex showed an infestation of 13%. In the biological trial, however, the infestation by the first generation was about 4% and the infestation by the second generation about 31%. The 2008 results were comparable to those of 2007.

**Key words:** codling moth, mating disruption, pheromone

**Introduction**

At the orchards of the research station codling moth (*Cydia pomonella*) is a serious pest, which can cause damage of economic relevance. The regions climate is advantageous for two complete generations of codling moth annually, and in some years even three generations are seen. This can lead to a very high population density, which needs intensive pest management. In the last eight years routine application against codling moth was done with the Carpovirusine formulation granulovirus (CpGV) with an average of eleven applications per year on the organic orchards. In 2003 and 2007 mating disruption was accomplished. High pest numbers in the last years have needed additional measures to augment the routine granulosevirus control. In order to find out about the CpGV-resistance in this orchard, the Julius Kühn Institute for Biological Control in Germany undertook sensitivity tests with the larvae. In the laboratory the mortality of newly hatched larvae exposed to different virus concentrations was determined (Jehle 2008). It was found that the codling moth strain from these orchards is less sensitive in relation to a sensitive strain of *C. pomonella*, by around a factor of ten. Based on this sensitivity data different mating disruption methods were examined to create an acceptable resistance management strategy.

Several alternative control methods using mating disruption against codling moth were demonstrated and evaluated in apple orchards in Austria that had been established in 1992. Alongside standard systems of mating disruption, the activity of the products Exosex CM and
Ecodian® in conjunction with different application systems and on several varieties were tested in 2007 and 2008.

**Material and methods**

In 2007 the trial was split into two sections. The first experiment included a treatment against the first generation with the spraying of diflubenzuron, which was applied on 22nd of May to decimate the outgoing population. The second generation being smaller in size was treated with Exosex, a mating disruption agent, as an extension to the conventional agent applied to the 1st generation. The Exosex dispensers were distributed on the 11th of July. In 2008 the tube dispensers of pheromone (Exosex CM) were assessed again. The trial was split into four fields, one left untreated (control), one where only the first generation was disrupted, one in which all generations of codling moth were treated with ExosexCM and additionally with Carpovirusine (thirteen times) and the last field was treated with compounds which are registered for use in integrated production (thiacloprid, chlorpyrifos) and also disrupted with Exosex CM. The Exosex CM is distinguished by the dispenser being impregnated with codlemon (87.5 mg per ha), which can be located throughout the orchard at a frequency of just 25 per hectare. Ecodian® dispensers, made of low-cost biodegradable material and easy to apply, were formulated with 10 mg of codlemone and placed at a rate of 2000 dispensers/ha. Ecodian® was tested on one hectare and one field was untreated. The assessments were done visually on 300 fruits per variant and variety, by examining pest damage on apples on windfall fruits, fruits on the tree and on all fruits at harvest followed by statistical evaluation. Also, pheromone traps were placed to monitor the flight of the codling moth.

**Results and discussion**

In 2007 the pheromone traps showed that the flight of codling moth was constant and comparable to the years 2006 and 2008 (fig.1). The results in 2007 on Topaz and Gala showed significantly higher rates of damaged fruits than Idared. The codling moth population was increasing at a significant rate in the ecological treatment, from 22% fruit injury in the first generation to 38% at harvest with the variety Idared. However, there was a significant difference between the varieties (Fig. 2). So, for instance, Topaz showed an infestation of 43% in the ecological treatment, whereas the infestation in the integrated treatment was 11%. It is well known that the mating disruption technique does not work well at high population densities (Cardé & Minks, 1995; Neumann, 1997; Casgrande & Jones, 1997), which was also the case in this trial. Conversely, in the trial with the Ecodian® treatment which was assessed on the 28th of August, only 1.7% damage was found, which means economical loss (Trautmann, 2008).

In 2008 the numbers of varieties were increased and the highest infestation occurred in Pilot with 18% damage, followed by Elstar, Gala and Golden Delicious, where the fruit injury was about 10% (Fig. 3).
In the organic trials the infestation was higher than in the comparative IPM trial. In the trials mating disruption success was dependent on moth density, with obvious effects on the different apple varieties, and also with either the ecological treatment or mating disruption. The attraction of the codling moth to apple volatile compounds which are known to elicit an antennal response has been previously tested and differences are known to exist between the varieties (Corarini et al. 2004).

Our data agree with the existing literature, which has shown that the mating disruption method is not sufficient alone, since too many fruits will be damaged. Particularly with an infestation in the previous year higher than 1-2 percent (Galli, 2006) or at an output population over 2000 larvae per hectare the chances are small that the employment of pheromone mating disruption alone is sufficient to control codling moth (Mani, 1996).
Acknowledgements

We thank the Institute for Biological Control of the Julius Kühn - Institute in Germany, for doing the resistance assessment with our codling moth strain in the frame of the EU-funded project “SustainCpGV”.

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Galli, P. et al. 2006: Der Apfelwickler, Obstbau; 30, 5, S. 259-290

Figure 3: Final damage assessment 2008
Mating disruption across the peach/apple interface

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Abstract: Our hypothesis is that deploying mating disruption against the oriental fruit moth, *Grapholita molesta* (Busck), across adjacent peach and apple blocks provides better control than if applied to only one of the two crops. CheckMate OFM dispensers were applied in mating disrupted peach blocks and CheckMate CM/OFM Duel dispensers were used in mating disrupted apple blocks. Where used, mating disruption was in addition to insecticide programs. Results confirm that it is easier to disrupt oriental fruit moth in peach than codling moth in apple.

*Grapholita molesta, Pheromone disruption*
Exploring the potential for using peripheral treatments with pheromone dispensers for controlling the grape berry moth (Lepidoptera: Tortricidae) by mating disruption

Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, 4902 Victoria Avenue North, Vineland Station, Ontario, Canada L0R 2E0

Abstract: The potential for using peripheral treatments with hand-applied pheromone dispensers for controlling Paralobesia viteana (Clemens) by mating disruption was examined in commercial vineyards in the Niagara peninsula, Ontario, Canada during 2007. Four 1 ha (100 x 100 m) experimental plots, each separated by 100 m, were established within each of three vineyards. Twenty-five synthetic sex pheromone-baited traps were deployed in each plot on a 20 x 20 m grid to indirectly measure the effect of pheromone treatments on the mate locating ability of male moths. The application of 500 dispensers/ha reduced the mean total number of moths trapped by 96% compared to the untreated control, indicating a high level of mating disruption. Trap catch was reduced by 87% when 80 or 160 dispensers were applied at intervals of 5 or 2.5 m, respectively, along the periphery of the 1 ha plots. The results provide impetus for additional research to determine if peripheral treatments with pheromone dispensers can be used to control P. viteana.

Key words: grape berry moth, Paralobesia viteana, pheromone, mating disruption, peripheral treatment

Introduction

The grape berry moth, Paralobesia viteana (Clemens) is the most important insect pest in commercial vineyards in eastern North America (Dennehy et al. 1990). Sex pheromone-mediated mating disruption can be used to control P. viteana (e.g. Trimble 2007), but there has been limited adoption of this technique because of the greater cost of using pheromone compared to using insecticide (Trimble 1997). One potential method of reducing the cost of mating disruption for controlling P. viteana may be the use of reduced rates of pheromone dispensers. For example, in Switzerland, Charmillot et al. (1996) disrupted mating of the European grape vine moth, Lobesia botrana Den. & Schiff. by using peripheral treatments with pheromone dispensers. The work described in this paper was carried out to explore the possibility of using peripheral applications of hand-applied pheromone dispensers for controlling P. viteana.

Material and methods

The effect of three pheromone treatments on the mate-locating ability of male P. viteana was tested using a randomized-complete-block experimental design in three commercial vineyards (i.e. blocks) in the Niagara peninsula, Ontario during 2007. The first vineyard (13.5 ha) was near Jordan, the second (12.8 ha) was near St. Catharines and the third (38.3 ha) was near Niagara-on-the-Lake. The vineyards were planted with white and red varieties of Vitis vinifera L. vines spaced at 1.2 m with 2.5–2.7 m between rows of vines. Four 1 ha (100 x 100 m) experimental plots, each separated by 100 m, were established within each of the three vineyards.
The activity and mate-locating ability of male *P. viteana* was monitored in each plot using 25 delta traps (21 x 20 x 12 cm, L x W x H) (Cooper Mill Ltd., Madoc, Ontario) that were positioned 1 m above the ground on a 20 x 20 m grid prior to first moth flight in the spring (15 May) (Fig. 1). Each trap was baited with a 9 mm-diameter, natural-rubber sleeve stopper (Chromatographic Specialties, Brockville, Ontario) loaded with 0.8 mg of Z-9-dodeceny acetate (Z9-12:OAc) and 0.2 mg of (Z)-11-tetradecenyl acetate (Z11-14:OAc) (Pherobank, Plant Research International, Wageningen, the Netherlands), the major and the most abundant minor pheromone compound of this species, respectively (Witzgall et al. 2000). Moths were counted and removed from the traps on Monday and Thursday from 22 May until 10 September. Stoppers were changed on 3 and 30 July at the end of the first and second of the three flights of *P. viteana* (Fig. 1).

![Number of *P. viteana* trapped per day in control plots at three vineyards in the Niagara peninsula, Ontario, 2007](image)

Figure 1. Number of *P. viteana* trapped per day in control plots at three vineyards in the Niagara peninsula, Ontario, 2007

Isomate® GBM Plus pheromone dispensers, each containing 221.5 mg of Z9-12:OAc (Pacific Biocontrol Corp., Vancouver, WA) were deployed after peak trap catch during the second flight of *P. viteana* (Fig. 1) on 6 July at the Jordan vineyard, on 11 July at the St. Catharines vineyard, and on 10 July at the Niagara-on-the-Lake vineyard. A plot received one of four treatments: 1) pheromone dispensers applied at equal spacing to the entire plot at the recommended rate of 500/ha; 2) dispensers applied to the periphery of the plot at intervals of 5, or 3) 2.5 m, and; 4) no treatment with pheromone dispensers (i.e. control plot). Dispensers were attached to the top trellis wire 110–120 cm above the ground within the grape vine canopy. The deployment of pheromone dispensers to the periphery at a spacing of 5 m was approximately equivalent to the distance between dispensers when applied to the entire plot at the 500 dispensers/ha application rate. The total number of moths trapped in a plot before and
after the pheromone dispensers were applied was transformed to $\sqrt{(x + 1)}$ and the significance of treatment on the mean total number of moths trapped was tested using analysis of variance (ANOVA). The significance of differences between means was tested using the Tukey multiple comparison test (SAS Institute 2007). The 80 x 80 m area within each experimental plot containing the grid of 25 delta traps was divided into a “border” and an “interior” zone. The border zone contained the 16 traps located on the edge of the plot and the interior zone contained the 9 traps located within the “edge” traps. The total number of moths captured in each trap in each plot before and after the pheromone dispensers were applied was transformed to $\sqrt{(x + 1)}$ and the significance of zone on the mean total number of moths captured was tested using the nonparametric Wilcoxon test (SAS Institute 2007).

**Results and discussion**

There was no difference in the mean total number of *P. viteana* trapped in each of the four experimental plots prior to application of the Isomate® dispensers (treatment, $F_{3,11} = 0.31, P = 0.82$; vineyard, $F_{2,11} = 5.77, P = 0.04$). After application of the dispensers, however, pheromone treatment significantly affected the mean total number of moths trapped (treatment, $F_{3,11} = 5.38, P = 0.04$; vineyard, $F_{2,11} = 12.32, P = 0.001$) (Table 1).

**Table 1.** Mean (±SD) total number *Paralobesia viteana* captured in pheromone-baited traps in treatment plots before and after application of pheromone dispensers.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before application of dispensers</th>
<th>After application of dispensers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.0±10.9a</td>
<td>78.8±10.9a</td>
</tr>
<tr>
<td>Periphery–2.5</td>
<td>14.6±8.0a</td>
<td>8.9±8.0ab</td>
</tr>
<tr>
<td>Periphery–5</td>
<td>10.6±4.5a</td>
<td>9.0±4.5ab</td>
</tr>
<tr>
<td>Full</td>
<td>9.5±11.3a</td>
<td>2.9±11.3ab</td>
</tr>
<tr>
<td>Total number trapped</td>
<td>192</td>
<td>539</td>
</tr>
</tbody>
</table>

Note: Moths trapped from 15–18 May to 6–10 July before application of dispensers and from 6–9 July to 10–18 September after application of dispensers. There were 25 traps in each treatment plot.

The application of 500 dispensers/ha reduced the mean total number of moths trapped by 96.3% compared to the untreated control, indicating a high level of mating disruption (Trimble 2007). There was no statistical difference in the mean total number of moths captured in the control plot and in the plots receiving either of the two peripheral treatments with pheromone dispensers (Periphery–5 and Periphery–2.5), however, the mean total number of moths trapped in these plots was reduced by 87% compared to the average number trapped in the control plots (Table 1). The failure of the multiple comparison test to detect a statistical difference between treatments with such large differences is likely due to the use of only three replicates (i.e. vineyards) in the this experiment, and to the large difference in the total number of moths trapped at each location, i.e., Jordan = 339, St. Catharines = 15 and, Niagara-on-the-Lake = 185.

There was no difference in the mean total number of moths captured in the peripheral and interior zones of any of the experimental plots before the application of pheromone dispensers. After the application of dispensers, there was no between-zone difference in five of the six plots receiving a peripheral zone treatment, suggesting that the peripheral treatment
with pheromone dispensers affected the mate seeking ability of male moths throughout the entire plot. A between-zone difference in the mean total number of moths captured occurred at the Jordan vineyard in the plot treated with dispensers at intervals of 2.5 m (Periphery–2.5), where the mean total number trapped in the border zone (0.7) was 69% smaller (\(Z = 2.3, P = 0.02\)) than the mean total number trapped in the interior zone (2.2). This suggests that the peripheral pheromone treatment had greater affect in the border zone than in the interior zone of this plot. There was also a between-zone difference in the mean total number of moths trapped in one of the tree control plots. At the Niagara-on-the-Lake vineyard, the mean total number of moths trapped in the interior zone (4.1) was 48% smaller (\(Z = 2.4, P = 0.02\)) than the number trapped in the border zone (7.9), suggesting that in this plot, there was greater between trap competition in the interior zone than in the border zone.

The results of this study demonstrate some potential for using peripheral treatments with pheromone dispensers for controlling \(P. \text{viteana}\) by mating disruption. Additional experiments using greater replication should be undertaken to confirm the current results and increase the likelihood of detecting significant treatment effects when using peripheral treatments. Any future experiments should also include the use of tethered, virgin-female moths (Charmillot et al. 1999) to measure the ability of male \(P. \text{viteana}\) to locate and mate with sexually receptive females.

**Acknowledgements**

We thank three Niagara peninsula grape growers for making their vineyards available for experimentation and Iris Roman for her able technical assistance.

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Control of codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), with EcoTape pheromone dispensers

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**Abstract:** A mating disruption approach using high densities of pheromone point sources has been developed for codling moth, *Cydia pomonella* (L.) (Lepidoptera Tortricidae), control. The EcoTape® device comprises a continuous adhesive tape integrated with 3-cm length dispensers at a separation of 0.6 m, loaded with 2.5 mg codlemone. Thus, in comparison with standard mating disruption, the content of dispensers is strongly reduced, whereas the density of point sources is increased (2,000 or 4,000 points/ha), with the purpose of increasing the competition between natural and synthetic sources. The release rate of new and field aged dispensers, measured directly by solid-phase micro-extraction (SPME), decreased over time but at the end of the season was still more potent than a calling codling moth female. Dispensers elicited close-range approaches in a wind tunnel irrespective of their field age. Traps lured with aged EcoTape dispensers were also able to catch a number of males in the field throughout the season comparable to that of traps loaded with reference dispensers. The results of field trials (2004-2007) showed that codling moth control can be obtained applying EcoTape dispensers. Our experiments demonstrated that EcoTape dispensers are a useful tool for efficient CM control throughout the season under the climatic conditions of the Trento Province (North Italy) and may satisfy some of the prerequisites for producing false-trail following effects.

*E8,E10-dodecadien-1-ol, Mating disruption, SPME, Wind tunnel, Field trial*
Two Spotted Mite, Tetranychus urticae Koch, Emerged as a New Pest in Persimmon Orchards and Approaches to Their Control

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Abstract: Oriental persimmon, Diospyros kaki Thunb., endemic to East Asia is one of the major fruit crops in Korea. We conducted a faunal survey of mites on persimmon trees in Korea from June to September 2006, focusing on herbivorous and predacious mites. Mites of Tetranychidae and Tenuipalpidae were dominantly collected as herbivores, while those of Phytoseiidae and Stigmaeidae were predominant as predators. All identified tenuipalpid mites were Tenuipalpus zhizhilashviliae Reck. Most of the collected tetranychid mites were found to belong to the genus Tetranychus. To clarify the species identity, additional collections of tetranychid mites during summer 2007 on sweet persimmon were made. The mites were identified as Tetranychus urticae Koch. Four phytoseiid species, Neoseiulus womersleyi (Schicha), Amblyseius eharai Amitai and Swirski, Phytoseius (Dubininellus) rubii Xin, Liang and Ke and Typhlodromus (Anthoseius) vulgaris Ehara were collected. Among them, A. eharai was the most dominant species. Seventeen populations of two spotted mites (TSM) were observed 3 times per month from May to October to decipher their fluctuations at the site of individual farmer’s orchard from Sacheon, Sancheong, and Jinju in Gyeongsangnam-do and Gwangyang, Gurye, and Suncheon in Jeollanam-do. Among them, only 2 sites were properly managed, 5 sites were required to control but the farmers had little information on the mite and its damage, though 10 orchards were not at risk of infestation. Numbers of TSM on 100 leaves reached more than 400 at orchards from Sacheon, Okgok, and Muncheok, showing remarkably discolored leaves.

Persimmon, Tetranychus urticae, Overwintering, Control
Observations on the relation between the induction and termination of diapause in codling moth in Dutch and Belgian populations.

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Abstract: Effective codling moth (CM) management requires accurate information on the phenological stage and development of the local CM population to be controlled. Several advisors and scientists in Europe explain local differences in pheromone trap catches from the hypotheses of “recalled diapause day length”. According to this hypothesis, individuals in the population remember the day length at which their diapause was induced, and terminate their diapause the following spring at the same day length. This would mean that events that have a quantitative impact on parts of the population shape the phenological development next year. This has the practical consequence that codling moth phenology is determined at a local scale and regional warning systems cannot provide the information necessary for local control. The aim of our work was to test if this hypothesis holds for CM populations in the Netherlands and Belgium. CM collected from orchards in the Netherlands and Belgium in 2007 consisted for 98% of univoltine individuals. For these individuals we found no relation between the date we collected them as fully grown larva during summer 2007, and their date of pupation in 2008. These results mean that the hypothesis of “recalled diapause day length” does not hold for the almost completely univoltine CM populations in the Netherlands and Belgium. Therefore, the phenology of our local populations can not be influenced by events in the previous year. Temperature relations and a normal distribution can be used to describe the spring pupation of a codling moth population.

Cydia pomonella, Diapause
Practical results of a stacked control strategy for codling moth (Cydia pomonella L.) management

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Abstract: Codling moth (CM) is an important pest in both organic and integrated apple production in the Netherlands and Belgium. Control of the pest became more difficult during the past ten years. A series of biological and chemical plant protection products (PPP’s) is available for the regulation of CM, but field trials throughout Europe have shown that season long application of the same PPP provides only 50 to 70% control. Random alternation of products is not likely to improve efficacy. However, the available PPP’s have different modes of action, and act at different life stages in the CM biology. When applied with respect to their individual mode of action, and scheduled according to the local biology of the CM population, the efficacy of PPP’s could be stacked, yielding a technically, economically and ecologically improved control. This approach was tested in commercial apple orchards in an extension project in 2007 and 2008. The penology of the CM populations was calculated with the RIMpro-Cydia model using weather data from on-farm weather stations. Combinations of pheromone confusion to reduce the total number of eggs deposited, fenoxycarb at 30% rate as an ovicide at the predicted peaks in egg deposition, and granulosis virus at a 50% rate in periods of predicted peaks in egg-hatching where used on the farms following the Stacked Control Strategy. Randomly chosen orchards in the same geographic region that did not take part in the extension project served as control group. In both years CM control in the Stacked Control Strategy orchards was more effective, and had a lower insecticide input and a lower environmental impact compared to the control group.

Codling moth, Cydia pomonella, Control strategy, RIMpro-Cydia
Biological aspects and predatory capacity of Chrysoperla externa (Hagen, 1861) (Neuroptera: Chrysopidae) fed on Planococcus citri (Risso, 1813) (Hemiptera: Pseudococcidae)

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Abstract: In the citrus mealybug complex, Planococcus citri is one of the most important pests and its control is effected by insecticides. The green lacewing Chrysoperla externa is an insect often found in citrus orchards and is a natural predator of this pest. This work deals with studies on the predatory capacity and some biological aspects of larvae fed the three instars and adult female of P. citri. The experiments were conducted at 25±1 °C, 70±10% of RH and 12-hour photophase with four treatments, represented by the development stages, and 30 replicates in a complete randomized design. It was found that the total predatory capacity of lacewing larvae was 231.2; 77.9; 32.6 and 21.2 for the three instars and adult females, respectively. The longevity of second and third instars of green lacewing larvae was lengthened when fed on adult mealybug females. The pupal stage was longer when it originated from larvae fed second and third instar larvae and adult females. The immature stage lasted from 19.8 to 22.9 days, and survivorship for this period was from 78.0 to 91.0%. A reduction in the number of consumed mealybugs was found in each instar, regardless the lacewing instar, however both nymphs and adult mealybug females were adequate prey for the larval development of C. externa.

Key words: citrus, lacewing, biology, predation

Introduction

The citrus mealybug Planococcus citri (Risso) (Pseudococcidae) is found in tropical, subtropical and temperate regions of the world (Llorens, 1990; Waterhouse, 1998) and is often reported causing injuries to fructiferous plants, coffee, soybean and ornamentals. Chemical control is the method most used to control this pest although alternative methods are currently under investigation. The mealybugs have several natural enemies, crysopids (Neuroptera: Chrysopidae) among them. These insects are found in several crops of economic interest feeding on aphids, scales, and eggs, larvae and pupae of Lepidoptera. Biological aspects of this predator have been studied in Brazil, specially Chrysoperla externa (Hagen), and its potential as a biological control investigated (Fonseca et al., 2001; Maia et al., 2004; Barbosa et al., 2006, 2008; Auad et al., 2007). However, development studies of this species and its efficacy on scale populations reduction are scarce.

The present study deals with biological studies of immature stages and predatory capacity of C. externa fed on P. citri nymphs and adult females.

Materials and Methods

One hundred C. externa eggs obtained from a stock rearing maintained in the laboratory (F3) were placed individually in Petri dishes, and kept at 25±1°C, 70±10% RH and 12-hour...
photophase. The mealybugs were collected on coffee plants cv. Mundo Novo and reared on potato *Solanum tuberosum* (L.) cv. Monalisa sprouts. Biological aspects of the immature stages and predatory capacity of this crysopid were evaluated for each instar by daily delivering a known number of first, second and third instar nymphs of *P. citri* and adult females under several densities (Table 1). The mean number of mealybugs furnished to each instar of the predator was above its daily consumption capacity. There were four treatments and 30 replicates under a completely randomized experimental design, with evaluations of the mean life span and survivorship on each instar and during the stages of larva, prepupa and pupa, larva-adult period, total predatory capacity.

The lifetime of the immature stages was obtained by punctual estimate given by the median, and data related to duration and survivorship obtained by the non-parametric method of Kaplan-Meier, which were compared by the Wilcoxon test (Colosimo & Giolo, 2006). The interpretations related to the mean lifetime of the first, second and third instars, larval stage and prepupa, pupa stages and larva-adult period were based on survivorship percent. Analysis related to the predatory capacity were made by estimating the consumption proportions of each instar of *C. externa* in relation to the total nymphs and adults of *P. citri* furnished.

Table 1. Number of *Planococcus citri* at different stages furnished to first, second and third instar *Chrysoperla externa* larvae. Temperature 25±1°C, 70±10% RH, 12-hour photophase.

<table>
<thead>
<tr>
<th>Development stages of the mealybug</th>
<th>Number of mealybugs furnished/instar of the predator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>60</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>40</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>15</td>
</tr>
<tr>
<td>Adult female</td>
<td>10</td>
</tr>
</tbody>
</table>

**Results and Discussion**

The mean lifetime was different for the second and third instars for *C. externa* larvae fed on adult female mealybugs, as compared to those fed on nymphs regardless the instar (Table 2). Gonçalves-Gervásio & Santa-Cecília (2001), in studies with *C. externa* fed on *Dysmicoccus brevipes* (Cockerell) nymphs in all three instars and adult females found duration of 4.2; 3.2; 5.4 and 12.8 days, respectively. Variations detected in the duration of each instar and larval stage gives evidence of the importance of diet on larval phase, in relation to results obtained in other studies, supporting results of Principi & Canard (1984) who detected the influence of food quality on insect development.

Higher survivorship was found in all three larval instars and total larval stage of *C. externa*, when fed with first, second and third instar of the prey as compared to being fed on adult females (Table 2). This may be due to the presence of a powdery secretion covering mealybug body as well as the hardening of its integument, increasing the difficulty on chrysopid feeding, as well as the larger size of the prey in relation to the predator. Awadallah et al. (1975) found that *Chrysoperla carnea* (Stephens) larvae had mouthparts tangled on wax secretion of *Icerya purchasi* (Maskell) nymphs and adults. The secretion by adult females of *P. citri* of a jelly substance from lateral ostioles may pose additional difficulties for *C. externa*
feeding. According to Willians (1978), this secretion, produced particularly by adult females, is a carrier of an alarm pheromone.

Table 2. Mean lifetime (T) in days and survivorship (S) in %, of three larval stage of *Chrysoperla externa* fed on *Planococcus citri*. Temperature 25±1°C, 70±10% RH, 12-hour photophase.

<table>
<thead>
<tr>
<th>Stages of the mealybug</th>
<th>Predator instar</th>
<th>Larval stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>S</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>3.8</td>
<td>90.0</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>3.8</td>
<td>89.0</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>3.8</td>
<td>98.0</td>
</tr>
<tr>
<td>Adult female</td>
<td>3.9</td>
<td>79.0</td>
</tr>
</tbody>
</table>

Wilcoxon test Value of *P*

|               | 0.9629 | 0.0001* | 0.0029* | 0.0984 |

The mean prepupa lifetime did not differ in relation to prey development stage, with a variation from 3.0 to 3.4 days, but did differ in the pupal stage originated from larvae fed on second and third stage nymphs and adult females in relation to those fed on first instar nymphs (Table 3). Survivorship in prepupae did not differ in function of the consumed food, with a variation of 98.0% to 100%. Nevertheless, there was a reduction in survival rate of pupae originated from larvae fed on second and third instars and adult females of the mealybug in relation to those of the first instar (Table 3). Thus, it is important to recognize the importance of the kind of prey and its development stage on the predator life-cycle. The mean larva-adult period of *C. externa* was 19.8 to 22.9 days in function to the stage of *P. citri* given to the larvae (Table 3). Results near these (23.8 days) were found by Gonçalves-Gervásio & Santa-Cecília (2001), with this chrysopid fed on *D. brevipes*.
Table 3. Mean lifetime (T) in days, survivorship (S) in %, of prepupa, pupa and larva-adult of *Chrysoperla externa* fed on *Planococcus citri*. Temperature 25±1°C, 70±10% RH, 12-hour photophase.

<table>
<thead>
<tr>
<th>Stages of the mealybug</th>
<th>Prepupa</th>
<th>Pupa</th>
<th>Period larva to adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>S</td>
<td>T</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>3.0</td>
<td>98.0</td>
<td>5.9</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>3.1</td>
<td>100.0</td>
<td>6.8</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>3.1</td>
<td>100.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Adult female</td>
<td>3.4</td>
<td>100.0</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Wilcoxon test

<table>
<thead>
<tr>
<th>Value of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0500</td>
</tr>
<tr>
<td>0.0004*</td>
</tr>
<tr>
<td>0.0014*</td>
</tr>
</tbody>
</table>

Survivorship for the larva-adult period of *C. externa* when fed with nymphs and adult females of *P. citri* were 89%; 91%; 89% and 78%, respectively. Adult females of the prey were related to lower survivorship of the predator, probably due to larger prey size, the presence of the wax powdery secretion covering mealybug body and the hardening of the integument.

In relation to predatory capacity, differences were found for total predation of all three instars and adult females of *P. citri* as a function of *C. externa* development stages, lower on first and higher on third instar. A 30X increase in larva consumption from first to third stage was detected, when fed on first instar mealybugs, and circa 8X when fed on second and third instars and adult females of *P. citri*. It was observed that there was, in general, an increase in consumption from the second day on of each instar and reduction from the fourth to the fifth day. This reduction was probably due to a reduction of the insect’s metabolism due to the proximality of a new ecdysis. Consumption, metabolism and growth have a tendency to reach a peak at the beginning or close to halftime of each instar and consumption efficiency may diminish along the stages.

For the chrysopid first stage, there was a higher second-instar mealybug consumption and lower third instar nymphs and females. Meanwhile, in relation to predation capacity in second and third stages, there was higher consumption of mealybugs nymphs of the first instar and lower consumption of those of the third instar and females. As for predatory capacity, there was a higher consumption of mealybugs of first instar relative to those of third instars and females of *P. citri* (Table 4). This is favorable in biological control programs since first and second instar larvae have greater mobility and capacity for spread on the plants. Differences in consumption found for all predator instars in relation to mealybug development stages were due to several factors, the size and density of prey among others, as reported by Fonseca et al. (2001), when fed *C. externa* larvae with aphid *Schizaphis graminum*.

Gravena et al. (1993) observed higher consumption of Parlatoria cinerea Doane & Hadden eggs than nymphs and adults by *C. externa*. Yet, Gonçalves-Gervásio & Santa-Cecília (2001) reported higher consumption of first and second instars relative to third instars and adult females of *D. brevipes*. Malleshaiah et al. (2000) detected that *C. carnea* were active predators of *P. citri*, with higher consumption of eggs and nymphs than adults.
Table 4. Total consumption during the three instars and adult females of *Planococcus citri* by larvae of *Chrysoperla externa*. Temperature 25±1°C, 70±10% RH, 12-hour photophase.

<table>
<thead>
<tr>
<th>Stages of the mealybug</th>
<th>Total number of consumed mealybugs/ instars predator</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; instar</td>
<td>6.5</td>
<td>28.5</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; instar</td>
<td>7.9</td>
<td>15.2</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; instar</td>
<td>2.5</td>
<td>5.8</td>
</tr>
<tr>
<td>Adult female</td>
<td>1.7</td>
<td>4.9</td>
</tr>
</tbody>
</table>

**Conclusion**

Nymphs of all three instars and adult females of *P. citri* were, in general, adequate prey for the development of immature stages of *C. externa*. Lower survivorship was observed in *C. externa* larvae fed on adult females of the mealybug. The development stage of both prey and predator influenced the number of predated mealybugs.

**Acknowledgements**

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**References**


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(Rondani) em diferentes temperaturas. Ciênc. agrotec. 25: 251-263.


**Effect of floral strips on the abundance of Hymenopteran parasitoids in apple and olive organic orchards**

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**Abstract:** Habitat manipulation techniques improve the availability of resources required by natural enemies to increase their effectiveness. This study focused on the effects of sown floral strips on Hymenopteran parasitoid abundance. The experiments were conducted during spring 2007 in one organic low-input apple orchard and five organic olive orchards located in southern France. The density and the diversity of parasitic wasps collected from sown floral strips were higher than those from naturally occurring flora or mowed plants. The family of parasitic wasps of Braconidae was strongly dominant, followed by Mymaridae, Eulophidae and Pteromalidae. Among the 26 studied flowering species in the apple orchard, the greatest diversity and density of parasitic wasps were collected from *Potentilla reptans*, *Achillea millefolium*, *Trifolium repens* and *Torilis arvensis*. In terms of the early flowering plants, the most important results were observed in *Euphorbia helioscopia*, *Senecio vulgaris* and *Veronica persica*. To give an idea of the functional role of these plants, we studied the parasitic wasps of the diapause larvae (cocoon) of codling moth *Cydia pomonella*. We recorded three emerged species: *Ascogaster quadridentata*, *Pristomerus vulnerator* and the hyperparasite *Perilampus fulvicornis*. However, none of these species have been observed on the 26 studied plants. Hence, this result may be suggesting that the studied plants do not have a functional role concerning these parasitoids. These studies may be advantageous for biological control programs in order to select flowering plant species attracting parasitic wasps specific to fruit pests.

**Keywords:** conservation biological control, habitat manipulation, floral strips, Hymenopteran parasitoid, organic orchard, olive, apple, codling moth, *Cydia pomonella*, cocoon

**Introduction**

The biological control of herbivorous pests can be improved by habitat manipulation in order to favour natural enemies, providing suitable microclimates, alternative preys, and floral resources for adult natural enemies (Landis *et al*., 2000; Rebek *et al*., 2005). In particular, establishing floral vegetation can provide adult parasitic wasps with essential nutrients and energy, and may improve longevity, fecundity, flight ability, and rates of parasitism in general (Jervis *et al*., 1993; Tooker and Hanks, 2000).

Although the availability of flowering plant has important implications for conservation biological control, little is known of the specific associations between parasitic wasps and flowering plant species (Jervis *et al*., 1993). This host-plant fidelity could be used in biological control programs for selecting plant species that attract parasitoid species (Tooker and Hanks, 2000).

The present study investigated the influence of floral strips on the abundance and the diversity of parasitic wasps (entomological role) in apple and olive organic orchards. To give an idea of the functional role of these strips, we studied the parasitic wasps of the diapausing larvae of codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae), a major worldwide pest of apple and pear (Mills, 2005).
Materials and Methods

Study orchards and floral strips
The studies were conducted during spring 2007 in southern France on five organic olive orchards, of 0.1 to 3 ha. In addition, one organic low-input apple orchard of 0.62 ha was surveyed; with 212 trees planted in 9 rows in 2001. In the olive orchards, 1 to 3 floral strips of 100-300m² were sown using a seed mixture of 49 floral species from autumn 2004 to autumn 2006. In autumn 2003, four floral strips of 1m width each were sown in the apple orchard.

Sampling method
The parasitic wasps were collected into tissue bags using aspirators. In the olive orchards, one aspiration bag is used per orchard on all the floral strips (2 minutes of aspiration per strip). Natural flora was considered as controls. One series of aspirations per orchard was performed. In the apple orchard, we studied the importance of 26 flowering species regarding the parasitic wasps (Table 1). One aspiration bag is used per species (three replicates per species and 5 seconds of aspiration per replicate). We chose the mowed plants (without flowers) as control. Each species was evaluated between three and seven times. The results of flowering species in apple orchard were compared for significance by a Mann-Whitney U test (P<0.05).

Study the parasitic wasps of the codling moth cocoons
212 corrugated cardboard bands (60 mm diameter) were wrapped around the trunks of apple trees in June 2006 (1band/tree). They were collected in October 2006 and in March 2007. 294 larvae were recovered. Each of them was put in a tube and observed until adult emergence.

Results and discussion

Hymenopteran parasitoid families
969 parasitic wasps divided into 21 families were collected from both floral strips and spontaneous flora in the olive orchards. The Braconidae family was the best represented with 306 parasitoid wasps (31.5% of all parasitoid wasps collected), followed by Eulophidae (16.4%), Mymaridae (14.7%) and Pteromalidae (8.9%). These families were observed in all the orchards. The remaining families were represented by 4.6% or fewer, each. In the apple orchard and during the observation period, a total of 1469 parasitic wasps divided into 22 families were collected from the 26 studied flowering species and the mowed plants (control). The majority of Hymenopteran parasitoids collected belonged to the Braconidae family with 423 parasitoid wasps (28.8%), followed by Mymaridae (22.8%) and Pteromalidae (8.7%). These families were listed on all flowering species and control and in all the observation dates. The remaining families were represented by 6.5% or fewer, each.

Braconids, which parasitize a wide range of hosts, are normally the parasitic wasps the most widespread in Europe. They are considered almost entirely beneficial and many species have been introduced or conserved in biological control programs (Tooker and Hanks, 2000).

Entomological role of floral strips
This study demonstrates that the density and the diversity of parasitic wasps collected from sown floral strips were higher than those from spontaneous flora (Figure 1) or mowed plants (Table 1), as found by similar studies (Jervis et al., 1993; Landis et al., 2000; Rebek et al., 2005). Floral strips are attractive for adult parasitic wasps which often visit flowers to feed on floral resources: pollen and/or nectar (Landis et al., 2000; Rebek et al., 2005).

In the apple orchard and among the 26 flowering species studied (Table 1), the greatest diversity and density of wasps was collected from Potentilla reptans, Achillea millefolium, Trifolium repens, and Torilis arvensis. In contrast, the lowest results were recorded on Melilotus officinalis, Malva sylvestris, and Tragopogon pratensis.
Figure 1. Diversity (A) and mean density (B) of Hymenopteran parasitoids collected from floral strips and spontaneous flora (control) in the five organic olive orchards.

Table 1. Mean (± SE) diversity and density of parasitic wasps per flowering species in the apple orchard. Means followed by different letters are significantly different (Mann-Whitney U test, \( P < 0.05 \)). \( (n) \): Number of assessments. (*) Early flowering species.

<table>
<thead>
<tr>
<th>Latin name</th>
<th>Family</th>
<th>Diversity</th>
<th>Density/species</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefolium</em></td>
<td>Asteraceae</td>
<td>7.00 ± 1.56 (a,b)</td>
<td>9.55 ± 3.34 (a)</td>
<td>3</td>
</tr>
<tr>
<td><em>Borago officinalis</em></td>
<td>Boraginaceae</td>
<td>2.71 ± 0.51 (d)</td>
<td>2.95 ± 1.18 (c)</td>
<td>7</td>
</tr>
<tr>
<td><em>Bromus catharticus</em></td>
<td>Poaceae</td>
<td>5.67 ± 0.90 (c)</td>
<td>3.66 ± 0.85 (c)</td>
<td>3</td>
</tr>
<tr>
<td><em>Bromus sterilis</em></td>
<td>Poaceae</td>
<td>4.17 ± 0.73 (c)</td>
<td>3.16 ± 0.82 (c)</td>
<td>6</td>
</tr>
<tr>
<td><em>Capsella bursa-pastoris</em></td>
<td>Brassicaceae</td>
<td>2.50 ± 0.29 (d)</td>
<td>1.83 ± 0.32 (c,d)</td>
<td>4</td>
</tr>
<tr>
<td><em>Cardaria draba</em></td>
<td>Brassicaceae</td>
<td>5.00 ± 0.72 (c)</td>
<td>4.61 ± 0.52 (b)</td>
<td>6</td>
</tr>
<tr>
<td><em>Cardus tenuiflorus</em></td>
<td>Asteraceae</td>
<td>6.40 ± 0.41 (b)</td>
<td>3.53 ± 0.31 (c)</td>
<td>5</td>
</tr>
<tr>
<td><em>Coreopsis tinctoria</em></td>
<td>Asteraceae</td>
<td>6.67 ± 0.68 (b)</td>
<td>3.44 ± 0.69 (c)</td>
<td>3</td>
</tr>
<tr>
<td><em>Crepis sancta</em></td>
<td>Asteraceae</td>
<td>3.34 ± 0.36 (c)</td>
<td>2.28 ± 0.40 (c)</td>
<td>7</td>
</tr>
<tr>
<td><em>Crepis vesicaria</em></td>
<td>Asteraceae</td>
<td>4.50 ± 0.29 (c)</td>
<td>2.66 ± 0.41 (c)</td>
<td>4</td>
</tr>
<tr>
<td><em>Euphorbia helioscopia</em></td>
<td>Euphorbiaceae</td>
<td>4.43 ± 0.90 (c)</td>
<td>3.95 ± 1.01 (c)</td>
<td>7</td>
</tr>
<tr>
<td><em>Galium aparine</em></td>
<td>Rubiaceae</td>
<td>6.33 ± 1.48 (b,c)</td>
<td>5.33 ± 1.60 (a,b)</td>
<td>3</td>
</tr>
<tr>
<td><em>Malva sylvestris</em></td>
<td>Malvaceae</td>
<td>2.00 ± 0.59 (d)</td>
<td>1.11 ± 0.41 (d)</td>
<td>3</td>
</tr>
<tr>
<td><em>Melilotus officinalis</em></td>
<td>Fabaceae</td>
<td>2.33 ± 1.36 (d)</td>
<td>1.11 ± 0.79 (d)</td>
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</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>Plantaginaceae</td>
<td>4.60 ± 0.83 (c)</td>
<td>2.80 ± 0.66 (c)</td>
<td>5</td>
</tr>
<tr>
<td><em>Poa trivialis</em></td>
<td>Poaceae</td>
<td>3.50 ± 0.42 (c)</td>
<td>2.22 ± 0.40 (c)</td>
<td>6</td>
</tr>
<tr>
<td><em>Potentilla reptans</em></td>
<td>Rosaceae</td>
<td>9.40 ± 1.27 (a)</td>
<td>17.00 ± 5.71 (a)</td>
<td>5</td>
</tr>
<tr>
<td><em>Sanguisorba minor</em></td>
<td>Rosaceae</td>
<td>4.40 ± 0.69 (c)</td>
<td>3.00 ± 0.60 (c)</td>
<td>5</td>
</tr>
<tr>
<td><em>Senecio vulgaris</em></td>
<td>Asteraceae</td>
<td>4.14 ± 0.89 (c)</td>
<td>3.57 ± 1.00 (c)</td>
<td>7</td>
</tr>
<tr>
<td><em>Sonchus oleraceus</em></td>
<td>Asteraceae</td>
<td>3.60 ± 0.69 (c)</td>
<td>2.53 ± 0.92 (c)</td>
<td>5</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em></td>
<td>Asteraceae</td>
<td>3.71 ± 0.55 (c)</td>
<td>2.43 ± 0.39 (c)</td>
<td>7</td>
</tr>
<tr>
<td><em>Torilis arvensis</em></td>
<td>Apiaceae</td>
<td>8.67 ± 1.71 (a)</td>
<td>4.44 ± 1.31 (b)</td>
<td>3</td>
</tr>
<tr>
<td><em>Tragopogon pratensis</em></td>
<td>Asteraceae</td>
<td>1.57 ± 0.42 (d)</td>
<td>1.14 ± 0.46 (d)</td>
<td>7</td>
</tr>
<tr>
<td><em>Trifolium repens</em></td>
<td>Fabaceae</td>
<td>8.00 ± 1.02 (a)</td>
<td>7.11 ± 2.65 (a)</td>
<td>3</td>
</tr>
<tr>
<td><em>Veronica persica</em></td>
<td>Scrophulariaceae</td>
<td>4.00 ± 1.00 (c)</td>
<td>3.33 ± 1.08 (c)</td>
<td>4</td>
</tr>
<tr>
<td><em>Vicia sativa</em></td>
<td>Fabaceae</td>
<td>4.83 ± 0.85 (c)</td>
<td>4.16 ± 1.01 (b,c)</td>
<td>6</td>
</tr>
<tr>
<td>Control (mowed plants)</td>
<td></td>
<td>1.64 ± 0.20 (d)</td>
<td>0.66 ± 0.09 (d)</td>
<td>11</td>
</tr>
</tbody>
</table>

In terms of the early flowering plants, the most important performance was remarked...
with *Euphorbia helioscopia*, *Senecio vulgaris*, and *Veronica persica*. Establishing these species, which start to flower early in the season in the orchards, is very important to attract the natural enemies and to develop their populations to satisfactory levels. These levels may be able to control the pests which arrive early, e.g. the rosy apple aphid (Wyss, 1995).

The popularity of *P. reptans* among the flowering species studied may be explained by its floral characteristics, such as the yellow colour, the depth of corolla tube and the floral odours which are effective attractants of parasitic wasps (Patt *et al*., 1997; Wäckers, 1994).

Many species of parasitic Hymenoptera should be able to reach the nectar in the flowers because they do not have mouthparts specifically adapted to extract pollen or nectar. Thus, they must feed on flowers with open structures, namely exposed nectaries and anthers, which are easily accessible to wasps of different sizes and with generalized mouthparts (Patt *et al*., 1997; Tooker and Hanks, 2000). The excellent examples of these plants in our study are *A. millefolium*, *T. repens* and *T. arvensis*. Flowers of several species of Fabaceae and Asteraceae have more concealed anthers and nectaries excluding some wasp species (Jervis *et al*., 1993). This fact could explain our results on *T. pratensis* and *M. officinalis*.

**Functional role of floral strips (parasitism of the codling moth cocoon)**

A total of 23 Hymenopteran parasitoids emerged from 294 larvae (parasitism rate: 7.82%). Three emerged species were recorded; two primary parasitoids *Ascogaster quadridentata* “Braconidae” (60.9%) and *Pristomerus vulnerator* “Ichneumonidae” (8.7%), and a hyperparasitoid *Perilampus fulvicornis* “Perilampidae” (30.4%). These parasitoid assemblages are similar to those reported previously (Mills, 2005).

Finally, it is notable that none of these species have been observed on the 26 studied plants or mowed plants (control). Hence, this result may suggest that the studied plants do not have a functional role concerning these parasitoids.

These findings support the concept that floral sources availability in orchards may be improving the parasitoids efficiency as biological control agents. This requires a judicious selection of flowering species to attract parasitoids specific to pests. Thus, it would appear that the functional role of flowering plants needs to be addressed in further studies.

**References**


Side effect of selected insecticides on *Aphidius colemani*, *Aphidoletes aphidimyza* and *Neoseiulus cucumeris* as model species of natural enemies

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**Abstract:** Side-effects of selected insecticides on model species of natural enemies, *Aphidius colemani*, *Aphidoletes aphidimyza* and *Neoseiulus cucumeris* were tested in laboratory conditions. Methoxyfenozide (Integro), indoxacarb (Steward 30 WG), pyridaben (Sanmite 20 WP), acetamiprid (Mospilan 20 SP), azadirachtin A (NeemAzal T/S) and spinosad (Spintor 480 SC) were tested against adults of *A. colemani* and larvae of *A. aphidimyza*. Propargite (Omite 570 EW) and Cyperkill 25 EC (cypermethrin) were also tested against adults of *N. cucumeris*. Mortality of tested species after 24 or 48 hours of exposure to residues of insecticides was evaluated. For insecticides with a low toxic effect, the effect on fecundity of *A. colemani* was tested. Methoxyfenozide had low toxic effect on all three insect species, causing mortality after 24 hours from 4.6% to 29.8%. Similarly, indoxacarb caused mortality after 24 hours from 11.1% to 25%. However, higher mortality of *A. colemani* was found after 48 hours of exposure to residues of methoxyfenozide and indoxacarb. Acetamiprid was highly toxic to *A. colemani* (100% mortality), medium toxic to *A. aphidimyza* (48.1% mortality) and no effect was found to *N. cucumeris* (2.3% mortality). Similar results were obtained with NeemAzal T/S. However, low toxicity to *A. colemani* was found when pure azadirachtin A was tested instead of formulated product NeemAzal T/S. In general, *N. cucumeris* exhibited the lowest sensitivity to all the insecticides. In contrast to this, *A. colemani* was highly sensitive to most of the insecticides.

**Key words:** *Aphidius colemani*, *Aphidoletes aphidimyza*, *Neoseiulus cucumeris*, insecticides, side-effect

**Introduction**

The success of integrated pest management (IPM) in orchards is closely connected with support of beneficial organisms in the environment. In order to protect habitats within and adjacent to agricultural areas, side-effect testing on nontarget organisms is required for registration of pesticides in the European Union (Council of the European Union, 1996). Hence, for most of the registered insecticides, selectivity to natural enemies of pests is declared. However, some of the insecticides are not entirely selective to some species of beneficial insects. Knowledge about the selectivity of insecticides is usually incomplete or the selectivity depends on the used rate of the insecticide. Repeat application of such less selective insecticides can lead to erosion of IPM.

The target of our study was to characterize side effects of new types of insecticides on selected species of natural enemies that is on the parasitic wasp *Aphidius colemani*, the predatory mite *Neoseiulus cucumeris* and the predatory gall midge *Aphidoletes aphidimyza*.

**Material and methods**

*Aphidius colemani*
The IOBC procedure (Candolfi et al., 2000) was used in our experiment to test side-effects of insecticides Integro (methoxyfenozide), Steward 30 WG (indoxacarb), Sanmite 20 WP (pyridaben), Mospilan 20 SP (acetamiprid), NeemAzal T/S (azadirachtin A), azadirachtin A and Spintor 480 SC (spinosad) on the parasitic wasp *A. colemani*. Wasps were held in groups of ten per replication. After treatment of glass plates, one-day-old wasps were exposed to dry residues of insecticides for 48 hours. The wasps were fed with honey via a cotton wool wick. Mortality of *A. colemani* wasps was evaluated after 24 and 48 hours. Effect of azadirachtin A and Integro as low toxic products were tested on fecundity of *A. colemani*. After 48 hours of exposure to dry residues of insecticides, the surviving wasps were sexed and females were introduced individually onto bean plants with defined numbers of nymphs of *Aphis fabae*. At the time of parasitism, most of the aphids (at least 80%) were last instar. Fecundity per female was calculated after 14 days as the number of mummified aphids per female and compared with untreated controls.

*Aphidoletes aphidimyza*
Side-effects of Integro (methoxyfenozide), Steward 30 WG (indoxacarb), Mospilan 20 SP (acetamiprid), NeemAzal T/S (azadirachtin A) and Spintor 480 SC (spinosad) against *A. aphidimyza* were tested in bioassay. Filter-paper was treated with insecticides and placed into Petri-dishes. Larvae of *A. aphidimyza* in groups of ten per replication were introduced into Petri-dishes, fed by *Acyrthosiphon pisum* and exposed to fresh residues of insecticides for 24 hours. After this period, mortality was evaluated.

*Neoseiulus cucumeris*
Side-effects of Integro (methoxyfenozide), Steward 30 WG (indoxacarb), Sanmite 20 WP (pyridaben), Mospilan 20 SP (acetamiprid), Cyperkill 25 EC (cypermethrin) and Omite 570 EW (propargite) were tested in bioassay. Filter-paper was treated with insecticides and placed into macro titre-plates. Mites in groups of ten per replication were introduced into the plates and exposed to fresh residues of insecticides for 24 hours. After this period, mortality of mites was evaluated.

Perfekthion (dimethoate) was used as a reference insecticide with high toxicity in the experiments with *A. colemani* and *A. aphidimyza*. All the insecticides were tested at rates registered for use in orchards or fields.

**Statistical analysis**
Corrected mortalities (Abbott, 1925) of the tested insects were calculated. XL-STAT program was used to evaluate the side effects of insecticides on *A. colemani* fecundity by ANOVA. In this case, fecundity per female was transformed as \( N_t = \log (N + 1 \times 10^6) \), where \( N \) is number of mummified aphids per female.

**Results and discussion**

*Side-effects of insecticides on mortality of A. colemani, A. aphidimyza and N. cucumeris*
In general, *A. colemani* was highly sensitive to most of the tested insecticides (Figure 1). In contrast to this, *N. cucumeris* exhibited the lowest sensitivity to most of the insecticides (Figure 2). New classes of insecticides represented by methoxyfenozide and indoxacarb had usually low toxicity. According to Anonymous (2006), inconsistent results were obtained when side-effect of indoxacarb was tested on *A. colemani*. In our experiments, low mortality of *A. colemani* was found after 24 hours of exposure to residues of indoxacarb, but the mortality considerably increased after 48 hours of exposition. Takahashi et al. (2005) found high
toxicity of spinosad to *A. colemani*. We confirm this result in our experiments. On the other hand, spinosad was not toxic to *A. aphidimyza*. Various side-effects were found for acetamiprid, which was highly toxic to *A. colemani*, medium-toxic to *A. aphidimyza* and was not toxic to *N. cucumeris* (Figure 1 and 2).

![Figure 1](image1.png)

Figure 1. Mortality of *A. colemani* after 24 and 48 hours of exposure to dry residues of insecticides

![Figure 2](image2.png)

Figure 2. Mortality of *A. aphidimyza* and *N. cucumeris* after 24 hours of exposure to residues of insecticides

Different toxicity was found, when NeemAzal T/S (azadirachtin A formulated) and azadirachtin A were tested against *A. colemani*. While azadirachtin A was not toxic to *A. colemani*, the formulated product NeemAzal T/S based on azadirachtin A was highly toxic (Figure 1). Low toxicity of NeemAzal T/S was found to *N. cucumeris*, while medium-toxicity was found to *A. aphidimyza*. 

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Side-effects of insecticides on fecundity of A. colemani

Based on the results from the side-effect testing of insecticides on mortality of A. colemani, Integro (methoxyfenozide) and azadirachtin A were selected as the only two insecticides with low toxicity to test their side-effect on fecundity of A. colemani. The Anova model (F(2,46) = 4.076; p = 0.024) showed differences in fecundity of A. colemani. When the fecundity in methoxyfenozide, azadirachtin and control treatments was compared by Tukey’s post hoc comparison, the fecundity in methoxyfenozide treatment was reduced significantly (p=0.018). In contrast to this, any effect of azadirachtin A on fecundity of A. colemani was not proved. According to Spollen and Isman (1996), a significant negative effect of azadirachtin A on fecundity of N. cucumeris was detected. In contrast to this, no effect of azadirachtin A on fecundity of Orius laevigatus was found (Angeli et al., 2005). Concerning methoxyfenozide, any data about the effect on fecundity of A. colemani has not recently been published.

According to our results, Integro (methoxyfenozide) and Steward (indoxacarb) proved to be suitable pesticides for using in integrated pest control programmes as low toxic products.

Acknowledgements

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References

Avian biodiversity: impacts of pest management strategies and landscape in South-Eastern French apple orchards

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Abstract: In French apple orchards, the predominant conventional management strategy has resulted in insecticide resistance in major pests like codling moth and an increased frequency of environmentally harmful insecticide applications. Organic agriculture as well as IPM represent alternatives to this situation.

Impacts on the avifauna of three different management strategies (organic, conventional and integrated) were studied during three years in 15 commercial apple orchards. These orchards were situated around Avignon and had similar contexts in terms of local and landscape features. Our results show that the avifauna differ significantly among the three management strategies with abundances of 46, 30.3 and 7.6 individuals/ha for the organic, integrated and conventional orchards respectively; species richness of 18.1, 14 and 7.6 breeding species/ha respectively and Shannon diversity indexes of 3.8, 3.3 and 2.6 respectively. The functional structure of bird communities is also affected, with a lesser proportion of insectivores in conventional orchards than in other orchards. Phytosanitary and environmental factors taken together explain 52% of the variability of the composition of bird communities. Phytosanitary treatments and local environment of the orchards had a similar explanatory power of 11% while environment at the landscape scale explained approximately 19% of the variability.

We have demonstrated an important impact of phytosanitary practices on all parameters used to describe bird communities. These results highlight the influence of fruit production on avian biodiversity and its consequences in terms of protection of species of agronomical or patrimonial interest.

Key words: Avian biodiversity, Apple orchard, Management strategy, Landscape

Introduction

In France, apple orchards constitute the main fruit culture covering about 54 000 ha. The most widespread management method, which is referred to as the conventional strategy, involves the exclusive use of chemical pesticides. Although pesticides allowed a significant increase in productivity, their intensive use was associated with negative externalities such as the development of insecticide resistances in the codling moth (Cydia pomonella), the major pest of apple orchards, and undesirable environmental impacts due to considerable potential for exposure of wildlife to frequent pesticides spraying. Organic and integrated pest management (IPM) strategies represent alternatives to this situation. Both strategies involve alternative practices such as insect mating disruption (saturation of an area with synthetic female pheromone) and the application of granulosis virus against codling moth. The aim of this study is to provide an integrated assessment of the environmental impact of farming practices in apple orchards from South Eastern France using bird communities as bioindicators. In this analysis, we attempted to dissociate the effects of pest management strategies on bird communities from effects of landscape elements surrounding the orchards.
Material and methods

Study Area and apple orchard selection
Impacts of pest management strategies on the avifauna were studied during three years from 2003 to 2005 in 15 commercial apple orchards around Avignon, of which 5 were organic, 5 under an IPM strategy and 5 conventional. These orchards had an average area of 0.8 ha and were chosen so that the three management strategies shared similar local and landscape features that might influence bird communities. Management strategies were characterized at the orchard level by analyses of the treatment calendars that were implemented in each of the studied orchards.

Bird data
They were recorded using a point count method with 20 min visits between mid April and mid May. Point counts were performed within 5 h after sunrise during the dawn peak in bird activity and were carried out under good weather conditions (windy and rainy days were excluded). All nesting birds heard and seen were recorded within each orchard and its surrounding hedgerows. For each species, the maximum count of individuals from the two visits was used. Bird counts were used to calculate the following parameters for each studied orchard: bird abundance (the number of birds per orchard), species richness (the number of species per orchard), and Shannon diversity indice \( H = - \sum \frac{n_i}{N} \log_2 \frac{n_i}{N} \), where \( n_i \) is the relative abundance of species \( i \) and \( N \) is the bird number of the orchard. These parameters were also calculated within ecological guilds such as insectivorous, granivorous, omnivorous and prey birds per orchard.

Landscape description
At the local scale, the following elements of focus orchards were recorded on the field: hedgerow presence / absence, structure (height, length), and flora species richness; ditch presence / absence. From these observations we calculated the maximal height of hedgerows, the difference between maximal and minimal height of hedgerows, the average ratio length of the hedgerow / perimeter of the orchard, calculated as the mean of these ratios on all the hedgerows surrounding the orchard, the number of ditches, the average species richness of hedgerows flora weighing individual hedgerow values by hedgerows lengths.
In a second step, we characterised the landscape context surrounding the orchards within 200 m wide buffers. For this purpose, focus orchards were mapped on a GIS (Arcview 9.1.) and surrounding hedgerows, others orchards, wooded and open areas, and habitations were mapped from 2004 aerial photographs (source IGN). From these features, we calculated the proportions of buffer areas covered with either orchards, wooded area, open areas or habitations (no dimension) and the ratio of the hedgerow length over buffer area (1/m).

Statistical analysis
Statistical analysis of data was performed using R (package Vegan). ANCOVA analyses were performed to compare both numbers of pesticides treatments and bird parameters among management strategies within years and across years. To investigate the part of the variation in bird communities that was explained (1) by the pesticide applications, (2) by focus orchards local characteristics and (3) by surrounding landscape features, we performed partial Canonical correspondence analyses (CCA) using the package Vegan.
Results and discussion

Apple orchard pest management strategies
Pesticide applications were mostly targeted towards two fungal diseases, apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*), and an insect pest, the codling moth. Chemical pesticides were never used in organic orchards. Compared to conventional orchards, the adoption of mating disruption in IPM orchards resulted in a decreased number of applications of neurotoxic insecticides that are known to have a high environmental impact. Nevertheless, the mean number of annual neurotoxic insecticide applications in orchards under IPM protection did not differ statistically from that in conventional orchards (Figure 1).

![Figure 1. Annual pesticide applications in organic, IPM and conventional apple orchards (mean number ± SD)](image)

Apple orchard management strategies and bird biodiversity
Global bird community differed among the management strategies with abundances of 46, 30.3 and 7.6 individuals/ha for the organic, integrated and conventional orchards respectively (LSMeans comparisons: organic vs IPM *P*=0.0959, organic vs conventional *P*=0.0008 and IPM vs conventional *P*=0.0568); species richness of 18.1, 14 and 7.6 breeding species/ha respectively (LSMeans comparisons: organic vs IPM *P*=0.0664, organic vs conventional *P*=0.0001 and IPM vs conventional *P*=0.0160), and Shannon diversity indexes of 3.8, 3.3 and 2.6 respectively. The functional structure of bird communities was also affected, with a lesser proportion of insectivores in conventional orchards than in other orchards. An increase in bird abundance and diversity has been recorded in other studies in orchards (Genghini et al., 2006) as well as in cereal fields under organic management, and sometimes under IPM, compared to conventional ones (Chamberlain *et al.*, 1999; Freemark and Kirk, 2001; Beecher *et al.*, 2002). These results show the positive effects of organic and IPM agriculture on bird communities and their functional structure. These effects could be attributed to the different pest management strategies, i.e. the type of chemicals and the number of applications that can affect bird communities and reproduction of insectivorous species through both a reduction of food resources and a direct toxicity of pesticides (Walker, 1983; Bouvier *et al.*, 2005; Boatman *et al.*, 2006).

Variance partitioning for bird community composition
However, other factors, and in particular the landscape characteristics of the studied orchards may influence the composition of bird communities. In the present study, studied parameters explained 52% of the variability of the composition of bird communities. Among the total
variability, phytosanitary treatments and local environment of the orchards had a similar explanatory power of about 11% while environment at the landscape scale explained about 19% of the variability. Year and interactions explained 11% of the total variability.

In conclusion, we showed that bird communities were both more abundant and more diverse in organic than conventional orchards, communities from IPM orchards being intermediate. Bird communities were also largely influenced by the landscape context of the orchards, which confirms the need to be cautious in the choice of orchards for comparisons of management strategies.

Acknowledgements

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References

Is the distribution of beneficial arthropods influenced by mixed hedgerows

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Abstract: Farming intensification in recent decades has led to an alarming level of degradation and loss of wildlife and its hedgerow habitat. The relationship between biodiversity and ecosystem functioning has emerged as a central issue in ecological sciences, but the situation regarding hedgerow function as a potential source of biological control agents against agricultural pests remains poorly understood. We evaluated possible effects of the arthropod community in a neighbouring hedge on the distribution of the pest psylla Cacopsylla pyri L. (Hemiptera: Psyllidae) in a pear orchard Pyrus communis L. over three consecutive years (1999 - 2001). We measured the diversity of the arthropod community in the hedge and in the orchard at increasing distances from the hedge using Shannon index of diversity, and the Hellinger distance and Mahalanobis index to highlight dissimilarities between population distributions. Our results showed a convergence between predator populations in the orchard and the hedgerow during Psylla proliferation. There was a decreasing diversity gradient as distance from the hedge increased. Beneficial arthropod exchanges occurring between the mixed hedgerow and the pear orchard during the pest proliferation period suggest that field border management can be used in an integrated pest management strategy aimed at reducing insecticide use.

Mixed hedge, Cacopsylla pyri, IPM, Arthropod Community, Shannon index of diversity
Changes of entomophauna in orchards under different pest management regimes

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Abstract: Integrated fruit production is facing problems with intensive pesticide use accompanied by the reduction of nature enemies in agroecosystems. This results in outbreaks of pests with high reproductive potential (aphids, psyllids, leaf midges, etc.). The side effect of plant protection products on the diversity of beneficials, as well as, the effect of pest control on selected pests (mining Lepidoptera, codling moth) were evaluated in different pest control regimes (conventional, integrated, biological). Insects were sampled before and after each application using the limb jarring method. During the first season (2006) fewer Heteroptera species (cca 10x) and Forficula auricularia nymphs (3x) were found in conventional and IPM variant in comparison with biological regime. In the 2nd experimental year (2007) this effect was very similar with a higher total number of Hymenoptera species (x 1.5) sampled in biological regime. In contrast, ladybirds, lacewings and Cantharidae species showed relatively stable abundance. Direct influence of particular treatments on entomophauna was not so evident when the number of individuals before and after applications was collected. In spite of this, the preliminary results suggest that a shift in insect populations develops, although this process is relatively slow and more apparent changes may be expected during the next experimental seasons. As far as the direct efficiency of control of mining Lepidoptera species is concerned the most effective appears to be IPM with the use of selective insecticides. Codling moth was successfully controlled in conventional and IPM variants, and in the biological regime with applications of CpGV.

IPM, Entomophauna, Orchards, Agroecosystems, Natural enemies
Arthropods and mycorrhizal fungi associated to the rhizosphere of grapevine in Sicily

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Abstract: To evaluate the variation of AM fungi and arthropod populations and their possible interactions in mycorrhizosphere of grapevine in Sicily, a research in different tillage systems was carried out: the first data on the endomycorrhizal fungi and arthropods are reported. One vineyard in Palermo in state of neglect and two vineyards in Alcamo (TP), one organically managed and the other traditionally managed, were investigated during 2007. The index of root mycorrhization (IM) and the whole population of both AM fungi and arthropods were evaluated. The IM was similar in soils traditionally and organically managed: high in winter and in spring and lower in summer; the vineyard in state of neglect, during all seasons, showed IM variable values. In all Sicilian vineyards the highest number of spores was detected in winter, whereas in spring AM populations decreased. With respect to the arthropods low Shannon’s index (H') was observed in all soils, while the BSQ values were found higher in vineyards traditionally and organically managed.

Arthropods, AM fungi, Grapevine, Sicily
Mixed deciduous hedgerows as sources of anthocorids and other predators of pear psyllids in the UK

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Abstract: Anthocorid predatory bugs are the key natural enemies of pear sucker but they often migrate into orchards too late and/or in too small numbers to affect adequate natural control of pear sucker populations. A 4 year study began at East Malling Research in 2008 to develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker in the spring. Part of this study is to identify woody species and species mixes for hedgerows/windbreaks that act as sources of pear psyllid natural enemies, especially early in the season. Three established hedgerows with a range of plant species compositions and structures adjacent to pear orchards in Kent, UK were identified and characterised. The aim was to identify species mixes that maximise anthocorid populations in the spring and foster their migration into pear orchards when pear sucker populations start to increase. The arthropods were beat sampled from the woody species and sweep net sampled from stinging nettles at 3-4 week intervals from April to September.

A large data base comprising more than 30,000 individuals, sampled and identified from 24 plant species, was constructed but not yet analysed. However, some trends in the data are obvious. 1) The largest numbers of anthocorids were found on hawthorn, goat willow and stinging nettle in the early season, while on downy birch, grey willow, stinging nettle, hazel, black alder, goat willow, field maple, blackthorn, rose and sycamore late in the growing season. 2) In the early growing season the highest numbers of anthocorids were found on the same plants that had the highest numbers of psyllids. 3) Later on, anthocorids were present mostly on plant species that had high numbers of aphids. 4) A large number of other predatory arthropods (mostly Miridae, Araneae, Dermaptera, Neuroptera, Cantharidae, Coccinellidae) also potential predators of pear psyllids were found on the hedge plants. 5) Cacopsylla pyri (L.) was discovered to be the most dominant psyllid species in the pear orchards, not Cacopsylla pyricola (Foerster), as previously reported for the UK.

Key words: Anthocoridae, Psyllidae, Aphididae, hedgerows, natural enemies, biocontrol, pear sucker

Introduction

Pear sucker is a devastating pest of pears which is currently out of control and causing serious widespread damage in many commercial pear orchards in the UK. Nymphs suck sap from leaves and fruits, excreting honeydew which turns black with sooty mould. This contaminates the foliage and fruits, ruining the crop. Attacks weaken the trees which suffer from severe depletion in fruit buds the following year or may even be killed. The pest transmits ‘pear decline’, a debilitating phytoplasma-caused disease of young trees. In the past, the dominant pear sucker species in the UK was known to be Cacopsylla pyricola (Foerster), whereas Cacopsylla pyri (L.) was considered dominant in other European countries (Ossiannilson, 1992).

Anthocorid predatory bugs (especially Anthocoris nemoralis (Fabricius)) are the key predators of pear sucker (Solomon et al., 2000). If the pesticide programme allows them to survive, they can naturally regulate populations of the pest (Solomon et al., 1989; 2000). However, only few anthocorids are able to overwinter in pear orchards because of the lack of

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sufficient food or shelter. Anthocorids migrate into pear orchards from early April. This migration is responsible for the major part of their population found during summer. If the early season influx is inadequate and/or too late, pear sucker populations increase to damaging levels in advance of those of anthocorid predators (Solomon et al., 2000).

Adjacent habitats including hedgerows and windbreaks can promote abundance of insect predators in adjacent orchards, but little is known about the significance of certain woody hedgerow species for conservation biological control. Hedgerows and windbreaks are the source of anthocorids in the spring (Solomon et al., 2000). To sustain anthocorids and other beneficial invertebrates, hedgerows must provide alternative, early season prey (e.g. psyllids, aphids and gall midge larvae) and shelter. Previous research has shown that early in the growing season anthocorids concentrate on a particular host plant. During the time that willow is flowering, *Anthocoris nemorum* (L.) and *A. nemoralis* occur on it in very large numbers (Anderson, 1962). The flowering period is usually late March or early April, and anthocorids leave the willow after only a week or so. Solomon et al. (1999) showed that various herbaceous flowering plants attract anthocorids and can be used to enhance predator populations in orchards. Sigsgaard & Kollmann (2007) showed that hedgerows containing flowering hawthorn or elderberry and herbaceous layers with stinging nettle held high numbers of anthocorids in the spring. It is likely that mixed hedgerows provide, in succession, a range of alternative prey, pollen and nectar sources in early spring.

In 2008 a study at East Malling Research aimed to develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker in spring. Part of this 4 year study is to identify woody species and species mixes for hedgerows/windbreaks that act as sources of pear psyllid natural enemies.

**Material and methods**

Results and discussion

A large database comprising >30,000 individual s identified from 24 plant species has been constructed and started to be analysed. Some trends in the data are obvious.

1) From the total of 8 anthocorid species collected, *A. nemorum* and *A. nemoralis* were the most abundant. The other 6 species (*Anthocoris confusus* Reuter, *Orius niger* (Wolff), *Orius laevigatus* (Fieber), *Orius majusculus* (Reuter), *Orius laticollis* (Reuter) and *Orius vicinus* (Ribaut)) were found in lower numbers (Table 1). The largest numbers of overwintered adult anthocorids were found on hawthorn, goat willow and stinging nettle early in the season (Figure 1). Mostly, *A. nemoralis* was found on hawthorn and goat willow, while *A. nemorum* was found on stinging nettle (Table 1). Later in the growing season, grey willow and goat willow were the most frequent hosts for *A. nemoralis*; downy birch, hazel, stinging nettle, field maple, grey willow, black alder, blackthorn, rose, sycamore and goat willow were good sources for *A. nemorum*; while Buddleia was host to more *Orius* species (Figure 3, Table 1).

Table 1. Mean numbers of anthocorid species collected from the plants.

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2) Of the 24 plants studied, 16 are host to some psyllid species (marked with * in Table 2). A total of 24 psyllid species were found (Table 2), 19 were associated with 15 of the studied plants (*Rhinocola aceris* (L.): field maple and sycamore; *Psylla alni* (L.) and *Baeopelma foersteri* (Flor): black- and grey alder; *Chamaepsylla hartigii* (Flor): downy birch; *Cacopsylla melanoneura* (Foerster) and *Cacopsylla peregrina* (Foerster): hawthorn; *Psyllopsis fraxinicola* (Foerster) and *Psyllopsis fraxini* (L.): common ash; *Cacopsylla mali* (Schmidberger): apple; *Cacopsylla pruni* (Scopoli): blackthorn; *C. pyri* and *C. pyricola*: pear; *Cacopsylla ambigua* (Foerster), *Cacopsylla brunneipennis* (Edwards) and *Cacopsylla moscovita* (Andrianova): goat- and grey willow; *Bactericera curvatinervis* (Foerster): white-,
goat- and grey willow; *Bactericera salicivora* (Reuter): white willow; *Trioza remota* Foerster: pedunculate oak; *Trioza urticae* (L.): stinging nettle (Ossiannilsson, 1992)).

In the early growing season the greatest numbers of psyllid adults were found on pear (22.2), stinging nettle (18.6), hawthorn (16.0) and field maple (11.0), while nymphs were on hawthorn (31.0) and goat willow (7.7) (Figure 2). Later in the growing season common ash, grey willow, apple and blackthorn also became good sources of psyllids (Table 2). On the pear trees, six psyllid species were found with *C. pyri* dominant (42.8), followed by *C. pyricola* (7.5) and *C. melanoneura* (2.8) (Table 2). Previously, *C. pyricola* was reported as the dominant pear sucker in the UK.

| Table 2. Mean numbers of most common psyllid species on the plants (adults and nymphs). |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| R. aceris | P. fraxini | P. fraxini | P. alni | C. ambiguous | C. fraxinipennis | C. mali | C. melanoneura |
| ACE | 19.0 | 11.0 | 2.0 | 1.0 | 0.5 |
| CAM* | 2.0 | 0.5 | 1.5 |
| ALN | 4.3 | 0.3 | 0.3 | 1.0 |
| INC* | 52.0 | 6.3 |
| PUB* | 0.5 |
| BUD |
| DAV |
| CAS |
| SAT |
| COR | 3.0 | 0.2 | 1.3 | 0.5 | 0.3 | 0.3 | 0.8 |
| AVE | 0.8 | 2.0 | 0.8 | 52.0 | 6.3 | 0.5 |
| CRA |
| MON* |
| FRA | 1.5 | 73.5 | 33.0 | 2.0 | 3.0 | 0.5 |
| EXC* |
| ILE | 2.0 | 1.0 | 2.0 | 3.5 | 1.5 | 0.5 |
| AQU |
| LON |
| MAL |
| DOM* | 7.5 | 3.5 | 0.5 | 2.0 |
| PRU | 1.0 | 0.5 | 0.5 |
| AVI |
| PRU | 3.0 | 0.5 | 1.5 | 1.0 | 4.5 | 1.0 |
| SPI* |
| PYR | 0.8 | 2.8 | 0.2 | 42.8 | 7.5 |
| COM* |
| QUE |
| ROB* | 2.5 | 1.0 |
| ROS | 0.5 | 4.5 | 3.0 | 3.0 | 0.5 |
| SP |
| SAL |
| ALB* | 0.5 | 1.0 | 0.5 |
| SAL |
| CAP* |
| SAL | 5.3 | 3.7 | 2.5 | 0.3 | 0.3 |
| CIN* |
| SAM | 0.2 | 0.7 | 1.7 | 0.2 | 0.5 | 0.3 |
| NIG |
| URT | 0.2 | 114.4 |
| DIO* |
| VIB |
| OPU* |
| Rare species omitted (*B. foersteri*: black alder (1.0), *C. hartigii*: downy birch (1.0), *C. moscovita*: grey willow (0.3), *B. salicivora*: white willow (0.5), *B. curvatinervis*: grey willow (1.3) and field maple (0.5), *Trioza* |
centranthi (Vallot): black alder (1.0), Trioza galii Forster: pear (0.2) and stinging nettle (0.2), Trioza sp.: grey willow (0.3), Cacopsylla sp.1: sweet chestnut (0.5), and Cacopsylla sp.2: goat willow (0.2).

3) The highest numbers of aphids were found on sycamore (943.5), downy birch (457.5), field maple (339.0), blackthorn (291.5), rose, (176.0), grey willow (135.3), stinging nettle (105.0), hazel (94.8), white willow (90.5) and black alder (78.0) (Figure 4).

4) A large number of other predatory arthropods, which may also be important predators of pear psyllids (mostly Miridae, Araneae, Dermaptera, Neuroptera, Cantharidae, Coccinellidae) were found on the hedgerow plants.

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Figure 1. Mean numbers of the overwintered anthocorid adults on plants during the early season (15-April – 22-May).

Figure 2. Mean numbers of psyllids on plants during the early season (15-April – 22-May).

Figure 3. Mean numbers of the new anthocorid generation on plants (adults: 16-June – 03-September; nymphs: 22-May – 03-September).
During the early season, the highest numbers of anthocorids were on hawthorn, goat willow and stinging nettle (Figure 1). These plants are hosts of some psyllids that were present on these plants from the beginning of the sampling period. Hawthorn is the main host of *C. melanoneura* and *C. peregrina* and nymphs were also collected from this plant. Goat willow was the host of numerous psyllids species, but *C. ambigua* and *C. brunneipennis* were found in greater numbers. Stinging nettle was the host of *T. urticae*, the psyllid collected in the largest number in this study (Table 2).

Aphid numbers were very low at the beginning of the growing season, and we suggest that psyllid eggs and nymphs may be among the most important prey for anthocorids in the early spring. During the second half of the season the anthocorids seemed to follow the growing aphid numbers, until late May when they are likely to be contributing to growth of anthocorid populations. It appears that the planting of strategic hedgerow species may increase the number of anthocorids available to predate psyllids in pear orchards. Future work will improve our understanding of these complex predator-prey relationships.

The dominant pear sucker species in the UK has been known *C. pyricola*, whereas *C. pyri* is considered to be dominant in other European countries (Ossiannilsson, 1992). In this study of three pear orchards, two had much higher numbers of *C. pyri* than *C. pyricola* (Table 2). Hence, the European *C. pyri* seems to have a more important role in British pear orchards than previously reported. This has implications for our understanding of pear sucker population dynamics and the formulation of Integrated Pest Management strategies.

**Acknowledgements**

We thank Michelle Fountain and Adrian Harris helping in project organisation. We also thank Elke Groll and Vanesa Alaiz Fuertes for insect sorting. This work is funded by many industrial partners, growers and research councils under a Defra HortLINK project.

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psyllid, *Cacopsylla pyricola*, in South East England by predators and pesticides. – Crop Protect. 8: 197-205.


Species diversity, dominance and frequency of leaf-eating Lepidoptera in plum biocenose in West Bulgaria

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Abstract: The mating disruption technique is one of the most selective methods of controlling Cydia funebrana Tr. and is under development in Bulgaria. In this connection, a pre-study was carried out to follow the dynamics and density of the leaf-eating and fruit-surface damaging lepidopteran larvae in an abandoned plum orchard of mixed varieties in Sofia region. Species belonging to eleven families were found during the eight years of observations. Permanent inhabitants in the plum biocenose were larvae of Gelechiidae, Tortricidae and Geometridae with index of constancy c=100. The next by frequency of occurrence were species of Coleophoridae and Noctuidae (c=87.50), followed by Yponomeutidae (c=75), Lycanidae (c=50), Lymantridae and Pieridae (c=25). The rarest were individuals of families Ypsolophidae, Chimabachidae and Lasiocampidae with c=12.50. In 1998 and 2002 the dominant species of all collected lepidopterian larvae was Recurvaria nanella (Denis & Schiffermüller, 1775) and Anarsia lineatella (Zeller, 1839) in 1999. Operophtera brumata (Linnaeus, 1758) dominated in complex of external lepidopteran larvae in 2000 and 2005, Neusphaleroptera nubilana (Hübner, 1799) in 2003, Hedia nubiferana (Haworth, 1811) in 2006 and Argyresthia spp. in 2007. Specimens belonging to 35 genera and 47 species were identified altogether. In spite of very rich biodiversity, the density of leaf-eating and fruit-surface damaging lepidopteran larvae was above economical threshold only during three of the eight years of investigation, so we consider it possible to develop plant protection programs for biological production of plum fruits in West Bulgaria.

Key words: Leaf-eating Lepidoptera, Plum orchard, West Bulgaria

Introduction

The mating disruption technique for controlling Cydia funebrana Tr. is under development in Bulgaria. In this connection, a pre-study was carried out to follow species diversity and densities of the surface lepidopteran larvae in plum orchard with aim to evaluate the most dangerous species which we have to monitor carefully and manage with ecologically safe methods.

Materials and methods


Results and discussion

Species belonging to thirteen families were found altogether in abandon plum orchard in Sofia region during the eight years observations. Permanent inhabitants in the plum biocenose
were larvae of Gelechiidae, Tortricidae and Geometridae with index of constancy $c = 100$. The next highest frequency of occurrence were species of Coleophoridae and Noctuidae ($c = 87.50$), followed by Yponomeutidae ($c = 75$), Lycanidae ($c = 50$), Lymantriidae and Pieridae ($c = 25$). The rarest were individuals of families Ypsolophidae, Chimabachidae and Lasiocampidae with $c = 12.50$.


Family Tortricidae was the richest of species (Table 1). *Hedia nubiferana* Hw. predominated in 1998 with index of dominance $c = 48.15$, in 1999, the pest was in same abundance as *Neushphaleroptera nubilana* Hb. The last mentioned leafroller dominated in all of the rest years of observations (Table 1). The other frequent representative of leafrollers in the plum biocenose were *Ancyiis achatana* Den.&Schiff. and *Hedya pruniana* Hbn (Table 1). These two leafroller species were typical for plum biocenose and were rare inhabitants in apple orchards, where 21 species were registered in abandoned apple orchard situated in the same region of our country (Velcheva, 2005; Peeva, Velcheva in press).

Table 1. Index of dominance and constancy of the species of external leaf-eating and fruit-surface damaging Tortricide in plum biocenose of abandon orchard.

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<td><em>Exapate congelatella</em> (Clerck, 1759)</td>
<td>1.83</td>
<td>25</td>
</tr>
<tr>
<td><em>Acleris variegana</em> (Denis &amp; Schiffermüller, 1775)</td>
<td>0.31</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Nonidentified</em></td>
<td>0.92</td>
<td>25</td>
</tr>
</tbody>
</table>

Lecheva (1999) identified 18 geometrid species in plums orchards in Bulgaria. During our investigation we identified ten species belonging to this family with very high dominance of
Operophtera brumata L. (Table 2).

Table 2. Index of dominance and constancy of the geometrid species in plum biocenose.

<table>
<thead>
<tr>
<th>Species Geometridae</th>
<th>Indices of dominance</th>
<th>Constancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operophtera brumata, (Linnaeus, 1758)</td>
<td>74.23</td>
<td>100</td>
</tr>
<tr>
<td>Erranis defoliaria (Clerck, 1759)</td>
<td>10.31</td>
<td>75</td>
</tr>
<tr>
<td>Alsophila aescularia (Denis &amp; Schiffermüller, 1775)</td>
<td>4.12</td>
<td>33.33</td>
</tr>
<tr>
<td>Agriopis marginaria (Fabricius 1776)</td>
<td>3.61</td>
<td>12.5</td>
</tr>
<tr>
<td>Rhinopra chloerata (Mabille, 1870)</td>
<td>2.06</td>
<td>25</td>
</tr>
<tr>
<td>Alsophila aceraria (Denis &amp; Schiffermüller, 1775)</td>
<td>1.03</td>
<td>12.5</td>
</tr>
<tr>
<td>Apocheima pilosaria (Denis &amp; Schiffermüller, 1775)</td>
<td>1.03</td>
<td>12.5</td>
</tr>
<tr>
<td>Agriopis bajaria (Denis &amp; Schiffermüller, 1775)</td>
<td>0.52</td>
<td>12.5</td>
</tr>
<tr>
<td>Licia hirtaria (Clerck, 1759)</td>
<td>0.52</td>
<td>25</td>
</tr>
<tr>
<td>Gymnoscelis rufifasciata (Haworth, 1809)</td>
<td>0.52</td>
<td>12.5</td>
</tr>
<tr>
<td>Nonidentified</td>
<td>2.05</td>
<td></td>
</tr>
</tbody>
</table>

The winter moth was not only the predominant geometrid pest, but also the most constant geometrid inhabitant in abandon plum orchard. It was present during all vegetation seasons of the study (Table 2). Alsophila aceraria (Den. & Schiff.), Agriopis bajaria (Den. & Schiff.), Licia hirtaria (Cl.) and Gymnoscelis rufifasciata (Haworth, 1809) were found during one vegetation season in very low density, so we may conclude that the appearance and development of these species depend in very high degree of the meteorological factors of particular year. This is the first finding of G. rufifasciata Hw. to feed on plums for our country.

Nine were the species of Noctuidae on plums trees (Table 3). The ten species Xestia c-nigrum (Linnaeus, 1758) was present with only egg masses in the tree crown. The richest complex was in 2002, when seven species were identified. In the same year we found the largest number of Noctuidae and in an apple orchard (Velcheva, Peeva, 2005). The peculiar for this year was very high temperature in February – 5.08°C above the norm.
Table 3. Index of dominance and constancy of the noctuid species in plum biocenose.

<table>
<thead>
<tr>
<th>Species of Noctuidae</th>
<th>Indices of dominance</th>
<th>constancy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eupsila transversa</em> (Hufnagel, 1766)</td>
<td>21.67</td>
<td>50</td>
</tr>
<tr>
<td><em>Orthosia cerasi</em> (Fabricius, 1775)</td>
<td>18.33</td>
<td>63</td>
</tr>
<tr>
<td><em>Conistra vaccinii</em> (Linnaeus, 1761)</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td><em>Orthosia gracilis</em> (Denis &amp; Schiffermüller, 1775)</td>
<td>13.33</td>
<td>50</td>
</tr>
<tr>
<td><em>Cosmia trapezina</em> (Linnaeus, 1758)</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td><em>Diloba caeruleocephala</em> (Linnaeus, 1758)</td>
<td>8.33</td>
<td>25</td>
</tr>
<tr>
<td><em>Orthosia cruda</em> (Denis &amp; Schiffermüller, 1775)</td>
<td>3.33</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Orthosia munda</em> (Denis &amp; Schiffermüller, 1775)</td>
<td>3.33</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Amphipyra pyramidea</em> (Linnaeus, 1758)</td>
<td>1.67</td>
<td>12.5</td>
</tr>
<tr>
<td>Nonidentified</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Larvae of family Gelechiidae were rather numerous -19.4% of all collected individuals but was represented only with four species *Recurvaria nanella* ([Denis & Schiffermüller, 1775], *Anarsia ineatella* (Zeller, 1839), *R. leucatella* (Clerck, 1759) and *Dichomeris derasella* (Denis & Schiffermüller, 1775). This is the record of *D. derasella* Den. & Schiff. on plums for our country.

We identified only *Coleophora hemerobiella* (Scopoli, 1763), *Coleophora coraciapennella (= nigricella)* (Hubner, 1796) on plums. Perhaps there are more species as we had numerous non-emerged and unidentified specimens. *Diurnea fagella* (Denis & Schiffermüller, 1775) was the only Chimabachidae; *Ypsolophia asperella* (Linnaeus, 1761) and *Satyrium pruni* (Linnaeus 1758) were the only representatives of Ypsolophidae and Lycanidae, respectively.

In spite of very rich biodiversity, the density of leaf-eating and damaging surface of the fruits lepidopteran larvae was above economical threshold during only three of the years - in 2000 and 2002 in April, and in 2007 in May. So, they are not a big threat to the fruit of plums and only need careful observations and forecasting. Biological insecticides are effective against most of the species in warm spring, so we consider developing plant protection programs for biological production of plum fruits possible in West Bulgaria.

References


Hazelnut quality and sensory evaluation in organic and conventional growing systems

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Abstract: Consumer acceptance of organic products requires the association of the production system with directly perceivable quality attributes. Up to now, organically grown hazelnuts have been scarcely characterised for specific quality traits. Nuts of two Italian hazelnut cultivars, ‘Tonda Gentile Romana’ and ‘Tonda di Giffoni’, grown in conventional and organic systems, were evaluated for technological traits, kernel chemical composition and sensory profile. Organic nuts showed a slightly lower oil and starch content, a lower incidence of total saturated fatty acids and a higher content of oleic acid in comparison to conventional ones. Crude protein content in the kernel was higher in organic nuts in ‘Tonda di Giffoni’. In both cultivars, the organic regime has positively influenced the content of polyphenols in the kernel. Sensory evaluation revealed differences associated with the growing system for the attributes of colour and oiliness of roasted kernels. The organic samples were the most appreciated for both cultivar.

Key words: Corylus avellana L., nut traits, chemical composition, quality, sensory evaluation

Introduction

During the last few years, Italian hazelnut growers have expressed a growing interest in the exploitation of indigenous varieties and in organic growing systems. This is common in the Monti Cimini hazelnut district (Central Italy) where both conventional and organic methods of production have been applied to the Italian cultivar “Tonda Gentile Romana”. Furthermore, consumers and food industry are becoming increasingly concerned about how, where and when foods are produced, and this has led to an increased consumer interest in organically grown fruits including nuts.

Currently nut quality is still identified with some morphological, physical and chemical traits requested by the food industry, which processes about 90% of production (Garrone and Vacchetti, 1994). Nevertheless a wider meaning of quality has been suggested based on the potential effect of some chemical components on taste, nutritional and health properties and on the storability of nut and processed products (Bignami et al., 2005; Kornsteiner et al., 2006; Cristofori et al., 2008). Since the influence of growing system on taste and chemical composition of fruits and vegetables has been often highlighted (Worthington V., 2001; Peck et al., 2006), but up to now organic dry fruits have been scarcely characterised, the quality of organic versus conventional nuts of the Italian cultivars “Tonda Gentile Romana” and “Tonda di Giffoni” has been examined by analysing their chemical composition and sensory attributes.
Material and methods

Samples
Nuts of 'Tonda Gentile Romana' (TGR) and 'Tonda di Giffoni' (TG), grown in conventional (con) and organic (org) systems were sampled from two different farms located in the same area of the Monti Cimini hazelnut district (Central Italy). Immediately after harvest, the nuts were dried to 5% moisture content, in the same manner as the current post-harvest treatments applied in commercial production, and then stored at –20 °C until analysed.

Nut traits and kernel composition
Nut and kernel weight, width, thickness and height, and shell weight were recorded on subsamples of 50 nuts for each cultivar. Nut and kernel shape and seed/nut ratio were then calculated. Oil in the kernel was determined by the Soxhlet method. Soluble sugars, organic acids, starch and fatty acid composition were analysed by gas-liquid chromatography (GLC) according to Cristofori et al. (2008). Total phenolic content was determined according to the Folin-Ciocalteu's procedure, calibrating against gallic acid (Scalbert et al., 1989). All analyses were performed in duplicate.

Sensory Evaluation
Roasted nuts were examined for their sensory profile by a panel including 15 members of the department staff and students trained on sensory evaluation of dry fruits. The evaluation was carried out in an equipped room, where anonymous samples were submitted to the panelists. The most important visual and taste descriptors: colour; roundness; shape regularity; peelability; taste intensity; aroma intensity; sweetness; oiliness; were evaluated. The assessors were requested to indicate the intensity of each attribute by placing a vertical line on an 11 cm unstructured scale line with anchored terms at both ends. The scores were then quantified as the distance from the origin and the vertical line.

Statistical analysis
Analysis of variance was performed to estimate the effects of cultivar, growing system and their interaction using the SYSTAT MGLH procedure (Wilkinson, 1998). The least significant difference (LSD $\alpha=0.05$) for the comparison of the means was then calculated.

Results and discussion

Nut traits and kernel composition
Significant differences among growing systems were observed for the nut traits with the exception of nut shape and kernel/nut ratio. ‘TG org’ was characterised by the highest kernel/shell ratio (Table 1). The nuts of both cultivars collected in the organic farm showed a higher incidence of defects, mainly empty nuts and shrivelled kernels (data not reported). The oil and crude protein content were not significantly affected by growing systems and cultivar (Table 2). Significant differences in fatty acid composition were observed between growing systems for stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids (Table 2). The main fatty acid was oleic acid (C18:1) whose content was higher in nuts collected in the organic farm for both cultivar. The soluble sugar content did not significantly differ among cultivars and growing systems, while starch was almost double in organic nuts in comparison to conventional ones (Table 3). The content of total organic acids was significantly higher in TG independent of the growing system (Table 3). The polyphenol contents in the kernel agreed with those found in previous research (Cristofori et al., 2008; Kornsteiner et al., 2006) and differed between growing systems in both cultivars, showing higher contents in the organic nuts, with values of
3.13 and 2.82 g GAE kg\(^{-1}\) d.w. respectively in “TGR org” and “TG org” (Table 3).

Table 1. Nut traits of TGR and TG obtained in organic and conventional growing systems

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Nut weight (g)</th>
<th>Kernel weight (g)</th>
<th>Shell weight (g)</th>
<th>Nut shape</th>
<th>Kernel %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGR con</td>
<td>1.98</td>
<td>0.93</td>
<td>1.06</td>
<td>1.02</td>
<td>46.21</td>
</tr>
<tr>
<td>TGR org</td>
<td>2.48</td>
<td>1.20</td>
<td>1.39</td>
<td>1.05</td>
<td>45.94</td>
</tr>
<tr>
<td>TG con</td>
<td>2.19</td>
<td>0.96</td>
<td>1.24</td>
<td>0.95</td>
<td>44.13</td>
</tr>
<tr>
<td>TG org</td>
<td>2.28</td>
<td>1.08</td>
<td>1.26</td>
<td>0.98</td>
<td>46.49</td>
</tr>
</tbody>
</table>

**Effects**

<table>
<thead>
<tr>
<th>Effects</th>
<th>l.s.d. (α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Growing system (GS)</td>
<td>0.10</td>
</tr>
<tr>
<td>C x GS</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2. Oil, fatty acids, and crude proteins (N x 6.25). Interaction C x GS not significant.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Oil (mg 100mg(^{-1}) d.w.)</th>
<th>Fatty acids (%)</th>
<th>Crude proteins (mg 100mg(^{-1}) d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C 18:0</td>
<td>C 18:1</td>
<td>C 18:2</td>
</tr>
<tr>
<td>TGR</td>
<td>66.84</td>
<td>5.71</td>
<td>2.39</td>
</tr>
<tr>
<td>TG</td>
<td>64.82</td>
<td>5.54</td>
<td>2.60</td>
</tr>
<tr>
<td>Con</td>
<td>66.84</td>
<td>5.92</td>
<td>2.73</td>
</tr>
<tr>
<td>Org</td>
<td>65.18</td>
<td>5.33</td>
<td>2.26</td>
</tr>
</tbody>
</table>

**Effects**

<table>
<thead>
<tr>
<th>Effects</th>
<th>l.s.d. (α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Growing system (GS)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Table 3. Contents of starch, soluble sugars, organic acids (mg mg100g-1 d.w.) and polyphenols (g GAE kg-1 d.w.) in the kernel. Interaction C x GS not significant.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Starch</th>
<th>Total soluble sugars</th>
<th>Total organic acids</th>
<th>Polyphenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGR</td>
<td>1.42</td>
<td>5.27</td>
<td>0.43</td>
<td>2.83</td>
</tr>
<tr>
<td>TG</td>
<td>1.44</td>
<td>5.19</td>
<td>0.62</td>
<td>2.60</td>
</tr>
<tr>
<td>Con</td>
<td>1.81</td>
<td>5.01</td>
<td>0.51</td>
<td>2.45</td>
</tr>
<tr>
<td>Org</td>
<td>1.04</td>
<td>5.46</td>
<td>0.54</td>
<td>2.98</td>
</tr>
</tbody>
</table>

**Sensory analysis**

The general appreciation expressed by the panelists showed differences related to the cultivar and growing system, with the organic samples being preferred for both cultivars (Table 4). The perceived differences for roundness, shape regularity and peelability were mainly related
to the cultivar, while the difference of colour and oiliness was dependent on the growing system, with organic nuts being characterized by a lesser colour and higher oiliness (Table 4).

Since the oil content was similar in both growing systems, other factors, such as the seed texture, should be involved in the higher perception of oiliness. Sweetness, aroma and taste intensity did not differ among treatments (data not shown).

Table 4. Sensory profile of roasted kernels. Interaction C x GS not significant.

<table>
<thead>
<tr>
<th>CULTIVAR</th>
<th>Colour</th>
<th>Roundness</th>
<th>Shape regularity</th>
<th>Peelability</th>
<th>Oily</th>
<th>Global preference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGR</td>
<td>38.9</td>
<td>59.1</td>
<td>53.5</td>
<td>37.2</td>
<td>41.3</td>
<td>53.4</td>
</tr>
<tr>
<td>TG</td>
<td>43.8</td>
<td>46.0</td>
<td>42.4</td>
<td>61.8</td>
<td>43.2</td>
<td>59.6</td>
</tr>
<tr>
<td>Con</td>
<td>48.2</td>
<td>51.3</td>
<td>45.0</td>
<td>49.7</td>
<td>38.6</td>
<td>51.6</td>
</tr>
<tr>
<td>Org</td>
<td>34.4</td>
<td>53.8</td>
<td>50.9</td>
<td>49.3</td>
<td>45.9</td>
<td>61.4</td>
</tr>
</tbody>
</table>

Effects

Cultivar (C) n.s. 7.1 8.9 7.3 n.s. 5.7

Acknowledgements

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References

Flash grazing of hogs in apple orchards for pest management

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Abstract: A project to develop and evaluate an orchard system for Upper Midwest (USA) fruit growers that integrates rotational swine grazing for control of insect and disease pests, while enhancing profit potential through sales of organic pork was investigated in 2007-2008. The impact of hog grazing on aborted apples for control of one of the most serious pests of organic apples, Conotrachelus nenuphar, was evaluated most extensively. The number of June Drop apples for two cultivars, Idared and McIntosh, was quantified as a mean of ca. 123 apples per tree for both years. Forty-seven percent of field-collected, aborted apples in 2008 had at least one C. nenuphar oviposition scar, and 15.7% of drops contained viable larvae. Twenty-seven two-month old Berkshire hogs (Ca. 20-30kg), grazed prior to predicted emergence of C. nenuphar larvae, consumed over 98% of dropped apples in 0.4ha plots in 2007. In 2008, 24 two-month old Berkshire hogs consumed over 99% of dropped apples. Hogs were rotated among 3 grazed plots, spending 2-3 days in each grazed plot per week for three weeks. A controlled feeding experiment demonstrated that ingestion of C. nenuphar larvae in apples by pigs was 100 percent lethal to the larvae. Spring egg-laying injury from C. nenuphar in 2007, prior to start of grazing, was 11% in grazed plots, 8% in non-grazed. Summer C. nenuphar feeding injury, following the start of grazing in 2007, was 4.9 fold higher in non-grazed control plots (p=2.081E-13). Spring C. nenuphar oviposition injury in 2008 was 8.7% in non-grazed plots and 4.1% in grazed plots (p=7.763E-05). Summer C. nenuphar feeding injury was 3.4 fold higher in non-grazed plots and 4.1% in grazed plots (p=1.326E-05). Rooting of young hogs (under 45kg) in the tree row soil, as they foraged through the orchard, averaged 4-6 inches in depth. Rooting by hogs larger than 45kg resulted in some exposure of tree roots and some destruction of sod in the drive rows. Overall, the health status of all animals was acceptable, and did not require the use of any pharmaceuticals. Apple pulp and discarded whole apples were provided continuously, about 450 kg per day since weaning, providing over 50% of their daily food intake. Anecdotal observation in 2007 suggested superior weed control and improved nutrient availability resulted from hog grazing/rooting. Data collected during the 2008 season on weed growth, nutrition, and control of codling moth (Cydia pomonella) and apple scab (Venturia inaequalis) will be reported on in this paper.
The sterile insect technique as a component of area-wide integrated pest management.

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Abstract: The benefit of integrated pest management (IPM) when applied on an area-wide (AW) basis is that all habitats are treated. Such programmes are successful if the pest is suppressed to below economic or environmental thresholds and its re-establishment is prevented. Apart from horticultural production areas AW-IPM programmes often impact urbanised and native vegetation areas and waterways. The requirement for the protection of humans, fauna and flora and their communities and eco-systems demands the use of biologically sensitive technologies in AW-IPM programmes. The sterile insect technique (SIT) is a form of biological control which uses releases of sterile mass-reared insects to suppress wild populations of the same species. Desired outcomes from SIT include a reduction in the use of toxic pesticides, improved production, quality and marketability of produce where only the target pest species is affected. To date a wide range of insect pests has been targeted, successfully, by SIT in diverse regions of the world but SIT is most effective when used as a component of AW-IPM programmes. SIT is recognised as a component of internationally accepted systems approaches to pest management. For example the FAO / International Plant Protection Convention’s International Standards for Phytosanitary Measures (ISPM) numbers 3, 9, 18 and 26 have provision for the transport or deployment of sterile insects for SIT purposes. In this paper we will discuss the requirements for a thorough understanding of the biology and behaviour of the target pest and its interaction with the geography, climate and host flora of an area under pest management and the means by which SIT can be an essential component to AW-IPM.

Pests, Sterile insect technique, Area-wide, Integrated pest management
Softpest: a website on the usage of pesticides & biocontrol agents in soft fruits

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Abstract: The usage of plant protection products and biocontrol agents in soft fruit production has always been an important subject for the IOBC/WPRS Working Group "Integrated Protection of Fruit Crops" Study Group "Soft Fruits". The usage of pesticides and biological control methods varies considerably between countries and it is very difficult to get a good overview on the range of products that are applied or in development in soft fruits. In order to share and facilitate the flow of information, the Study Group "Soft Fruit" initiated a survey on the availability and usage of active ingredients and biocontrol agents in the different European countries in 2007. First, the most important pests and diseases in strawberry and raspberry production were identified. Then members of the different countries listed available products on the domestic market and indicated their usage in the field. So far 15 countries have contributed to the survey. The received data are accessible on the website http://www.any3.ch/IOBC/Softpest/index.html.

Key words: Insecticides, fungicides, biological control, database
Introduction

Since the first meeting of the IOBC Study Group "Soft Fruits" in Vienna in 1997, the availability and usage of pesticides in soft fruit production has often been the subject of heated debates at our workshops (Jörg 1998; Gajek & Jörg 2003; Gallie et al. 2003; Umpelby 2003). During a discussion round at the Dundee meeting in 2001, participants agreed to create and maintain a database specific to soft fruits. This database should cover diseases, pests, weeds as well as chemical, biological and other non-chemical control methods (Umpelby 2003).

However, it was not until 2007 at the East Malling meeting that the Study Group members decided to provide the basis for the development of a database on the usage of pesticides and biological control agents (=BCAs) in soft fruits. The approach of the Study Group "Soft Fruits" is briefly presented in the following chapter.

Approach

Members from 14 IOBC/WPRS (West Palearctic Regional Section) countries as well as one representative from Canada have chosen to focus their first efforts on strawberries and raspberries. In a first step, a series of key pests and diseases have been identified. Then members of the different countries listed available products on the domestic market and indicated their usage in the field. In 2008, a worksheet circulated among a limited numbers of key collaborators in each country who verified the validity of the collected data. Data have been compiled and are now available on the website Softpest:


Table 1. Information on active ingredients and BCAs are available for the following crops, pests and diseases on the Softpest website (state December 2008).

<table>
<thead>
<tr>
<th>Crops</th>
<th>Pests</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td>Aphids</td>
<td>Botrytis</td>
</tr>
<tr>
<td></td>
<td>Miridae</td>
<td>Colletotrichum</td>
</tr>
<tr>
<td></td>
<td>Tarsonemids</td>
<td>Phytophthora</td>
</tr>
<tr>
<td></td>
<td>Tetranychids</td>
<td>Podosphaera</td>
</tr>
<tr>
<td></td>
<td>Thrips</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vine Weevil</td>
<td></td>
</tr>
<tr>
<td>Raspberry</td>
<td>Anthonomus</td>
<td>Botrytis</td>
</tr>
<tr>
<td></td>
<td>Aphids</td>
<td>Cane diseases</td>
</tr>
<tr>
<td></td>
<td>Byturus</td>
<td>Phytophthora</td>
</tr>
<tr>
<td></td>
<td>Cane midge</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetranychids</td>
<td></td>
</tr>
</tbody>
</table>

The website allows retrieval of information by crops, pests, diseases, active ingredients and/or BCAs (Table 1). Data are displayed in a simple table giving an overview of what is applied in the participating countries (Figure 1). Currently, more than 140 pesticides or BCAs are listed. The full list of collaborators and additional information on pesticides' and BCAs' special usage or restrictions can also be found on the website.
Results

Table of results obtained on the Softpest website for the query "Strawberry – Tarsonemids"

Perspectives

In the near future, the Study Group intends to integrate more countries, crops, pests and diseases in the database. However, it should be noted that the website does not intend to replace national pesticides lists. The website should simply facilitate the exchange of information among scientists, advisory services and everybody interested in the availability and usage of pesticides and BCAs in soft fruits.

References


Organic Raspberry Production in Serbia

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Abstract: In Serbia, the first raspberry plantings maintained according to the organic production procedure were established in 1999. So far, the production has reached some 3,000 t/yr. This programme is promising, provided strict observance of regulations EC 2092/91, EC 834/2007 and EC 889/2008 is performed. Organic production plantings are established in well-drained, loose soils containing high quantity organic matter. The incorporation of 20 t/ha of manure into the soil is a regular cultivation practice. Considering the control of diseases and pests, the following control measures are applied: setting up plantings on suitable terrains, application of appropriate cultivation practices, healthy planting material, and application of sulphur and copper fungicides. The control and monitoring of pests was aided by the use of visual inspections (Rebell traps and Moerick vessels) and pheromone traps (monitoring of Resseliella theobaldi). The incidence of gray mold caused by Botrytis cinerea is a major problem in seasons with high rainfall rates. Unfortunately, efficient biological fungicides on raspberry have not yet been registered.

Key words: raspberry, organic production, Serbia

Introduction

Raspberry production in Serbia is largely concentrated in central and western parts, the total raspberry production ranging from 40,000 – 75,000 tons. According to the available data, it is estimated that the total annual export of organically produced raspberry in Serbia amounts to 3,000 t, which accounts for 5% of the total raspberry production.

The first organic raspberry plantings were registered in 1999. Five years later, the production amounts to some 3,000 t. During the recent period, no appeals have been lodged regarding the observance of the regulations, which supports the assertion that this programme is prospective provided that all requirements strictly conform to EC 2092/91, EC 834/2007 and EC 889/2008. The programme, which is similar to the traditional manner of production, presupposes the appropriate selection of the region for growing (the upland regions) and highly conscientious producers who are willing to co-operate on this project (Mišić et al., 2004).

Environmental Conditions

Temperate-to-mountain climate characterized by a moderately warm summer and a moderately cold winter is most suitable for raspberry production. Meteorological indicators point to a rather sub-humid climate. The data suggest that the annual precipitation rate ranges from 750 – 960 mm, 550 mm of which is over the period of vegetative growth. Mean annual air temperature is 10°C, mean air temperature over the vegetative period is 15.3°C, the sum of the active air temperatures exceeds 3,200°C, and the cloudiness rate is 58% (Petrović and Milošević, 2005).

The appropriate selection of a terrain suitable for raspberry growing is utterly important, as raspberry develops sturdy but nonetheless shallow root system accompanied by a multitude
of canes. Raspberry will fare best in deep, moderately cold, medium hard, well-drained soils of the above-mentioned soil types. This fruit species is not susceptible to slightly acid soil types (except to extremely acid soils), and the most suitable soils are pH 5 - 6 which consist 8 – 10 mg P₂O₅ and 18 - 20 mg K₂O per 100 g of aerated soil with about 5% of humus.

Specific Conditions

Raspberry is grown on small private households, i.e. 0.10 – 0.30 ha in size. About 90% of the crop is intended for freezing, whereas the remainder is realized on the fresh market or is processed. The vicinity of cold storage plants and other processing facilities enables freezing and storage of produced raspberries and other small fruit crops (Nikolić et al., 2008).

In raspberry areas of Serbia, raspberry performs best at altitudes between 400 and 800 m, but it is also successfully grown at 200 – 1,000 m. On the sites lying at an altitude of 800 m, with the annual precipitation of 900 mm (with half the amount of rainfall during the growing season), raspberry favours most northwest and northeast exposures. With the increase of altitudes (over 800 m) southern exposures are preferred. Pre-cultivation of leguminous plants, maize and small grains are favourable for a raspberry planting, whereas potato, tomato and small fruits (raspberry, blackberry and others) are not preferred, being the potential hosts to some highly damaging fungal diseases (cane blight, root rot).

Nursery Material

Raspberry has been successfully propagated via in vitro micropropagation, an essential stage in the worldwide approved scheme (OEOO/EPPO, 1998, PM 4/10) of healthy raspberry planting material production. The buds from the root cuttings of the virus-free planting material have been used as the initial explants. The aseptic culture has been established on the Murashiga and Skoog medium. The rooted plants have been planted in the peat: zeolite = 2:1 substrate and acclimatised under a glasshouse “mist” system. This method of raspberry propagation under completely controlled conditions accompanied by the continuous testing for the presence of viruses and Phytophthora spp. under in vitro and ex vitro conditions enables the obtainment of healthy planting material (Milenković et al., 2006).

Soil Cultivation

The inter-row cultivation is performed by disking, and in-row cultivation is carried out manually, by hand hoeing and weeding. An increase in the organic matter content in the soil is the key precondition for achieving the basic principle of organic agriculture. The conversion period is used maximally in order to increase the content of organic matter in the soil by applying manure, compost, green manure and other measures. The incorporation of 1 t of manure results in the soil enrichment by: 10 kg N, 5 kg P₂O₅ and 10 kg K₂O. The manure application rate is limited to 170 kg N/ha annually due to the possibility of N leaching. Manure application rates are 10-30 t/ha. Humus content in the soils of typical production regions ranges from 2.5 to 4%. The incorporation of 20 t of manure every year (respecting maximum permissible pure nitrogen incorporation rate of 170 kg per hectare) is recommended as a regular measure for increasing the organic matter content and maintaining fertility.

Green Manure

An increase in the soil organic matter and improvement in its structure are also achieved through green manuring. The procedure involves planting, growing till the blooming stage and eventual ploughing of herbaceous plants into the soil. The plants are grown as pre-crops, intercrops or after-crops. Barley, common vetch, lupine and alfalfa are cultivated as pre-crops.
Autumn-planted after-crops are as follows: oil seed rape, a mixture of hairy vetch, rye grass and clover, a mixture of hairy vetch and winter barley. The green material is shredded and ploughed into the soil at the blooming stage. In the orchards on sloping terrain plants of spontaneous flora are maintained; these plants are reaped and the remains are spread along the inter-row space.

**Pests and Diseases**

In Serbia, the most serious raspberry diseases caused by pathogenic fungi are: gray mold (*Botrytis* spp.) on fruits, cane blight (*Leptosphaeria coniothyrium*) on the trunk, midge blight, raspberry leaf spot (*Sphaerulina rubi*), and phytophthora root rot (*Phytophthora fragariae var. rubi*). Spur blight has been most commonly attributed either to the pathogen *Didymella applanata* or low winter temperatures. Raspberry root rot has been observed almost in all productive regions (Milenković and Sretenović, 2006). It is manifested in sudden wilting of plants, often during harvest. Various pathogenic fungi such as *Fusarium* spp., *Alternaria* spp., *Phoma* spp., and *Leptosphaeria coniothyrium* develop on the spots where the periderm has been modified and vascular tissue radially damaged by the larvae of raspberry cane midge.

Small raspberry aphid, *Aphis idaei* van der Goot, is a pest widely spread in all raspberry growing regions in Serbia. Large raspberry aphid, *Amphorophora idaei*, is sporadically found, its population pressure being low. Raspberry fruit worm, *Byturus tomentosus* F. is not a serious raspberry pest in Serbia, mainly populating higher terrains. Raspberry gall midge *Lasiosiptera rubi* Heeger is largely controlled by pruning. Raspberry cane midge, *Resseliella (Thomasinia) theobaldi* Barnes (Diptera, Cecidomyiidae) causes dieback of raspberry canes (Milenković, 2005). The results of research at East Malling Research (UK) have provided successful utilization of the pheromone traps for the monitoring of this pest (Cross and Hall, 2006). Strawberry blossom weevil, *Anthonomus rubi* Hrbst., is the most serious pest in the region of Arilje, our largest raspberry growing region.

The experiences of raspberry producers in Switzerland show the beneficial effect of compost dressings (40 l/ 1 m) which ensure high yields, general improvement of a planting and substantially reduced employment of control measures (Weidmann, 2005).

**Direct Control Measures**

More recent practice in Serbia has relied on the application of copper and sulphur-based chemicals for the reason of incomplete registration system. Over 2004 – 2005 chemicals based on natural pyrethrin (in the control of leaf aphids and strawberry blossom weevil) and the toxin *Bacillus thuringiensis* (in the control of damaging moths) have been applied for experimental purposes. Monitoring of raspberry fruit worm *Rebell bianco* traps have been used successfully. Raspberry pests are an important problem; therefore there is a case for importing biologically based insecticides and parasitoids, and damaging insect predators.

The principal conception of the organic production prevails recommending that producers primarily focus on the application of all other available measures (growing resistant cultivars, application of appropriate cultural practices, physical measures and introduction of beneficial living organisms, etc.) which will ensure lowering of the pressure of damaging agents to an acceptable level. The application of the stated chemicals is recommended in extreme cases, when the situation really requires such measures.

**Biological Control Measures**

Biological measures for controlling harmful organisms include fostering of development and planned introduction of beneficial organisms into a raspberry planting. The root rot of raspberry, as already underlined, is a serious cultivation-related problem. The use of compost and the employment of growing practices in elevated rows contribute to the maintenance of
optimum moisture regime in the zone around the root system. The beneficial fungus *Trichoderma* *spp.* is an antagonist (development inhibitor) of *Phytophthora fragariae* var. *rubi* and is used combined with manure and compost.

The biological equilibrium and the prevention from insect damage in a raspberry planting are enhanced by ladybirds, common green lacewings, parasitic Cecidomyiidae, hoverflies, parasitic wasps and predatory mites.

**References**


Is organic hazelnut cultivation profitable?

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Abstract: In order to analyze the economic results of hazelnut cultivation in an organic regime, the two methods of production commonly used in the Monti Cimini hazelnut district (central Italy) were used. These systems of production, due to the different levels of input that they require, can be considered to be “extensive” and “intensive”. Costs and productive values were evaluated for the two techniques and, afterwards, a comparison with conventional management was carried out, referring to a standard method of production which allows average yields of 2.7 t/ha to be achieved. The examination of the costs of production for these systems highlighted a substantial homogeneity in variable costs, although remarkable variations were observed in the different categories (raw materials, mechanization, work). On the contrary, the produce which can be sold varies greatly according to the different orchard management forms. A comparison of gross margins showed that the results achieved by conventional management is intermediate between those of the two organic techniques. This result justifies the contrasting opinions of hazelnut producers on the relative convenience of the two management forms. The only certainty is that, because of public aid, organic hazelnut production is able to guarantee better economic results.

Key words: Corylus avellana L., cultural account, organic agriculture

Introduction

In the last fifteen years, that is to say since organic agriculture has been given rules and aid, supporters and opposers of organic management have presented their views through debates which involve environmental, technical and, of course, economical aspects. Hazelnut cultivation in the intensive hazelnut production area of Monti Cimini is no exception and, even if a good number of farmers have decided to switch to organic management, the debate on the efficiency of the productive technique and the economical convenience, as compared to the conventional regime, is still open.

This paper contributes to this debate by focusing on two main topics: the cultivation characteristics of organic hazelnut in the area of Monti Cimini, and the evaluation of the economic results of organic management and its comparison with conventional production.

The organic hazelnut cultivation in Monti Cimini

In the Monti Cimini district, there are about 10,000 hazelnut farms covering almost 18,000 hectares and producing 40,000 tons of hazelnuts. The number of organic hazelnut farms is 350 with a area share of organic farming of about 13%.

As far as the territorial localization of organic hazelnut cultivation is concerned, a very high presence in border areas of the district emerges. Such a presence becomes lower progressively towards the areas which are more specialized and which characterize the core of the district.
During the last years, a remarkable technical evolution concerning all aspects of the agronomical management of organic hazelnut plantations, that is to say from soil management, to pruning, to fertilisation and defence, has taken place. In general, organically-managed cultivation requires an integrated technical-agronomical practice, which has the aim of keeping the plant in an harmonic condition in terms of vegetative vigour and allows a better resistance to possible environmental adversities (Caporali, 2003). In the Monti Cimini area, it is possible to identify two different techniques for organic management of hazelnut orchards, which are called “extensive” and “intensive” and are characterized by different level of inputs and, obviously, by a different yield. In particular, the yield of the “extensive” technique ranges between 1.2 and 1.8 ton/ha, while the yield of the “intensive” technique varies between 2.2 and 2.6 ton/ha. The extensive or the intensive technique is chosen by the farmer on the basis of his predisposition towards innovation, of territorial suitability and of the mechanization limits due to soil characteristics.

Hazelnut prices and economic results

With particular reference to the last decade, hazelnut prices have shown an extremely uncertain trend, mainly due to the Turkish hazelnut production. Indeed, the amount of Turkish hazelnuts on the market, 5 to 6 times the Italian production on the average, depends on factors which are hardly predictable, such as climate conditions and political choices made by the sector’s institutions (Dono and Franco, 2001).

Obviously, the price of organic hazelnuts is directly linked to the conventional hazelnut price, as can be seen in figure 1, in which the monthly trend of the conventional hazelnut price is shown and the average price at the beginning of the harvesting (September-November) is compared to the organic one.

![Figure 1. Prices (€/kg) of conventional and organic “Tonda Gentile Romana”](image)
It can be seen that the latter is 15-20% higher and that this difference rises as the conventional hazelnut price lowers. It is important to highlight that such premium price is effective only at the beginning of the harvest, while, during the following months, conventional hazelnuts, following the market trend, can undergo further price reductions or, in some cases, such as in 1997-98 and 2000-01, they can even reach a higher price than organic hazelnuts.

In order to estimate the economic result of organic hazelnut cultivation, the two different techniques which are employed (extensive and intensive) were considered. Afterwards, in order to compare results with the conventional management, a representative technique (Dono and Franco, 2003) was considered. In normal conditions, the latter allows average yields of 2.7 t/ha, with a yield of unshelled product slightly higher (42%) than the one which can be obtained with an organic management (40%).

The data elaboration (table 1) highlights a substantial homogeneity of variable costs, which are slightly higher than 1,000 €/ha for all the considered techniques. Comparing the organic techniques, the extensive one is characterized by a raw material cost which is 45% lower, but a greater number of workers is needed due to the less efficient harvesting modality. The comparison between conventional and intensive organic techniques shows that in the organic management the raw material costs are 20% higher, as a consequence of the higher price of the technical tools, whereas the mechanization costs are slightly lower, due to both the small number of interventions and the shorter amount of time dedicated to harvesting, which is linked to the lower yield.

<table>
<thead>
<tr>
<th></th>
<th>Organic “extensive”</th>
<th>Organic “intensive”</th>
<th>Conventional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (ton/ha)</td>
<td>1.5</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Price (€/kg)</td>
<td>2.45</td>
<td>2.45</td>
<td>2.25</td>
</tr>
<tr>
<td>Product value</td>
<td>3,675</td>
<td>5,880</td>
<td>6,075</td>
</tr>
<tr>
<td>Organic support</td>
<td>550</td>
<td>550</td>
<td>0</td>
</tr>
<tr>
<td>CAP payment</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>Revenues</td>
<td>4,465</td>
<td>6,650</td>
<td>6,315</td>
</tr>
<tr>
<td>Labour costs</td>
<td>765</td>
<td>700</td>
<td>665</td>
</tr>
<tr>
<td>Mechanization costs</td>
<td>130</td>
<td>150</td>
<td>185</td>
</tr>
<tr>
<td>Inputs costs</td>
<td>140</td>
<td>250</td>
<td>205</td>
</tr>
<tr>
<td>Variable costs</td>
<td>1,035</td>
<td>1,100</td>
<td>1,055</td>
</tr>
<tr>
<td>Gross margin</td>
<td>3,430</td>
<td>5,550</td>
<td>5,260</td>
</tr>
</tbody>
</table>

On the contrary, according to the different types of management, the yield which can be sold varies in a substantial way. As the table shows, it depends on the product value as well as on the aid which is given to organic farms.

Comparing the gross revenues of the three processes, it can be observed that the conventional management result is positioned between the results obtained with the two organic techniques. This result justifies the contrasting opinions among hazelnut farmers on the opportunity of adopting one management technique or the other. The only certainty is that only public aid allows the organic hazelnut production to achieve better economic results.

Conclusions
The organic hazelnut production in the Monti Cimini district represents a phenomenon which cannot be overlooked. Yet, after a period of fast development, the choice of converting to organic production has shown a progressive slowdown. This evolution represents the consequence of different phenomena: firstly, technical aspects, such as the possibility of facing with success the phytopathological attacks; secondly, market conditions, such as the risk of demand saturation and the consequent loss of a satisfactory premium price; thirdly, the policy evolution, in particular the uncertainty concerning the stability of public aid.

This research addressed in depth such aspects, making some of them clearer: the evolution of organic hazelnut prices, the definition of production techniques and yields, the market situation and, above all, the comparison of the economic results of conventional and organic techniques. On the other hand, other important issues remain unresolved: the demand evolution, the future policy directions concerning agro environmental measures, the experimentation and availability of phytosanitary products allowed in order to fight different pathologies.

Nevertheless, even if the conducted research allowed to obtain remarkable results concerning the knowledge on the sector and the identification of possible strategies for its competitive growth, one fundamental element in the analysis of organic hazelnut production cannot be ignored: the individual attitude towards innovation and respect for the environment. Indeed, the refusal of the organic “challenge” is often a conservative attitude regarding traditions and behaviours which are part of the cultural background of farmers, and in general of the entire population of the Monti Cimini area.

Acknowledgements

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References

Further observation on hazelnut yielding and fruit quality under organic and conventional management.

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Abstract. In some previous contributions, our Institute has pointed out some difficulties in the organic management of filbert orchards. A further two years of investigation showed that the main negative aspect of organic management is the high rate of nuts affected by bugs. Therefore another important problem is the reduction in productivity.

To validate the conclusions of previous works, the comparison between organic and conventional management was carried on in three typical hazelnut orchards named “Alta Langa”, “Langa” and “Monregalese”. In these areas conventional and organic orchard management were chosen to record productivity and nut quality traits. In the years 2007-08, both total and average (t/Ha) production were recorded for each hazelnut orchard, and 3 kg samples of nuts were taken from whole nut yielding. Each sample was studied through the standard marketing surveys. In particular fruit and kernel weight, Curculio holes, fruit empty and insect kernel damage percentage, has been considered. The results were statistically analyzed through conventional-organic comparisons, and tested with the "t" test.

Keywords: Hazelnut yielding, fruit quality, organic management, bug kernel.

Introduction

Starting from 2002 our Institute began to be make comparative observations on hazelnut orchards conventional or organic managed, in three different locations. The first year only yielding was recorded instead in the following years also the fruit quality was considered. From the published results (Roversi & Sonnati, 2006; Roversi & Castellino, 2007; Roversi, Ughini, Malvicini & Sonnati, 2008) clearly appears an important lack in yielding and a lesser quality of nuts obtained in organic managed hazelnut orchards. In particular the insect damaged kernel percentage results were significantly higher with organic management than conventional one. Also the percentage of kernel without any defects and so suitable for agro-industry purposes, was higher for the nuts obtained in conventional orchard.

Year after year, the difference between the quality of nuts obtained between organic and conventional, decreases and becomes often not significant. One district (Monregalese) seems more suitable for hazelnut orchard organic managed. Unfortunately the loss in yield was not improved.

In order to resolve the cause of low yield and the lower nut quality of the organic management a further 2 years of observations and analysis was done.

Material and methods

In 3 typical hazelnut orchard areas, Alta Langa, Langa and Monregalese, a conventional managed and an organic managed orchard were choses. For each district the orchards are under the same climatic and soil conditions, and plots of similar age (20-25 years) were considered for observations and fruit sampling. The main difference between organic and conventional management was that use of any pesticide, chemical fertilizers and hormonal
suckers control was prohibited according to the “Protocol of Production for organic hazelnut orchards”.

In 2007-08, average production (t/ha) was recorded for each orchard, and 3 kg samples of nuts were taken from the total nut production. Each sample was studied through the standard marketing surveys as shown in the tables. Defects of fruits we considered, Curculio holes, while for the kernel the percentage of mouldy, shrivelled, whitened, rancid, undersized and twin were considered, both for the nuts produced in organic and in conventional orchards. Because its value was not as high and the difference between location and orchard management were not so high, they were summarized and considered together as total defects. Because among the different defects fruit empty and kernel whitened were the most frequent, they were considered separately and presented only as figures. Bug damage is not considered in this total. The data was statistically analyzed by ANOVA and conventional-organic were compared using a "t" test.

**Results and Conclusions**

**Yielding**
Generally speaking, the yield was higher in the Monregalese district for both years and independently from orchard management. As observed in the previous 5 years (Roversi, Ughini, Malvicini & Sonnati, l.c) the organic orchard yielding is lesser (cfr. Fig. 1) than conventional. In 2007 the minimum production was 25.8 % (Alta Langa) and the maximum reached 84 % for Langa orchards. In the second year (2008), the results showed a similar lack of production (32.7 % to 64.8 % for Alta Langa and Langa respectively). Because the supply of mineral fertilizers is prohibited in organic orchards. The lack of production could be explained by poor nutritional status of their plants. In fact, in the organic hazelnut orchards, the use of only organic fertilizers could not satisfy the plants nutritional needs.

**Fruit quality**

**Fruit weight**
The fruit weight ranged from a minimum of 1.9g (conventional orchard in 2008, in Monregalese district), to 2.6g in the same district. ANOVA (data not reported) shows that fruit weight was significantly affected by location and year.
The difference between fruit weight obtained in orchards with different management regimes, were not significant, with the exception of Alta Langa and Langa, in 2008, when the nuts obtained from conventional orchards were significantly higher than those obtained by organic production.
On the contrary in the Monregalese area the weight of nuts produced in 2008 was significantly ($p \leq 0.007$) higher in the organic (2.04 g) than in the conventional orchard (1.89 grams).
This was also observed in 2003 (Roversi & Sonnati, l.c.), 2004 (Roversi & Castellino, l.c.) and 2006 (Roversi et al) and suggests that Monregalese could be an area in which organic management will be suitable without negative influence on the weight of nuts.

**Kernel weight**
The kernel weight value (see table 2-4) was between 0.84g (in Monregalese for conventional production, in 2008) and 1.14g (Monregalese conventional production in the previous year). The kernel weight values were not significantly influenced by the different managements.
Kernel fruit ratio
The kernel fruit ratio percentage was variable between years and less so in 2007 than in the following year. The kernel fruit ratio is higher in the conventional than in the organic production orchard, but all the difference between percentages were not significant. The values recorded in Langa appear high in both years for nuts obtained in the conventional orchard.

Insect damage
Damage due to insects (2007-2008) was less than in the previous 4 years (cfr. Roversi, Ughini, Malvicini, Sonnati) with the noticeable exception of the organic management for Monregalese in 2008. In particular, in the first year of fruit analysis the percentage of kernels with insect damage was zero in Alta Langa and very poor in the other 2 other districts. There was no significant difference between the nuts from organic and conventional production. In the following year, the percentage was higher in the organic production than in the conventional, but significantly only in Alta Langa. In particular the values were: 0.3 % for conventional production and 14.7 % for the organic. Unfortunately, the active substances of natural origin are (Guidone & Tavella, 2007) ineffective against curculio and so this damage is one of the most important problem of hazelnut organic management.

Total defects
The total defects were higher for the organic production in Alta Langa and Langa, in 2007 and 2008, but not significantly. In Monregalese in both years of observation, the total percentage of damage was higher in the conventional production, but not significant. The percentage of empty fruit could be influenced by mineral nutrition and floral biology (Roversi, et al., l.c.).

Kernel without any defects
From a commercial point of view, kernel fruit ratio, it is very important and could determine the price of the product stock. Generally the percentage of kernel without defects is higher in the product obtained from conventional orchards but significant only for Alta Langa in 2008, with the value of 96.44% and 71.44 % respectively. In the Monregalese district, there was no significant difference between organic and conventional production..

Tables and graphs
Tab. 1 – Values of some fruit parameters for Alta Langa production, as related to management and years.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2007</th>
<th></th>
<th>2008</th>
<th></th>
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<tr>
<td></td>
<td>Con</td>
<td>Org</td>
<td>p</td>
<td>Con</td>
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<tr>
<td>Fruits weight</td>
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<td>2.45</td>
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<td>2.30</td>
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<td>Kernel weight</td>
<td>1.14</td>
<td>1.12</td>
<td>.939</td>
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</tr>
<tr>
<td>Kernel/fruit ratio</td>
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<td>45.56</td>
<td>.935</td>
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<tr>
<td>Bug</td>
<td>.00</td>
<td>.00</td>
<td>-</td>
<td>.31</td>
</tr>
<tr>
<td>Total defects</td>
<td>9.40</td>
<td>10.73</td>
<td>.571</td>
<td>3.25</td>
</tr>
<tr>
<td>Without any defects</td>
<td>90.60</td>
<td>89.27</td>
<td>.571</td>
<td>96.44</td>
</tr>
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</table>

Tab. 2 – Values of some fruit parameters for Langa production, as related to management and years.
Tab. 3 – Values of some fruit parameters for Monregalese production, as related to management and years.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>2007</th>
<th>2008</th>
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<tr>
<td></td>
<td>Con</td>
<td>Org</td>
</tr>
<tr>
<td>Fruits Weight</td>
<td>2.12</td>
<td>2.08</td>
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<tr>
<td>Kernel weight</td>
<td>1.03</td>
<td>.93</td>
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<tr>
<td>Kernel/fruit ratio</td>
<td>48.42</td>
<td>44.75</td>
</tr>
<tr>
<td>Bug</td>
<td>.00</td>
<td>2.06</td>
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<tr>
<td>Total defects</td>
<td>11.25</td>
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</tr>
<tr>
<td>Without any defects</td>
<td>88.75</td>
<td>79.40</td>
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</table>

Fig. 1 – Nut average yield (tons/ha) as related to location, orchard management and year.
Fig. 2 - Percentage of empty fruits as related to years and orchard management, in Alta Langa district.

Fig. 3 - Percentage of empty fruits as related to years and orchard management, in Langa district.

Fig. 4 - Percentage of empty fruits as related to years and orchard management, in Monregalese district

Fig. 5 - Percentage of whitened kernel as related to years and orchard management, in Alta Langa district.
Conclusions

Fruit and kernel weight appear generally higher, but not significantly, for conventional production than organic one. Just in the Monregalese district, in 2008, the results of organic management are better than conventional, but significantly only for fruit weight.

There was no effect on the kernel fruit ratio.

In contrast with previous years the insect damage was higher in the kernels obtained in organic orchards but not significant.

With time (years) net differences in quality/yield between the two management practices tend to disappear.

The percentage of kernels without defects, and so suitable for agro-industry purposes, are higher for the fruit obtained in conventional orchard for the all locations and years, with the exception of Monregalese in 2007.

- Differences in production could be due to factors other than conventional and organic management.

References

Nazionale sul Nocciolo, Giffoni Valle Piana, 5 Ottobre: 28-42.
Codling moth proof hail nets

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Abstract: Single row hail nets (3x7.4mm) modified to wrap up whole tree canopies, named Alt’Carpo, were evaluated as a way of control of orchard lepidopteran pests. A two years study was conducted in a 10 rows experimental apple orchard in Southern France, together with large field trials in commercial orchards. The experimental orchard was insecticide free in year 1 and pesticide free in year 2. Eight rows were protected with the nets, two rows were unprotected. The nets allowed an 80% reduction of fruit injury when compared to the unprotected rows, which suffered over 70% codling moth injury. However this efficacy was lower than in commercial orchards, especially those covered with 2.2x5.4mm nets in which fruit injury did not exceed 0.1%. The outer females, issuing from unprotected rows, were proved able to lay eggs on leaves or apples touching the 3x7.4mm nets. Virgin females or synthetic lures baited traps poorly captured wild or marked and released males under the nets, while the traps placed in unprotected rows captured over 30% of the released males. Moreover, significant rates of males released under the nets were captured outside while only 1 out of 300 males released in the control rows was observed to pass through the net, proving the need for flying over the canopy for sex encounter. Despite the known alteration of communities in protected crops, no significant effect of the net was observed on rosy aphid and scab injuries on leaves or fruits. The agronomic, economic and environmental consequences of replacing chemical insecticides by synthetic barriers are discussed.

Protected crop, Cydia pomonella, Mating behaviour, Environmental impact, Pest and disease management
Building up, management and evaluation of orchard systems: a four-year experience in apple production

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\textsuperscript{1} INRA (National Institute for Agricultural Research), UERI Gotheron, F-26320 Saint-Marcel-lès-Valence, France; \textsuperscript{2} INRA, UMR 1115 PSH-EPI, Agroparc, F-84914 Avignon Cédex 9, France

Abstract: Three apple orchard systems were planted in 2005 to assess agronomic and environmental effects of different pest management regimes: organic farming (OG), conventional supervised (SV) and low-input (LI) systems. Three apple cultivars presenting different susceptibility to scab were planted in each system: Ariane (Vf-resistant), Melrose (low-susceptibility) and Smoothee 2832T\textsuperscript{®} (susceptible), creating nine « system x cultivar » situations. Decision rules were defined within the framework of each system, and their possible interactions were integrated. Starting from planting, the survey included pest and disease assessments, and agronomic and environmental parameters. The OG system was the slowest to produce commercial yield, whereas the SV one showed the highest performances. Although globally low, pest and disease fruit damage at harvest was the highest in the OG system. The treatment frequency index (TFI) was the highest in the SV system, and in Smoothee plots within each system. Two-fold more treatments were applied in any SV plot and in Smoothee OG compared to Melrose LI. The LI system presented the lowest TFI and the lowest environmental impact of pesticides calculated by the I-phy\textsubscript{ARBO} fuzzy expert system. Apart from Smoothee, I-phy\textsubscript{ARBO} in the OG system scored between LI and SV. From the first four years of the experiment, the importance of the cultivar in the management of the orchard diseases (and to some extent pests) is outlined whatever the system, with a high variation in the number of treatments. This experimental design proved to be a functional tool permitting the conception of decision rule patterns, and also to assess the agronomic, environmental and economical performances of the systems.

Key words: orchard system, apple, decision rule, agronomic evaluation, environmental evaluation, pest and disease management

Introduction

Apple production, which ranks first among fruit tree production in France, largely relies on the recurrent use of pesticides to control a high number of pests and diseases. Such pesticide applications have detrimental effects on the environment (Aubertot \textit{et al.}, 2005) and on human health. Many studies have focused on the development of alternative methods to avoid the use of chemicals against one target pest or disease. However, only two experimental designs located in the U.S.A (Reganold \textit{et al.}, 2001; Peck \textit{et al.}, 2006) and in Switzerland (Bertschinger \textit{et al.}, 2004) address the global pest and disease complex through system approaches that include various pest and disease management regimes. The aim of our research programme was to assess the agronomic performances and the environmental impact of different apple systems within the frame of conventional and organic agricultures. The designing, planting and evaluation of apple orchards aiming at such purposes started in 2005 in an experimental INRA (National Institute for Agricultural Research) unit in Southern France.
Material and methods

Experimental systems and experimental design
The three studied systems (Table 1) were mainly defined by their pest and disease management regimes: organic farming (OG), conventional supervised (SV) and low-input (LI) systems. Three apple cultivars presenting different levels of susceptibility to scab were planted in each system: Ariane (Vf-resistant), Melrose (low-susceptibility) and Smoothee 2832T® (Golden mutant, susceptible), creating nine « system x cultivar » situations. Decision rules were defined within the framework of each system, and their possible interactions were integrated. The orchards were planted in January 2005 under similar soil, climate and environmental conditions. Except in the OG system which excluded synthetic inputs for fertilization and thinning, cultural practices other than protection were similar in the three systems.

Assessments
Starting from planting, the survey included pest and disease assessments, agronomic and environmental parameters or indices:
- yield, fruit quality, fruit damage at harvest;
- the treatment Frequency Index (TFI) = \( \sum_{i=1}^{n} \frac{\text{dose of compound applied}}{\text{registered dose}} \) for each of the n treatments of the orchard during the season;
- IphyARBO fuzzy expert system (Sauphanor et al., 2008);
- earwig (Forficulidae) abundance (numbers of earwigs observed in 10 traps per system), as an indicator of the effects of pest management on a natural enemy arthropod community.

Table 1. Experimental systems and their main principles

<table>
<thead>
<tr>
<th>Systems</th>
<th>Organic(^1)</th>
<th>Low input(^2)</th>
<th>Supervised(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>General background</td>
<td>Fruit production / evolving framework</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific strategies</td>
<td>Management of bottlenecks</td>
<td>Minimise pesticides</td>
<td>Efficiency &amp; performance</td>
</tr>
<tr>
<td>Alternative methods</td>
<td>Always preferred</td>
<td>Always preferred</td>
<td>NO unless no other way</td>
</tr>
<tr>
<td>Sanitation practices, mechanical and labour input</td>
<td>Always used</td>
<td>Always used</td>
<td>NO unless cost&lt;other methods or for resistance management</td>
</tr>
<tr>
<td>Decision rules to apply pesticides based on:</td>
<td>Local conditions, treatment thresholds if usable</td>
<td>Local conditions, treatment thresholds as available</td>
<td>Regional conditions, treatment thresholds if consistent</td>
</tr>
<tr>
<td>Pesticide choice</td>
<td>Organic registered compounds</td>
<td>Selective compounds</td>
<td>Highly efficient compounds</td>
</tr>
</tbody>
</table>

\(^1\)European regulations (EEC 91/2092, appendices and modifications); \(^2\)IOBC guidelines; \(^3\)French IPM National guidelines (National Apple Board) as specific frameworks of the systems beside French regulations.

Results and discussion

Agronomic performances
The OG orchards were the slowest to set fruits. The SV system produced the highest yield in 2007 but adverse conditions (frost) at the chemical thinning period lowered yield in 2008 (Figure 1). Fruit damage due to pest and disease remained low at harvest (although it may vary according to years), which attests to the efficacy of the experimented pest and disease management regimes. Pest and disease fruit damages were similar for both LI and SV systems.
whereas the highest values were observed in the OG system.

![Cumulative yield and average percent 1st grade fruits (2006-2008 period)](image)

**Environmental impact**

The mean TFI was the highest in the SV system, and in Smoothee plots within each of the systems. Two-fold more treatments were applied in any SV plot and in Smoothee OG compared to Melrose LI. The LI system displayed the lowest environmental impact as measured by TFI (Figure 2) or Iphy\textsubscript{ARB} (data not presented).

![Mean Treatment Frequency Index (TFI) according to categories of compounds](image)

*The French « Plan Interministériel de Réduction des Risques liés aux Pesticides » has listed 47 compounds to be banned by the end of 2009 because of their harmfulness (http://www.ecologie.gouv.fr/Plan-interministeriel-de-reduction.html).

![Earwigs were seldom observed in the SV system whereas both OG and LI presented high numbers from May onwards, with similar patterns for these latter systems (Figure 3).](image)
Figure 3. Earwig abundance in 2008

From the first years of experiment, the importance of the cultivar in the management of orchard diseases (and to some extent pests) is outlined whatever the system, with a high variation in the number of treatments. This experimental design proved to be a functional tool permitting: i) the conception of decision rule patterns based on various degrees of integrated pest and disease management; and ii) the assessment of the agronomic and environmental performances of the studied systems through a longitudinal survey of various parameters. Other tools and measures, including economical performance, Life Cycle Assessment and soil and aerial communities, will be developed or continued in collaboration with partner research teams, for a global assessment of the systems.

Acknowledgements

This work was partly supported by the Ecoger and the ADD-GEDUPIC programs funded by the French National Agency for Research. Dispensers for mating disruption in the OG and LI systems were provided by Sumi Agro France. The authors wish to thank the staff members of Gotheron unit in charge of the management of the orchards.

References

Effect of different type row mulches on the success of biological control of strawberry tarsonemid mite

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Abstract: Organic experimental strawberry fields were established to study the effect of mulching materials on growth, yield, fruit quality and mites. Black plastic, flax fibre mat, fresh green mass, barley straw, buckwheat husks, pine woodchips and birch woodchips were used for mulching. Strawberry tarsonemid mite was recorded in the autumn of the planting year and biological control of mites was started in the spring by introduction of Neoseiulus cucumeris which kept the strawberry tarsonemid mite under control. Small numbers of Anthoseius rhenanus and Euseius finlandicus were also introduced, but these species were rarely found afterwards in folded leaf samples. In the third year, one release of N. cucumeris took place at the beginning of June. In late August strawberry tarsonemid mite population growth was unacceptable in black plastic and barley straw mulches whereas in green mass and buckwheat husk mulches the mite was controlled by predatory mites during the whole season. Faster vegetative growth in green mass and buckwheat husk mulches in organic farming is proposed to enhance biological control of strawberry tarsonemid mite.

Strawberry, Organic farming, Organic mulches, Phytonemus pallidus, Phytoseiidae, Neoseiulus cucumeris
The OrganicA Project: Organic Disease Management in Orchards with ‘Newer’ Cultivars

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Abstract: Although there is significant interest in organic apple production in the New England region of the USA, there are few certified organic orchards, in part, because of disease challenges associated with ‘McIntosh’, the predominant cultivar grown in the region. However, recent shifts in consumer preference for ‘newer’ cultivars have led to the planting of different apple cultivars which have different disease susceptibility. A long-term research project was initiated in 2006 to examine the opportunities and challenges of organic apple production within the two production systems growers are using to change to new cultivars: planting a new orchard with young trees purchased from a nursery and/or “top-grafting” an established, older orchard to new cultivars. The cultivars being studied in replicated plots in each orchard system are: ‘Zestar!’; ‘Ginger Gold’, ‘Honeycrisp’, ‘Macoun’, and ‘Liberty’, a scab-resistant cultivar. Both orchard systems are being managed with approved, organic practices and materials. Standard foliar disease assessments for apple scab, caused by Venturia inaequalis, and other diseases are being conducted to determine differences in disease incidence and severity among the cultivars. Based on initial foliar disease assessments during the establishment years of the orchards, ‘Honeycrisp’ appears more resistant to apple scab than the other scab-susceptible cultivars ‘Zestar!’; ‘Ginger Gold’, and ‘Macoun’, but appears more susceptible to cedar apple rust, caused by Gymnosporangium juniperi-virginianae, than ‘Liberty’ and ‘Zestar!’”. ‘Macoun’ and ‘Zestar!’ exhibited a higher incidence of necrotic leaf spots than the other cultivars. This research is on-going and will document disease challenges and the economic costs, returns, and risks associated with these five cultivars being grown under organic production practices within the two orchard systems.

Key words: Organic apple production, apple scab, cedar apple rust, apple diseases, integrated pest management

Introduction

Although there is significant interest in organic apple production in the New England region of the USA, there are few certified organic orchards, in part, because of disease challenges associated with ‘McIntosh’, the predominant cultivar grown in the region. However, recent shifts in consumer preference for ‘newer’ cultivars have led to the planting of different apple cultivars which have different disease susceptibility. A long-term research project was initiated in 2006 to examine the opportunities and challenges of organic apple production within the two production systems growers are using to change to new cultivars: planting a new orchard with young trees purchased from a nursery and/or “top-grafting” an established, older orchard to new cultivars (Berkett et al., 2007) Reported herein are results of disease assessments conducted over the ‘establishment years’ of the five cultivars in the two apple production systems which are being investigated.
Material and methods

Two orchards, located at the University of Vermont Horticultural Research Center in South Burlington, Vermont, USA, are involved in this research project (Figs. 1, 2, 3). Orchard 1 is an orchard planted in April 2006 with ‘Ginger Gold’, ‘Liberty’, and ‘Macoun’, and ‘Zestar!’ on Bud. 9 rootstock and ‘Honeycrisp’ on M.26; cultivars are arranged in a completely randomized design with three-tree replications. Orchard 2 was an existing orchard planted in 1988 with ‘McIntosh’ and ‘Liberty’ trees on M.26 rootstock which was ‘top-grafted’ in April 2006 to the same five cultivars. Since the original cultivar (i.e., original ‘McIntosh’ or ‘Liberty’ interstock) may affect growth of the new ‘top-grafted’ scion, a randomized complete block experimental design, with two-tree replications, was used to block any effect on new scion growth.

Environmental conditions within the orchards were monitored with a Davis Vantage Pro Wireless Weather Station (Davis Instruments Corp.) and primary scab infection periods were determined using “revised” Mills criteria (MacHardy & Gadoury, 1989), with the exception that all wetting periods including those starting at night were used in infection period determinations. In 2006, 2007, and 2008, there were six primary infection periods of varying durations per growing season and numerous secondary infection periods during the remainder of each growing season.

In each of the three growing seasons, multiple applications of lime sulfur and/or sulfur at standard label rates were used for disease management. In 2006, a combination of liquid lime sulfur and sulfur was applied on June 22 and July 1 and sulfur on July 14. In 2007, seven sprays of liquid lime sulfur and five sprays of sulfur were applied. Eight liquid lime sulfur and five sulfur applications were made in 2008. Due to the small size of the trees in 2006, sprays were applied with a Rears 'Nifty Fifty' Hydraulic Handgun Sprayer (Rears Manufacturing Inc., Eugene, OR) mounted on the three-point hitch of a small tractor. Sprays were applied in a sufficient volume of water to achieve near-drip conditions, and the volume was increased during each season as foliage emerged. Beginning in 2007, all sprays were applied with a Rears Pak-Blast airblast sprayer in a sufficient volume of water to achieve near-drip conditions. Travel speed and water rates differed between the two orchards because of tree size and foliage density differences.

Disease incidence of apple scab (*Venturia inaequalis* (Cooke) Wint.), cedar apple rust (*Gymnosporangium juniperi-virginianae* Schwein.) and necrotic leaf spots, which resembled frogeye leaf spots caused by the black rot fungus (*Botryosphaeria obtusa* (Schwein.) Shoemaker), was assessed on all leaves on two vegetative terminals per tree on at least five three-tree replications per cultivar in Orchard 1 and on four vegetative terminals per tree on eight two-tree replications per cultivar in Orchard 2 in August of each year. Analysis of variance and mean comparisons using Tukey’s HSD Test ($P \leq 0.05$) were performed on the data. Since the orchards are in the ‘establishment phase’, no apples were produced in 2006 and 2007, with a very minimal crop being produced in 2008. Future disease evaluations will include fruit.

Results and discussion

Results are presented in Tables 1, 2, 3 and 4. Based on these initial foliar disease assessments during the establishment years of the two organic apple orchards, ‘Honeycrisp’ appears more resistant to apple scab than the other scab-susceptible cultivars ‘Zestar!’ , ‘Ginger Gold’, and ‘Macoun’, but appears more susceptible to cedar apple rust, caused by *Gymnosporangium juniperi-virginianae*, than ‘Liberty’ and ‘Zestar!’ . ‘Macoun’ and ‘Zestar!’ exhibited a higher incidence of necrotic leaf spots than the other cultivars.
This research is on-going and will document disease challenges along with the overall economic costs, returns, and risks associated with these five cultivars being grown under organic production practices within the two orchard systems.

Figure 1. Orchard 1 - Planted with five cultivars in 2006

Figure 2. Orchard 2 - An eighteen year old orchard was “top-grafted” with five cultivars in 2006.

Figure 3. Orchard 2 - 2008 growth in “top-grafted” orchard.
Table 1. 2006 Orchard 1 - Percent Vegetative Terminal Leaves Infected, August 9-11, 2006

<table>
<thead>
<tr>
<th></th>
<th>Scab</th>
<th>Rust</th>
<th>Necrotic Leaf Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginger Gold</td>
<td>41.3</td>
<td>25.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Honeycrisp</td>
<td>2.7</td>
<td>13.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Liberty</td>
<td>0.0</td>
<td>0.3</td>
<td>3.1</td>
</tr>
<tr>
<td>Macoun</td>
<td>28.4</td>
<td>1.5</td>
<td>10.8</td>
</tr>
<tr>
<td>Zestar!</td>
<td>34.1</td>
<td>0.7</td>
<td>10.2</td>
</tr>
</tbody>
</table>

Numbers within columns followed by the same letter do not differ significantly, Tukey’s HSD Test (P ≤ 0.05)

Table 2. 2007 Orchard 1 - Percent Vegetative Terminal Leaves Infected, August 7, 2007

<table>
<thead>
<tr>
<th></th>
<th>Scab</th>
<th>Rust</th>
<th>Necrotic Leaf Spot</th>
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<tbody>
<tr>
<td>Ginger Gold</td>
<td>0.3</td>
<td>24.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Honeycrisp</td>
<td>0.0</td>
<td>39.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Liberty</td>
<td>0.0</td>
<td>14.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Macoun</td>
<td>0.9</td>
<td>25.2</td>
<td>8.9</td>
</tr>
<tr>
<td>Zestar!</td>
<td>0.2</td>
<td>14.5</td>
<td>6.5</td>
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Numbers within columns followed by the same letter do not differ significantly, Tukey’s HSD Test (P ≤ 0.05)

Table 3. 2007 Orchard 2 - Percent Vegetative Terminal Leaves Infected, August 8-14, 2007

<table>
<thead>
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<th></th>
<th>Scab</th>
<th>Rust</th>
<th>Necrotic Leaf Spot</th>
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</thead>
<tbody>
<tr>
<td>Ginger Gold</td>
<td>0.4</td>
<td>35.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Honeycrisp</td>
<td>0.1</td>
<td>36.6</td>
<td>7.1</td>
</tr>
<tr>
<td>Liberty</td>
<td>0.0</td>
<td>17.9</td>
<td>9.4</td>
</tr>
<tr>
<td>Macoun</td>
<td>0.8</td>
<td>18.6</td>
<td>20.8</td>
</tr>
<tr>
<td>Zestar!</td>
<td>0.1</td>
<td>12.6</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Numbers within columns followed by the same letter do not differ significantly, Tukey’s HSD Test (P ≤ 0.05)

Table 4. 2008 Orchard 2 - Percent Vegetative Terminal Leaves Infected, August 20-26, 2008

<table>
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<tr>
<th></th>
<th>Scab</th>
<th>Rust</th>
<th>Necrotic Leaf Spot</th>
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</thead>
<tbody>
<tr>
<td>Ginger Gold</td>
<td>11.2</td>
<td>39.6</td>
<td>15.1</td>
</tr>
<tr>
<td>Honeycrisp</td>
<td>0.6</td>
<td>22.2</td>
<td>14.7</td>
</tr>
<tr>
<td>Liberty</td>
<td>0.0</td>
<td>7.1</td>
<td>14.6</td>
</tr>
<tr>
<td>Macoun</td>
<td>1.2</td>
<td>8.6</td>
<td>25.0</td>
</tr>
<tr>
<td>Zestar!</td>
<td>2.1</td>
<td>5.4</td>
<td>20.1</td>
</tr>
</tbody>
</table>

Numbers within columns followed by the same letter do not differ significantly, Tukey’s HSD Test (P ≤ 0.05)

Acknowledgements

Major funding for this project is from a grant from the USDA Integrated Organic Program.

References

Project. http://www.uvm.edu/organica/
Investigation on survival and viability of cankers of Nectria galligena following removal from apple trees and pulverisation on the orchard floor

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Abstract: The risk of pulverised excised canker prunings to apple trees was evaluated in two orchard trials. Cankers (Nectria galligena) on one year shoots were collected from apple trees cv. Gala in two orchards and distributed among sprout net bags. Cankers on two, three or older wood were similarly collected and pulverised with a tractor-trailed standard orchard pulveriser before placing in sprout net bags. Both sets of bags were pegged out in two orchard sites, either in the tree row or the grass alley way between trees in February 2005. The bags were sampled at monthly intervals and the state and viability of the cankers assessed. The pruned out cankers whether pulverised or unpulverised continued to produce perithecia for at least 16 months after removal from the trees. Conidia were only found in the first two samples. Perithecia were produced more abundantly on pruned out cankered one year shoots. Pulverised prunings decayed more rapidly in the grass alley way than in the tree row. This study shows that pulverised canker prunings could be a source of inoculum of N. galligena and hence a risk to apple trees for more than a year after pulverising.

Key words: Apple, canker, Nectria galligena, prunings, pulverisation

Introduction

Canker, caused by the fungus Nectria galligena, is one of the most important diseases of apple and pear. The fungus attacks trees in the orchard, causing cankers and die back of young shoots, resulting in loss of fruiting wood and increasing pruning costs. Nectria also causes a fruit rot that can result in significant losses as high as 10% or more in stored fruit. The fungus produces two spore types, conidia in the spring and summer and ascospores in the autumn and winter. These enter shoots and branches on the tree through wounds, either natural such as leaf scars or artificial such as pruning wounds. Thus inoculum and points of entry on the tree are available all year round and the only limiting factor is rain, which is essential for spore production, spread, germination and infection. Autumn leaf fall is usually the main infection period and wet autumns are usually followed by a high incidence of shoot dieback due to canker the following spring and summer. Currently canker is controlled by a combination of cultural methods to remove canker lesions and the use of protectant fungicides. Effective fungicides are limited. Generally copper fungicides are used at autumn leaf fall and before bud-burst to protect leaf scars and bud-scale scars and carbendazim is applied during the spring and summer.

Up until the 1970s it was normal orchard practice to remove and burn prunings, eliminating any pruned out cankers. However, most prunings, including cankers, are now pulverised in the tree alley ways. The effect of this practice on canker survival and viability and risk to apple trees is not clear. Previous studies by van der Scheer (1981) and Swinburne and Souter (1984) have indicated a minimal risk. The purpose of this study was to investigate the effect
of pulverising on survival and viability of canker prunings on the orchard floor and the potential risk to apple trees.

Materials and methods

Site
Two orchard sites were selected for the study. One orchard site was located at Rocks Farm, East Malling (TL161) and consisted of a solid block of cv Gala on M9 rootstock, planted in 1999. The second orchard was located at Elverton Farm, Teynham (Marsh Gala) and consisted of Gala with Cox pollinators both on M9 rootstock. In both orchards canker had been a significant problem since planting.

Collection and preparation of cankers
The orchard (TL161) was visited in February 2005. Approximately 110 cankered one year old shoots were collected from the Gala trees in each of four pairs of rows and placed in black sacks, giving a total of about 440 cankered shoots. Similarly approximately 110 cankered two, three, four and older wood was collected from each of the same rows and placed in black sacks, giving a total of around 440 lumps. At least 10 one-year old cankered shoots were placed in each of 44 sprout nets, giving four replicates of 11 nets. These were then placed back out in the orchard, in the tree row of each of 4 rows, 11 bags per row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling. The older cankers were spread out on a concrete pad and pulverised by a tractor-trailed standard orchard pulveriser twice, in two different directions. All pulverised prunings were then collected up and divided into four equal replicate lots. Each lot was then divided up into 11 sprout nets, giving 44 in total. These were then placed back out in the orchard, in the tree row in each of four rows, 11 bags per row. The bags were held in place with metal pins and the positions noted so that they could be relocated for future sampling. A similar procedure was followed for canker collection in the second orchard (Marsh Gala) as that at East Malling. One-year old cankers and mature cankers were laid out in sprout nets in the orchard, but were placed in the grass alleyway rather than the tree row.

Assessments
Initially at monthly intervals the orchards were visited and the state of the cankers assessed in terms of decay. One of the labeled bags containing pieces of canker from the pulverised wood was collected from each replicate in the orchard. In the laboratory each canker piece was examined carefully for signs of conidial masses or perithecia. Where present they were checked for spores. Estimates were made of numbers of fruiting bodies present. Cankers on one-year old pruned wood were similarly collected and similarly assessed.

Results and discussion
Rain fall was frequent throughout the period of the study at both sites such that conditions were favourable for canker sporulation throughout the spring and early part of the summer with conditions becoming less favourable in July and August which were exceptionally hot in 2005.

Unpulverised one-year cankered shoots
Netted samples of one-year old cankered shoots and pulverised cankers on older wood were collected from the orchard and checked for sporulation at roughly monthly intervals from March 2005 until July 2005. Thereafter samples were collected and examined at longer intervals until June 2006 (Table 1). The netted one-year shoots from TL161 mostly retained
their bark throughout the assessment period. The dead wood was rapidly colonised by various saprophytic fungi, including *Botryosphaeria* and *Diaporthe* which were present on many of the twigs examined from the first assessment in March 2005 onwards. By the final assessment in June 2006 the shoots were becoming very dry, rotted and easily broken, although the original cankered area was still obvious and perithecia easily visible. On average more than 60% of cankers were sporning at each sampling. Perithecia containing mature or immature ascospores were present in abundance on these cankered shoots at each sampling. This is surprising as perithecia are normally associated with mature cankers rather than those on young shoots. Conidia were present in samples checked in April and May 2005 but were not observed in subsequent samplings. The results for netted one-year shoots from Marsh Gala were similar to those for East Malling. Assessments were only continued up to October 2005 as later samples could not be located in the orchard. In contrast to the East Malling site conidia were present in samples up to the final assessment in October 2005. Perithecia however, were usually more abundant.

**Pulverised older cankers**

The netted pulverised cankers from TL161 had mostly been debarked during the pulverising process and as with the one year shoots by June 2006 were becoming very dry and colonised by various saprophytic fungi. The original cankered areas were less easily distinguished with time. Initially most perithecia or conidia observed were associated with the barked areas of the prunings, but in later samplings perithecia were equally found on debarked areas also. Numbers of cankers observed with conidia or perithecia present were on average half of that recorded on the one year old unpulverised prunings (Table 1). Conidia were only recorded on samples collected in April 2005. Perithecia were recorded on the pulverised prunings at every sampling and 43% of cankers were still producing perithecia 16 months after pulverising (Table 1). The netted pulverised cankers from Marsh Gala had mostly been debarked during the pulverising process. In contrast to the TL161 site, the netted samples had been placed in the grass alleyway. Consequently at each sampling the pulverised prunings were much wetter, were more colonised by saprophytic fungi and generally decaying more rapidly. It was also noted that the grass was rapidly growing over the netted samples, increasing the general decay of the wood. This more rapid decay was reflected in the incidence of perithecia found on the prunings which was usually less than half of that found on pulverised prunings at the TL161 site. At the last sampling in December 2005 perithecia were found on less than 10% of cankers (Table 1). Conidia were only observed on cankers during the first two samplings in March and April 2005.

**General discussion and conclusions**

It is clear from this study that pruned out cankers pulverised or unpulverised can produce perithecia for at least 16 months after being removed from the trees. The cankers continued to produce conidia for a much shorter period of time, but it is the perithecia that pose the risk to canker infection on trees as ascospores can be shot out from perithecia during wet weather similar to the release of scab ascospores from fruiting bodies (pseudothecia) surviving on leaf litter. These ascospores of *N. galligena* then can be carried by air currents up to infect trees. Perithecia were produced more abundantly on pruned out cankered young shoots. These are often just dumped in the tree row rather than the alleyway and left unpulverised. Decay of prunings appears to take place more slowly in the tree row. Pulverised prunings left in the grass alleyway appeared to decay more rapidly and were also overgrown by the grass. Despite this perithecia could still be found almost twelve months after the pulverising.
Previous studies by van der Scheer (1981) and Swinburne and Souter (1984) have indicated a minimal risk from cankered prunings dumped in the grass alleyway. However, this study shows that pulverised cankers can continue to pose a threat to apple trees for more than a year after pulverising. Ideally in areas where conditions favour canker it would be desirable to return to the practice of collecting prunings and burning to minimise the risk. This however, may not be practical. The best alternative would be to dump all prunings, including young shoots, in the grass alleyway and pulverise. Decay is more rapid and repeated mowing of prunings would increase the break down.

Table 1 Percentage of cankers with perithecia present on one-year-old unpulverised wood or on pulverised older wood at various sample dates from orchard sites at East Malling (TL161) or Teynham (Marsh Gala) in 2005 or 2006

<table>
<thead>
<tr>
<th>Wood age</th>
<th>Orchard site</th>
<th>Date cankers sampled and assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>One-year-old</td>
<td>TL161 East Malling</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>Marsh Gala Teynham</td>
<td>0</td>
</tr>
<tr>
<td>Pulverised older cankers</td>
<td>TL161 East Malling</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>Marsh Gala Teynham</td>
<td>0</td>
</tr>
</tbody>
</table>

Acknowledgements

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References

Inventory of European canker in southern Sweden and Nectria galligena as a soil pathogen.

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Abstract: In recent years pomme fruit growers in southern Sweden have reported that they have been experiencing severe outbreaks of European canker and the problem seems to be increasing. The growers mainly import new trees from nurseries in Belgium or Holland. Soon after the trees have been planted the trees are heavily affected by canker disease. To assess the extent of the outbreaks a survey has been started which will include orchards from all parts of the Scania province. In swedish fruit orchards it is a common management practice to leave pruned branches containing canker on the orchard floor and cut them into small pieces with a heavy duty lawn mower. The wood chips eventually get incorporated into the soil and the fungus might infect the trees via the roots. We are currently conducting experiments investigating the canker fungus’ ability to survive in soil and infect apple tree roots.

European canker, Nectria galligena, Apple, Soil pathogen, Integrated control
Integrating scab control methods with partial effects in apple orchards: the association of cultivar resistance, sanitation and reduced fungicide schedules.

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Abstract: To preserve the environment, consumer health and reduce the economic impacts of apple scab, it is crucial to improve disease control while reducing the number of treatments and the impact of fungicide spraying. To reach this goal, the planting of cultivars with partial resistance to the disease, associated with an integrated control strategy, may be an attractive alternative. However, to decrease the risks for growers, cultivars with a high partial resistance level are required, and several methods of control must be associated. The application of such a strategy must be simple and reliable. The thresholds for chemical spraying must be defined and validated, taking into account the cultivar resistance level and the sanitation practices applied. Since 2006, we have studied within an experimental orchard the association of the cultivar 'Reine des Reinettes' (which presents good partial resistance) with:

1 A sanitation practice: reduction of leaf litter.
2 A chemical schedule: fungicide spraying only if a medium or high Mill’s risk is recorded or expected.

The results obtained in 2006 and 2007 showed that, with only 5 to 6 sprays per season (on average, twice as many sprays were applied in conventional orchards in the Loire Valley), scab control was efficient with less than 2% of scabbed fruits.

Key words: partial resistance, apple scab, control strategy, sanitation practices, fungicide treatments, Venturia inaequalis.

Introduction

Repeated fungicide treatments against apple scab reduce the auxiliary fauna and can favour the development of some pests (Sauphanor et al., 2005). Moreover, resistance to several active ingredients has appeared in Venturia inaequalis populations (Parisi et al., 1994; Köller et al., 2004; Köller et al., 2005). It is, therefore, crucial to improve disease control while reducing the number of treatments and the impact of fungicide spraying.

The alternatives to intensive chemical control are numerous (Carisse & Dewdney, 2002). In our study we focus on the association of three methods with partial effects: sanitation; partial resistance; and Integrated Pest Management (IPM) adapted to partial resistance.

Sanitation has been used in several countries for many years, and its efficiency in reducing scab epidemics is recognized (Sutton, et al., 2000; Holb, 2006; Gomez et al., 2007). One of the best results seems to be obtained by removing of the autumn leaf litter.

Partially resistant apple cultivars show low levels of disease, confirmed by recent data (Brun et al., 2008). In this work, the old French cultivar ‘Reine des Reinettes’ appeared to be of very low susceptibility: without fungicide protection, the level of scab incidence on fruits
and leaves was significantly lower on ‘Reine des Reinettes’ than on ‘Gala’ (a cultivar susceptible to scab). In 2003, characterized by dry climatic conditions the disease didn't develop on 'Reine des Reinettes'. However, when climatic conditions are favourable for scab, partial resistance must be combined with other control methods.

Schedule recommendations for low susceptibility cultivars were established in 1980’s (Olivier, 1986; Lefeuvre, 1995), but fungicide resistances, that don’t allow applying curative treatments with the majority of active ingredients, rendered difficult the practical application of these recommendations. A way to take into account the current fungicide resistance context is to apply some preventive treatments and when necessary choose “stop-applications” of contact fungicides.

In this study, we combined partial resistance ('Reine des Reinettes', X2640) and sanitation (removal of the autumn litter) with an adapted fungicide schedule on an experimental orchard. The efficiency for control of apple scab was studied for two years (2006, 2007). The aim was to be able to recommend reliable methods limiting the number of sprays.

Material and methods

The orchard.
The orchard in the Loire Valley (France), was planted in 1999 and restructured in 2005, when 3 of the plots were over grafted in order to obtain 6 monoculture plots of 'Reine des Reinettes'. Each plot (810 m²) contains 6 rows of 13 trees surrounded by non host plant hedge. The distance between plants within the row is 1.25 m and 4 m between the rows. The orchard contains 16 plots of which 6 are used for this trial: 3 treated plots and 3 untreated plots of 'Reine des Reinettes'.

Treatments and assessments.
Leaf litter was removed in autumn 2005 and 2006 from the treated plots only. The fungicide schedule was based on Olivier's model of treatment threshold (Lefeuvre, 1995), and the plots were protected by a “stop-spray” or a curative fungicide application when the moderate and severe Mill’s risks (Mills & Laplante, 1951) were detected with the Pulsowin 3.1 software (Pulsonic, Orsay, France). However, preventive treatments were applied in case of forecast of a rainy period when a high ascospore stock was present. The 78 trees of each plot were observed on 12-06-2006 and on 6-06-2007. The scabbed trees (from 1 lesion observed) were counted. At harvest around 26 Kg of apples per row (for the rows 2 to 5 per plot) were picked, and the percentage of scabbed fruits was assessed. After harvest the percentage of scabbed leaves (25 to 42 shoots per plot) was observed and the quantity of inoculum was calculated (Gagoury & MacHardy, 1986), in October 2005, 2006 and 2007.

Results

In 2006 and 2007, the number of fungicide applications was 5 and 6, respectively. This level is half of the number of sprays applied in commercial plots of susceptible cultivars in the INRA experimental station for the two years (Table 1).

In June 2007, the percentage of scabbed trees remained under 15% in treated plots, while in untreated plots it reached 74% (Figure 1-A). At harvest, the incidence on fruits was 1.0 and 0.2% in 2006 and 2007 respectively (Figure 1-B). In untreated plots this number was around 3.5% for the two years.

Table 1: Number of fungicide sprays applied in 2006 and 2007 in the experimental and commercial plots (average) of the experimental station
<table>
<thead>
<tr>
<th></th>
<th>2006</th>
<th>2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental plots</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Average commercial plots</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>% of reduction</td>
<td>55</td>
<td>57</td>
</tr>
</tbody>
</table>

Figure 1: Development of disease in treated and untreated plot (cv 'Reine des Reinettes'), - A and B : * Statistically significant effect of the treatments (logistic modelling).

In 2005, before the beginning of the trial, the level of disease in autumn (figure 1-C) was higher in treated plots than on untreated plots (9.9% and 5.3% of scabbed leaves, respectively). This order was reversed in 2006 and 2007. The incidence reached 21% of scabbed leaves in 2007, while this number remained under 10% in treated plots.

**Discussion**

The disparities of disease levels between the untreated plots were very important. This variability was very pronounced at harvest 2007, where no fruit was scabbed in one of the three treated plots. This fact explains the lack of significant effect of treatments in spite of the high differences in average scab incidence on fruits between the two modalities. Nevertheless, during the two years, the attacks of scab on leaves and fruits were limited in all treated plots. The incidence remained very low and constant in contrast to the results observed in untreated plots. The number of unhealthy fruits remained tolerable for commercialisation with a quantity of fungicide treatments strongly reduced compared to the quantity applied on average in commercial orchards of Loire Valley.

We can conclude that cv 'Reine des Reinettes' showed a good level of partial resistance, and that the chemical spraying schedule associated with sanitation was very efficient to enhance this partial scab resistance during these 2 years. The association of these three methods showed good efficacy, even in difficult conditions (climatic conditions in 2007 were favourable to scab).

In the future we will try to reduce the number of fungicide applications. For this goal, the Mill's contamination threshold (Mills & Laplante, 1951) for application decision can be modified: as Olivier and Lefeuvre proposed, if the autumn inoculum is low, only the severe Mill's threats will be treated. The schedule strategy will be based on these recommendations.
Acknowledgments

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Application of thermotherapy and chemotherapy in vitro for elimination of some viruses infecting fruit trees and small fruits

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Abstract: In vitro culture is known currently as a technique used to eliminate viruses from plants. In this study thermotherapy and chemotherapy in vitro were applied to eliminate ACLSV and PNRSV from myrobalan, PNRSV from ‘Empress’ plum, PDV from ‘Early Rivers’ sweet cherry, ACLSV from apple ‘Jonagold’ and pear ‘Pierre Corneille’, and RVCV from ‘Norna’ raspberry. Shoots were placed in a growth chamber where the temperature was raised gradually to 36°C and kept at this level for 4 weeks for thermotherapy. Chemotherapy was conducted using 10-100 mg l⁻¹ Virazole® (ribavirin) applied into the proliferation medium. Combining both methods was also used. ELISA assays for ACLSV, PNRSV and PDV were conducted one year after therapy. The rooted raspberry plants were planted in a greenhouse and observed for possible RVCV symptoms. Thermotherapy in vitro was highly effective for PNRSV and ACLSV elimination but it was not efficient for obtaining PDV-free sweet cherry and RVCV-free raspberry. Efficiency of chemotherapy varied depending on concentration of Virazole®, virus and species of infected plant. Virazole® at concentration 25-100 mg l⁻¹ was effective in eliminating ACLSV from myrobalan and PNRSV from plum but was not successful in eliminating PNRSV from myrobalan and PDV from sweet cherry shoots. Combining thermotherapy and chemotherapy contributed to elimination of all studied viruses from most treated shoots.

Keywords: in vitro culture, thermotherapy, chemotherapy, ACLSV, PNRSV, PDV, RVCV

Introduction

In vitro culture is known not only as a method of micropropagation of plant material and maintenance of gene bank accessions but currently as a technique used for eradicating viruses from plants. Thermotherapy, chemotherapy, shoot-tip culture, micrografting or combinations of these methods in vitro are useful tools for elimination of different viruses from fruit trees (Cieślińska, 2002; Cieślińska, 2007; Cieślińska and Zawadzka, 1999; Deogratias et al., 1989; Hansen and Lane, 1985; Howell et al., 2001; Gella and Errea, 1998; Manganaris et al., 2003; Snir and Stein, 1985; Spiegel et al., 1993; Stein et al., 1991).

The objective of the present work was to use thermotherapy and chemotherapy in vitro and combinations of these both methods to eliminate Apple chlorotic leaf spot virus (ACLSV) from apple ‘Jonagold’, pear ‘Pierre Corneille’, and myrobalan, Prunus necrotic ring spot virus (PNRSV) from myrobalan and ‘Empress’ plum, Prune dwarf virus (PDV) from ‘Early Rivers’ sweet cherry and Raspberry vein chlorosis virus (RVCV) from ‘Norna’ raspberry.

Material and methods

The study was done on ‘Jonagold’ apple and ‘Pierre Corneille’ pear infected with ACLSV, myrobalan infected with a complex of ACLSV and PNRSV, ‘Empress’ plum infected with PNRSV, ‘Early Rivers’ sweet cherry infected with PDV, and ‘Norna’ raspberry infected with RVCV. The infection of the fruit trees was confirmed by positive reaction in enzyme-linked immunosorbent assay (ELISA) using specific antisera. RVCV presence in raspberry plant was
verified by observation of characteristic symptoms and the results of biological indexing.

The apical and axillary buds isolated from infected trees were put in the tubes and multiplied using Murashige and Skoog (MS) (1962) medium with the addition of 0.25 µM l\(^{-1}\) indole-3-butyric acid (IBA), 2.5 µM l\(^{-1}\) 6-benzylaminopurine (BA), 30 g l\(^{-1}\) sucrose and solidified with 7 g l\(^{-1}\) of agar. The cultured shoots were placed in a growth chamber at under a day/night temperature regime of 24/21°C with a 16 h photoperiod.

Temperature was gradually increased from 24°C to 36°C over a week and kept at 36°C for the following four weeks in the course of thermotherapy. Untreated shoots (control) were maintained at 22°C. After thermotherapy the surviving shoots were transferred to MS rooting medium with addition of 2 mg l\(^{-1}\) IBA, and 4-5 weeks later they were potted in a greenhouse. Chemotherapy was conducted using Virazole® (ribavirin) (ICN Pharmaceuticals, Inc., USA), a synthetic broad-spectrum antiviral nucleoside (1-ß-D-ribofuranesyl-1,2,4-triazole-3-carboxamide), added into the proliferation medium at concentrations of 10, 25, 50 and 100 mg l\(^{-1}\). Four weeks after initiating the experiment, shoot tips (1cm long) were excised and transferred to a Virazole®-free medium and one month later to the rooting medium. Rooted plantlets were potted in greenhouse. Combining of the both methods, thermotherapy and chemotherapy was also used. DAS-ELISA assays for ACLSV, PNRSV and PDV were conducted one year after therapy (Clark et al., 1976). Shoots of healthy and infected plants of the same species maintained in vitro were used as the negative and positive controls. The rooted raspberry plants were planted in a greenhouse and observed for possible RVCV symptoms.

**Results and discussion**

Depending on the plant species the survival rate of the shoots treated with thermotherapy was 67-100%. Virazole® at concentrations 10-25 mg l\(^{-1}\) did not affect significantly the survival rate but 50-100 mg l\(^{-1}\) of this compound was phytotoxic for pear, plum and raspberry shoots, especially when combined with thermotherapy (Figure 1).

![Figure 1. Effect of thermotherapy (T) and Virazole® (V 10-100 mg l\(^{-1}\)) on survival rate of apple, pear, raspberry and *Prunus* sp. shoots](image)

These shoots showed chlorosis and apical necrosis and most of them died during the therapy. Survival rate of shoots depended on species and cultivar of plants and a kind of virus. It is
known that pome fruit trees tolerate heat better than stone fruit trees (Gella and Errea, 1998; Stein et al., 1991). The use of alternating temperature 28°C/38°C in thermotherapy *in vitro* for 18-20 days was less harmful to shoots and in many cases allowed elimination of PNRSV from peach (Spiegel et al., 1995).

Deogratias et al. (1989) showed that all shoots of 'Noire de Meched' sweet cherry infected with ACLSV, treated with 32-34°C for three weeks, died at the end of the treatment period. However, in the same experiment 30% of 'Van 2D' sweet cherry shoots infected with PDV and PNRSV survived thermotherapy. 90-100% of apricot shoots infected with ACLSV, 70% of sweet cherry shoots with PDV and PNRSV and 42-90% of peach shoots with PNRSV and ACLSV survived thermotherapy (Gella and Errea, 1998).

Thermotherapy effect strongly depended on species of treated shoots (Table 1). High temperature enabled elimination ACLSV from 24% of pear 'Pierre Corneille', 67% of apple ‘Jonagold’ and myrobalan shoots. Thermotherapy was also effective in elimination of PNRSV from myrobalan and plum (58% and 50%, respectively), but it was not successful method of PDV and RVCV elimination.

Table 1. Effect of *in vitro* treatment on the elimination of viruses in apple, pear, *Prunus* sp. and raspberry shoots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of virus free plants/number of tested plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>apple</td>
</tr>
<tr>
<td>Control</td>
<td>0/25</td>
</tr>
<tr>
<td>Thermotherapy</td>
<td>16/22</td>
</tr>
<tr>
<td>Virazole® [mg l(^{-1})] 10</td>
<td>6/25</td>
</tr>
<tr>
<td>25</td>
<td>15/25</td>
</tr>
<tr>
<td>50</td>
<td>21/23</td>
</tr>
<tr>
<td>100</td>
<td>13/13</td>
</tr>
<tr>
<td>Thermotherapy + Virazole® [mg l(^{-1})] 10</td>
<td>10/25</td>
</tr>
<tr>
<td>25</td>
<td>18/22</td>
</tr>
<tr>
<td>50</td>
<td>15/15</td>
</tr>
<tr>
<td>100</td>
<td>12/12</td>
</tr>
</tbody>
</table>

Depending on the period of heat treatment, it was possible to eliminate PNRSV, ACLSV and PDV from shoots of several *Prunus* sp. fruit trees shoots (Barba et al., 1992; Deogratias et al., 1989; Gella and Errea, 1998; Snir and Stein, 1985; Stein et al., 1991). However it was difficult to eliminate ACLSV and PPV from apricot shoots using thermotherapy (Llácer, 1995). Janečkova (1995) eliminated complex of *Apple mosaic virus*, *Apple stem grooving* and ACLSV from apple cultivars by combination thermotherapy *in vivo* with the segment bud cultures *in vitro*. In this study, thermotherapy was not an effective method for RVCV elimination from raspberry. Baumann (1982) produced raspberry plants free from RVCV by a combination of thermotherapy and meristem tip culture and Sobczykiewicz (1986) by meristem tip culture alone.

Efficiency of chemotherapy varied depending on virus, species of infected plant and
concentration of Virazole®. This compound was effective in elimination of PNRSV from ‘Empress’ plum – the higher the concentration the greater the chemotherapy efficiency. Independent of concentrations of Virazole®, chemotherapy was not effective in elimination of PNRSV from myrobalan and PDV from ‘Early Rivers’ sweet cherry. Deogratias et al. (1989) showed that Virazole® at concentration 25, 50 and 100 mg l⁻¹ was effective in eliminating ACLSV from 50-100% of *Prunus mahaleb* shoots. However, in the same experiment it was necessary to apply 50 and 100 mg l⁻¹ of this compound to eliminate PNRSV and PDV. It was possible to obtain RVCV-free raspberry shoots after using Virazole® in concentrations of 50 and 100 mg l⁻¹. No symptoms of vein chlorosis were observed for two years whereas raspberry plants still infected with RVCV were stunted and showed chlorosis of the minor veins. Phytotoxic influence of Virazole® on apple, pear, *Prunus* sp. and raspberry shoots was observed with increasing concentration of this compound. Most treated shoots showed chlorosis and necrosis and finally died at the end of the therapy. Some phytotoxicity of higher Virazole® concentrations was observed by Deogratias et al. (1989) on cherries in the course of elimination of PDV, PNRSV and ACLSV, and Hansen and Lane (1985) who treated apple shoots to eliminate ACLSV. Combining thermotherapy and chemotherapy contributed to elimination of all studied viruses from most treated shoots.

**Acknowledgements**

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**References**


**In vivo antagonism of Acremonium byssoides endophyte in Vitis vinifera, towards Plasmopara viticola**

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**Abstract:** The endophytism of *Acremonium byssoides* in *Vitis vinifera* was recently ascertained in Sicily. In particular, the hyphomycete was observed in leaves of three vine cultivars (Regina Bianca, Catarratto and Insolia). Moreover, in the leaves of cultivar Insolia inoculated with *P. viticola*, the *A. byssoides* showed an antagonistic activity (hyperparassitism and antibiosis) towards asexual and sexual structures of the oomycete.

In spring 2002 and 2007 “Insolia” vines, infected by the endophyte, suffered repeated attacks by *P. viticola*, that lasted until the formation of gametic structures of pathogen. The aim of our researches was to ascertain *in vivo* the effect of *A. byssoides* on viability of oospores, the only means of *P. viticola* overwintering. The “mosaic spotted” leaves were collected in October from vines colonized (cv. Insolia) or not (cv. Catarratto) by *A. byssoides* and exposed to natural climatic conditions. The oospores viability was assayed by germination tests. The differentiated oospores in endophyte-free leaves showed the highest mean germination value, whereas the other ones were degenerated and did not germinate at all.

This study shows that the interaction between *A. byssoides*, *V. vinifera* and *P. viticola* could assume a determinant role to contain the mildew infections in our environment.

**Key words:** grapevine, endophytism, antagonism, downy mildew

**Introduction**

Recent studies confirmed the endophytism of *Acremonium byssoides* in symptomless leaves of different cultivars of *Vitis vinifera* (Regina Bianca, Catarratto, Insolia). Moreover, in leaves of cv. Insolia inoculated *in vitro* with *Plasmopara viticola*, the endophyte inhibited the pathogen infection by invading mycelium (Fig. 1a), sporangiophores (Fig. 1b), sporangia and sexual spores (Fig. 1c) of the oomycete (Burruano *et al.*, 2008).

![Image](image.png)

Figure 1. a) *P. viticola* mycelium invaded by *A. byssoides*; b) sporangiophores bridled by the endophyte hyphae; c) hyphomycete insightful a gametic structure of the oomycete.

In spring 2002 and 2007, natural infections of *P. viticola* were detected on plants of cv.
Insolia, lasting during summer and autumn until the formation of the pathogen gamic structures. In order to define new defensive strategies against downy mildew primary infections, an investigation was carried out to ascertain in vivo the A. byssoides effect on viability of oospores, the only overwintering structures of P. viticola.

Materials and methods

In the 30th October of each year (2002 and 2007) leaves with numerous “mosaic spots” were harvested from vines colonized (cv. Insolia) or not (cv. Catarratto) by A. byssoides and growing in Palermo and S. G. Jato, respectively. Foliar fragments 1 cm² wide were obtained. The occurrence of P. viticola spores within the mesophyll was detected by stereomicroscopy, whereas their nuclear stage was assessed using DAPI (4,6-diamidino-2-phenylindole) fluorochrome (Burruano et al., 2000). Foliar fragments were put to overwinter under environmental climatic conditions (Burruano et al., 1989).

The germinative efficiency was evaluated by means of germination assays from February to April-May. In particular, every 15 days foliar fragments were taken for manual isolation of spores. Some spores were treated with DAPI while the remaining ones were put to germinate into drops of water in moist chamber at 20 ± 2° C. For each test the oospore viability was daily checked by counting the germinated oospores; at the end of germination phase, the total number of oospores was counted to calculate the germination percentage (Burruano et al., 2000).

Results

In 2002, at the harvest, the spores showed three different nuclear stages (oogonia, oospheres and oospores; fig. 2a; 2b and 2c) with different percentage in relation to the tested cultivar. In particular, oogonia 42.3%, oospheres 33.5% and oospores 17.9 % were observed in A. byssoides-free grapevines (cv. Catarratto; Fig. 3a), while oogonia 65.3%, oospheres 16.6% and oospores 11.6% in plants colonized by the hyphomycete (cv. Insolia; Fig. 3b). Moreover in both cases degenerated spores (fig. 3d) were occasionally detected.

![Figure 2](image-url) Figure 2. Nuclear stage of P. viticola spores: a) oogonia; b) oosphera; c) oospora; d) degenerated spore.

The nuclear staining showed a different dynamic of the nuclear stages in examined samples. In endophyte-free fragments, in fact, the uninucleate spore percentage varied between 7 and 18.1 from February to March and strongly reduced in the following months (Fig. 3a). On the contrary, in the colonized leaves, the mononucleate oospores detected at the harvest degenerated, in fact their amount reached 100% already at the second assay (Fig. 3b).

In 2007 the percentages of the nuclear stages at the harvest were the following in endophyte-free leaves: 42.4% of oogonia, 13.4% of oospheres and 28.8% of oospores (Fig. 4a); whereas in the colonized ones oogonia were 64.2%, oospheres 10.5% and uninucleate
oospores 3.3% (Fig. 3b). In the first case, the high amount of mononucleate oospores detected in February (29.1%) reduced at first assay of March (10.5%) and increased in the two following ones (17.7 and 18.0% respectively), reaching the lowest value (6.5%) in the last assay (Fig. 4a). In fragments colonized by the endophyte instead the mononucleate oospores amount was very low, ranging from 8.4% (first test on February) to 0% (in April); at the same time the degenerated and endophyte-invaded spores increased continuously (Fig. 4b).

As regards the germination assay, in 2002 the oospores of endophyte-free cultivar (Catarratto) germinated constantly reaching the highest value (18.1%) in March (Fig. 2a). In contrast no germination occurred in A. byssoides colonized fragments (cv. Insolia; Fig. 2b). In 2007, the germination tests showed a similar trend: oospores of Catarratto leaves during all tests reached the highest percentage in March (15.4%), whereas the oospores of Insolia didn’t germinate (Fig. 4a-b).

Figure 3. Percentage of gamic structures and oospores germination observed in Catarratto (a) and Insolia (b) in October-May 2002-03.
Conclusions

Our researches demonstrated \textit{in vivo} the antagonism activity of \textit{A. byssoides} towards the oomycete gamic structures. In fact, as regards the endophyte colonized leaves, the constant decreasing of the uninucleate spores, the increasing of the degenerated ones and total lack of germination confirmed the antibiosis and hyperparasitism activity of endophyte against the pathogen (Burruano \textit{et al.}, 2007). \textit{A. byssoides} can be considered one of the extrinsic factors that influence the maturation of \textit{P. viticola} spores. In southern and island environments, characterized by hot and dry climate, the primary infections of \textit{P. viticola} occur during May-June, causing 2-3 infective cycles until the middle of July. Usually the infections start again at the first autumnal rains, with “mosaic spots” which sporulate regularly and allow the differentiation of gamic structures, assuring the pathogen survival (Burruano \textit{et al.}, 2006). In these environments, hence, the interaction between \textit{A. byssoides} and \textit{P. viticola} could have a decisive role in the containment of \textit{P. viticola} primary and secondary infections.

References


Preliminary investigation on the endophytic communities in *Olea europaea* L. in Sicily

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**Abstract:** An investigation was carried out in order to study the composition of the endophytic community of olive (*Olea europaea* L.) in Sicily (Italy). One olive-yard in San Cipirello (Palermo) and another one in Racalmuto (Agrigento) were sampled, similar for plant age, cultivars and agricultural management. Isolation assays were carried out on samples collected from each locality in spring, summer and autumn during both 2007 and 2008.

Numbers of fungal and bacterial isolates differed between the sampled sites. Prevailing fungal genera in almost all samplings were *Alternaria*, *Cladosporium*, *Diplodia*, *Phoma*, *Septoria*, *Stemphylium* and its teleomorph *Pleospora*. Isolation frequencies were dependent on the sampling site. Our preliminary results show a constant composition of endophytic assemblage of *O. europaea* in Sicily, even if the degree of infection varies depending on both geographical and environmental factors. Further studies will be carried out in order to complete fungal and bacterial identification and to analyse the interactions between endophytes, host and environment.

**Key words:** *Olea europaea*, fungi, bacteria, endophytism.

**Introduction**

Endophytes are microorganisms living all or part of their life-cycle asymptotically inside plants, even though, after incubation or a latency period, they may cause diseases (Rakotoniriana *et al*. 2008). They may establish a variety of interactions with their hosts, ranging from mutualism to commensalism and antagonism (Schulz and Boyle 2005). The stability or the variability of the asymptomatic interaction depends on numerous factors such as environmental stresses, host senescence, endophyte virulence and host defense response (Schulz *et al*. 1999). Endophytic infection may result in advantages to the host plant such as enhanced vegetative growth, drought tolerance, higher resistance to insects and herbivores and protection against pathogens (Rakotoniriana *et al*. 2008).

Many studies have been carried out on endophytes of agricultural crops, particularly on endophytic fungi, considering their assemblages *in planta*, interactions with host plants, production of secondary metabolites, and potential in biological control of pests and diseases (Wang *et al*. 2007).

This research was undertaken in order to investigate the composition of endophytic microflora of *O. europaea* L. in Sicily and to evaluate its variability in time and space.

**Materials and methods**

One olive-yard in San Cipirello (Palermo, Northern Sicily) and another one in Racalmuto (Agrigento, Southern Sicily) were investigated. Similar plant age, cultivars (Biancolilla, Nocellara del Belice) and agricultural management were studied at both sites. In both sites healthy trees were present together with plants showing different degrees of foliar chlorosis,
sometimes associated with necrotic spots and withering twigs starting from their apex.

In 2007 and 2008 seasonal epidemiological investigations were carried out to evaluate the incidence of both symptoms and its devolopment. At the same time, samples of both asymptomatic and symptomatic organs were randomly collected and processed within 24 h.

Prior to surface sterilization, leaves and twigs were thoroughly washed in running tap water to remove dust particles from their surfaces and dried with blotting-paper. Thereafter, leaves were surface sterilized (5 min 5% NaClO, 1 min and 30 s 95% EtOH, 1-3 min 5% H2O2, rinse in H2O), small segments of approximately 2 × 2 mm were cut and sets of seven-nine segments per leaf were plated in Petri dishes containing 2% malt extract agar (MEA). As regards twigs, after flame-sterilization and de cortication, segments of approximately 2 × 2 mm were sterilely cut and placed on 2% MEA in Petri dishes; seven fragments were plated per dish. All the dishes were incubated at 20 ± 1 °C. Fungi and bacteria growing out from the plant fragments were individually subcultu red on 2% MEA and 1.5% Plate Count Agar (PCA), respectively. The efficacy of the foli ar sterilization procedure was ascertained following the method of Schulz et al. (1998).

The overall colonization rate (CR) was calculated as the total number of tissue segments yielding ≥1 isolate divided by the total number of tissue segments incubated. The isolation frequency (IF) of a single endophyte was calculated as the number of segments from which the endophyte was isolated divided by the total number of segments incubated per organ ×100. The species diversity was calculated by the Shannon index, and two-way ANOVA was used to test whether there were significant differences ($P < 0.05$) in CR between different seasons and localities. All statistics were calculated with Microsoft Excel for Windows version 2003.

Fungal isolates were identified on the basis of both macro- and microscopic features, by means of an appropriate atlas (Barnett 1965; Watanabe 2002) and identification keys (Goidanich 1994). Bacterial identification was carried out by microscopic observation and physiological tests (Scortichini 1995).

**Results and discussion**

A total of four epidemiological assays were carried out from spring 2007 to spring 2008. In the olive-yard of S. Cipirello, chlorosis incidence varied strongly over the course of the year, reaching the lowest value (12.8%) in spring 2007 and the highest (97.9%) in the following summer; the incidence of twigs withering increased progressively from 6.4% (first assay) to 46.8% in autumn, then decreasing to 36.2%. In Racalmuto incidences of the two symptomatologies showed the same trend, reaching the highest value in spring 2008 (89.2% chlorosis, 90.8% twigs withering) (Fig. 1a). Correlation between symptoms was medium ($R=0.45$) in the first case and high in the second one ($R=0.96$).

The numbers of isolated endophytes were different in relation to the sampling site and season. The overall colonization rates of endophytes were always higher in leaves than in twigs; the CR of leaves reached the highest value in autumn 2007 in S. Cipirello (0.78) and in the spring of the same year in Racalmuto (0.92); in twigs the highest CR occurred in spring and autumn 2007, respectively (Fig. 1b). No significant differences in CR between different seasons and localities were observed. In plants of S. Cipirello the Shannon index showed a similar diversity in endophytic assemblages of twigs and leaves ($H'=0.95$), whereas in Racalmuto species diversity was higher in leaves ($H'=0.97$) than in twigs ($H'=0.88$). Endophytic assemblages in olive-trees showed a similar composition at both sites.
Figure 1. a) Seasonal incidences of foliar chlorosis and withering twigs in San Cipirello (Sc) and Racalmuto (Ra); b) colonization rates of endophytes in different tissues of O. europaea collected in different seasons in San Cipirello (Sc) and Racalmuto (Ra).

All fungal isolates (Table 1) were divided into 56 morphological groups, 33 of which were identified at genus level, whereas 19 were unidentified due to sterility and the remaining 4 (2 Ascomycetes and 2 Coelomycetes) due to contamination or death of cultures. Considering both the incidence and the IF% of each endophyte, the most common genera were Alternaria, Cladosporium, Phoma, and Septoria followed by Camarosporium spp., Stemphylium and its teleomorph Pleospora. Fungal genera commonly associated with foliar symptoms were Alternaria, Cladosporium and Phoma, followed by Septoria, Diplodia and Camaropsorium. Endophytic bacteria were detected mainly from twigs. Two genera were identified so far: Bacillus spp. and Pseudomonas spp.

Even if the IF of the detected endophytes varied with organ (organ specificity or preference), season and sampling site, our results show a certain degree of recurrence of some genera in tissues of the host species. This could suggest a characteristic endophytic community of O. europaea with a relatively constant composition regarding the prevalent genera. Moreover, leaves seemed to harbour a more diverse fungal assemblage. Further studies will be carried out in order to complete fungal and bacterial identification at the species level and to investigate the interaction between endophytic microorganisms, host and environment.
Tab. 1. Isolation frequencies (%) of endophytes detected in twigs (T) and leaves (L) of *O. europaea* in San Cipirello (Sc) and Racalmuto (Ra).

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<td>1.5</td>
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<td>2.5 4.0</td>
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<td>0.2 0.3 4.2</td>
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<td>- 0.5 2.0 15.7 3.2 14.6</td>
<td>- 1.5 5.9 0.6 3.2</td>
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<td>- 0.3 1.7 0.4 5.2</td>
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<td>8.5 3.2</td>
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<td>1.8 0.6 31.8</td>
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Population variability of strawberry powdery mildew (Podosphaera aphanis) in different geographical regions

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Abstract: Strawberry powdery mildew, caused by Podosphaera aphanis, is one of the most important diseases worldwide. Mildew lesions were sampled from a number of cultivars at several sites in the UK; a limited number of lesions was also sampled from China, the USA, Italy and Israel. SSR markers were developed and used to genotype sampled isolates for determining population variability; the ITS region of 20 samples selected from different countries was sequenced. Both SSR and ITS data indicated that there were significant differences between samples from the USA and the other countries. In the UK, there was significant population differentiation between mildew samples from different cultivars at the same sites, or between mildew samples from the same cultivar at different sites.

Keywords: rDNA ITS, SSR, population differentiation, strawberry, powdery mildew

Introduction

Powdery mildew, caused by Podosphaera aphanis Braun & Takamatu (= Sphaerotheca macularis (Wallr.:Fr.) Lind = S. humuli (DC.) Burrill), can infect leaves, leaf petioles, flower trusses, flowers and fruit, and is a serious disease of strawberries (Fragaria x ananassa) (Miller et al. 2003; Blanco et al. 2004; Amsalem et al. 2006). Increasingly, both June-bearing and everbearing strawberries are being grown under protection, which has led to more severe epidemics than under open field conditions (Xiao et al. 2001). Lack of cultivars with durable resistance necessitates routine applications of fungicides. Breeding for durable resistance to mildew is hampered by observations that resistance is not consistent over time or vary greatly with environmental conditions (Mcnicol and Gooding 1979; Nelson et al. 1995).

Population variability of powdery mildews in general has been little studied due to the fact that there is insufficient fungal genomic DNA of suitable quality for molecular analysis because of its obligate biotrophic nature. Molecular markers can be used to quantify fungal population variation in relation to host and geographical location for studies of fungal population genetics, ecology and evolution (Xu 2006; Bonin et al. 2007; Dutech et al. 2007). Such information can then be used to infer the underlying evolutionary forces acting on the population and, hence, to estimate the likely spread of new traits, such as virulence or fungicide insensitivity, into the population. For example, molecular techniques were used to investigate sources of overwintering inoculum (Miauzzi et al. 2003; Cortesi et al. 2005; Núñez et al. 2006) and mating types (Peros et al. 2005) in grape powdery mildew, and race structure in apple mildew (Urbanietz and Dunemann 2005).

Currently, there is limited knowledge on population variability of strawberry powdery mildew. In one study, differences in the sensitivity to control agents and in the ITS sequences between a small number of mildew samples from Italy and Israel were assessed (Pertot et al. 2007). The two sampled populations responded similarly to those tested control agents and were nearly identical (99-100% similar) based on sequence analyses of a 359 bp fragment of the ITS 1 and 2 region and 6 random fragments totalling 2800 bps. Another study suggested that there were no indications of significant isolate × cultivar (race-specific) interactions but isolates appeared to differ considerably in their virulence (Xu et al.).
This paper reports a study aiming to determine population variability of *P. aphanis* in relation to host cultivars and geographical regions in the UK. In addition to samples from the UK, a number of mildew samples were also obtained from China, the USA, Israel, and Italy. Several SSR markers were developed; but only two were successfully used to genotype field samples. Based on the SSR results, a number of mildew samples were selected from different regions for ribosomal DNA ITS sequencing for further comparison.

**Materials and Methods**

**Sampling mildew isolates:**
It was not possible to sample mildews based on a hierarchical design, i.e. from many cultivars at many sites, because (1) very few common cultivars were grown at different sites and (2) powdery mildew may not have developed on those varieties of interest at all sites. Instead, the following sampling plan was used to sample leaves with mildew lesions in the UK from June to August 2006: samples were obtained from (1) cv. Elsanta (the most popular June-bearer) at six sites, and (2) several cultivars at two sites (Table 1). Many samples were also obtained from a single site in California, USA, and Qingdao, China in May-June 2007. In addition, a few samples were also obtained from Italy (Trentino) and Israel (Volcani Centre).

In order to remove plant DNA whilst increase mildew DNA, a leaf disc (diameter of 0.5 cm) with a single lesion was cut in the field and placed immediately into a centrifuge vial containing 2 ml of 95% ethanol. The vials were then shaken manually for 10-20 seconds and kept under ambient conditions for 24 h before the disc was removed and the vial was left open for ethanol to evaporate. For Italian and Israeli samples, leaves with lesions were taken, dried under ambient conditions and shipped. Discs with a lesion were then cut out from these dried leaf samples and treated with ethanol as described.

**DNA extraction**
600 μl cell lysis solution (5mM TRIS, 10mM EDTA, 0.5% SDS) and 5 μl of 20 mg/ml proteinase K was added to each dried sample vial. Tubes were incubated overnight at 55°C and vortexed vigorously for 1 min. After samples were cooled to room temperature, 200 μl of ice-cold protein precipitation solution (3 M ammonium acetate) was added and tubes centrifuged at 13000 rpm for 10 min. The supernatant was decanted and mixed with 600 μl isopropanol, and precipitate centrifuged at 13000 rpm for 10 min. The pellet was washed in 70% ethanol and again centrifuged at 13000 rpm for 10 min. The pellet was air-dried and re-suspended in 50 μl water.

**SSR isolation and sample genotyping**
Development of microsatellites followed an enrichment protocol previously developed for fungi (Harvey 2006), which produces a 30-40% enriched library. The enrichment led to development of six SSR primer pairs that produced unambiguous polymorphic markers in initial mildew screens. PCR annealing temperature is 60°C for all six primer pairs. However, only two pairs (coded SM4 [(GT)9] and SM10 [(AC)18]) produced consistent band patterns for field samples whilst others produced many null alleles. Each forward PCR primer was tagged with a different fluorescent dye: SM4F [6FAM-CTAGCTTTGCAACACTCGAGGT] (SM4R [TGTAGGTATTGGGAAAAGTCGG]), and SM10F [6FAM-CCTGGCTAAACACAGACGATAA] (SM10R [TTCACATGTAGACCATCCTTG]). Fluorescently labelled products were analysed on a semi-automated ABI 3100 sequencer. Data were collected and allele sizes determined using GENESCAN and GENOTYPER software (Applied Biosystems).
ITS sequencing
The ITS regions of 20 mildew samples, selected from different regions, were sequenced. The universal primers Ek18F (AGAGGAAATGAAAGTCAACAAG) and Ek28R (ATATGCCATGTCAGCGGG) were used to PCR across the entire ITS1 and ITS2 regions from at least 3 distant samples from each country. Bands were cut from gels, cloned and sequenced, and the sequences matched to the GenBank database using the BLASTN program (http://www.ncbi.nlm.nih.gov/blast).

Data analysis
A total of 484 samples were screened for SM4 and SM10. In many cases, two alleles were recorded at the same locus on a single leaf disc sample; this was assumed to have resulted from two fungal genotypes that colonised the same disc. In a few cases, two alleles were recorded at two SSR loci; only two fungal genotypes were assumed to be present, randomly selected from possible four genotypes. Any alleles with an overall frequency less than 1% were excluded.

A single population was defined as samples from a single combination of site and cultivar. Analysis of molecular variance (AMOVA) was used to test whether were significant differences in fungal populations among the UK, China and the USA, among the six sites in the UK (cv. Elsanta), and among cultivars at the same site. The significance of population differentiation was based on 10,100 permutations. Pairwise comparisons of populations (based on $G_{ST}$) were assessed on the basis of 1023 permutations. Arlequin version 3.1 (Excoffier and Schneider 2005) was used to carry out these analyses. Relationships among fungal populations were further examined with cluster analysis using UPGMA (Sneath and Sokal 1973) with MEGA software (Tamura et al. 2007). Nei’s $D_A$ index (Nei et al. 1983) was used as a genetic distance measure for cluster analysis: $D_A = \frac{1}{r} \sum_{j=1}^{r} \left( 1 - \sum_{i=1}^{m_j} \sqrt{x_{ij}y_{ij}} \right)$, where $x_{ij}$ and $y_{ij}$ are the frequency of the $i$th allele at the $j$th locus in the two populations respectively, and $r$ and $m_j$ is the total number of loci and number of alleles at the $j$th locus, respectively. $D_A$ is less affected by the diversity within populations than $G_{ST}$ is.

All ITS sequences from the selected mildew samples were compared with those of Sphaerotheca or Podosphaera in GenBank before being included in further analysis. Relationships among the sequences were analysed using MEGA4 (Tamura et al. 2007) based on aligned 479 bp. Four sequences from GenBank were included in the analysis: DQ139429 (P. pannosa isolate from host Prunus laurocerasus), DQ139433 (P. pannosa isolate from host Rosa sp.), AB026136 (P. aphanis from host F. grandiflora in Japan- (Takamatsu et al. 2000)) and AF073355 (P. aphanis from host F. x ananassa (= F. grandiflora) in Victoria, Australia - (Cunnington et al. 2003)). Both parsimony and neighbour joining analyses were performed with inserts and deletions included.

Results
General summary of SSR data
Of 484 mildew samples screened, 390 produced reliable band patterns for SM4 and SM10. There were 44 and 39 samples where two different alleles were present in the same sample for SM4 and SM10, respectively. Of these 83 samples, 13 had two different alleles for both SM4 and SM10: 12 were from cv. Elsanta at three sites in the UK. For SM4, there were four different alleles, representing 13, 20, 21 and 23 repeats. For SM10, there were six different alleles, representing 9, 12, 13, 14, 15 and 16 repeats; the first two alleles only occurred in a few samples and hence were excluded from further statistical analysis.

Table 1 gives the summary of allele frequencies for 15 populations. For SM4, the
The predominant allele is B (20 repeats), with an overall frequency of 77%. This was true across all populations except the samples from California and cv. Yixiang of China. This is the only allele detected in four populations (Table 1): two from China and two from the UK. Allele A (14 repeats) was found mostly in the samples from California; alleles C (21 repeats) and D (22 repeats) were found mostly in samples from cv. Elsanta at Ullingswick in the UK and cv. Yixiang of China, respectively (Table 1). For SM10, the predominant allele is B (14 repeats) across all populations, with an overall frequency of 84%. In five populations, only allele B was detected (Table 1). In three populations, only a single allele was detected at both SM4 and SM10: cvs. Fenxiang and Qinxiang (China), and on plants of trial 1159 at Wisbech (UK). All six Italian samples had the same genotype for SM4 and SM10 (BB) as did those 23 Israeli samples (BD).

Table 1 Summary of origins of strawberry powdery mildew (*P. aphanis*) samples and allele frequencies of the two microsatellite loci

<table>
<thead>
<tr>
<th>Region</th>
<th>Cultivar</th>
<th>Location</th>
<th>No samples</th>
<th>SM4</th>
<th>SM10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Latitude</td>
<td>Longitude</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>USA</td>
<td>California</td>
<td>Chandle  r</td>
<td>17</td>
<td>0.94</td>
<td>0.06</td>
</tr>
<tr>
<td>China</td>
<td>Qingdao</td>
<td>Fenxiang</td>
<td>23</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Qingdao</td>
<td>Qinxiang</td>
<td>21</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Qingdao</td>
<td>Yixiang</td>
<td>39</td>
<td>0.00</td>
<td>0.49</td>
</tr>
<tr>
<td>UK</td>
<td>Ightham</td>
<td>Elsanta</td>
<td>51:17:06N</td>
<td>0:16:28E</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Ightham</td>
<td>Opal</td>
<td>51:17:06N</td>
<td>0:16:28E</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Ightham</td>
<td>Pearl</td>
<td>51:17:06N</td>
<td>0:16:28E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Peterboro ugh</td>
<td>Elsanta</td>
<td>52:28:37N</td>
<td>0:21:47W</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Takely</td>
<td>Elsanta</td>
<td>51:48:50N</td>
<td>0:16:07E</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>West Peckham</td>
<td>Elsanta</td>
<td>52:08:37N</td>
<td>0:23:37W</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Ullingswick</td>
<td>Elsanta</td>
<td>51:15:08N</td>
<td>0:21:14E</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Wisbech</td>
<td>Elsanta</td>
<td>52:37:15N</td>
<td>0:09:58E</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Wisbech</td>
<td>T1159a</td>
<td>52:37:15N</td>
<td>0:09:58E</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Wisbech</td>
<td>T1276a</td>
<td>52:37:15N</td>
<td>0:09:58E</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Wisbech</td>
<td>T931a</td>
<td>52:37:15N</td>
<td>0:09:58E</td>
<td>31</td>
</tr>
</tbody>
</table>

*Note:* Breeding lines not commercially released yet.
AMOVA
AMOVA indicated significant ($P < 0.001$) differences among samples from China, the UK and the USA ($P < 0.001$), accounting for 23.8% of the total variation. Separate AMOVA for Chinese samples showed that samples from cvs. Fenxiang and Qinxiang differed ($P < 0.001$) from samples from cv. Yixiang. Similarly, samples from three cultivars at Ightham or four cultivars at Wisbech in the UK differed significantly ($P < 0.001$), both accounting for about 8.3% of the total variation. However, there were no significant differences between samples from Ightham and Wisbech. Mildew samples from cv. Elsanta differed significantly ($P < 0.001$) among the six sites in the UK, accounting for 5.0% of the total variation. Samples from China (cv. Yixiang) and the USA differed significantly from all other populations based on pairwise comparison of $G_{ST}$. The dendrogram based on $D_A$ is shown in Fig. 1. California isolates appeared to be most distant from all other isolates whilst isolates from the UK and China are well mixed together.

![Dendrogram](image)

Figure 1. A dendrogram derived from a cluster analysis of molecular data of two SSR loci depicting the observed differences between 15 populations of *P. aphanis* using the UPGMA method (Sneath and Sokal 1973). The optimal tree with the sum of branch length = 0.655 is shown, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007) using the Nei $D_A$ genetic distance (Nei et al. 1983).

**ITS analysis**
Since SSR results suggested significant genetic differences among isolates from the USA, China and the UK, 20 samples were selected for sequencing of the rDNA ITS region to confirm these differences. All the samples produced a sequence of either 588 bp or 589 bp, which closely matched *Sphaerotheca* or *Podosphaera* in GenBank. The topology produced is the same for both distance and parsimony-based analyses using the neighbour joining
algorithm (Fig. 2). Outgroup sequences comprised two GenBank sequences - DQ139429 and DQ139433 (both *P. pannosa*). Two GenBank sequences (AB026136 and AF073355 – both *P. aphanis* from Japan and Australia, respectively) formed an ingroup with the new sequences, relative to the outgroup sequences.

All strawberry samples formed a single well-supported group, with the exception of the three Californian samples and AF073355 (Fig. 2). These differences represent two transitions and a single base insertion or deletion. Among the main strawberry group, one sequence (the same as AB026136) existed in 12 samples, including samples from the UK, China, Israel and Italy. Five other sequences also existed in this group, each consisting of two unique substitutions, with the China P2 sample also having a unique single base deletion. California P5 has 2 unique substitutions.

Figure 2. A bootstrapped neighbour joining tree produced from 479 bp of the ITS1 and ITS2 sequences from strawberry mildew (*P. aphanis*). Sample names specify the location from which the sample was isolated.
Discussion

Both SSR and ITS sequencing data suggested that there were significant differences in samples from different countries, particularly those from the USA compared with those from Eurasia (the UK, Italy, China and Israel). Nevertheless, there were one or more UK populations that were not significantly different from the California population in pairwise comparisons. However, the present finding has to be treated with caution because (1) only two SSR markers were used and (2) only a limited number of samples were from China, the USA, Israel and Italy. Based on a limited number of samples, Italian and Israeli samples of strawberry powdery mildew were shown to be nearly identical (Pertot et al. 2007).

There is some evidence for fungal adaptation to host cultivars. In the UK, at both sites there were significant differences in the mildew samples from different cultivars. Similarly, the Chinese populations from two cultivars at the same field were identical in the two loci but differed from the third population on a different cultivar at the same site. Mildew samples from the two UK sites also did not differ significantly, indicating that adaptation to host cultivars takes place at the two sites but has not led to appreciable population differentiation yet between them. However, a recent study indicates the lack of strong race-specific interactions between strawberry cultivars and powdery mildew (Xu et al. 2008). Therefore, such adaptation to host cultivars may have not lasted long enough to result in any significant consequences on fungal pathogenicity. Alternatively, the lack of race-specific interactions could also have resulted from regular introduction of new cultivars, leading to short co-adaptation time. Research with more samples and/or more molecular markers is needed to further our understanding on this. There is also some evidence of genetic differentiation among different regions based on the mildew samples from cv. Elsanta in the UK. This could result from genetic drift and/or founder effect.

ITS sequences have been used consistently as an accurate tool for differentiating between populations and species of certain fungi in situations where classical morphology cannot be utilized. For the well-defined powdery mildew taxa identified using morphology or host range, the differences in rDNA ITS sequences are always identical or within 99% similar within each taxa (Saenz and Taylor 1999; Hirose et al. 2005; Cook et al. 2006; Kiss et al. 2006; Inuma et al. 2007). In the present study, there was more than 99% similarity in the ITS sequence among P. aphanis samples with one common sequence occurring in 12 of 20 samples, suggesting there were from a same taxa. Similarly, in a study with a limited number of samples, several P. aphanis samples from Israel and Italy were shown to have an identical ITS sequence whereas other pairs showed 99% sequence similarity (Pertot et al. 2007). As in the present study, they found the identical ITS sequences between their strawberry samples and those from strawberry in Japan - AB026136 (Takamatsu et al. 2000). However, it was also recently suggested that morphologically indistinguishable powdery mildews that differ in one to five single nucleotide positions in their ITS region can be considered as different taxa with distinct host ranges (Jankovics et al. 2008). Based on this judgement, mildew from China and the USA could be considered as a separate species. Further studies are necessary to resolve this taxonomy issue, particularly by cross-inoculation studies.

Acknowledgements

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Evaluation of fruit genetic resources for disease resistance

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Abstract: A field survey throughout Switzerland established an inventory of fruit genetic resources. The decentralised collections network was subsequently completed and the characterization of the accessions is ongoing. Considering international standards, guidelines for the phenotypic description of the fruit genetic resources were developed and practically applied. Apple accessions from the Swiss pool of genetic resources were tested for fire blight (Erwinia amylovora) resistance in the greenhouse. Results of the fire blight screening confirm significant differences between accessions. Additionally, the accessions were analysed with SCAR markers surrounding a QTL for fire blight tolerance. Moreover, young trees of 600 accessions were planted to evaluate their scab (Venturia inaequalis) and powdery mildew (Podosphaera leucotricha) resistance in a field trial. The project aims at defining accessions useful for cultivation as standard trees for cider and juice production and that are an important landscaping and ecological factor. Promising accessions are being used for breeding.

Key words: fruit genetic resources, fire blight, Erwinia amylovora, breeding, apple

Introduction

In close collaboration with NGO’s the Swiss government is supporting and implementing structures for a sustainable conservation and utilization of plant genetic resources. The national campaign plant (NCP) is coordinated by the Swiss Commission for the conservation of plant genetic resources (www.cpc-skek.ch). Kellerhals and Egger (2004) reported about a field inventory carried out to detect as many still existing Swiss fruit genetic resources as possible. This inventory allowed subsequently a complete and safe conservation of fruit genetic resources in Switzerland. 2800 accessions were selected for long term conservation or further testing.

Here we report on the characterization of the rich fruit genetic resources in order to allow for their use in fruit-growing and breeding. Around 800 accessions are being described and scientifically photographed within the project. Further evaluation of selected accessions includes the testing of 600 apple accessions for their scab and mildew susceptibility in the field, the testing of 160 apple and pear accessions for their fire blight susceptibility in the quarantine glasshouse and the molecular characterization of at least 250 apple and 250 cherry accessions with SSR-markers.

Fire blight is one of the most serious disease and causes great losses in pome fruit cultivation. It is also a threat for genetic resources collections (Peil et al., 2004). Among the Malus genetic resources a broad range of resistance levels towards fire blight can be found (Aldwinckle et al., 1976). Since fire blight first appeared in Switzerland in 1989, it has steadily spread from the original epicentre in the north-eastern cantons to the south-western regions (Duffy et al., 2005). In 2007, the so far most significant outbreak took place and several of the decentralised genebank collections suffered from losses. A wide range of molecular markers related to different disease resistance genes or QTL’s is nowadays available for apple scab, mildew and fire blight resistance (Calenge et al., 2005, Khan et al., 2007). In this study we included molecular markers related to a fire blight resistance QTL.
Materials and methods

The screening of heritage varieties for relative fire blight tolerance was conducted in the quarantine glasshouse of ACW in Wädenswil. Scion material was grafted onto M9 rootstock. In spring 2007 and 2008, trees were planted in plastic deep-pots 60 cm from Stuewe & Sons (Corvallis, US) with a length of 35.5 cm and diameter of 7 cm and then grown in the glasshouse for several weeks prior to inoculation. For each variety, 6 to 10 replicate trees were inoculated by puncturing the distal tip of shoots 15-30 cm long with a syringe containing an *E. amylovora* solution of $10^6$ cfu/ml. In 2007 strain CFBP1430 (INRA, France) and in 2008 strain FAW 611 (ACW, Switzerland) was used. Disease development was evaluated weekly for three weeks by measuring the expansion of the necrotic lesion from the shoot tip in relation to the total shoot length. Artificial fire blight infections were performed in 2007 and 2008 each with 40 different accessions, including heritage varieties still grown for apple juice and cider production, and with commercial standard varieties. 39 apple accessions tested in 2008 were analysed for the presence or absence of molecular markers flanking a fire blight resistance QTL which was described by Calenge et al. (2005) and Khan et al. (2007). The two SCAR markers AE 10-375 and GE-8019 were used to spot accessions that carry both of these markers and conclusively also the fire blight resistance QTL. Molecular analysis was performed according to methods described by Frey et al. (2004).

Results

The results of fire blight testing in the glasshouse revealed a wide range of tolerance and susceptibility among the apple accessions tested in 2007 and 2008 (results with 30 out of totally 40 accessions tested in 2008 are presented in Fig. 1).

![fire blight glasshouse screening of 30 traditional apple cultivars with 'Gala' as standard, year 2008 (Scoring 3 weeks after inoculation, Number of plants in brackets, bars represent standard deviation).](image)

Some accessions displayed good resistance whereas others displayed no resistance and were highly susceptible. The apple variety ‘Schneiderapfel’ was highly resistant in our trials. Unfortunately ‘Schneiderapfel’ is a triploid cultivar and can therefore hardly be used in breeding as a parent. Other cultivars such as Ohio Reinette and Danziger Kantapfel are being used in crosses to develop new apple cultivars with high fruit quality and increased fire blight
tolerance. Comparison of the fire blight test results with the same accessions performed in 2007 and 2008 revealed a satisfactory reliability of the test, although two different *E. amylovora* strains had been used Fig. 2.

![Figure 2: comparison of fire blight glasshouse screening with selected cultivars tested in 2007 and 2008 (Scoring 1, 2 and 3 weeks after inoculation).](image)

Both SCAR markers AE and GE and therefore the resistance QTL was present in 4 out of 39 tested accessions, namely Dettighofer, Bernecker Wildling, Schweizer Orangenapfel and Sternapi. These accessions had an average necrosis length of 39.8 % of the total shoot length compared to 44.5 % for the average of the accessions carrying none or only one of the flanking SCAR markers.

**Discussion**

Forsline and Aldwickle (2002) have screened the USDA Apple Collection at Geneva N.Y. (USA) including apple germplasm from Asia and Europe for natural occurrence of fire blight. Considerable variability in resistance was observed in seedlings from almost all collections of all species. For fire blight no major resistance genes were found. However, recently Peil et al. (2007) reported evidence for a major fire blight resistance gene of *Malus robusta*. Dondini et al. (2004) found QTLs linked to fire blight in pear and Calenge et al. (2005) and Khan et al. (2007) found major additive QTLs for fire blight resistance based on work with two related apple progenies. While testing selected accessions for markers flanking the Fiesta LG7 QTL (AE and GE) in our study we detected only a slight difference in susceptibility or resistance, respectively, between the group of accessions carrying the flanking SCAR markers and the group not carrying them or only one of them. However, planned work with a larger number of accessions might show more conclusive results. The information on the differential resistance and susceptibility of the Swiss apple and pear genetic resources is important for breeding and for replanting traditional varieties. This will enable informed decision making as to which varieties to replant and which to avoid due to fire blight susceptibility. Further tests are needed to confirm these results under natural disease pressure especially related to flower infection.
Acknowledgements

We acknowledge financial support for the project NCP 03-21 by the National Campaign Plants (NCP), a Swiss Federal Office for Agriculture’s program and for the collaboration with the NGO Fructus (www.fructus.ch) and all the other project partners. Moreover, we acknowledge support by Beatrice Frey for the molecular analysis.

References


Activity of Physpe (*laminarin*) in control of strawberry diseases

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Abstract: Strawberry plants are susceptible to many pathogens, such as *Botrytis cinerea*, *Mycosphaerella fragariae* and *Sphaerotheca macularis*. Phytochemicals are intensively used to limit pathogen infections on strawberry plantations in Poland. Resistance problems, residues in fruits and protection of the environment require alternative strategies. In the present study the efficacy of Physpe (laminarin) in control of main strawberry diseases in field conditions was tested. Experiments were conducted in 2006 and 2008. Laminarin reduced *B. cinerea* infection by approximately 50 to 80%, depending on the experimental site. Its effectiveness in reduction of leaf spot symptoms was about 50% and almost 80% in reduction of powdery mildew. The use of Physpe in program with fungicides sprays could be acceptable for commercial use and gives possibilities to reduce the number of chemical treatments against main strawberry diseases.

Keywords: strawberry, grey mould, leaf spot, powdery mildew, control strategy, laminarin

Introduction. Grey mould (*Botrytis cinerea*), leaf spot (*Mycosphaerella fragariae*) and powdery mildew (*Sphaerotheca macularis*) are the most important strawberry diseases in Poland. Severity of these diseases depends on susceptibility of strawberry cultivars (Muller, 1965), weather conditions (Jarvis, 1964) and level of inoculum. Some strawberry cultivars commonly planted in Poland, like Senga Sengana, Kent, Malling Pandora, Kama are seriously affected by *M. fragariae* and others, Elsanta, Marmolada, Honeoye and Camarosa, by *S. macularis* (Meszka and Bielenin, 2007, Łabanowska et al., 2004). The most susceptible cultivars to grey mould are Senga Sengana, Marmolada and Pegasus cultivars. Control of strawberry diseases has still been based mainly on chemicals, but resistance problems, residues in fruits and protection of the environment require alternative strategies (Aziz et al., 2003). They involve biological agents as antagonists of pathogens or activation of plant defense mechanism using elicitors, such as laminarin (Physpe). Laminarin (linear β-1,3 glucan) is a product extracted and purified from the brown alga *Laminaria digitata* (Aziz et al., 2003). Application of this compound causes a stimulation of the natural resistance of plants against diseases.

The aim of this research was to evaluate Physpe (laminarin) efficacy for control of main strawberry diseases in field conditions.

Materials and methods

Physpe was tested on commercial strawberry plantations in 2006 and 2008. The experiment was conducted on Kent and Senga Sengana cultivars for grey mould and leaf spot control and Marmolada for powdery mildew control. Depending on disease, different standard fungicides were used: piraclostrobin+boscalid (Signum 33 WG) and folpet (Folpan 80 WG) against grey mould, tetraconazol (Domark 100 EC), tiophanat methyl (Topsin M 500 SC) and trifloxystrobin (Zato 50 WG) against powdery mildew and leaf spot. Tested product - Physpe and standards, Domark 100 EC and Folpan 80 WG, were applied in two programmes: I - 3-treatments during blossom and II - 6 treatments (3 during blossom and 3 before harvest). Other standard fungicides were used only during blossom. Applications were made at 5-7
days intervals, using a motor knapsack sprayer at 600 l/ha. The biological effectiveness of control of strawberry leaf spot and powdery mildew was evaluated based on the number of affected leaves (100 leaves in each of four replications) and intensity of disease symptoms, using 6-degree scale, for leaf spot evaluation: 0- healthy leaf, 1- (up to 1% of leaf surface with spots), 2- (1-5%), 3- (5-20%), 4- (20-50%) and 5- (above 50%) and for powdery mildew: 0- healthy leaf, 1- (up to 10% of leaf surface with spots), 2- (10-20%), 3- (20-50%), 4- (50-80%) and 5- (above 80%). Grey mould infestation was evaluated during harvest, based on the number of affected fruits, both in field and after 5 days of storage at 4.5°C. One hundred fruits from each from four replicates, were randomly sampled from each plots, twice during the harvest. The total yield was calculated for each plot.

Results and discussion

In 2006, Physpe used at two doses, 1.0 and 2.0 l/ha, gave good control of all tested diseases. The intensity of grey mould on the non-protected strawberry plants of Kent cv. was medium and total number of infected fruits, in the field and after storage in 4,5°C, was 21.5%. The effectiveness of laminarin in control of grey mould was 80% and 89%, respectively, for the two doses (Figure 1a). In 2008 the grey mould on the control strawberry plants of Senga Sengana cv. was observed on 11-44% of fruits, depending on the location (Figure 1b). The effectiveness of Physpe used at 1.0 l/ha in control of grey mould was from 60-90% after 3 treatments and from 83-97% after 6 treatments, depending on the plantation (Figure 1b). Additional 3 applications of Physpe before harvest increased effectiveness only, when the severity of grey mould was high. Yield obtained from plots treated with Physpe was about 15-35% higher than from control plots and the same as from plots treated with standard fungicides. Effectiveness of Physpe in control of grey mould was similar as all standard fungicides on plantation I were lower than standards on plantation II and the same as Folpan 80 WG but lower than Signum 33 WG on plantation III (Figure 1b). Aziz et al. (2003) noted that laminarin reduced the development of *B. cinerea* on grapevine by approximately 50 and 75%, depending on the dose used. Laminarin has also protected tobacco from soft rot disease, *Erwinia carotovora* (Klarzyński et al., 2000).

In our study Physpe gave good control of strawberry diseases. In 2006 it effectively reduced leaf spot (59%) and powdery mildew (69%), when intensity of both diseases was high. On control plants 58% of leaves were affected by *M. fragariae* and 54% by *S. macularis* (Table 1).

In 2008, the severity of powdery mildew was lower. On untreated plants symptoms were observed on 36% of leaves (Table 1). Tested product showed good efficacy in control of disease on cv. Marmolada, ~80% after 3 treatments and 81% and 89% (depending on location) after 6 treatments. Laminarin, as showed by Aziz et al.(2003), also reduced the severity of downy mildew of grapevine (*P. viticola*). They noted that compound used at 0.5-1.0 g/l reduced the percentage of affected leaf surface from 28% on control plants to 7% on laminarin treated plants.

In 2008 the intensity of leaf spot on the non-protected strawberry plants of Senga Sengana cv. was medium to high, 47% and 74% of affected leaves, respectively for I and II location. Physpe treatments reduced leaf spot severity by 50% (Table 1). The results of several studies indicated that laminarin could be effective against a wide spectrum of plant pathogens.
Figure 1. Efficacy of Physpe in control of grey mould on strawberry of Senga Sengana cv.

a) 2006 season

b) 2008 season

Conclusions

1. Physpe showed good effectiveness in control of grey mould and powdery mildew and reduced intensity of leaf spot symptoms.
2. Efficacy of Physpe was not evidently increased with higher spray frequency.
3. The use of Physpe in control of strawberry diseases can enable the reduction of chemical control in strawberry protection programmes.

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Table 1. Efficacy of Physpe in control of strawberry leaf spot and powdery mildew

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Rate per 1 ha</th>
<th>Leaf spot</th>
<th>Powdery mildew</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Percent of affected leaves</td>
<td>Effectiveness in %</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>57.5 c</td>
<td>-</td>
</tr>
<tr>
<td>Physpe (3 sprays during blossom + 3 before harvest)</td>
<td>1.0 l</td>
<td>23.4 b</td>
<td>59.3</td>
</tr>
<tr>
<td>Domark 100 EC-standard (3 sprays during blossom)</td>
<td>0.6 l</td>
<td>0.1 a</td>
<td>99.8</td>
</tr>
<tr>
<td>Domark 100 EC-standard (3 sprays during blossom)</td>
<td>0.6 l</td>
<td>0.1 a</td>
<td>99.8</td>
</tr>
<tr>
<td>Domark 100 EC-standard (3 sprays during blossom + 3 before harvest)</td>
<td>0.6 l</td>
<td>0.1 a</td>
<td>99.8</td>
</tr>
<tr>
<td>Zato 50 WG- standard (3 sprays during blossom)</td>
<td>0.25 kg</td>
<td>17.8 bc</td>
<td>62.3</td>
</tr>
<tr>
<td>Topsin M 500 SC –standard (3 sprays during blossom)</td>
<td>2.5 l</td>
<td>24.0 c</td>
<td>49.2</td>
</tr>
<tr>
<td>Domark 100 EC-standard (3 sprays during blossom)</td>
<td>0.6 l</td>
<td>14.5 a</td>
<td>80.3</td>
</tr>
<tr>
<td>Domark 100 EC-standard (3 sprays during blossom + 3 before harvest)</td>
<td>0.6 l</td>
<td>11.9 a</td>
<td>83.8</td>
</tr>
<tr>
<td>Zato 50 WG- standard (3 sprays during blossom)</td>
<td>0.25 kg</td>
<td>18.7 a</td>
<td>74.6</td>
</tr>
<tr>
<td>Topsin M 500 SC –standard (3 sprays during blossom)</td>
<td>2.5 l</td>
<td>34.2 b</td>
<td>53.5</td>
</tr>
</tbody>
</table>

* Means in columns followed by the same letter do not differ at 5% level of significance according Newman-Keuls test
Prediction of Xanthomonas arboricola pv. pruni infection on peaches

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Abstract. X. arboricola pv. pruni (Xap) is present on Prunus spp. in some European countries, and it is listed as an A2 quarantine pest by EPPO; its importance in Northern Italy has increased in the last decade. An empiric model predicting Xap infection has been developed in late ‘90s. Occurrence of the first seasonal infection was monitored in peach orchards of Romagna, in 1992 to 2008, and compared to model predictions: an infection was predicted when there were at least 3 successive rainy days, with temperature between 14 and 19°C; symptom’s onset was expected after one to four weeks of incubation. Xap symptoms appeared in 10 out of 17 years; first seasonal symptoms become visible between 19 May and 12 July. These infections were always correctly predicted by the model, with an average incubation period of three weeks. Five infection periods were predicted by the model that did not result in actual infection. In five years the disease did not appear at all. In four of these years the model did not predict infection all season long, while in one year it wrongly predicted two possible infection periods. Sensitivity, specificity and accuracy of the model showed that one would have somewhat more confidence in predictions of non-infections than in predictions of infections. In a practical use of the model, this would led to some unjustified alarms.

Keywords: disease modelling, bacterial spot, weather, infection period, validation

Introduction

Bacterial spot of stone fruits, caused by Xanthomonas arboricola pv. pruni (since now cited as Xap) occurs in most countries where stone fruits are grown. Xap is present on Prunus spp. in some European countries, and it is listed as an A2 quarantine pest by EPPO. It is more common and most severe in areas where stone fruits are grown in light, sandy soils and the environment is humid or moist and warm during the growing season. The most common hosts include peach and nectarine, Japanese plum, apricot and almond (Ritchie, 1995). Bacterial spot symptoms were observed on both peach leaves and fruits as necrotic spots and watersoaked respectively, while on plum twigs cankers were also described.

According to the traditional description of disease cycle on peach, the pathogen invades twigs via fresh leaf scars in autumn. These infections are then expressed as cankers in spring or as black tip in the following winter, or in the early spring. Overwintering bacteria associated with these symptoms are believed to be the most important inoculum for primary infections. However bacteria may overwinter in terminal buds; in Ontario (Canada) both terminal and axillary buds have been reported as overwintering sites for Xap (Dhanvantari, 1971). Xap have also been detected in epiphytic association with both twigs and buds on peach or plum (Shepard and Zehr, 1994). The bacterium was found on all symptomless organs sampled during a 13-month period during 1984-85; therefore, the pathogen can persist year-round on surfaces of both peach and plum trees, even in absence of symptoms of bacterial spot.

The occurrence of infection depends entirely on the environmental conditions. Frequent
periods of moisture during late bloom to a few weeks after petal fall are very conducive to primary infection on fruits and leaves of peach or nectarine. Wind-driven rain may increase disease severity. Similar environmental conditions throughout the growing season allow for the continuation of secondary infections, while few infections occur during hot dry conditions (Ritchie, 1995). Battilani et al. (1999) found that infection occurs when three or more rainy days occur with air temperature of 14 to 19°C.

The increasing prevalence of the bacterial spot in North Italy induced to verify the possibility of using the simple rule of Battilani et al. (1999) for predicting infection periods of Xap.

**Materials and methods**

**Model description**
A simple empiric model predicting Xap infection was developed from a 3-year study carried out in two peach orchards located southeast of lake Garda in the Veneto region (North Italy) in late '90s (Battilani et al., 1999). An infection period is predicted when there are at least three successive rainy days, with air temperature between 14 and 19°C; symptom’s onset is expected after one to four weeks of incubation.

**Data collection**
Occurrence of the first seasonal infection of Xap was monitored in peach orchards located in Romagna (North Italy), in 1992 to 2008. Starting from bud break, plants were carefully inspected at least once per week to observe the first seasonal appearance of disease symptoms.

Weather data were supplied by the regional agrometeorological network for the nearest automatic station (not farther than 15 km); from the year 2001 the regional network supplied interpolated data for the grids (5x5 km wide) which comprised the considered orchards (Bottarelli and Zinoni, 2002). The model was ran for each orchard using the weather data as input variables, the occurrence of any infection periods between bud break and first seasonal Xap onset was determined, as well as the correspondent period of expected appearance of the disease symptoms (Fig. 1).

**Model validation**
Total model predictions were distinguished in: i) accurate infections, when a predicted infection corresponded to the actual appearance of the disease in the orchard; ii) accurate non-infections, when the model did not predict an infection and no disease appeared in the orchard; iii) wrong infections, when the model predicted an infection but the disease did not appear; iv) wrong non-infections, when the model did not predict an infection that actually occurred. All the possible combinations of observed (O) versus predicted (P) infections were organized in a 2x2 contingency table, where the two groups O-,P- (no observed and no predicted infection) and O+,P+ (yes observed and yes predicted infection) were the right estimates, while the two groups O-,P+ and O+,P- were the wrong ones.

Sensitivity, specificity and accuracy of the model predictions were evaluated by means of the Bayesian analysis (Yuen and Hughes, 2002). To assess the advantages rising from the model in practice, the probabilities that an infection period result or not in a Xap infection were determined as P(O+,P+) and P(O-,P-) following Madden (2006), and compared with the correspondent prior probabilities, P(O+) and P(O-), respectively.
Figure 1. Air temperature (T) and rainfall registered in the peach orchard of Villanova, year 2006: ● is an infection period predicted by the model, ←→ is the expected period of disease appearance, ▼ is the first seasonal onset of Xap symptoms.

Results and discussion

Symptoms of Xap appeared in 10 out of 17 years; first seasonal symptoms become visible between 19 May and 12 July (Table 1). These infections were always correctly predicted by the model, with an average incubation period of three weeks (minimum 10 days, maximum 29 days); in 1996, two infection periods (15 and 28 May) were associated with the disease onset of 12 July (Table 1). Five infection periods (two in 1995, one in 1996, 1997, and 2002) were predicted by the model that did not result in actual infection (Table 1).

In five years (1993, 1999 to 2001, and 2003) the disease did not appear at all. In four of these years the model did not predict infection all season long, while in one year (1999) it wrongly predicted two possible infection periods on 22 May and 21 June. In 2004 and 2007, the disease did not appear in the considered orchards all season long, but traces of Xap symptoms were observed in neighbouring orchards at the end of May; in both cases the model predicted only one infection period in early May.

Comparison between predicted infection periods and observed disease symptoms (Table 2) showed that the model had very high sensitivity, because it correctly predicted all the actual infections, giving a true positive proportion TPP=1. The model also showed low specificity, because 7 out of 20 predicted infection periods were wrong because the disease did not appear, giving a false negative proportion of FPP=0.64. Overall accuracy of the model, which considers accurate versus total predictions, was high (=0.71) while the Youden’s index, which is the difference TPP-FPP was low (J= 0.36) because FPP was high (=0.64). Likelihood ratios of infection ($LR(+)=$TPP/FPP=1.57) and no infection ($LR(-)=FNP/TNP=0$) showed good accuracy, because an accurate model has, in general, large $LR(+)$(above 1) and small $LR(-)$(close to 0) (Madden 2006).

The prior probability of an infection to be predicted without the model was $P(O+)=13/24=0.54$. Using the model, the predicted posterior probability of an infection when one is predicted raised to $P(P+,O+)=0.65$, which is higher than the prior probability. The probability of no infection was $P(O-)=11/24=0.46$, while the correspondent posterior probability was $P(P-,O-)= 1.00$. Therefore, the probability that there will not be an infection when an infection is not expected is more than two-fold increased compared to the prior
probability of a non-infection when no model is used. Finally, the posterior probability of an infection given that a non-infection is predicted is \( P(P-,O+)=0 \), meaning that there is no probability that a random unknown observation is actually an infection when a non-infection is predicted by the model.

In conclusion, this validation work showed that the model predicting infection periods of \( Xap \) of peaches is accurate and robust. Because of the properties of the model and the prior probability of an infection, one would have somewhat more confidence in predictions of non-infections than in predictions of infections. In a practical use of the model, this would led to some unjustified alarms.

Table 1. Peach orchards considered for monitoring the epidemics caused by \( Xanthomonas arboricola \) pv. \( pruni \), and comparison between the first seasonal onset of the disease and the infection periods predicted by the model.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Variety</th>
<th>First seasonal ( Xap ) onset</th>
<th>Predicted infection periods(^d)</th>
<th>Days of incubation(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>Alfonsine</td>
<td>Elegant Lady</td>
<td>19 May</td>
<td>30 April</td>
<td>19</td>
</tr>
<tr>
<td>1993</td>
<td>Alfonsine</td>
<td>Elegant Lady</td>
<td>No</td>
<td>(^c)</td>
<td>-</td>
</tr>
<tr>
<td>1994</td>
<td>Alfonsine</td>
<td>Elegant Lady</td>
<td>5 July</td>
<td>13 June</td>
<td>22</td>
</tr>
<tr>
<td>1995</td>
<td>S. Alberto</td>
<td>Elegant Lady</td>
<td>3 July</td>
<td>10 May / 19 May / 6 June</td>
<td>27</td>
</tr>
<tr>
<td>1996</td>
<td>Ravenna</td>
<td>Several(^a)</td>
<td>12 July</td>
<td>3 May / 15 May / 28 May</td>
<td>28 / 15</td>
</tr>
<tr>
<td>1997</td>
<td>Ravenna</td>
<td>Several</td>
<td>12 June</td>
<td>7 May / 2 June</td>
<td>10</td>
</tr>
<tr>
<td>1998</td>
<td>Ravenna</td>
<td>Several</td>
<td>23 June</td>
<td>25 May</td>
<td>29</td>
</tr>
<tr>
<td>1999</td>
<td>Villanova</td>
<td>Elegant Lady</td>
<td>No</td>
<td>22 May / 21 June</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>Villanova</td>
<td>Elegant Lady</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2001</td>
<td>Villanova</td>
<td>Elegant Lady</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2002</td>
<td>Villanova</td>
<td>Elegant Lady</td>
<td>10 June</td>
<td>4 May / 12 May</td>
<td>29</td>
</tr>
<tr>
<td>2003</td>
<td>Villanova</td>
<td>Big top</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2004</td>
<td>Villanova</td>
<td>Big top</td>
<td>31 May(^b)</td>
<td>5 May</td>
<td>26</td>
</tr>
<tr>
<td>2005</td>
<td>Villanova</td>
<td>Big top</td>
<td>8 June</td>
<td>18 May</td>
<td>21</td>
</tr>
<tr>
<td>2006</td>
<td>Villanova</td>
<td>Big top</td>
<td>25 May</td>
<td>29 April</td>
<td>26</td>
</tr>
<tr>
<td>2007</td>
<td>Villanova</td>
<td>Big top</td>
<td>25 May(^b)</td>
<td>5 May</td>
<td>20</td>
</tr>
<tr>
<td>2008</td>
<td>Ravenna</td>
<td>Several</td>
<td>1 June</td>
<td>20 May</td>
<td>12</td>
</tr>
</tbody>
</table>

\(^a\) observations were carried out in a varietal collection of peaches; \(^b\) the disease did not appear in the considered orchard but in the neighbouring ones; \(^c\) the disease did not appear all season long; \(^d\) last day of a predicted infection periods: in italic the infection periods that resulted in actual disease onset; \(^e\) number of days elapsing between a predicted infection period (in italic) and the first seasonal disease onset.
Table 2 – Comparison between *Xap* infections predicted by the model and observed in the orchards of Tab. 1, and correspondent properties of the model.

<table>
<thead>
<tr>
<th>Predicted</th>
<th>Observed</th>
<th>Yes (P+)</th>
<th>No (P-)</th>
<th>Total</th>
<th>Accuracy</th>
<th>Likelihood ratio (LR)</th>
<th>Posterior probability (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (O+)</td>
<td>13</td>
<td>TPP =1.00</td>
<td>0</td>
<td>13</td>
<td>0.71e</td>
<td>LR(+) =1.57</td>
<td>P(P+,O+) =0.65</td>
</tr>
<tr>
<td>No (O-)</td>
<td>7</td>
<td>FPP =0.64</td>
<td>4</td>
<td>11</td>
<td>0.36f</td>
<td>LR(-) =0.00</td>
<td>P(P-,O+) =0.00</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>4</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a True Positive Proportion (sensitivity); b False Negative Proportion; c False Positive Proportion; d True Negative Proportion (specificity); e overall accuracy: (11+4)/24; f Jouden’s index (J): TPP-FPP

References


Monitoring of virus and phytoplasma diseases by laboratory diagnostic methods (DAS-ELISA, PCR, RT-PCR) in apple and pear after sanitation process

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Abstract: Sanitation of apple cultivar (‘Rubinstep’) and pear cultivars (‘Astra’, ‘Bohemica’, ‘David’, ‘Elektra’, ‘Erika’) was carried out by in vitro thermotherapy and chemotherapy. In the course of sanitation, the plant material was periodically tested to verify the suitability of selected methods. The presence of pathogens in selected initial trees was detected by PCR, RT-PCR and DAS-ELISA before the beginning of sanitation in 2005. Twenty clones of apple cultivar ‘Rubinstep’, 20 clones of pear cv. ‘Elektra’, 19 clones of pear cv. ‘Erika’, 20 clones of pear cv. ‘Astra’, 20 clones of pear cv. ‘Bohemica’, and 12 clones of pear cv. ‘David’ were tested after chemotherapy in years 2007-2008. Fifteen clones of pear cv. ‘Elektra’, 6 clones of cv. ‘Lada’ and 10 clones of cv. ‘Rubinstep’ were tested after thermotherapy in 2008. The occurrence of viruses Apple chlorotic leaf spot virus (ACLSV), Apple stem grooving virus (ASGV), Apple stem pitting virus (ASPV), Apple mosaic virus (ApMV) and phytoplasmas Candidatus ‘Phytoplasma pyri’ (CPP) and Candidatus ‘Phytoplasma mali’ (CPM) were monitored. The clones, which remained infected with viruses or phytoplasmas after therapy, were later discarded. Those in vitro clones that proved to be pathogen-free after repeated testing were further multiplied and in vitro rooted. The results presented here are preliminary.

Key words: sanitation, thermotherapy, chemotherapy, apple, pear, ApMV, ACLSV, ASPV, ASGV

Introduction

Apple chlorotic leaf spot virus (ACLSV) and Apple stem grooving virus (ASGV) both occur frequently in rosaceous fruit trees. Infection is often latent, although several economically important diseases are associated with the viruses (Németh 1986). Field grown fruit trees are affected by many pathogens of viral nature. Some of these pathogens are well identified and characterized (Németh 1986). Apple chlorotic leafspot virus (ACLSV), Apple stem grooving virus (ASGV), Apple stem pitting virus (ASPV) and Apple mosaic virus (ApMV) are common viruses affecting cultivated species of the Malus genus and other species of the Rosaceae family (Németh 1986; Knapp et al. 1998; Desvignes et al. 1999; Polák & Zieglerová 2001).

Certification schemes of European and Mediterranean Plant Protection Organisation (EPPO) have been established in Europe to guarantee the two requirements that propagative material must meet and maintain throughout the different steps of production: trueness of plant cultivar type and sanitary status. Among the pathogens main consideration is given to viruses, phytoplasmas and viroids because they are transmitted in propagative way and cannot be eliminated by pesticide treatments (Barba 1998). Sanitation by thermotherapy and chemotherapy is the most common way to obtain healthy plant material for the first step of certification scheme for fruit trees (pre-basic material).
Material and methods

Thermotherapy

Actively growing shoot tips of apple and pear cultivars were cut from shoots sprouting in laboratory conditions for *in vitro* culture initiation. This initial plant material was disinfested with mercuric chloride (0.15 %) for 1 min. and rinsed with sterile distilled water. Explants were cultured in Erlenmeyer flasks each with 25 ml of MS medium (Murashige & Skoog 1962) gelled with agar (0.7 %). The heat treatment was applied after one month of cultivation on the same MS medium but with 1.5 mg.l⁻¹ BAP for multiplication. The cultures were placed in a heat chamber, where the temperature was raised gradually to 34 or 39° C. Between 5 and 31 day of thermotherapy, according to growth vigour and viability of particular explant, the apical part of the axis about 1-2 mm in length comprising the apical meristem plus one or two leaf primordia, which developed during the high temperature period, was transferred to a fresh multiplication MS medium with 1.5 mg.l⁻¹ BAP and returned to standard growth conditions in a growth chamber.

A period of about 6 months was necessary to obtain well-established actively growing cultures of particular clones. Then several leaves from each *in vitro* cultivated clone were sampled and tested by RT-PCR for the abovementioned viruses and by PCR for phytoplasmas *Candidatus* ‘Phytoplasma pyri’ and *Candidatus* ‘Phytoplasma mali’. Those *in vitro* clones that proved to be virus-free by RT-PCR testing were further multiplied and *in vitro* rooted. *In vitro* clones with positive results of testing were discarded (Paprstein et al. 2008).

Chemotherapy

The chemotherapy was carried out on MS medium (Murashige & Skoog, 1962) with ribavirin. Thermolabile antiviral ribavirin (Duchefa, Biochemie B.V.) in concentration 200 mg.l⁻¹ was filter sterilized (25 mm, Acrodisc Syringe Filter 0.2 μm, Pall Gelman, USA) and added to the medium after autoclaving. Explants were sampled from actively growing *in vitro* culture and submerged to medium with ribavirin. Flasks with explants were placed to the cultivation room with the same conditions as for induction of multiplication. Twenty apical shoot tips (2 – 4 mm) were sampled from every cultivar after one month, cultivated and tested for viruses ApMV, ACLSV, ASGV and ASPV (Paprstein et al. 2008).

Testing

The presence of viruses in selected initials tree was detected by ELISA and RT-PCR before the beginning of sanitation. Virus pathogens ApMV, ACLSV, ASGV were detected by ELISA and ACLSV, ASGV and ASPV were tested by RT-PCR. *Candidatus* ‘Phytoplasma pyri’ and *Candidatus* ‘Phytoplasma mali’ were tested by PCR.

The virus detection was done by RT-PCR. The extracts of total RNA were obtained and transcribed using a commercial kit (Qiagen). The products of transcription by ASGV, ASPV and ApMV were amplified by 35 cycles, by ACLSV by 39 cycles in a thermocycler (Kundu 2002). The phytoplasma detection was done by PCR. The extracts of total DNA were obtained using a commercial kit. The DNA of phytoplasma was amplified by 35 cycles in a thermocycler. PCR products were diluted with sterile distilled water (1:39) prior to amplification by nested-PCR using R16F2/R2 (Gundersen & Lee 1996) and fU5/rU3 (Lorenz *et al*. 1995) primer pairs. Final R16F2/R2 amplicons (10 μl) were digested with *Rsa*I and *Bfi*I 16 hours at 37°C. Products of PCR and RT-PCR were visualized by Sybrgreen under ultraviolet light on agarose gel.
Results and discussion

Results of testing of initial field-grown trees before beginning of therapies are summarized in Table 1. During testing of initial plant material, ACLSV was detected by ELISA in apple cultivar ‘Rubinstep’ and by RT-PCR in pear cultivar ‘Bohemica’. ASGV was detected by RT-PCR in apple cultivar ‘Rubinstep’ and pear cultivars ‘Elektra’, ‘Erika’ and ‘Lada’. ASPV was detected by RT-PCR in apple cultivar ‘Rubinstep’ and pear cultivars ‘Astra’, ‘David’, ‘Elektra’ and ‘Lada’. \( \textit{Candidatus \text{ Phytoplasma pyri}} \) was proved by PCR in cultivar ‘Elektra’.

Table 1. Results of virus detection in initial field grown trees before sanitation by thermo – and chemotherapy (+ positive, - negative, n - not tested)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>ApMV</th>
<th>ACLSV</th>
<th>ASGV</th>
<th>ASPV</th>
<th>CPM/ CPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA/RT-PCR</td>
<td>ELISA/RT-PCR</td>
<td>ELISA/RT-PCR</td>
<td>ELISA/RT-PCR</td>
<td>PCR</td>
</tr>
<tr>
<td>apple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubinstep</td>
<td>- / n</td>
<td>+ / -</td>
<td>- / +</td>
<td>n / +</td>
<td>-</td>
</tr>
<tr>
<td>pear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astra</td>
<td>- / n</td>
<td>- / -</td>
<td>- / -</td>
<td>n / +</td>
<td>-</td>
</tr>
<tr>
<td>Bohemica</td>
<td>- / n</td>
<td>- / +</td>
<td>- / -</td>
<td>n / -</td>
<td>-</td>
</tr>
<tr>
<td>David</td>
<td>- / n</td>
<td>- / -</td>
<td>- / +</td>
<td>n / +</td>
<td>+</td>
</tr>
<tr>
<td>Elektra</td>
<td>- / n</td>
<td>- / -</td>
<td>- / +</td>
<td>n / -</td>
<td>-</td>
</tr>
<tr>
<td>Erika</td>
<td>- / n</td>
<td>- / -</td>
<td>- / +</td>
<td>n / +</td>
<td>-</td>
</tr>
<tr>
<td>Lada</td>
<td>- / n</td>
<td>- / -</td>
<td>- / +</td>
<td>n / +</td>
<td>-</td>
</tr>
</tbody>
</table>

The numbers of negative clones after chemotherapy and thermotherapy are summarized in Table 2. Cultivar ‘Bohemica’ had 16 clones (80 %) negative from 20 beginning clones after chemotherapy, cultivar ‘Astra’ had 16 clones negative (80 %) and cultivar ‘David’ had 6 clones negative (50 %) from 12. Cultivar ‘Elektra’ had 14 clones (70 %) negative from 20 tested, cultivar ‘Erika’ had 17 (89.4 %) negative from 19 tested. In the case of apple cultivar ‘Rubinstep’, all 20 clones were free of tested viruses after chemotherapy.

Table 2. Number of negative clones of apple and pear cultivars after chemotherapy and thermotherapy (in parentheses percentage of negative clones)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Number of clones</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Negative</td>
</tr>
<tr>
<td>Bohemica</td>
<td>20</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>Astra</td>
<td>20</td>
<td>16 (80.0)</td>
</tr>
<tr>
<td>David</td>
<td>12</td>
<td>6 (50.0)</td>
</tr>
<tr>
<td>Elektra</td>
<td>20</td>
<td>14 (70.0)</td>
</tr>
<tr>
<td>Erika</td>
<td>19</td>
<td>17 (89.4)</td>
</tr>
<tr>
<td>Rubinstep</td>
<td>20</td>
<td>20 (100.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Number of clones</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Negative</td>
</tr>
<tr>
<td>Elektra</td>
<td>15</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td>Lada</td>
<td>6</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Rubinstep</td>
<td>10</td>
<td>6 (60.0)</td>
</tr>
</tbody>
</table>

The best results of chemotherapy were achieved in the case of apple cultivar ‘Rubinstep’, where 100 % of clones were negative after sanitation. The best results of thermotherapy were noted
also in the case of apple cultivar ‘Rubinstep’.

The results show, that the system of therapy presented in this paper was partially successful. The mixed infection of three viruses ACLSV, ASGV and ASPV was completely eliminated by chemotherapy from apple cultivar ‘Rubinstep’. By thermotherapy, only ACLSV was completely eliminated from all clones of cultivar ‘Rubinstep’. The remaining viruses were eliminated only in 60 % of total amount of clones.

Other results show, that the elimination of viruses from some cultivars can be difficult. It is often associated with mixed infections, which are difficult to eliminate (da Camara Machado et al. 1998, Knapp et al. 1995).

With exception of cultivar ‘David’, comparison of both methods of sanitation proved that chemotherapy was more efficient. The success of virus elimination by chemotherapy in cultivar ‘David’ was only 50 %.

After sanitation process, the presence of ApMV was identified in cultivars ‘Astra’, ‘David’ and ‘Elektra’. However this virus was not identified in initial field-grown trees. This result can be caused by low concentration of virus in initial plant material. The more sensitive method RT-PCR detected ApMV in \textit{in vitro} cultures after sanitation process.

Table 3. Number of clones with occurrence of viruses and phytoplasmas in clones of apple and pear cultivars after sanitation process

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Tested clones</th>
<th>ApMV</th>
<th>ACLSV</th>
<th>ASGV</th>
<th>ASPV</th>
<th>CPM/ CPP</th>
<th>Negative clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Astra</td>
<td>20</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Bohemica</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>David</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Elektra</td>
<td>20</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Erika</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Rubinstep</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Thermotherapy</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Elektra</td>
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<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Lada</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Rubinstep</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

The results show, that both methods of sanitation were efficient. It is necessary to repeat the testing to exclude the possibility of latent virus infection. Laboratory methods and testing on woody indicators will be used for following testing of this material.

**Acknowledgements**

This work was supported by The Ministerium of Education, Youth and Sports of the Czech Republic (MSM2527112101).

**References**


Eutypa dieback as an important disease in red currant (Ribes rubrum) and gooseberry (Ribes uva-crispa) in the Netherlands

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Abstract: Over decades, growers in the Netherlands have problems with a disease that causes dying branches and stem cankers in red currant. For many years it was assumed that this disease was related to fungi such as Nectria cinnabarina, Phomopsis spp. and the insect Synanthedon tipuliformis. However, recently it was found by Applied Plant Research and the Plant Protection Service that the causal organism is the fungus Eutypa lata. The disease is considered of major economic importance, especially as red currant growing is rapidly expanding in the Netherlands. E. lata was identified with three detection methods (visual, plating and DNA). Symptoms of Eutypa do not usually appear until currant plants are at least three to four years old. These cankers are always associated with old pruning wounds. Eventually, the entire branch is killed. High disease incidences and annual losses of 10% - 30% of the productive branches are reported. In some cases entire fields have to be replanted. Eutypa is well known as one of the most destructive diseases of grapes. The importance of this disease in currant growing was not known. Research is focusing on the evaluation of control measures; e.g. chemical and biological control treatment of pruning wounds, and disease management such as sanitation practices. Also, the epidemiology of Eutypa is studied. Recently, high densities of ascospores of Eutypa were found in a spore trap placed in a red currant field in the Netherlands. In the subsequent field survey, fruiting structures (stromata) and ascospores were found on dead infected red currant wood.

Eutypa lata, Canker, Control strategies, Currants
Chlorantraniliprole (DPX-E2Y45, Rynaxypyr®) (Coragen®20SC and Altacor®35WG) - a new diamide insecticide for control of codling moth (Cydia pomonella) and other top fruit Lepidopteran pests

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Abstract: Chlorantraniliprole (DPX-E2Y45, Rynaxypyr®) is a new compound from DuPont belonging to a new class of selective insecticides (anthranilic diamides) featuring a novel mode of action (group 28 in the IRAC classification). By activating the arthropod ryanodine receptors it stimulates the release and depletion of intracellular calcium stores from the sarcoplasmic reticulum of muscle cells causing impaired regulation, paralysis and ultimately death of sensitive species. Extensively tested in the field since 2002, it is registered in the USA, Australia, Canada, China and it is close to market introduction in all the main top fruit producing countries. The product general features have been presented in previous, referenced papers. It has very low toxicity for mammals (both acute and chronic), high biological activity on the sensitive species with strong ovicidal efficacy and good residual properties, excellent performance on codling moth and other chewing pests, stability of performance across the different climatic and farming conditions, no cross-resistance detected to any existing insecticide and minimal impact on pollinator and beneficial arthropod species. Published studies indicate that chlorantraniliprole may have significant mating disruptive effects on C. pomonella adults when both males and females are exposed to the residues equivalent to the recommended field rate. This paper focuses on the product features that best fit IFP (Integrated Fruit Protection) criteria and may enhance IFP options while ensuring higher efficacy standards. After reviewing some toxicity data, examples from field/semi-field and laboratory tests are provided regarding comparative performance assessment, minimal impact on beneficial arthropods and bees and a possible reduction in the number of applications versus current standards.

Insecticide, Chlorantraniliprole, Rynaxypyr®, Ryanodine receptor, Beneficial arthropods, Mating disruption, Codling moth, Bees
No evidence in codling moth for cross-resistance between chemical insecticides and Cydia pomonella granulovirus

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1 JKI, Institute for Biological Control, 64287 Darmstadt, Heinrichstr. 243, Germany; annegret.schmitt@jki.bund.de; 2 INRA, Agroparc, 84914 Avignon, France; 3 DLR Rheinpfalz, Laboratory for Biotechnological Crop Protection, Breitenweg 71, 67435 Neustadt/Wstr., Germany

Abstract: Codling moth larvae from 23 orchards located in five European countries were tested for their susceptibility/resistance to the Cydia pomonella granulovirus (CpGV-M) in standardized laboratory bioassays. Farmers observed in several of these populations reduced susceptibility to CpGV-M treatment. For each C. pomonella strain, the percentage of larvae surviving CpGV-M concentrations of 104 to 106 OB/ml were calculated 14 days after start of the trial and used for prediction of percentage of resistant individuals in the collected population. The mortality was corrected using Abbott’s formula, with the average mortality determined in the controls of all 14-day trials performed (mortality due to other reasons than virus). In general, the results from the bioassays were in accordance with the observations in the field. Most orchards from which the farmer reported failure of the CpGV-M treatment contained resistant codling moth populations. The percentage of resistant individuals in a population ranged roughly from 30 to 90%. However, in some apparently susceptible populations there were also hints for the presence of a very small fraction of resistant individuals. Several of these European populations were tested for susceptibility to eight insecticides including different classes of insect growth regulators and neurotoxic compounds. High mortality was recorded to most insecticides, independent of resistance to CpGV. A reduced susceptibility to azinphos, diflubenzuron, and tebufenozide was recorded in several populations. Overall, there was no indication for the occurrence of cross-resistance between CpGV-M and insecticides in the tested populations. First laboratory tests showed that populations of C. pomonella resistant to CpGV-M were susceptible to new CpGV strains. This study was funded by the EU, CRAFT project 32857; Further information can be found under www.sustaincpgv.eu.

Codling moth, Cydia pomonella Granulovirus, Chemical insecticides, Resistance
Can delayed flight activity serve as an indicator for insecticide resistance?

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Abstract: Together with the codling moth, Cydia pomonella, the summer fruit tortix moth, Adoxophyes orana, is a major pest insect in apple orchards of western Switzerland. Whereas codling moth developed simple, cross and multiple resistances to various classes of insecticides over the last decade, A. orana seemed to be still susceptible to these pesticides. However, since 2004, fruit growers indicate more and more failures of conventional control schemes against summer fruit tortix moths. Using laboratory bioassays we established that A. orana shows resistance to insect growth regulators and to insect growth inhibitors. This resistance becomes manifest in the slower development of A. orana larvae. Field observations showed that the flight of resistant moth populations is delayed. Delayed flight activity might therefore serve as a reliable indicator of insecticide resistance in summer fruit tortix moths. In conclusion, the key to successfully managing insecticide resistance is to reduce selection pressure. This can be achieved by incorporating cultural, biological and pheromonal control practices, by minimising the use of insecticides and by the alternate use of insecticides with different modes of action.

Key words: Torticidae, pomiculture, pesticide use, resistance management

Introduction

The summer fruit tortix moth, Adoxophyes orana F. v. R. and the codling moth, Cydia pomonella (L.) (both Lepidoptera, Torticidae), are the two major pest insects in apple orchards of western Switzerland. The first resistant C. pomonella was detected in 1996 and since then several other cases have been discovered all over the country (Charmillot et al., 2005). In some populations the effectiveness of commonly used insecticides is nearly zero and these insects evolved cross-resistance to nearly all insecticide classes applied (Ioriatti et al., 2007; Reyes et al., 2007). As a consequence, mating disruption and granulosis viruses were successfully implemented as alternative control strategies against codling moth. In orchards where these two strategies have been implemented, insecticides are regaining their efficiency (Charmillot et al., 2007). Whereas codling moth developed simple, cross and multiple resistances to various classes of insecticides, A. orana seemed to be still susceptible to these pesticides. However, over the past years fruit growers indicated more and more failures of conventional control schemes against summer fruit tortix moths (Charmillot et al., 2005; Salamin et al., 2007).

In this paper we study the efficacy of commonly applied insecticides against A. orana using field observations and laboratory bioassays.
Material and methods

Laboratory bioassays
In 2005, larvae of *A. orana* were collected in different orchards in the canton Vaud, Switzerland. Three years later, another 140 larvae were collected in a pear orchard in the Valais, Switzerland. In all these orchards, fruit growers indicated the failure of commonly used insecticides. These larvae were exposed to eight different insecticides (chlorpyrifos-methyl, fenoxycarb, tebufenozide, methoxyfenozide, hexaflumuron, lufenuron, Spinosad and indoxacarb). Untreated leaves of apple and pear were dipped for about one minute in a solution containing an insecticide dose that kills 99% of individuals of our susceptible *A. orana* strain (Figure 1a). After dipping, leaves were placed under a ventilated hood for drying. Three to four of these leaves were put in a small plastic container of 20x20x15 mm together with one collected *A. orana* larva. Containers were stored in a climate chamber (25°C, 70% RH, 18/6 h D/N) and after one week, the mortality of exposed larvae was assessed.

Field observations
The flight of *A. orana* was studied by the use of pheromone traps. Traps were exposed in apple and pear orchards in western Switzerland and they were assessed every one to two weeks.

Results and discussion

Figure 1. Efficacy of different active ingredients applied to larvae of *A. orana* at a discrimination dose of 99% mortality (=DD), b) males caught by pheromone traps in the Vaud in 2005 and c) in the Valais 2008.
Tested larvae of *A. orana* showed an increased resistance to insect growth regulators and insect growth inhibitors (Figure 1). Besides, moths were caught significantly latter in orchards where moths had reduced insecticide susceptibility (Figure 2). This might be explained by an adaption of *A. orana* to the usual application scheme or by a slower development of resistant larvae.

In conclusion, we believe that a delayed flight activity can serve as a first indicator for identifying insecticide resistant moth populations. Early detection of insecticide resistance is of paramount importance for implementing integrated management schemes, such as the adoption of cultural, biological and pheromonal control practices, the reduction of insecticide use and the alternate use of insecticides with different modes of action.

**References**


Cydia pomonella (Lep: Tortricidae) resistance and cross-resistance to various classes of insecticides in Central Europe

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Crop Research Institute, Department of Entomology, Drnovská 507, Prague 6, 161 06, Czech Republic

Abstract: Insecticide bioassays were used to investigate resistance of Cydia pomonella (L.) to insecticides with various types of active ingredients. The efficacy baselines of selected insect growth regulators (fenoxycarb), insect growth inhibitors (diflubenzuron, teflubenzuron), organophosphorous insecticides (phosalone) and neonicotinoids (thiacloprid) against the eggs, first- and fifth-instar larvae of sensitive laboratory strains of codling moth were determined. The lethal concentration ratio quantified the relation between the efficacy of selected insecticides against fifth-instar larvae found by topical application and against first-instar larvae found by diet-treated bioassay. According to concentration-mortality baseline, 50% lethality concentration values and 90% lethality concentration values were determined for all the tested insecticides. The bioassay was used to monitor the resistance of codling moths collected in 2003 – 2005 in two apple orchards with different intensities of chemical control. Resistance ratios to the tested insecticides were determined for both field populations of codling moth. For the population of codling moth from an apple orchard in Velké Bílovice, cross-resistance to fenoxycarb, teflubenzuron and phosalone was detected after the topical application of insecticides to fifth-instar larvae. The population of codling moth from Prague-Ruzyn was slightly resistant to phosalone and teflubenzuron. No resistance to diflubenzuron was detected in either tested population. This work was funded by the Czech Science Foundation, the Czech Republic, grant 522/04/P181. Partial funding was also obtained from the Ministry of Agriculture, the Czech Republic, project 0002700603.

Cydia pomonella, Resistance, Insecticides
Efficacy of clothianidine against the strawberry root weevil (*Otiorhynchus ovatus*) on strawberry plantations.

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Abstract: The strawberry root weevil (*Otiorhynchus ovatus*) feeds on roots and, therefore, it is a very dangerous pest on older strawberry plantations. The efficacy of two clothianidine formulations (Apacz 50 WG (clothianidine 50%) and TI 435 1 GR (CAGR 8; Santana 1 GR) (clothianidine)) were tested against the strawberry root weevil on strawberry plantations. Both insecticides belong to neonicotinoid group. Granular formulation (TI 435 1GR) incorporated into soil at the rate of 10 and 15 kg/ha in the spring, before strawberry blossom, reduced significantly the number of weevil larvae. In two trials TI 435 1 GR applied at the higher rate (15 kg/ha) decreased the number of larvae by 72%. The efficacy of this insecticide used at the lower rate (10 kg/ha) against weevil larvae was 61.3 and 78.7%. Results obtained with TI 435 1 GR were similar to those obtained with standard insecticide – Diazinon 10 GR (80 kg/ha). Apacz 50 WG applied as a spray treatment at the rate of 0.15 and 0.20 kg/ha before strawberry blossom reduced weevil larvae by 74.5 - 99.6%. Apacz 50 WG applied at the rate of 0.15 and 0.20 kg/ha just after strawberry harvest reduced the pest abundance by 72.1-96.3%. Reduction of the pest at this time is very important because after harvest adults of the strawberry root weevil feed on leaves and females lay eggs. The results obtained with Apacz 50 WG were similar or better than those obtained with standard the insecticides; Diazinon 10 GR or Dursban 480 EC (chlorpyrifos).

Keywords: Strawberry root weevil, *Otiorhynchus ovatus*, strawberry, neonicotinoids, clothianidine, Apacz 50 WG, TI 435 1 GR (Santana 1 GR), chemical control

Introduction

The strawberry root weevil (*Otiorhynchus ovatus*) is a very important pest causing great losses on strawberry plantations in many regions of Poland as well as in other countries (Penman and Scott, 1976, Łabanowska, 1994). The larvae live and feed on strawberry roots. The level of pest population is higher on older plantations than on young ones. The largest damage is caused by older larvae, from the end of April until the beginning of June, when they need the most food. The infested plants are weakened, their leaves are smaller and many plants dry out. The adults feed on leaves mainly in July, whilst females lay eggs on the soil surface under plants. Insecticides containing chlorpyrifos and diazinon could be applied as spray-treatments to control the pest before strawberry planting or after harvest (Łabanowska and Olszak, 2003, Łabanowska, 1994), and acetamiprid as Mospilan 20 SP could be used after harvest to control adults. The experiments to find biological control for the strawberry root weevils are carried out in many countries (Wilson et al., 1999, Tkaczuk et al., 2005).

Materials and methods

Experiments were conducted in 2004-2005 at the Research Institute of Pomology and Floriculture in Skierniewice, in central Poland. They were carried out on commercial strawberry plantations, cv. Senga Sengana in a randomized block design with four replicates. Plot sizes ranged from 10.5 m² to 50 m². The spray-treatments were applied before strawberry blossom or after fruit harvest, when adults were feeding on leaves and females laid eggs.
Apacz 50 WG was used as foliar and soil spray-treatment at the rate of 750 l/ha solution. A knapsack motor sprayer ‘Stihl’ was used for spraying. The granule insecticide was incorporated by hand into the soil near the plants, in the strawberry rows only. The efficacy of treatments was estimated in June or early July by counting larvae, pupae and adults (weevils). After spring treatments larvae were counted in the same year, but if they were applied after harvest, the larvae were counted in the following year, at the end of harvest. Six plants per plot, i.e. 24 per treatment were removed with soil. The roots and sieved soil were checked for the pest’s presence. Data was transformed according to the formula $y = \log(x+1)$, where $x$ is the number of specimens and analysed using analysis of variance. Significant differences of means were tested with Duncan’s multiple range “t” test at 0.05 significance level.

Results and discussion

Control of the strawberry root weevil in the spring
Clothianidine as Apacz 50 WG (0.15 and 0.20 kg/ha) applied as a spray before blossom of strawberry reduced the number of larvae under plants by 74.5%; 86.7% and 98.5%, depending on the insecticide rate and experiment (Tab. 1). The efficacy of clothianidine was much higher than acetamiprid (Mospilan 20 SP) as a reference insecticide. These results confirmed the data obtained earlier with Apacz 50 WG used against the Colorado beetle (Wachowiak and Mrówczyński, 2005). Clothianidine as TI 435 1 GR (10 and 15 kg/ha) applied before blossom of strawberry, reduced the pest abundance by 61.3 – 72.4%, similarly as a reference insecticide, Diazinon 10 GR.

Control after harvest
Clothianidine containing Apacz 50 WG used as a spray-treatment of plants and soil after harvest as well as granular formulation TI 435 1 GR used to the soil near the plants after harvest, reduced the number of pest in the following year. Apacz 50 WG (0.15-0.20 kg/ha) efficacy was estimated as 72.1% - 96.3% but TI 435 1 GR (10 and 15 kg/ha) showed 75.3 – 77.1% reduction of the pest. The results with clothianidine were similar to those obtained with reference insecticides – Dursban 480 EC and Pyrinex 480 EC (chlorpyrifos) and other neonicotinoids as imidaclorpid, thiacloprid and thiametoxam in the control of the strawberry root weevil (Łabanowska 2007, Łabanowska, Olszak 2003). Clothianidine will be useful to control of strawberry root weevil on strawberry in IPM programme.

Conclusions

Apacz 50 WG and TI 435 1 GR (Santana 1 GR, CAGR 8) containing clothianidine applied as foliar and soil treatments before blossom of strawberry or after fruit harvest showed good control of the strawberry root weevil (Otiorhynchus ovatus) on strawberry plantations. The results obtained with clothianidine were similar to data obtained with standard substances (diazinon, chloropyrifos and acetamiprid).
Table 1. Efficacy of clothianidine (Apacz 50 WG and TI 435 1 GR) applied in spring against the strawberry root weevil

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate kg/ha</th>
<th>No. of larvae, pupae and adults per plant</th>
<th>Efficacy in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>-</td>
<td>8.5 e*</td>
<td>-</td>
</tr>
<tr>
<td>Apacz 50 WG*</td>
<td>0.15</td>
<td>0.1 a</td>
<td>98.47</td>
</tr>
<tr>
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<td>0.20</td>
<td>0.03 a</td>
<td>99.63</td>
</tr>
<tr>
<td>Mospilan 20 SP</td>
<td>0.60</td>
<td>1.6 b</td>
<td>80.74</td>
</tr>
<tr>
<td>b. Złota, Date of spray-treatment – 7.05.2004; Date of counting – 8.07.2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
<td>-</td>
<td>11.9 d</td>
<td>-</td>
</tr>
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<td>0.15</td>
<td>3.0 b</td>
<td>74.53</td>
</tr>
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<td>Apacz 50 WG*</td>
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<td>1.6 a</td>
<td>86.67</td>
</tr>
<tr>
<td>Mospilan 20 SP</td>
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<td>5.6 c</td>
<td>52.76</td>
</tr>
<tr>
<td>c. Skierniewice, Date of soil-treatment – 21.05.2004; Date of counting – 6-7.07.2004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check</td>
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<td>2.9 b</td>
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<td>TI 435 1 GR**</td>
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<td>1.1 a</td>
<td>61.31</td>
</tr>
<tr>
<td>TI 435 1 GR**</td>
<td>15.0</td>
<td>0.8 a</td>
<td>72.39</td>
</tr>
<tr>
<td>Diazinon 10 GR</td>
<td>80.0</td>
<td>0.6 a</td>
<td>80.06</td>
</tr>
</tbody>
</table>

Table 2. Efficacy of clothianidine (Apacz 50 WG and TI 435 1 GR applied after harvest of strawberries against the strawberry root weevil

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate l/kg/ha</th>
<th>No. of larvae, pupae and weevils per plant</th>
<th>Efficacy in %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>a. Złota, Date of treatment – 22.07.2004; Date of counting – 8.07.2005</td>
<td></td>
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<tr>
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<td>-</td>
<td>0.85 b</td>
<td>-</td>
</tr>
<tr>
<td>Apacz 50 WG*</td>
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<td>0.03 a</td>
<td>96.30</td>
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<tr>
<td>TI 435 1 GR**</td>
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<td>76.29</td>
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<tr>
<td>b. Czyżew, Date of treatment - 22-26.07.2005; Date of counting - 10-11.07.2006</td>
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<td>Check</td>
<td>-</td>
<td>35.84 b*</td>
<td>-</td>
</tr>
<tr>
<td>Apacz 50 WG* Apacz 50 WG</td>
<td>0.15</td>
<td>10.01 a</td>
<td>72.06</td>
</tr>
<tr>
<td>TI 435 1 GR**</td>
<td>10.0</td>
<td>8.84 a</td>
<td>75.33</td>
</tr>
<tr>
<td>TI 435 1 GR</td>
<td>15.0</td>
<td>8.20 a</td>
<td>77.11</td>
</tr>
<tr>
<td>Diazinon 10 GR</td>
<td>80.0</td>
<td>14.57 a</td>
<td>59.35</td>
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<tr>
<td>Dursban 480 EC</td>
<td>2.5</td>
<td>8.92 a</td>
<td>75.12</td>
</tr>
<tr>
<td>Pyrinex 480 EC</td>
<td>2.5</td>
<td>10.31 a</td>
<td>71.23</td>
</tr>
</tbody>
</table>

* spray-treatment; ** soil-treatment ***Number followed by the same letter do not differ at P= 0.05, according to Duncan’s t-test

Acknowledgements

I would like to thank Elżbieta Paradowska, Małgorzata Tartanus, Bożena Pawlik and Stanisław Lesiak for their technical help in conducting the experiments.
References

Trials for the development of alternative control strategies against the codling moth (Cydia pomonella) in pome fruits in Austria in 2007

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Abstract: The development of future alternative control strategies against the codling moth, Cydia pomonella (Tortricidae, Lepidoptera), is an important subject for the pome fruit production both nationally and internationally. The reasons are not only the increasing resistance of C. pomonella against plant protection products including virus products, but also the expiration of the authorization of important plant protection products especially for integrated production. In Austria great problems are expected from 2008 onwards due to the loss of the most commonly used organophosphate insecticide against the codling moth at present. Therefore, in 2007 control trials against the codling moth also suitable for integrated production were carried out by the Institute of Plant Health (AGES) in coordination with the chambers of agriculture of Lower Austria and Styria. Trials were conducted according to the EPPO-guideline PP 1/7(3) comprising 8 variants including one untreated control. Four plant protection products with Fenoxycarb, Methoxyfenozid, Chlorpyrifos and Indoxacarb as active ingredients were used in different numbers of applications and combinations. The untreated control plots showed very high infestation levels (66% infestation). Although the other treatments resulted in different efficacy levels in the reduction of the pest, the economic damage threshold (1% infestation) was exceeded in every treatment. Because the infestation levels of the codling moth and resistance problems increased during the last years it can be concluded that more effective control strategies have to be developed to ensure the quality and quantity of pome production for the future.

Key words: Cydia pomonella, pomefruit, control strategy, IPM
Microencapsulation and PBO: a tool in resistance management of the green peach aphid

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Abstract. Insecticide resistance can be a serious threat to the application of Integrated Pest Management. The Green Peach-Potato Aphid, \textit{Myzus persicae} (Sulzer) is a serious pest in peach orchards. Insecticide treatments have selected many populations that have different degrees of insecticide resistance due to different resistance mechanisms. These resistance mechanisms can interfere with many classical insecticide classes, but, fortunately, till now, there is no clear evidence for resistance to neonicotinoids. The severity of this problem is also increased by the reduction of the available active ingredients that can lead to an abuse of a single group of insecticides. Many populations of \textit{M. persicae}, both in Italy and in Europe, over-express a carboxylesterase (E4/FE4) that reduces in various degrees the efficacy of several insecticides by hydrolysis and/or by sequestering. Recently, many authors have demonstrated that piperonylbutoxide (PBO) can efficiently interfere with esterase activity overcoming insecticide resistance. Several microencapsulated products (in polyurea or cyclodextrin) with PBO and various active ingredients have been tested in laboratory bioassays against a susceptible and against an esterase resistant population of \textit{M. persicae}. A comparison was done with the commercial formulated products alone or mixed with PBO. The results achieved with the different formulation are discussed in term of increased mortality, application rate as well as offsprings reduction. According to the results, the use of these types of microencapsulation together with PBO could be an interesting tool to be included in resistance management strategies against the green peach-potato aphid.

Key words: esterases, resistance management, piperonyl-butoxide.

Introduction

The green peach-potato aphid, \textit{Myzus persicae} (Sulzer), is a serious pest in peach orchards and in many herbaceous crops because:

\begin{itemize}
  \item its population abundance grows rapidly above the economic thresholds;
  \item it transmits plant viruses;
  \item it is resistant to many insecticide treatments.
\end{itemize}

Many \textit{M. persicae} populations have resistance mechanisms that can interfere with many insecticides classes even if, till now, there is no clear and absolute evidence for resistance to neonicotinoids (Nauen & Denholm, 2005; Foster \textit{et al.}, 2008). The severity of this problem is increased by the reduction of the available active ingredients, potentially leading to the abuse of single insecticides. For the above reported reasons insecticide resistance is a serious threat to successful application of Integrated Pest Management in agricultural crops and any tool and/or strategy trying to overcome resistance have to be exploited.

Many populations of \textit{M. persicae} over-express a carboxylesterase (E4/FE4) that reduces the efficacy of several insecticides by hydrolysis and/or by sequestration (Devonshire
et al., 1998). Recently, it has been demonstrated that the synergist piperonylbutoxide (PBO) can efficiently interfere with esterase activity in *M. persicae*, overcoming insecticide resistance (Bingham et al., 2008).

The aim of the work is to evaluate the efficacy of different microencapsulated insecticides together with PBO against resistant *M. persicae* populations.

**Materials and methods**

**Insects**

*M. persicae* populations used in the present study were originally collected in peach orchards in Emilia-Romagna (Northern Italy) then maintained as colonies of parthenogenetic females for several years on pea seedlings (cv “Meraviglia d’Italia”) at 21 °C and 16:8 L:D photoperiod, without any selection pressure with insecticides. Before use in bioassays the populations were sub-cloned from a single winged female and a few specimens of the progeny assayed for total esterase activity and acetylcholinesterase insensitivity according to a protocol previously described (Devonshire et al., 1992; Mazzoni & Cravedi, 2002). The biomolecular tests to asses the presence or absence of the mutation in the paratype sodium channel gene producing the “kdr” phenotype conferring target-site resistance to pyrethroids have been performed according to Cassanelli et al. (2005).

**Bioassays**

A series of different insecticide products, listed in tables 1 & 2, have been tested in leaf-dip bioassays in comparison with a commercial formulation alone or mixed with PBO against an esterase resistant strain (without “MACE” or “kdr” mutations). A few tests, for comparison, have been carried out also using a susceptible strain and a “kdr” strain. Mortality data recorded 24 hours after the treatment were analyzed using probit analysis.

Table 1. Microencapsulated products containing “bifentrin” (PU= polyurea; CD= cyclodextrin).

<table>
<thead>
<tr>
<th>Product</th>
<th>a.i. (%)</th>
<th>PBO (%)</th>
<th>Ratio (a.i.:PBO)</th>
<th>capsule type</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN32-2/16</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>PU</td>
</tr>
<tr>
<td>EN32-2/16/P</td>
<td>10</td>
<td>50</td>
<td>1 : 5</td>
<td>PU</td>
</tr>
<tr>
<td>EN32-2/17</td>
<td>8.8</td>
<td>19.9</td>
<td>1 : 2.3</td>
<td>CD</td>
</tr>
<tr>
<td>EN32-2/20</td>
<td>9.9</td>
<td>-</td>
<td>-</td>
<td>CD</td>
</tr>
<tr>
<td>EN32-2/21</td>
<td>7.4</td>
<td>17</td>
<td>1 : 2.3</td>
<td>CD</td>
</tr>
</tbody>
</table>

**Results and discussion**

The bioassay procedure adopted did not allow the insect to reach a total mortality applying commercial formulations of both insecticides against the resistant aphids but the efficacy, above all against the “kdr” strain, has been increased by a “tank mix” with PBO. Much more interesting results have been achieved with micro-encapsulation in polyurea and above all in cyclodextrin. Indeed for both active ingredients a good reduction of the LC$_{50}$ has been recorded using cyclodextrin microencapsulation in comparison with the corresponding commercial formulation: 250 times lower in the case of bifentrin and 90 times in the case of $\alpha$-cypermethrin (Graphs 1 – 2). Moreover a lower number of offspring was produced by
females surviving the highest concentrations (data not shown).

Table 2. Microencapsulated products containing “α-cypermethrin” (PU= polyurea; CD= cyclodextrin).

<table>
<thead>
<tr>
<th>Product</th>
<th>a.i. (%)</th>
<th>PBO (%)</th>
<th>Ratio (a.i.:PBO)</th>
<th>capsule type</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN32-1/40</td>
<td>6.1</td>
<td>23.8+6.1</td>
<td>1 : 4.9</td>
<td>PU</td>
</tr>
<tr>
<td>EN32-1/52</td>
<td>1.3</td>
<td>6.4+19.3</td>
<td>1 : 19.8</td>
<td>PU</td>
</tr>
<tr>
<td>EN32-1/36</td>
<td>7.8</td>
<td>18.3</td>
<td>1 : 2.4</td>
<td>CD</td>
</tr>
<tr>
<td>EN32-1/37</td>
<td>11.7</td>
<td>27.6</td>
<td>1 : 2.4</td>
<td>CD</td>
</tr>
<tr>
<td>EN32-1/46</td>
<td>7.4</td>
<td>18.4</td>
<td>1 : 2.5</td>
<td>CD</td>
</tr>
</tbody>
</table>

Graph 1. LC$_{50}$ of α-cypermethrin products estimated using “probit analysis”. In the case of cyclodextrin products the LC$_{50}$ is reduced more than 90 times in comparison with the commercial formulation.

Conclusions
Although a small scale experiment, some of the microencapsulated products used demonstrated properties overcoming esterase resistance, improving the performance against “kdr” strains and allowing a reduction in application doses. The use of microencapsulation together with PBO could be an effective tool to be considered in resistance management strategies against the green peach-potato aphid. The evaluation of different active ingredients is in progress.
Graph 2. LC$_{50}$ of bifentrin products estimated using “probit analysis”. In the case of EN32-2/21 (cyclodextrin) the LC$_{50}$ is reduced more than 250 times in comparison with the commercial formulation.

References


Susceptibility to abamectin of pear psylla, Cacopsylla pyri (L.) (Hemiptera: Psyllidae) in pear orchards of north-east Spain

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Abstract: \textit{Cacopsylla pyri} (L.) (Hemiptera: Psyllidae) is a key pest of pear orchards in the fruit growing area of north-east Spain. Chemical control is the most common method used against pear psylla, but the number of insecticides registered to control it has been reduced in the last years. The high selection pressure with abamectin, applied repeatedly over the whole area, can result in the appearance of resistance, as has happened with other products. With the aim of monitoring future changes in the susceptibility of \textit{C. pyri} to abamectin, we used topical application bioassays in adults, and residual application in nymphs to obtain current data on the susceptibility in the area. We collected 15 populations from different orchards in Lleida, Huesca and Girona, where heavy use of insecticides (including abamectin) is the common practice. The bioassays were carried out from October 2004 to September 2006. To check the evolution of abamectin treatments in the last years we analyzed the records of the treatments from the different orchards. We obtained the current data, LC\textsubscript{50} and LC\textsubscript{90} of all the populations (adults and all instars nymphs). No evidence of a high level of resistance has been found. However there are a few populations that presented a lower susceptibility, as well in adults as in nymphs. The populations with the lowest level of susceptibility in nymphs were the same that presented the lowest level of susceptibility in adults and they came from the fields with the highest number of insecticide applications.

\textit{Cacopsylla pyri}, Pear psylla, Abamectin, Resistance, Bioassays, Treatment records
Plant infusions to limit the development of pests or diseases: results on Aphis pomi

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Abstract: We started research on physiomedicalism in 2003, in order to limit the development of pests or diseases in an environment-friendly manner. The potential of indigenous medicinal plants is largely explored and used for human and veterinary medicines, but lately work has started to look at their potential for providing pesticides for use on cultivated plants. Our preliminary tests target has been Aphis pomi in apple orchards. To ensure the feasibility of growers using them in the future our preparations are home-made, from dry medicinal plants. From the literature, six plants were selected and then tested to see if they would limit the development of Aphis pomi: Artemisia absinthium L., Artemisia vulgaris L., Saponaria officinalis L., Mentha x piperata L., Salvia officinalis L., Tanacetum annuum L. The best results of 2006 and 2007 trials were obtained with the infusions of Mentha x piperata and Artemisia vulgaris. Results are discussed.

Organic farming, Physiomedicalism, Plant infusions, Aphis pomi
Comparison of susceptibility and nychtemerals rhythms between reared insects of Mediterranean fruit fly (Ceratitis capitata) and wild population of Algeria treated with a fenthion insecticide.

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Abstract: Fenthion toxicity was studied with topical application and lethal dose LD 50 and DL 80 were assessed on various C.capitata Wiedemann populations. Toxicity was lower in wild individuals than in reared insects, among which individuals irradiated at 90 Gy gamma ray were significantly more susceptible. A nychthemeral variation in the susceptibility to this insecticide was characterized, with some peculiarities related to the origin of the insects and the LD considered.

Ceratitis capitata, Tephritidae, Wild population, Insecticide, Fenthion, Irradiation, Lethal dose, Nychthemeral variation.
Preliminary resistance screening of abamectin on pear psylla (Hemiptera: Psyllidae) in Northern Italy

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Abstract: Civolani S.1, Peretto R.1, Chieco C.1, Chicca M.1, Leis M.1, Pasqualini E.2 1 Department of Biology and Evolution - University of Ferrara (I) 2 DiSTA (Department of Agroenvironmental Science and Technologies) - University of Bologna Preliminary resistance screening of abamectin on pear psylla (Hemiptera: Psyllidae) in Northern Italy . In northern Italy (Emilia-Romagna Region), integrated pest management (IPM) has been adopted for several years to control pear psylla, Cacopsylla pyri L. (Hemiptera: Psyllidae), a relevant pest of pear (Pyrus spp.) orchards. After the outlawing of amitraz in 2005, the most common active ingredient now used for control is abamectin, a mixture of avermectin B1a and avermectin B1b. After the development of C. pyri resistance to different active ingredients in several European growing areas, an evaluation using a range of laboratory tests (topical application on adults, spray application on eggs, leaf dip test on young and old larvae) were carried out during 2007 and 2008 to assess C. pyri susceptibility to abamectin, using populations of this pest which had been obtained, from several orchards where a range of control strategies were being applied. The results are discussed.

Cacopsylla pyri, Abamectin, Resistance, Pear
Strategies and timing of protection practices against *Cydia pomonella* in apple orchards

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Abstract: The understanding of actual farmer practices is essential to identify the constraints for the adoption of new integrated pest management strategies. From data collected in 2006 in 71 randomised pear and apple orchards in a small production area in south France, our objective was to comprehend the management practices against codling moth (*Cydia pomonella* L.). We first investigated the timing and frequencies of insecticide applications in relation with national and regional recommendations. The orchards were classified according to three management strategies: conventional with major use of chemical insecticides, MD associating mating disruption with chemical pesticides and organic orchards. For each plot and day, the probability of applying an insecticide was described by a logistic model taking into account the main variables that influence farmers’ decisions to make the application. The protection strategies significantly affected the number of insecticides applied against *C. pomonella*, the application frequencies during the risk periods of each generation of the pest and the choice of active ingredients. Farmers followed the application guidelines more closely within MD protection strategy.

Key words: *Cydia pomonella*, pesticide management practices, application frequency, mating disruption, organic orchards, timing of spray application.

Introduction

Codling moth (*Cydia pomonella* L.) is a major pest of apple and pear orchards. Pest management practices against *C. pomonella* were analyzed according to three protection strategies as described by Simon et al., 2007: conventional (Conv) based on a major use of chemical insecticides, mating disruption (MD) associated with chemical insecticides and organic (Organic) production based on a large use of microbiological control of codling moth with granulosis virus (CpGV). The timing and frequency of insecticide spray applications during the three generations of codling moth were investigated by logistic modelling in relation with national and regional guidelines. We also examined the alternations of active ingredients to reduce the selection of insecticide resistance (Boivin et al, 2005).

Material and methods

Sources of data

Data were collected in 2006 in 71 randomised apple and pear orchards in a small production area near Avignon, in south eastern France. The schedules of pesticide applications were recorded by inquiry and fruit damages were assessed by visual inspection of 1000 fruits per orchard in early July (end of G1).

Six risk periods (Fig. 1) were defined according to the rate of egg hatching of each of the three generations of *C. pomonella* in this area according to Boivin et al. (2005).
Modelling

In each orchard k at day d, the probability for a farmer to apply an insecticide $P$ (Insecticide) was modelled using a logistic function that integrated variables that influence farmers’ decisions such as the risk period, the tree species and the history of previous dates of pesticide applications.

$$P\,(\text{Insecticide} = 1/H_d) = \exp(g_d) / [1+\exp(g_d)]$$

with

$$g_d = a_0 + \theta \ast S + \beta_1 \ast \Delta t_{1d} + \beta_2 \ast \Delta t_{2d} + \beta_3 \ast \Delta t_{3d} + \beta_4 \ast \Delta t_{4d} + \beta_5 \ast \Delta t_{5d} + \beta_6 \ast \Delta t_{6d}$$

where $S$ is tree species and $H_d$ is the treatment history of the orchard, meaning that $\Delta t_{jd}$ measured the part of elapsed time within risk period j since the last insecticide application and present time d. Model parameters were afterward rendered in terms of mean time lag between treatments with an estimation of their confidence intervals at $\alpha = 95\%$.

Results and discussion

Number of insecticide applications

The number of insecticide applications against $C.\,pomonella$ was affected by the protection strategy in apple, but not in pear orchards. MD strategy significantly reduced the number of insecticide applications (41%) when compared to conventional orchards (Fig.2).

Timing of insecticide applications

The mean time lag between two applications was expected to increase in low risk periods
according to the national recommendations. The model indicated that (i) in conventional and organic orchards, the application frequencies did not significantly differ between high risk and low risk periods and (ii) in MD orchards, the application frequencies correctly followed the recommendations, apart from the high risk period G3 corresponding to fruit harvest (Fig. 3).

![Figure 3. Mean and confidence intervals of time lag between treatments for each risk period (§ precise values exceeded 70 days).](image)

**Alternation of active ingredients**
The alternation of insecticide classes in apple orchards (Fig. 4) was very limited in conventional (85% organophosphates, mostly azinphos-methyl and chlorpyriphos-ethyl) and in organic orchards (92% granulosis virus (CpGV)).

![Figure 4. Alternation of insecticide classes in apple orchards according to protection strategies](image)

**Fruit damage**
Fruit damage was lower in MD than in organic and conventional orchards, where 50% and 38% orchards, respectively, had more than 2% infested fruits (Fig. 5).
Conclusions

The number of insecticides applied against *C. pomonella* was significantly affected by the host plant species and by the protection strategy.

Conventional orchards received over 12 treatments against this species, mostly organophosphates, and no relation was found between the application timing and the intensity of pest damage risk. However the conventional protection strategy did not completely prevent the fruit infestation, due to the widespread occurrence of insecticide resistance in this area.

Similarly, the 15 CpGV applications (frequency of 6-9 days) did not prevent fruit damage in organic orchards also due to CpGV resistance in this area (Sauphanor et al., 2006).

Mating disruption allowed a 41% reduction of insecticides applied against *C. pomonella*, with satisfying protection in the orchards of our sample. In these orchards, farmers also better alternated the active ingredients and adapted the timing of applications to risk periods.

The understanding of farmer practices is an essential issue to identify the determinants to the adoption of integrated pest management strategies. Constraints were linked to windy or rainy climatic conditions and to spatial separation between orchards (Kaine and Bewsell, 2008), in addition to time consuming technical acts such as thinning and harvesting.

Acknowledgements

This research was supported by INRA ECOGER program (‘Eco des Vergers’ project) and French National Research Agency program (‘Gedupic’ project).

References


Figure 5. Fruit damage by *C. pomonella* in apple orchards according to protection strategies.
Insecticide resistance of Cydia pomonella (L.) (Lepidoptera: Tortricidae) eggs and first larval instars in Spanish field populations

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Abstract: To know the efficacy of insecticides on Codling moth (Cydia pomonella (L.) (Lepidoptera: Tortricidae)) Spanish field populations of this insect were collected from orchards with heavy damage and the mortality caused by the LC90 of a susceptible strain (S_Lleida) was recorded. Five ovicides and 7 larvicides were tested on eggs and first instar larvae (L1), respectively, from field populations. Commercial and technical products were used for L1 and eggs, respectively. Eggs were topically treated (0.1 µl/egg) and L1 were exposed to semia rtificial diet treated on its surface (2 µl/cm2). Every insecticide showed an efficacy significantly lower than its efficacy for S_Lleida for at least one population. The majority of the field populations were significantly less sensitive to the insecticides than S_Lleida was (96 % and 70% for ovicides and larvicides, respectively). Fenoxycarb and thiacloprid were the most effective ovicides, and lambda cyhalothrin, alpha cypermethrin and chlorpyrifos-ethyl were the most effective larvicides. For three field populations, an inverse relationship between the efficacy of azinphos-methyl and chlorpyriphos-ethyl was observed. To know the role played by detoxification mechanisms, esterase (EST), mixed-function oxidase (MFO) and glutathione-S-transferase (GST) activity was evaluated on L1. Seventy percent of field populations showed a MFO activity significantly higher than the susceptible one, but only one of them also showed higher EST and GST activity.

Cydia pomonella, insecticide resistance, field populations, eggs, L1, detoxication enzymatic activity.
Molecular detection of pest resistance to insecticides

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Abstract: Insecticide resistance occurs at three levels in insects: i) stopping penetration through barrier tissues ii) conjugation, storage, and metabolisation in internal tissues iii) modification of the molecular target site. The detection of these biological adaptations is often realized by the use of bioassays. This technique allows characterizing the resistance level of a population to a given compound, but is not informative on the mechanism. Therefore, it limits the potential of investigation and resistance management becomes more difficult. Molecular detection can be useful, enabling the identification of target mutations, and the modifications in the expression or the structure of detoxifying enzymes. Acetylcholine esterase and the sodium channel are two important molecular targets of organophosphates (OPs), carbamates and pyretroids, respectively. The study of gene sequences allows the development of molecular tools in order to screen field populations.

We have already developed some molecular tools to detect pyrethroid resistance in *Cydia pomonella*. We are now investigating the molecular structure of target sites in other pest species, including *Cydia molesta* aiming to define new molecular tools for resistance detection. The first results are presented and discussed.

Key words: resistance, insecticides, molecular detection, acetylcholine esterase, sodium channel

Introduction

Insecticide resistance involves three main mechanisms in insects i) reducing the penetration through barrier tissues ii) insecticide conjugation, storage, and metabolisation in internal tissues iii) modification of the molecular target of the insecticide. The detection of these biological adaptations is usually obtained using biotests, which allow the determination of the resistance levels to the different compounds, regardless of the mechanism. This technique requires numerous insects at a defined developmental stage for each insecticide tested. Molecular detection may therefore be an interesting tool, allowing a genotype characterization at the individual insect level and making it easy to identify target mutations, expression or structural modifications of detoxification enzymes that may or may not lead to cross resistances.

This study focused on the codling moth *Cydia pomonella* and the oriental fruit moth *Cydia molesta*. The acetylcholine esterase and sodium channel sequences of the first species are already published whereas those of the second are not. Firstly, we developed routine molecular tests to detect kdr mutations in the sodium channel of codling moth field populations. We then tried to identify mutations on two molecular targets of insecticides in *C. molesta*.

Material and methods

*Insects*

Three *C. pomonella* populations were collected in treated orchards with different insecticide programs (reduced treatments for 1, 2 or 3 years). A fourth population originating from an untreated experimental orchard was considered as a reference.

Acetylcholine esterase gene was sequenced in six *C. molesta* individuals, one coming
from a susceptible reference strain and five from treated orchards expected to host OP-resistant populations.

**Routine molecular tests in C. pomonella**

The genetic variability of a 169 bp fragment in the sodium channel gene was analysed by PCR-RFLP. This fragment encompasses the mutation L1014F which is linked with pyrethroid resistance (Brun-Barale et al., 2005).

Rapid genomic DNA extraction was obtained from an adult leg with 10% Chelex 100 (Bio-rad) solution and 10mg/ml proteinase K (Walsh et al. 1991). After a five-fold dilution, this extract was used as a DNA template for PCR amplifications.

PCR amplifications were carried out in a 12 µl reaction volume containing, 1X GoTaq buffer (Promega), 200µM of each dNTPs, 0.208µM of each fluorescent labelled or not CPNa F forward primer (5'-TAGAGAGCATGGGATGCG-3'), 0.416µM of reverse primer CPNa R (5'-AATTTCCTGAGCCCTTGATCG-3') (Franck et al. 2007), 1.5 mM of MgCl₂, 0.1 mg/ml of BSA, 0.75 Unit of GoTaq and 2µl of DNA template. Thermal conditions were as follows: 94°C for 3min, followed by 35 cycles of 94°C for 30s, 55°C for 60s and 74°C for 45s.

A quantity of 5 µl of the PCR product was subsequently digested with 1 unit of the restriction enzyme Tsp509I (NEB) in 25 µl reaction volume at 65°C for 16 hr. Using a licor IR4200 sequencer, 62 samples could be run in one acrylamide-bisacrilamide (6.5%) gel.

**Characterization of the mutations**

900 pb of acetylcholine esterase and 1343 pb of sodium channel genes were sequenced for the first time in oriental fruit moth, in order to find mutations potentially involved in OP or pyrethroid resistance.

Total DNA extraction from oriental fruit moth was based on hexadecyl-trimethyl-ammonium bromide (CTAB) protocol by Murray & Thompson (1980). Adult were ground in 200µl of proteinase K (0.3 mg/ml) and incubated over night at 56°C. Lysis occured at 65°C during 1 hour by adding 300µl of lysis buffer (TRIS-HCl 200mM, EDTA 50mM, NaCl 2M, CTAB 2%) and 100µl of sarcosyl 5%. Proteins were precipitated and separated by chlorophorm-isoamylalcohol (24:1) treatment. Nucleic acids were precipitated at -20°C after adding one volume of isopropanol. DNA pellet was washed in ethanol and resuspended in 60µl of miliQ water. PCR amplifications were carried out in a 25 µl reaction volume containing, 1X GoTaq buffer (Promega), 200µM of each dNTPs, 0.4µM of each primer: ACE 1S (5'-CCCAGACCTGGTAAAGCTG-3') and ACE 1R (5'-TGCTCTCTGGTAATGCTACG-3') for ace-1 or Super KdrF(5'-GGCCGACAGTTAATTTACA-3') and Intron 2 SKdr R (5'-GCAAATACATGCTCTTA-3') following by Kdr mol F (5'-GTGGAACCTACCGACCTTC-3') and CgD 2 (5'-CAAGGCTAAGAAAAAGTGAAG-3') (Brun-Barale et al., 2005) for para voltage-dependant sodium channel, one Unit of GoTaq and 2µl of DNA template. Thermal conditions were as follows: 94°C for 3min, followed by 35 cycles of 94°C for 30s, 55°C for 60s and 74°C for 2min. ACE 1S, ACE 1R primer were designed on codling moth cydpom-ace1 sequence, noted in the GenBank database under the reference DQ267977 (Cassanelli et al. 2006). Super Kdr F and intron 2 SKdr R primer were designed on codling moth sodium channel gene (AY763097). Kdr mol F primer was found on the C molesta sequence determined previously, and CgD 2 is defined by Brun-Barale (2007).

PCR fragments obtained (1080 pb for ace-1, 1245 pb (super Kdr F/intron 2 Skdr R) and 250 pb (Kdr mol/CgD 2) for para) were purified after visualisation from 1% agarose gel with the QIAquick® Gel Extraction Kit and directly sequenced (Genome expess). Data analysis were performed with the BioEdit free software.
Results and discussion

**PCR-RFLP diagnostic test**

Table 1 shows the proportion of kdr mutations found in four populations of *C. pomonella* subjected to different crop protection programs.

Table 1. Proportion of *C. pomonella* individuals carrying a kdr mutation in the sodium channel

<table>
<thead>
<tr>
<th>% individuals</th>
<th>Number of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ss</td>
</tr>
<tr>
<td>susceptible</td>
<td>93.5</td>
</tr>
<tr>
<td>trt stop 3 year ago</td>
<td>86.0</td>
</tr>
<tr>
<td>trt stop 2 years ago</td>
<td>80.0</td>
</tr>
<tr>
<td>trt stop 1 years ago</td>
<td>82.3</td>
</tr>
</tbody>
</table>

The kdr mutation was present in the four *C. pomonella* populations, even in the absence of selection pressure (6.5% heterozygotes in the susceptible population). So, the fitness cost of this mutation is presumably low making resistance management difficult, as attested by the high rate of mutation several years after relaxing the chemical protection. Due to the widespread nature of the kdr mutation, the use of pyrethroids against this species may rapidly lead to the selection of homozygous resistant individuals.

**Sequence analysis**

Sodium channel: Using primers designed for *C. pomonella* we successfully amplified part of the sodium channel gene in *C. molestia* and sequenced it. Few differences were detected between these two tortricidae in exons (11 mutations), but sequenced introns showed many differences in size and composition compared to *C. pomonella*. Protein sequences were strictly similar in susceptible codling moth and oriental fruit moth.

Acetylcholine Esterase: The same method was used for this gene. A longer part of ace-1 sequence was obtained. No differences of sequence were found among the five individuals sequenced. 77 point differences were found between the DNA sequences of *C. molestia* and *C. pomonella*, 3 of them being responsible of protein variation (Figure 1). These 3 amino acids are well conserved in other species. They are presumably not involved in the enzyme conformation and activity.
C. pomonella R 121
C. molesta 1

C. pomonella R 181
C. molesta 12

C. pomonella R 241
C. molesta 72

C. pomonella R 301
C. molesta 132

C. pomonella R 361
C. molesta 192

C. pomonella R 421
C. molesta 252

Figure 1. Alignment of protein sequences of C. molesta (consensus sequence of 5 individuals) and C. pomonella (Cassanelli et al. 2006) resistant cydpom-ace1 allele. *MACE mutation (F290 in Torpedo AChE)

The discovery of these sequences initiated the research into mutations in resistant populations.

Acknowledgements

We would like to acknowledge Alan Knight for providing us with wild populations of C. pomonella. We thank Pierre Franck and Benoit Sauphanor for critical review of the manuscript.

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Walsh, P.S., Metzger, D.A., Higuchi, R. 1991: Chelex (R)100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 507.
New isolates of CpGV overcome virus resistance of codling moth

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Abstract: Since 2004 codling moth (Cydia pomonella) populations with resistance towards the Mexican isolate of Cydia pomonella granulovirus (CpGV) have been found in Austria, France, Germany, Holland, Italy and Switzerland. In the following years Andermatt Biocontrol developed Madex Plus and several other new virus isolates, which can overcome the resistance. The new isolates were selected on virus resistant codling moth populations in the laboratory. The virus isolates were tested on sensitive and virus-resistant codling moth populations in laboratory bioassays and in field trials. All tested new virus isolates showed a good efficacy on sensitive codling moth larvae comparable to or better than the Mexican isolate. Also all the new virus isolates gave good control of Mexican isolate-resistant codling moth populations. Andermatt Biocontrol is thus able to offer products based on new virus isolates that present the solution against virus resistance.

Codling moth, Cydia pomonella, Granulovirus, CpGV, Resistance, New isolates
Evaluation of technical scenarios for the peach-brown rot system using a virtual fruit model simulating quality and storage potential

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Abstract : Improving fruit quality while reducing pesticide and water use supports both consumers’ requirements and environmental and health concerns. This objective promotes some alternative technical scenarios that use more cultural than chemical control for pest management. Our study focused on the peach-brown rot system (\textit{Monilinia laxa}). It aims at determining sets of cultural options providing an optimal trade-off between revenue build-up, consumers’ requirements and environmental impacts. We used a modelling approach to simulate technical scenarios by using a virtual fruit model describing the seasonal changes in peach fruit quality traits during final swelling under the influence of climatic, biotic and cultural factors. We defined 243 virtual scenarios based on agronomical and epidemiological inputs (time and intensity of thinning, irrigation, cultivar choice and disease control). Virtual scenarios were evaluated on a multi-criteria profile of performance integrating storage potential, organoleptic and environmental factors, according to different objectives of profitability, water saving and no pathogen entry (cuticular crack) on fruits. Scenarios including water stress during final swelling are promising while requiring an evolution of market standards.

Key words : brown rot, cuticular crack, fruit quality, irrigation, modelling, peach, profitability, thinning, storage potential, water saving.

Introduction

Integrated fruit production (IFP) objectives are to produce high quality fruits in an economically sustainable way by minimizing pesticides and resources use to preserve both health and the environment (Cross & Dickler, 1994). The question of the reduction of pesticides points out the necessity to study the interactions between fruit-trees and pests under the influence of cultural practices in order to find some alternatives for pest management. But how can we design technical scenarios integrating agronomical and environmental considerations? A modelling approach is well adapted in a first step to evaluate a large range of production systems to better target an experimental stage. Moreover, the complexity of fruit quality profiling also requires this approach to represent underlying processes, their interactions and the influence of environmental (climatic or biotic) and cultural variations (irrigation and crop load) on their expression. We focused our work on the peach-brown rot system (\textit{M. laxa}). Thinning and irrigation are the main cultural practices modifying peach fruit growth (Naor \textit{et al.}, 1997). By using and improving an existing virtual fruit model (Lescourret & Génard, 2005), we constructed various technical scenarios reflecting various cultural practices and evaluated them on their ability to provide a correct trade-off by considering a multi-criterion profile of quality. Results are presented.

Materials and methods

\textit{Description of the integrated virtual fruit predicting outputs of agronomical interest}
The virtual peach fruit model simulates the seasonal changes in several peach fruit quality traits during the final swelling of a “mean” fruit on the shoot-bearing-fruit (Lescourret & Génard, 2005). The model runs at a daily time step. It was developed by integrating three existing process-based models describing fruit dry mass growth, sugar accumulation and water accumulation, respectively (Figure 1). It was improved by integrating (i) the variation of fruit surface conductance and its components (cuticle, stomata and cuticular cracks) in relation with the fruit growth (this variable being implicated in fresh mass and sugar content) and (ii) an epidemiological function predicting the storage potential according to fruit growing conditions and the inoculum pressure of \( M. \text{ laxa} \). The integrated model is fully described in Gibert (2007).

**Construction of 243 virtual technical scenarios**

Virtual technical scenarios were developed to reflect various cultural practices (cultivar choice, time and intensity of thinning, irrigation level, disease control). These agronomical and epidemiological factors were described through a parameter characterising the susceptibility to cuticular cracking (SCC), the fruit dry mass at thinning (FDMT), the shoot:fruit ratio (fruit crop load, FLC), the sequences of leaf and stem water potentials (IL) and the inoculum density (ID). For each factor, three distinct levels were applied to simulate contrasted situations, that is altogether 243 virtual scenarios detailed in Table 1. Irrigation levels were considered to vary with the fruit crop load (Naor et al., 1997) when water was limited.
Table 1. Details about the construction of the 243 virtual technical scenarios.

<table>
<thead>
<tr>
<th>Agronomical and epidemiological factors</th>
<th>Levels of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptibility to cuticular cracking (SCC)</td>
<td>Low</td>
</tr>
<tr>
<td>Fruit dry mass at thinning (FDMT)</td>
<td>Small</td>
</tr>
<tr>
<td>Fruit crop load (FCL)</td>
<td>Low</td>
</tr>
<tr>
<td>Irrigation level (IL)</td>
<td>Water stressed during pit hardening</td>
</tr>
<tr>
<td>Inoculum density (ID)</td>
<td>Low</td>
</tr>
</tbody>
</table>

**Definition of multi-criteria profile of performances**

The five quality traits of interest were studied at harvest for each virtual scenario: the sweetness index ($SI$; Kulp et al., 1991), the flesh dry matter content ($FDMC$), the ratio of flesh per fruit fresh mass ($FFFM$), the cuticular crack density ($CCD$) indicative of shrivelling during storage, the fruit fresh mass which corresponds to a commercial grade and the probability of fruit infection by *M. laxa*. These two later variables were associated into the revenue calculation ($R$; €/tree). Each quality traits were separated into four classes described by colours in Table 2.

Table 2. Description of classes for quality traits constituting the profile of performances

<table>
<thead>
<tr>
<th>Quality traits</th>
<th>Classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetness index (%; $SI$)</td>
<td>Very low: $\leq 5.5$</td>
</tr>
<tr>
<td>Flesh dry matter content (%; $FDMC$)</td>
<td>Very low: $\leq 10$</td>
</tr>
<tr>
<td>Flesh per fruit fresh mass ratio (%; $FFFM$)</td>
<td>Very low: $\leq 90$</td>
</tr>
<tr>
<td>Cuticular crack density (%; $CCD$)</td>
<td>Very low: $\leq 3$</td>
</tr>
<tr>
<td>Revenue (€/tree; $R$)</td>
<td>Very low: $0$</td>
</tr>
</tbody>
</table>

**Evaluation of scenarios according to distinct objectives: profitability, water saving and no pathogen entry**

We analysed and classified the different scenarios by considering the profile of performances at harvest in relation with distinct objectives corresponding: (i) to the present market standards or profitability, which only consider the fruit commercial grade, (ii) to a will of water saving or (iii) to an avoid of the chemical protection by deciding to produce fruits presenting no cuticular crack, that is no pathogen entry.
Results and discussion

Results of the evaluation of the scenarios according to the distinct objectives mentioned above are presented in Table 3. The most beneficial scenarios corresponded to what is currently done and recommended: big commercial grade that is very interesting in terms of revenue for growers, irrigation restricted during a short period (pit hardening) and a lot of applications of fungicide to control the disease pressure. Fruits from these scenarios present a poor quality profile: big size, slightly sweetened and presenting a short shelf life (very high $\text{CCD}$). In comparison, an other set of cultural options only differing by a long but moderate water restriction during the final swelling leads to a better trade-off for the fruit quality profile. It improved the sweetness index, the flesh dry matter content and its post-harvest shelf life. Moreover, this scenario could be very attractive for producers and consumers since they contribute to water saving. Fruits without any cuticular crack required a normal crop load, to choose small fruit at thinning date, and a water restriction during the final swelling. Although they are no lucrative nowadays (commercial grade out of standards), this scenario produced fruits of good quality (high $\text{SI}$, $\text{FDMC}$, $\text{FFFM}$). Moreover, it could be adopted to a large range of situations of production, i.e. different cultivar susceptibility to cracking and various inoculum densities. Such scenarios, though economical in terms of water use and fungicides while preserving fruit quality and environment, do not meet the present standards but could be considered in the future, in case of segmentation of supply proposed to consumers by retailers (Parker, 1993), or for technical practices based on IFP guidelines.

The model presented here gave the opportunity to evaluate several technical scenarios on their profile of agronomical performances according to a large point of view combining the present market standards, which only consider the fruit commercial grade and IFP requirements that include the consumers’ satisfaction and environmental concerns.

Table 3. Virtual scenarios selected for their adequacy with objectives of profitability, water saving and fruits presenting no cuticular crack (no pathogen entry)

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Inputs</th>
<th>Criteria of performances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{SCC}$</td>
<td>$\text{ID}$</td>
</tr>
<tr>
<td>Profitability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water saving</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pathogen entry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgements

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References


Codling moth (Cydia pomonella L.) egg-laying behaviour on host and non-host Malus sp. and leaf surface metabolites.

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Abstract: Cydia pomonella (C. pomonella) is the main pest of Malus domestica (M. d.). Malus floribunda (M. f.) which is used in orchards to cross pollinate trees, shows no C. pomonella damage. We observed on single trees without any alternative that 60 % of females may lay eggs on M. d. (41 eggs) vs. 0 % on M. f.. After collecting and analyzing, by gas chromatography, leaf surface metabolites, we were able to test the known active metabolite pattern on females to confirm the tree observations. Acceptance and egg-laying was reduced by the M. f. metabolite pattern. The gravid female behavior was observed on trees in no-choice controlled conditions. On both Malus. sp. females preferred to land on the upper side of corymb leaves and on the fruits. Then females generally remained on the site where they had landed. The behavioural difference to both Malus sp. was observed at the stage of ovipositor scanning, which was linked to egg-laying. Scanning was dramatically reduced on M. f. and the locomotion speed was lower. Host and non-host characters belonged to the egg-laying stage and non volatile metabolites.

Key words: Cydia pomonella, Malus domestica, egg-laying, sugars, behaviour.

Introduction

For some Lepidoptera species the correct choice of the host plant by the female is critical. The female has to choose the food source of her offspring without any direct access to feeding sites of larvae which are relatively immobile after hatching (Renwick and Chew, 1994; Schoonhoven et al., 1998).

C. pomonella is one of the most important insect pests in orchards, especially in apple (M. d.) production. However, there is no damage to M. f. used as a source of pollen in orchards. An egg-laying stimulating blend including glucose, fructose, sucrose, sorbitol, quebrachitol and myo-inositol has already been identified from the surface of apple (Lombarkia and Derridj, 2002). The ratios between these metabolites within the blend are mainly responsible for the resistance of an apple tree cultivar (X65-11) to C. pomonella egg-laying (Lombarkia and Derridj, 2008). This poses the questions: i) where is the localized M. f. resistance, ii) what is the metabolite blend concerned, iii) at which behavioural step is resistance perceived by the moth?

Material and methods

Collect of leaf surface metabolites and chemical analysis in GC-FID
Metabolites were collected on both Malus sp. during the egg-laying periods at twilight at the period of maximum egg-laying for the second flight. The collecting process consisted of spraying ultra-pure water on leaves and fruit (Fiala et al., 1990). Four replicates per species consisting of one cluster per tree were sampled. A cluster composed of four leaves for M. d. and six for M. f. The collecting method was the same as described in Lombarkia and Derridj (2008).
Egg-laying behaviour on artificial substrate
The six metabolite blends at similar concentrations to those that occur on the corymb leaves of both *Malus* sp. were presented in no-choice conditions to single gravid female per cage. Cages were lined with nylon cloths impregnated with ultra-pure water (control), see method by Lombarkia and Derridj (2002).

Apple trees
Both *Malus* sp. were two years old, two meters high, cultivated in greenhouse, and tested at the stage J “growth of fruits” following Baggiolini stages. A single tree was used for each *Malus* sp.. *M. d.* Reine des Reinettes variety was used for behavioral observations. It is codling moth susceptible. *M. f.* (Baugène clone) is a good pollinating variety (INRA Angers 1985) and resistant to scab.

Behavior observations
The behavioral observations on trees were carried out in a climatic chamber: 23 ±2°C, 70 ±10% (r.h.) and 16:8 (L:D). The egg-laying of *C. pomonella* starts just after the scotophase (Lombarkia, 2002). We fixed the beginning of the scotophase at 1700 h, so that tree physiology corresponded to the insect egg laying. The duration of the observation was fixed at one hour of darkness from 1700 to 1800 h, during which more than 1/3 of females layed eggs on the tree. During the observation periods, the climatic chamber was lit with red light (60 W). On each species five gravid females having already laid eggs during 48 hours in boxes were released together, in no-choice conditions, on a single tree placed in a large mesh (1 mm) cage (1.5 m x 1.5 m x 3 m). Females were released on four successive days from 1700 to 1800 h solar time. Ten minutes before the beginning of the experiment, the tree was transferred from the greenhouse to the climatic chamber for the observation and after the insect test placed back in the greenhouse. A tape recorder (Pearlcor S 928) with cassette-deck (TDK, MC-60) was used to record the female behavior. The data processing was carried out on a laptop. Different behavioral parameters were directly recorded or calculated: walking, stopping, ovipositor scanning while walking, ovipositor scanning while stopping, locomotion speed, distance covered.

Statistical analysis
Means of speed of locomotion were compared by Mann-Whitney test (P < 0.05). The χ² test was used to compare the proportions of soluble carbohydrates and sugar alcohols (within each group) between the two *Malus* sp. The comparison of the proportions of egg-laying females was made using the χ² test (P < 0.05). The Mann-Whitney test (P < 0.05) was used to compare the number of eggs laid per female on each substrate.

Results and discussion

Leaf surface metabolites and chemical analysis in GC-FID
Chemical analyses of the *M. f.* metabolite pattern showed a difference from *M. d.* (Derridj et al., 1999). Opposite ratios between the two chemical groups gathering three soluble carbohydrates (61.7 ±11.9) on *M. d.* vs. (29.9 ±2.7) on *M. f.* and of the three sugar alcohols (38.3 ±11.9) on *M. d.* vs. (70.1 ±2.7) on *M. F.*. Furthermore, *M. f.* metabolites were higher than *M. d.* (ratio: 8).

Egg-laying on artificial substrate
A blend of six synthetically produced *M. f.* metabolites showed a reduction of egg-laying behaviour (10 ±0.00) and stimulation (0.8 ±0.5) vs. *M. d.* (43.3 ±2.8 and 10.3 ±3.3) and the water control (50 ±1.8 and 6.7 ± 1.6). These results suggest that the non-acceptance for egg-
laying on *M. f.* could be related to higher ratios and concentrations of sugar alcohols.

**Egg-laying behaviour on trees**

Four main sequential behaviors of females were highlighted:

- **Landing** on the apple tree surface, **Visiting** (staying on a site), **exploring** the site by walking, stopping, ovipositor scanning while walking or stopping, **Acceptance** or **non-acceptance** for egg-laying.

![Figure 1: Malus sp. tree sites for landing and then visiting after landing by females (%).](image)

The upper side of the corymb leaves and fruits were the preferred sites for landing and then visiting for both *Malus* sp. (Figure 1). 66.7% of females which layed eggs scanned with their ovipositor while walking vs. 22.2% which did not lay eggs. Their speed of locomotion was also higher (0.6 ± 0. vs. 0.3 ± 0.03).

The stimuli, which generated the egg-laying, may have been perceived by receptors on the legs and the ovipositor by scanning while walking. The examination of the plant surface by ovipositor scanning in *C. pomonella* is necessary for the egg-laying. In *Acrolepiopsis assectella* (*Acrolepiidae*) and *Plutella xylostella* (*Plutellidae*) examination of the plant surface by the antennae and the ovipositor before the egg-laying are typical (Justus and Mitchell, 1996; Thibout and Auger, 1996). Spencer (1996) observed that *Plutella xylostella* (*Plutellidae*) examines the surface with the ovipositor only after meeting stimuli for egg-laying with its tarsi or its antennae.

In addition to primary metabolites, other cues are available on the plant surface, *(E,E)-α*-farnesene, in particular, influence landing and egg-laying of codling moth (Bengtsson *et al.*, 2001; Ansebo *et al.*, 2004; Witzgall *et al.*, 2005). This study shows that on *M. f.*, which is a non host plant, *C. pomonella* lands in the same way as on its host plant. The non-host is perceived in contact with plant surface, before scanning with the ovipositor. This may mean that there is a deterrent detected by legs or there is no positive stimulation for ovipositor scanning. The lower locomotion speed on *M. f.* may mean there is a deterrent effect. Results with the synthetically produced blend of the *M. f.* surface highlighted the importance of non volatile primary metabolites in ovipositor scanning behavior and confirmed their early effect at the acceptance stage. These behavioral observations may help in recognizing new methods for apple tree protection based on surface metabolites and the plant resistance.
References


Apple resistance to arthropod herbivores: genetic basis and modification by environmental factors

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Abstract: Arthropod herbivores reduce the quantity and quality of apple yield. Resistant apple varieties hold promise to increase the sustainability of pest management in orchards, but little is known on the genetic basis of apple resistance to most arthropod herbivores. Knowledge on the apple genome and QTL (quantitative trait locus) analysis is now facilitating the identification of gene regions associated with resistance. 160 F1-progeny plants of a cross of the apple varieties 'Fiesta' and 'Discovery' were surveyed at three different sites in Switzerland. Herbivore infestation per genotype as a measure of resistance was quantified for the apple aphids Dysaphis plantaginea, Dysaphis cf. depecta and Aphis pomi, the apple rust mite Aculeus schlechtendali, and the codling moth Cydia pomonella. The influence of the environmental factor 'drought stress' on apple resistance to a chewing and a sap-feeding herbivore (caterpillar; aphid) was studied in laboratory experiments considering different intensities of pulsed drought stress. Significant QTLs for resistance to D. plantaginea, D. cf. depecta, A. schlechtendali, and C. pomonella were detected. SSR alleles associated to the QTLs may be applied to identify and breed resistant apple cultivars. Environmental factors such as within-canopy variation in climate, and neighbourhood-effects affected herbivore distribution in the field. In the laboratory, pulsed drought stress resulted in non-monotonic resistance responses of apple trees. Low-stress plants showed the highest and high-stress plants the lowest resistance. The studies revealed the genetic basis of apple resistance to different arthropod herbivores and the modifying influence of environmental parameters that may impede QTL detection.

Key words: Apple Malus x domestica, aphids, caterpillars, deficit irrigation, host plant resistance, individual tree genotypes, interactions, pest management, QTLs, pulsed drought stress

Introduction

Apple (Malus x domestica Borkh.) is the most relevant fruit crop in the temperate region. Arthropod herbivores have a negative impact on the quantity and quality of apple yield and require control. The use of resistant apple cultivars may help to limit insecticide input and to increase the sustainability of pest management in apple orchards. Host plant resistance has a genetic basis, and increasing knowledge on the apple genome and QTL (quantitative trait locus) analysis helps to identify gene regions associated with resistance (Liebhard et al., 2003). QTL-based approaches to determine and characterize host-plant resistance against insects are commonly used in annual crops, however detailed analysis of the genetic basis of arthropod resistance in apple has received little attention (Bus et al., 2008; Stoeckli et al., 2008a). Contrary to diseases like scab or mildew, the variability in susceptibility to arthropod pests is generally low between the main apple cultivars. The detection of QTLs of minor effect is impeded by natural microsite-dependent variation of population density, additional to environmental parameters modifying or masking the expression of plant resistance.

Within-tree variation in the distribution of leaf- (Unsicker & Mody, 2005) or fruit-damaging arthropods (Stoeckli et al., 2008b) has to be considered for sampling protocols aiming at quantifying the field resistance of particular tree genotypes. Effects of microsite- or management-dependent tree growth characteristics, and of the position of the studied
genotypes within an orchard in relation to other genotypes (neighbourhood effects) may also interfere with the characterization of the genetic basis of a genotype’s resistance.

Water availability is an environmental parameter of paramount importance for plant growth and development. Temporary drought events are characteristic for many parts of the world, and frequency and intensity of extreme drought is predicted to increase in the future (Christensen et al., 2007). Water deficit may cause drought stress, which may have strong effects on plant resistance to arthropod herbivores. However, the influence of drought stress on plant resistance is not easily predictable, as both decreasing and increasing plant resistance as a consequence of drought stress has been observed (Huberty & Denno, 2004). Future studies, for example on stress intensity, are needed to better understand the effects of the environmental parameter drought stress on plant resistance (Mody et al., 2009).

The goals of the presented studies were (1) to elucidate the postulated genetic basis of apple resistance to different species of arthropod herbivores by QTL analysis, and (2) to assess genotype-independent factors influencing the distribution of pest insects and plant resistance in the field and in the laboratory.

**Material and methods**

**QTL analysis and field experiments**

Resistance QTLs were investigated in the field in a segregating F1 cross of the apple varieties 'Fiesta' and 'Discovery' (Stoeckli et al., 2008a; 2009a,b). Progeny plants representing 160 genotypes were surveyed at three different sites in Switzerland (cantons Ticino, Valais and Zurich). Herbivore infestation per genotype as a measure of resistance in the field was quantified in two consecutive years for the rosy apple aphid (*Dysaphis plantaginea*), the leaf-curling aphid (*Dysaphis cf. devecta*) and the green apple aphid (*Aphis pomi*), for the apple rust mite (*Aculus schlechtendali*), and for the codling moth (*Cydia pomonella*). QTL analyses based on herbivore infestation data were carried out with MapQTL® 4.0 (van Ooijen et al., 2002). The genetic linkage maps for both 'Fiesta' and 'Discovery' (single parent maps), used in QTL analysis, were calculated with 251 apple genotypes and were already published (Liebhard et al., 2003). Kruskal–Wallis tests and interval mapping (IM) were used for QTL analysis. Logarithm of odds (LOD) threshold values were determined by 1000-fold-permutation tests at a significance level of 95% (genome-wide).

The same trees were also investigated to assess the possible importance of within-tree variation in herbivore distribution, of tree growth characteristics and of neighbourhood effects for the quantification of infestation by different apple pest arthropods. Within-tree variation of codling moth infestation was characterized based on a survey of 40’000 apples from 12 sectors of each of the 160 different apple genotypes, considering canopy aspect (north, east, south, and west) and canopy height (bottom, middle, and top) (Stoeckli et al., 2008b). Effects of shoot growth characteristics on aphid population development were repeatedly studied for population growth of the green apple aphid in sleeve cages attached to 200 apple trees of different genotype (Stoeckli et al., 2008c). Neighbourhood effects were assessed for apple aphids and rust mites by quantifying the relationship between infestation levels of neighbouring trees (Stoeckli et al., 2008a; 2009a).

**Laboratory experiments on the effects of drought stress on plant resistance**

Drought stress effects on apple resistance to a chewing and a sap-feeding herbivore (*Spodoptera littoralis* caterpillars; *Aphis pomi* aphids) were studied in laboratory experiments considering control conditions and two intensities of pulsed drought stress (Mody et al., 2009). 'Control' plants were maintained in constantly humid soil, 'low stress' plants were watered for the first time when leaves started drooping (about one week after start of the experiment), and 'high stress' plants before irreversible necrosis occurred. Herbivore
experiments started after approximately three weeks of stress, i.e. 3 – 4 drought cycles for high stress plants and 6 – 7 drought cycles for low stress plants. Plants were watered during herbivore feeding to simulate different natural stress conditions with alternating dry and wet periods and insect feeding on plants that had previously been stressed, but were not while feeding actually occurred. As measures of resistance, plant acceptability (S. littoralis feeding preference in arena experiments; resistance by antixenosis) and plant suitability (S. littoralis growth rate and A. pomi population development; resistance by antibiosis) were quantified.

Results and discussion

QTLs for herbivore resistance in apple
We identified QTLs for herbivore resistance in apple. The detected QTLs highlight the genetic basis of arthropod resistance in apple. Apple genotypes amplifying QTL-relevant markers differed significantly from genotypes not amplifying the markers for the aphid species D. plantaginea and D. cf. devecta (Stoeckli et al., 2008a), the rust mite A. schlechtendali (Stoeckli et al., 2009a), and the codling moth C. pomonella (Stoeckli et al., 2009b) (Fig. 1). The detected markers may facilitate the breeding of resistant apple cultivars by marker assisted selection. They may also be used for screening existing cultivars for resistance to important pest arthropods.

Environmental factors related to apple infestation by arthropod pests in the field
In the field, additional environment-effects on herbivore distribution were identified. The infestation of apple fruits by the codling moth varied within apple tree canopies for first but not second generation larvae, with north-facing apples showing lower infestation than south- or east-facing fruits (Stoeckli et al., 2008b). Population growth of the green apple aphid was positively related to the length and growth of apple shoots (Stoeckli et al., 2008c). Neighbourhood effects appeared to influence the infestation of apple trees by the rosy and the green apple aphids, but not by the leaf-curling aphid and the rust mite (Stoeckli et al., 2008a; Stoeckli et al., 2009a). These genotype-independent determinants of herbivore distribution may mask QTLs, and they may help to explain difficulties in QTL detection for the studied herbivore species.

Figure 1. A significantly lower herbivore infestation was found for the subpopulation of the F1 apple cross amplifying the marker closest to the QTL compared to the apple genotypes not

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amplifying the marker. Herbivore infestation was analyzed by Mann-Whitney U-test. Herbivore species, parent and linkage group (LG), closest marker to the QTL, and $P$-value (* $P<0.01$, ** $P<0.001$, *** $P<0.0001$) are presented (data assembled from Stoeckli et al., 2008a; 2009a,b).

**Experimental drought stress and apple resistance**

Water stress treatments had a strong, non-monotonic effect on feeding preference and performance of the caterpillars and on performance of the aphids (Mody et al., 2009). Plants experiencing low water stress were less attractive to the caterpillars (repeated measures ANOVA, $F_{2,58}=25.0$, $P<0.0001$), indicating increased resistance by antixenosis, and they were also less suitable for development of caterpillars (ANOVA, $F_{2,84}=8.2$, $P<0.001$) than either control or high stress plants (Fig. 2), pointing to an increased resistance by antibiosis. Aphid population development was also lowest on plants experiencing low water stress (ANOVA, $F_{2,33}=9.3$, $P<0.001$), but the best performance was detected for nonstressed control plants (Fig. 2).

The study on drought stress underlines the important role that the intensity of pulsed drought stress plays in mediating interactions between herbivores and their host plants, and it helps to resolve the still existing conflicting evidence of both beneficial and detrimental effects of drought stress on plant resistance to insect herbivores (Mody et al., 2009). The study also suggests that non-monotonic plant responses to pulsed drought stress may be more strongly considered as means of cultural pest control. In particular for crops that show no reduced yield under moderate water stress, a controlled application of pulsed deficit irrigation may help to reduce crop damage by increased crop plant resistance to insect herbivores (Daane & Williams, 2003).

![Figure 2. Herbivore responses to stress treatments (mean ± 1 SE) as measure of plant resistance (caterpillar: L3-L5 larvae of *Spodoptera littoralis*; aphid: *Aphis pomi*). Caterpillar preference: feeding preference in bioassays conducted with leaves still attached to plant in arena experiments. Caterpillar performance: relative growth rates obtained while feeding on an experimental tree over seven days. Aphid performance: aphid population growth in 14 days on an experimental tree. Water stress treatments: C: control, nonstressed plants; LS: low stress; HS: high stress.](image-url)
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References


Peach breeding for multiple resistances to pests and diseases contributes to integrated fruit production

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Abstract: In spite of the worldwide decline in peach production, a constant stream of new varieties are being provide to fruit growers. For the greater part most of these new varieties being produced by private peach breeders, and as a consequence very few are selected on the basis of their resistance to pests or diseases, while the demand of consumers continues to be directed towards a quality fruit product which is free of pesticide residues. Within the framework of one INRA multidisciplinary group (Avignon-Bordeaux), we have developed for several years a wide applied breeding program aiming at improving the resistance of the peach tree to three of its main enemies: the green peach aphid (Myzus persicae), peach powdery mildew (Sphaerotheca pannosa var. persicae) and sharka disease (Plum Pox Virus). This work is globally conducted in a research context oriented towards varietal innovation including fruit quality and durable resistances building. In this way, two complementary approaches have been preferentially held for respectively improving the genetic gain by time unit and a better understanding of peach-enemies relationships. First, a genetic approach integrating the quantitative trait loci or major genes mapping for the development of molecular assisted selection. Second, a functional approach (i) leaning on the study of the insect behaviour and the plant metabolites involved in the resistance to M. persicae (ii) coupled to a candidate-genes research mainly developed for PPV resistance. Whole of first results and perspectives are discussed.

Prunus persica, Myzus, Powdery mildew, Sharka, Genetic linkage map
GEP, a tool to help decision making for pest control advisers in Lleida (Spain)

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Abstract: GEP is a new tool developed by the University of Lleida, IRTA and the Catalan Plant Protection Service to furnish Pest Control Advisers (PCAs) with up-dated information on the spatial distribution of pests in the fruit growing area of Lleida. It is the consequence of the work carried out since 1998, which has been regularly presented in the IOBC WG meetings. The Pest Control Advisers maintain and check the net of pheromone traps, send the results to the Plant Protection Service and the UdL, and receive back the processed information within 3 days. The system has been improved by the use of Google EarthTM maps.

GEP, Advisory system, Spatial distribution
MRV-Carpocapsa: a phenological model as decision support system for Codling Moth (*Cydia pomonella* L.) in Emilia-Romagna (Italy) - Effectiveness assessment in 1998-2008.

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Abstract: A warning Service for pests and diseases of the most important crops was set up in Emilia-Romagna region (Italy) in 1997. Integration of information obtained by forecasting models and fields surveys is used to develop warnings concerning the risk of pest/disease attack. For the control of *Cydia pomonella* is available a phenological time–distributed delay model. Biological parameters were defined in 1991 in lab-trials. On the basis of hourly temperature, the model can simulate the development of the first and second generation. It gives as output the cumulating percentages of egg-laying, egg-hatching, pupation and adult emergence as well as the age structure of the population. The model has been fully tested over 1992-1998 and therefore has been effectively used for ten years in Emilia-Romagna to optimize control strategies in IPM.

From the first application in 1998, it was executed steadily a quality control of simulated data by their comparison to that observed in orchards. As the pheromone traps do not always describe population dynamics properly, it has been chosen to assess the oviposition activity. Weekly field observations were carried out over 1998 – 2008 in an untreated orchard near Bologna.

The eggs were examined for the exact phase of embryonic development determination. Then the egg laying dates were estimated taking into account of specific degree-days for each embryonic phase. Results from the comparison between the simulated data and those observed in field are reported. Altogether, actual and simulated oviposition curves agree fairly well over the last eleven years despite the different climatic condition recorded in this period.

Key words: *Cydia pomonella*, phenological model, warning Service

Introduction

Warnings for *Cydia pomonella* control in the IPM orchards of the Emilia-Romagna region are based on the integration of information obtained by a phenological time–distributed delay model, named “MRV-Carpocapsa”, and field surveys.

The phenological trend of the target stages such as eggs and larvae, otherwise difficult to obtain using field surveys, is more precisely reported by the model on a daily basis. This information is particularly useful for establishing the most appropriate protection measures and their timing. The reliability of the simulated data is one of the main requirements for an efficient warning service using forecasting models. That is why, from the first model application in 1998, a quality control of simulated data has steadily been executed by their comparison to that observed in orchards. Monitoring focused on the oviposition activity as the pheromone traps do not always describe population dynamics properly. Besides the eggs represent the target stage, along with neonate larvae, for chemical and biological applications.
Materials and methods

Model description
The MRV-Carpocapsa model includes a time distributed delay model (Manetsch, 1976). Such a model is suitable to describe the flux of individuals belonging to the same population through the different phenophases. Each individual in a cohort goes through a series of stages and sub-stages at different times so as to simulate the variability due to genetic factors, food, microclimate and so on. In other words, it can describe the age class distribution and genetic variability of poikilo thermal organism populations. The model is based on temperature response at each development stage. The underlying relationship between temperature and development rate is implemented with the non-linear Logan’s function for eggs, larvae and pupae (Logan et al., 1976). A linear equation is used for female adults. Furthermore, the fecundity rate, as a function of age, is expressed by a modified Bieri’s function (Bieri et al., 1983). The number of the sub-stages representing the dispersal in time of the individuals coming out a given stage, or coefficient H, is calculated as the ratio of the squared permanence-time in a given stage and its variance. The model does not take into account any mortality factor, only the natural death of adults.

All of the biological parameters were defined in 1991 through individual-based laboratory experiments at a different constant temperature (4-6 temperatures between 12.6°C and 34°C) and under controlled conditions as close as possible to those found in nature (Butturini et al., 1992).

The model can simulate the development of the first and second generation on the basis of measured hourly temperature. The calculations start at the beginning of the year with a cohort of overwintering larvae. At each hourly time-step, the instantaneous development of each stage and the fecundity are calculated. A ‘delay’ is then assigned to the calculated development, therefore simulating the variability due to the above factors. At the end of each day, the model outputs the cumulative percentages of individuals that have already reached a given stage (egg-laying, newborn larvae, pupating larvae and emerging adults) and the percentages of the individuals that are still in each development stage (egg, larva, pupa, adult).

The model has fully tested for the first and second generations in the period 1992-1998 in several areas of Emilia-Romagna, by comparing both predicted flight and oviposition with the actual data obtained respectively by pheromone traps and field sampling on fruits (Tiso et al., 1999).

Quality control methodology
Field observation - Data were collected in the period 1998 – 2008 from an abandoned pear and apple orchard near Bologna (Italy). A random sample of a hundred fruit cluster was monitored weekly during the whole first and second generation to compare actual egg-laying with the analogous simulated data. As the embryogenesis has usually already begun at the time of sampling, eggs were closely examined for white, red ring and black head stages determination.

Egg-laying dates were estimated taking into account of specific degree-days for each embryonic phase (Wyniger, 1956).

Data analysis - To make comparison with the model possible the following were calculated:
- the total number of field observed eggs (back calculated egg-laying) per generation. The end of the generation was determined at the date of last egg-laying or when a clear decrease of catches and egg-laying occur. It did not take into account any case in which the number of eggs was less than 20 per generation.
- the cumulated percentage of egg-laying on each date
- the differences between the cumulated percentages of actual and predicted egg-laying (expressed in days, + and – sign respectively indicating an anticipation or a delay of the simulation)

**Results and discussion**

In order to interpret the distribution of the differences (in days) between the cumulative percentages of simulated and actual egg-laying, the first and second generation set data (132 and 142 respectively) was displayed with a Box-and-Whisker plot (Fig.1).

The results show some differences between the two generations of the insect: for the first generation, there is a greater dispersion of data and greater frequency of simulated data giving early times compared to those observed; for the second generation, there is a prevailing time-lag with the simulated data compared to observed data.

Observation-simulation pairs of data for each generation were tested using Pearson’s correlation coefficient (Fig.2). The values of Pearson coefficient are 0.902 for the first generation and 0.946 for the second generation. The results show that there is significant correlation (P < 0.01) between simulated and observed data for both generations.

The period between 1998 and 2008 revealed weather trends which, at times, deviated considerably from the norm. In the years 2000 and 2007, for example, fifty percent of cumulative egg-laying in the first generation was very early (May 13th and May 9th, respectively), whereas in the year 2004 a considerable time-lag was observed (May 31st). The graph in Fig. 3 shows that the model is capable of representing field-data satisfactorily even in abnormal years.

The results were good owing to the significant correlation between the simulated data and observed data for both generations of the insect. They were particularly satisfactory in view of the greatly-differing climatic conditions in the years in which the work was carried out. This highlights even further the good forecasting capacity of the model.

| Table 1. Difference in days between observed and simulated data |
|-------------------|-------------------|
| generation        | 1st | 2nd |
| Number of data set| 132 | 142 |
| median            | 5   | -1  |
| min               | -15 | -13 |
| max               | 23  | 6   |
| mean              | 5.95| -1.47|
| SD                | 7.848| 4.322 |

+ and – signs indicating an anticipation or a delay of the simulation
Fig. 2b. - Correlation between predicted and observed data for the first and second generation
Figure 3. 50% egg-laying - Comparison between the model (white dot) and the observations (black dot) for the first and second generation

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Development of a dynamic population model as a decision support system for Codling Moth (Cydia pomonella L) management

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Abstract: In 2004 RIMpro-Cydia was developed as a dynamic population model that simulates the within-year biology of a local codling moth population. The model is meant to be used by growers and advisors to optimize the control of codling moth populations in organic and integrated managed orchards. The model is based on literature data and unpublished research data. Fractional boxcar trains are used to mimic the dispersion in the developmental processes. The model is run in real time on the data input of local weather stations, starting on 1 January. The output of the model was compared with the results of field observations in four years in untreated orchards. The progress in egg deposition as predicted by the model was in general agreement with the field data. The start of the egg deposition period was predicted well. The end of the egg deposition period was predicted when, in the field, about 10% of the eggs were still to be laid in some years. There was no consistency in the relation between cumulated pheromone trap catches and the cumulative egg deposition as calculated from the field data.

Codling moth, Cydia pomonella, Simulation model
Impact of flower strip establishment in apple orchards on natural enemy populations

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Abstract. Composite flower strips were established in 2006 in three commercial apple orchards in Quebec. Strips were composed of the Canadian goldenrod (Solidago canadensis) and the common yarrow (Achillea millefolium), two native plants known to attract beneficial organisms. The aim of the project was to reduce pesticide application treatments directed against orchard pests, more specifically the European red mite (Panonychus ulmi), the two-spotted spider mite (Tetranychus urticae), the green apple aphid (Aphis pomi), the European apple sawfly (Hoplocampa testudinea), the tarnished plant bug (Lygus lineolaris) and the white apple leafhopper (Typhlocyba pomaria). In the present trial, populations of potential natural enemies of these pests were monitored in 2008, using sticky white traps, in both control and managed areas. The most common predator species were Coccinellidae, Syrphidae, Neuroptera (Chrysopidae and Hemerobiidae) and Aranea. Results varied according to the species of natural enemy, the treatment (control versus floral strip) and the distance to the flower strip.

Key words: habitat management, Achillea millefolium, Solidago canadensis, Aranea, Chrysopidae, Coccinellidae, Hemerobiidae, Syrphidae, predator.

Introduction

Habitat management in agriculture can provide alternative food (preys, hosts, pollen, nectar), refuges, oviposition and breeding sites to natural enemies (Altieri, 1994; Andow, 1991). Such management practices aim to increase the diversity and abundance of natural enemies in order to control pests and consequently reduce insecticide applications. Several studies deal with pest management in apple orchard but no one treatment was sufficient to efficiently control pest populations (Brown & Glenn, 1999; Wyss, 1996). In this study, we chose to establish in apple orchards a flower strip composed of two native plants which have been reported to attract beneficial organisms. The goal is to increase significantly the abundance of natural enemy populations and to establish permanently a biodiversity able to reduce apple’s damages.

We hypothesize that the management of a flower strip will increase the abundance of natural enemies in apple orchard.

Material and methods

Flower strips

Strips of composite flower were established perpendicularly to apple trees rows in 2006 in three commercial apple orchards in Quebec (Canada) (45N; 71O). Flower strips (2m x 20m) were composed of Canada goldenrod (Solidago canadensis) and common yarrow (Achillea millefolium). Each orchard contained two or four flower strips. Each managed area (with flower strip) was paired to an unmanaged area (control) for comparison, and had similar apple
tree age, size, rootstock and variety. Buffer zones of 10 to 15m were established on each side of the treatment areas to minimize edge effect.

**Sampling of natural enemies**
Natural enemy populations were monitored weekly from May to September in 2008 in the two central apple tree rows, with a sticky white trap hung (150cm from ground level) on each of the two apple trees located at 0, 10 and 30m from the treatment areas. Two traps were also placed 50cm above the ground within each flower strip and control treatments. Sticky white traps were kept in an icebox until the identification of insects in the laboratory. The main groups monitored were composed of Syrphidae, Coccinellidae, Chrysopidae and Hemerobiidae adults, and Aranea immature and adults. A paired t-test was used to compare managed and unmanaged areas at each distance from the treatment.

**Results**

**Natural enemies assemblage**
In 2008, 1868 individuals of natural enemy were collected on sticky white traps. The mean number per trap and per week was significantly higher at 0m in managed areas (0.772 adults) than in control areas (0.544) (df = 7; T = -1.960; \(P = 0.0454\)).

**Syrphidae**
Syrphidae constituted the most important family of natural enemies with 1224 captured adults. They were more abundant within the control (1.508 adults/trap/week) than within the flower strips (0.844 adults) (df = 6; T = 2.483; \(P = 0.0238\)) (Fig. 1).

![Figure 1. Mean abundance per trap and per week of Syrphidae adults captured on sticky white traps in three commercial apple orchards. * indicates statistically different values by Paired T-test (\(P \leq 0.05\)).](image)

**Coccinellidae**
Coccinellidae was the second most important family of recovered natural enemies with 389 individuals belonging to more than 10 species. Four species represented 83% of all Coccinellidae captured. They were more abundant within the flower strips (mean of 0.554 adults/trap/week) than within the control (mean of 0.336 adults) (df = 6; T = -2.604; \(P = 0.020\)) (Fig. 2).
Aranea and Neuroptera

No differences were observed between managed and unmanaged areas concerning the abundance for both Aranea and Neuroptera captured on sticky white traps.

Discussion

Our study shows that management of a flower strip has a significant positive impact on natural enemies. They were more abundant in managed orchards, near the flower strip, than in unmanaged area. However, this impact was restricted to the edge (<10m) of the orchard. Results about impact of such management techniques differ according to the studies, revealing either floral diversity increases natural enemy’s abundance (Kinkorova & Kocourek, 2000), or has no significant effect on natural enemies (Prokopy, 2003; Steffan-Dewenter & Leschke, 2003).

Syrphidae were significantly more abundant on the control groundcover than in the flower strip. Nevertheless, no difference was observed at any distance in the orchard between treatments. Our results contradict previous studies which noted that abundance of Syrphidae increased with flower density and proximity (Kohler et al., 2008; Pontin et al., 2006).

Coccinellidae were more abundant in the flower strip than in the control, confirming that *A. millefolium* and *S. canadensis* efficiently attract these predators. They may be attracted by pollen and nectar from these plants (Spellman et al., 2006; Price et al., 1980). In spite of this attractiveness, no effect was observed within the orchard.

Concluding, samplings have to be continued during the next years in order to establish 1) if the impact would be confirmed and increased, 2) if the impact would spread in the entire orchard or be restricted to the vicinity of the strips.

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COSMOS, a spatially explicit model to simulate the epidemiology of Cosmopolites sordidus in banana fields.

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Abstract: A stochastic individual-based model called COSMOS was developed to simulate the epidemiology of Cosmopolites sordidus in banana fields, based on simple rules of local movement of adults, egg-laying of females, development and mortality, and infestation of larvae inside the banana plants. The biological parameters of the model were estimated from literature. The model was validated at the small-plot scale. Simulated and observed distributions of attacks were similar in twelve out of 18 plots (Kolmogorov-Smirnov test). An exhaustive sensitivity analysis using the Morris method, showed that dispersal and demographic parameters of adults were the most influential parameters.

Keywords: Banana weevil; Curculionidae; Individual-based model; Life-history traits; Musa, West Indies

Introduction

Understanding the epidemiology of pests is of special importance for designing innovative control systems. The spatial component of epidemiology is crucial to understand the spread of damage from a localized inoculum or when pest dispersal is limiting (Winkler and Heinken, 2007).

In this work, we took as case study Cosmopolites sordidus (Coleoptera: Curculionidae) (Germar. 1825), a major pest of banana cropping systems. This tropical insect has a long development time and life span, low mortality rate and limited dispersal abilities. Larvae bore into corm of banana plants (Gold et al., 2001). Based on these characteristics, we chose (i) a spatially explicit approach to understand how local movements influence spatial distribution and damage of this pest in a homogeneous habitat, (ii) an individual-based approach assumed to help explaining observed population patterns, considering that different behaviours at the individual level allow the emergence of population level properties (Grimm and Railsback, 2005).

As sensitivity analyses are key steps of modelling processes, we performed an exhaustive sensitivity analysis using the Morris method to ascertain the importance of each biological parameter, in relation to their level of uncertainty as indicated by the literature.

Material and methods

According to the COSMOS model, the adults disperse with stochastic movement in a field that is figured by a grid with one plant per cell. Females lay eggs on banana plants according to the phenological stage of plants and to density-dependence, and larvae emerging from these eggs bore into the corm of the plants. Stage duration of juveniles and phenologic stages of...
banana plant are temperature-dependent. Plants pass through three distinct stages: maiden sucker, preflowering, post-flowering until harvest. Just before flowering, a new sucker of the mother plant is selected and grows simultaneously in the same cell. Delay between two consecutive harvests, corresponding to a cropping cycle, is approximately 200 days.

Damage caused by C. sordidus on banana plants was measured on 18 plots during two cropping cycles at the station of the CIRAD, Neufchâteau, Guadeloupe. At harvesting of the first and second cropping cycles, damage caused by larvae inside the corm was evaluated for each banana plant, separately.

For each of the 18 plots used for model evaluation, the model was initialized using populations of individuals distributed in the plot, estimated according to the attacks recorded at the end of the first cycle for each plant, i.e. the attacked circumference. Then, the model simulated the epidemiology of C. sordidus during the second cropping cycle. We used the Morris (1991) method to evaluate sensitivity of the model. Twenty biological parameters of the insect were tested. According to the Morris method, the mean $\mu$ and the standard deviation $\sigma$ of the absolute values of the elementary effects of each parameter are used as sensitivity measures to ascertain parameter importance.

For each plot used for model evaluation, smoothed distributions of attacks were plotted using the 100 replicates of each simulation, and compared to observations. Each replicate of simulation was compared to observations using the Kolmogorov-Smirnov test (Mellin et al., 2006). We defined the index $I_{ks}$ as the ratio of significant ks tests ($P < 0.05$) over the 100 replicates of a simulation.

**Results**

**Model evaluation**

Distributions of simulated attacks were not significantly different from observations for 12 plots out of 18 ($I_{ks}>0.75$) and for three plots, $I_{ks}$ was between 0.50 and 0.75. For the three last plots, $I_{ks}$ was under 0.50 (Figure 1).

Fig. 1. Observed and simulated distribution of banana damage for an example of 4 plots infested by C. sordidus in Guadeloupe.
Sensitivity analysis

Eight parameters played a significant role in the magnitude of mean and standard deviation of the distribution of attacks: dispersal parameters (DC1, DC2), demographical parameters (FH, MRL, ML and PE), threshold density effect (DE) and diameter of gallery (G; Fig. 2). The part of DC1, DC2 and DE was more important in the explanation of standard deviation of attacks than in that of the mean.

![Graph showing sensitivity analysis](image)

Fig. 2. Analyses of the COSMOS model sensitivity to biological insect parameters using the Morris method, focusing on two main parameters of the distribution of attacks: (a) Mean, (b) Standard deviation.

### Table 1: Model parameters of importance in sensitivity analyses

<table>
<thead>
<tr>
<th>Description</th>
<th>Code</th>
<th>Range used for sensitivity analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg Proportion of eggs removed by predators</td>
<td>PE</td>
<td>0.33-0.68</td>
</tr>
<tr>
<td>Larva Mortality Rate for larvae</td>
<td>MRL</td>
<td>0.32-0.64</td>
</tr>
<tr>
<td>Larva Diameter of gallery (in cm)</td>
<td>G</td>
<td>0.8-1.2</td>
</tr>
<tr>
<td>Adult Number of individuals necessary for density-dependent effect on fecundity</td>
<td>DE</td>
<td>10-33</td>
</tr>
<tr>
<td>Adult Number of eggs per week per female for groups of less than 20 females</td>
<td>FH</td>
<td>1.7-3.2</td>
</tr>
<tr>
<td>Adult Proportion of individuals moving to the next row every 3 time steps (%)</td>
<td>DC1</td>
<td>1.5-6.6</td>
</tr>
<tr>
<td>Adult Proportion of individuals moving to the second row every 3 time steps (%)</td>
<td>DC2</td>
<td>0-3</td>
</tr>
<tr>
<td>Adult Maximal lifespan of adult stage (days)</td>
<td>ML</td>
<td>520-900</td>
</tr>
</tbody>
</table>

Discussion and conclusions

With an IBM that includes local movements and individual variability of aging and mortality, according to tests performed for a wide range of levels of attack at initialization, including low levels of attack, we reproduced, with good accuracy, levels and distribution of attacks of *C. sordidus* to banana plants. Thus, we estimate that COSMOS reflects real population trends and is an interesting tool to predict the epidemiology of *C. sordidus* at the field scale.

The basic principles of the epidemiology of *C. sordidus* were successfully integrated in the COSMOS model. Further steps should involve integration in this model of management practices able to influence the epidemiology of this pest and to contribute to Integrated Pest Management (Huffaker and Gutierrez, 1999, p. 682), such as the use of resistant varieties, traps and biological control agents, as suggested by Gold et al. (2001). Furthermore, to design
IPM schemes at the farm scale, the next step will be to scale up the model to a group of fields and to account for interfaces between fields.

References


Effects of thermoperiodic conditions on the developmental rate of codling moth larvae of resistant and non-resistant strains to chemical and viral (CpGV) insecticides.

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Abstract: The developmental rate of codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae), is supposed to be directly proportional to air temperature between the lower and upper developmental thresholds. However, some review papers suggest that insect species have a higher developmental rate when reared in thermoperiodic conditions as compared with constant temperatures. Thus, in this study, the developmental rate and the zero temperature threshold of codling moth for the larval stage were determined in thermoperiodic conditions for strains resistant and non-resistant to chemical and viral (CpGV) insecticides. Two methods were used to determine the zero development temperature for the four C. pomonella strains: (i) the x-intercept method and (ii) the thermal unit test. Our study supports the “thermoperiod hypothesis” and suggests that the effect of thermoperiod on the developmental rate of C. pomonella larvae should be taken into account in the development of phenological models.

Keywords: thermoperiod, codling moth, developmental rate, insecticide resistance

Introduction

The development of poikilotherms is highly constrained by temperature. For insects, most studies about the relationship between temperature and developmental rate have been conducted in laboratory experiments using constant temperatures. Less well understood is the form of the relationship that best describes variation in developmental rate with thermoperiod (i.e. daily fluctuations in temperature). Some review papers suggest that insect species have a higher developmental rate when they are reared in thermoperiodic conditions as compared with constant temperatures (Howell & Neven, 2000). For the codling moth, Cydia pomonella (L.) (Lepidoptera: Tortricidae), the “thermoperiod hypothesis” seems to be corroborated by some physiological time data obtained for non-insecticide resistant strains (El Idrissi, 1980; Howell & Neven, 2000).

The codling moth is a worldwide pest at the larval stage in apple and pear orchards that requires frequent control treatments. The relationship between temperature and developmental rate of codling moth has been evaluated in a number of studies. The developmental rate of C. pomonella is supposed to be directly proportional to air temperature between the lower and upper developmental thresholds of 9.7-10°C and around 32-35°C, respectively, with a maximum developmental rate at approximately 30°C. To test the “thermoperiod hypothesis”, the developmental rate and the zero temperature threshold of codling moth were determined for the larval stage in thermoperiodic conditions for strains resistant and non-resistant to chemical and viral (CpGV) insecticides.
Material and methods

Insect material
The codling moths used in the study were descendants of eggs taken from the PSH INRA Avignon strains. Four strains were used: (i) Sv (susceptible strain), (ii) Rdfb (resistant to diflubenzuron), (iii) RΔ (resistant to deltamethrin), RGv (resistant to *C. pomonella* granulovirus).

Experimental setup
For each strain and for the following synchronous thermoperiods 10°C:15°C, 15°C:20°C, 20°C:25°C, 25°C:30°C with a constant photoperiod (L16:D8) and a constant relative humidity (70%), a minimum of one hundred neonate larvae were individually placed in (i.e. cups containing larval diet (Stonefly heliothis diet, Ward’s, New York, USA). Air temperature in chambers and temperature in microhabitats (i.e. cups) were monitored at 6-minute intervals with stand-alone data loggers (Hobo U8, Onset Computer Corporation, Bourne, U.S.) and thermocouples connected to a CR21X datalogger (Campbell Scientific, Logan, U.K.), respectively. Each day, all larvae were checked for pupation.

Data analysis
For each thermoperiod, the observed mean temperature was used to compare our results with those obtained with a constant temperature regime. Finally, two procedures were used to determine the zero development temperature for the four *C. pomonella* strains: (i) the x-intercept method i.e. an extrapolation of the best-fit linear approximation of the reciprocal of development time (1/di where di is the time for the development) and (ii) the thermal unit test. The thermal units (TU) were determined by using the equation TU = di X (ti-b) where di is the development time, ti is the temperature of insect rearing and b is the base temperature. Thus, according Howell & Neven (2000), the correct b value should give the same number of TU at each thermoperiod as long as the rearing thermoperiods used were between the lower and upper temperature thresholds.

Results and discussion
Table 1 gives for each strain the mean and median development times. Many significant differences were observed between strains at each thermoperiod and between thermoperiods for each strain (see Table 1). The zero temperature threshold of development (T0) obtained with the x-intercept (xi) and the thermal unit (TU) are Sv (T0 (xi)= 11.99°C; T0 (TU)= 11.54°C), Rdfb (T0 (xi)= 11.48°C; T0 (TU)= 11.93°C), RΔ (T0 (xi)= 10.34°C; T0 (TU)= 10.74°C) and RGv (T0 (xi)=11.57°C; T0 (TU)= 11.28°C). Our results suggest for the T0 a significant difference between strains. A priori, the resistance status to insecticides could be a reason for such differences, but in the literature the T0 has been reported to be as low as 8.0 or as high as high as 13.3 °C (Ammari El Idrissi, 1980; Williams & McDonald, 1982; Piticairn *et al.*, 1991; Butturini *et al.*, 1992; Howell & Neven, 2000; Kührt *et al.*, 2006).

The x-intercept and thermal unit methods are probably not the best methods to determine the T0 for any insect species since the assumption of linearity between temperature and rate of development is not true for low and high-end temperatures (Schoolfield *et al.*, 1981; Shaffer & Table 1. Physiological development time of resistant- and non-resistant codling moth larvases to chemical and viral (CpGV) insecticides at different thermoperiods
**Thermoperiod (12:12) (°C)** | **Strains** | **Caterpillar (N)** | **Mean ± SD (Days)** | **Median (Days)**
--- | --- | --- | --- | ---
15:20 | Sv | 109 | 41,6 ± 4,8 | 41
 | Rd.fb | 105 | 48,9 ± 6,1 a | 49
 | RA | 126 | 44,4 ± 5,1 b | 43
 | RGV | 105 | 38,9 ± 4,1 d | 38
20:25 | Sv | 100 | 23,3 ± 2,1 b | 23
 | Rd.fb | 89 | 26,0 ± 5,1 a | 26
 | RA | 113 | 24,7 ± 4,2 b | 24
 | RGV | 100 | 23,1 ± 2,0 b | 23
25:30 | Sv | 103 | 15,4 ± 2,4 c | 15
 | Rd.fb | 101 | 17,3 ± 1,8 a | 17
 | RA | 102 | 17,7 ± 4,2 a | 17
 | RGV | 102 | 14,5 ± 1,7 c | 14

* : Value with common letters were not significantly different at p ≤ 0,05 (Kolmogorov- Smirnov two sample test).

Figure 1. Developmental rate (days⁻¹) of PSH INRA Avignon strains and for the strains of some other researchers as a function of mean daily temperature observed during the thermoperiods. Gold, 1985; Georges *et al.*, 2005). Fig. 1 shows the development rates of our strains compared with other strains from literature data. Thermoperiods had a major effect on the development rate in comparison to literature data obtained with constant temperature regimes with the same daily mean and with a similar experimental design as Ammari El Idrissi (1980) (see Fig. 1). Regardless of the strains, for two thermoperiods (i.e. 20-25°C, 25-30°C), the development rate was higher (i.e. a shorter development time) compared with literature data (see Fig. 1).
Our study supports the “thermoperiod hypothesis” suggesting that insect development times decrease under thermoperiodic conditions compared with constant temperature regimes with the same daily mean. Finally, our study suggests that a non-linear model of codling moth development will be more appropriate to determine the zero temperature threshold associated with insecticide resistance status in codling moth populations and should be used in phenological models.

Acknowledgements

We thank Sandrine Maugin for technical assistance. A. Scomparin was supported by a scholarship provided by UNESP/Jaboticabal (FUNEP) of Brazil and D.G. Biron by a Haigneré fellowship of INRA. This work was funded by MICCES program of INRA.

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Raspberry cane midge (Resseliella theobaldi): 3 years of flight monitoring in Swiss raspberry crops and control trials

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Abstract: The raspberry cane midge Resseliella theobaldi is a major pest of Swiss raspberries. The midge population dynamics have been studied for 3 years in the Valais region using a sex pheromone identified and synthesised by EMR and NRI. Four generations were observed in the low altitude and three in the mountains. Based on these observations and in order to find a substitute to diazinon, the only registered insecticide, an efficacy trial was conducted in 2008. Besides diazinon, two insecticides were tested. Only thiacloprid and diazinon showed a significant difference compared to control. The trial will be repeated in 2009.

Key words: Soft fruits, raspberry, monitoring, Resseliella theobaldi

Introduction

Resseliella theobaldi (Barnes) is a major pest of Swiss raspberries (Antonin, 1998; Baroffio, 2007). It belongs to the gall midge family, Cecidomyiidae, 2 to 2.5 mm long and reddish brown. Cane withering is due to splits in the canes, to the egg laying in the splits, to the larvae feeding on the cortex of the canes and to the secondary fungi infection in the splits. Damages due to the midge blight are detectable in primocanes fruiting raspberries in July (violet spots). In the summerfruiting raspberries, damages are detectable only in the second year with fruiting canes withering (Nilsson, 2008; Rivière, 2008).

The sex pheromone of Resseliella theobaldi has been identified by EMR und NRI and has proved to be useful for pest monitoring. Jerry Cross coordinated the monitoring trial (EMR, UK) in nine European countries between 2006 and 2008 to compare the flight phenology under different climates (Cross, 2009). In order to find a substitute to diazinon, the only registered insecticide, an efficacy trial was conducted in 2008 in Switzerland.

Materials and methods

The monitoring trial has been realized between 2006 and 2008 in two Swiss locations: Bruson (1060 m high) uncovered (open field plantation) (Glen Ample, HimboTop and Zeva2) and Ardon (480 m high) covered with agryl in winter (Glen Ample and Heritage). On each site, two pheromone traps were deployed in the centre of the culture, separated by at least 30m. Traps were baited with a standard lure and replaced every month. Sticky base was replaced every week. Males were caught and counted on the sticky base every week from April to October during three years. Traps, lure and base were supplied by East Malling Research and Agralan (UK).

The first year of the efficacy trial was realized in 2008 in Ardon. Four different treatments were applied on the 2nd June with four replicates each on Heritage. Per replicate, 10 canes were examined and the number of larvae in the natural splits was scored 24 days
after treatment. The treatments were: thiacloprid, spinosad, diazinon and no treatment (Table 1).

Results and discussion

Monitoring trial

The flight pattern shows four generations in Ardon (480 m.) and three in Bruson (1060 m.) (Fig. 1). In both plots, population level increased during the three years (2006-2008).

![ARDON 2006-2008](image1)

![BRUSON 2006-2008](image2)

Figure 1. Pheromone trap catches of midges in the two fields Ardon (480 m.) and Bruson (1060 m.) during 3 years (2006 – 2008).

In the lower altitudes, the peak for first generation catch was between 30 April and 7 May. The second-generation peak was between 28 May and 4 June. The midge cycle is longer in Bruson at 1060 m. a.s. The first generation peak was between 14 May and 20 May. There were 50 days between the two peaks. The second-generation peak was between 24 June and 8 July. This information will allow finding the optimal date for a chemical treatment.
**Efficacy trial**

The treatment made in Ardon on the 2 June 2008 was 2 days before the second generation peak. The canes in the control plot had an infestation of 3 larvae per cm split. Diazinon and thiacloprid treatments gave significantly different results from those of the control plot with respectively 0.2 and 0.4 larvae per cm split in cane (Tab.1)

Table 1. Results of the efficacy trial in Ardon 2008. Average of 4 replications for each treatment. (Fisher LSD / p 95%)

<table>
<thead>
<tr>
<th>Products</th>
<th>Active ingredients</th>
<th>Concentrations</th>
<th>Results: Nr larvae/cm split</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>3.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Alanto</td>
<td>Thiacloprid 40%</td>
<td>0.02 %</td>
<td>0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Audienz</td>
<td>Spinosad 44%</td>
<td>0.02 %</td>
<td>0.76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diazinon</td>
<td>Diazinon 25%</td>
<td>0.1%</td>
<td>0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Conclusions**

The pheromone traps are a good indicator for estimating the presence and the quantity of midges flying in the raspberry culture. The traps will help to determine the right treatment window to be as efficient as possible to target the highest midge number. The first year of the efficacy trial showed the interesting alternative to diazinone with thiacloprid. Trials will be continued in 2009.

**Acknowledgements**

We thank the grower in Ardon, B.Huber who let us work in his plot, Agralan for providing traps, Delphine Rivière, Cathy Eckert and Philippe Massardier for their advice and experiences from France and Jerry Cross who coordinates this project.

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Management of Oriental Fruit Moth and Codling Moth with spray application of microencapsulated sex pheromone

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Abstract. Codling moth (Cydia pomonella L.) and Oriental Fruit Moth (Grapholita molesta (Busck)) are the main pests of apples and peach, respectively. Various formulations of synthetic sex pheromones of both species have been developed in order to manage these pests in apple and peach orchards. The most common use of sex pheromones has been with hand-applied dispensers, but their application is labour intensive and growers are interested in alternative approaches. Two sprayable microencapsulated formulations of sex pheromone have recently been commercialized. They can be applied either alone or mixed with different chemicals. Our studies, conducted in 2007 and 2008, showed that this method was very effective for both codling moth and oriental fruit moth and achieve the same results as insecticides and hand-applied pheromone dispensers.

Keywords: sprayable sex pheromone, Oriental Fruit Moth, Grapholita molesta, Codling Moth, Cydia pomonella, peach, apple

Introduction

Codling moth (Cydia pomonella L.) and Oriental Fruit Moth (Grapholita molesta (Busck)) are the main pests of apple and peach, respectively. Various formulations of synthetic sex pheromones of both species have been developed in order to manage these pests in apple and peach orchards. The most common use of sex pheromones has been with hand-applied dispensers, but their application is labor intensive and growers are interested in alternative approaches. Two sprayable microencapsulated formulations of sex pheromone have recently been commercialized. It is important to understand the suitability of the new technique since it has, in theory, a useful versatility.

Materials and methods

Apple
The apple orchard (2001, Golden Delicious) is located in Busca (CN) in North-West of Italy. It is 9.5 hectares and it has been divided into 3 plots: one (1.4 ha) was sprayed with the micro-encapsulated pheromone (Check Mate CM-F, Suterra) starting before the first flight, while the second (0.8 ha) started before the second flight as indicate in Figures 3 and 5. The third plot (7.3 ha) was protected with pheromone dispensers (Isomate CLR, Shin-Etsu). In 2007, an ovicide (Flufenoxuron, 30/4) and a larvicide (Azinfos-metil, 17/7) were applied on the first generation in the whole orchard. In 2008, an ovicide (Flufenoxuron, 10/5) and a larvicide (Azinfos-metil 2/7) were applied in the whole orchard.

Peach
The 2007 peach orchard (2001, Big-Top) is located in Manta (CN) in North-West of Italy. It is 4 ha divided into two plots: one (1 ha) was sprayed with the micro-encapsulated pheromone
(Check Mate CM-F, Suterra), while the other was protected with two larvicides as indicated in Figure 1. The 2008 peach orchard (2002, Summer Rich) is located in Villafalletto (CN) in North-West of Italy. It is 8 ha divided in two plots: one (1 ha) was sprayed with the microencapsulated pheromone (Check Mate CM-F, Suterra) and, in the rest, dispensers for mating disruption (Isomate CLR, Shin-Etsu) were applied (Figure 2). The ovicide Teflubenzuron was applied in both plots at the end of April.

**The rule of interventions**

The dose of spray pheromone is 100ml/ha, interventions are scheduled with an interval of 15 days. The rule applied in case of rain is to repeat the treatment at half dose (50ml/ha) if the rain falls in the first 10 days since spray application, if after 10 days to apply the next at full dose.

**Results**

**Peach**

The curves of male catches and the sprays applied are reported in Fig. 1 for 2007 and in Fig. 2 for 2008. No damage at harvest was recorded in both years. Microencapsulated sex pheromone of *G. molesta* has been applied in a half peach orchard whereas in the other half the usual integrated pest management was applied.

![Diagram](https://via.placeholder.com/150)

**Figure 1.** Mean number of Oriental Fruit Moth adult males in nectarine orchards close to experimental plots and interventions made in 2007. No damage at harvest was recorded in either plot.
Figure 2. Mean number of adult males of codling moth per trap caught in peach orchards close to experimental plots and interventions made in 2008. No damage at harvest was recorded in either plot. The two 1/2 dose applications of spray pheromone were applied according to the rule that, in case of rain earlier than 10 days from last spray, spray was repeated at half dose.

**Apple**

Microencapsulated sex pheromone of *C. pomonella* was applied in an apple orchard following two different schedules: in the first, sprays were applied starting before the first flight and in the second, sprays were applied starting before the second flight. In both cases, sprays were applied at a fifteen days interval, resulting in a total of 10-11 interventions in the first schedule and 7-8 in the second. In the other part of the orchard, Isomate dispensers were applied before the first flight. The curves of male catches and sprays applied are reported in Fig. 3 for 2007, and in Fig. 4 for 2008. No damage at harvest was recorded in both years.

Figure 3. Mean number of adult male of codling moth catch in apple orchards near experimental plots and interventions made in 2007.
Discussion

Our studies, conducted in 2007 and 2008, showed that this method is very effective for both codling moth and oriental fruit moth and gives the same results as insecticides and hand-applied pheromone dispensers, although the pest population was low in both years.

The starting period in peach orchards can be just prior to the second flight, since the first generation usually causes little damage. In northern Italy this schedule permits saving 2-3 sprays. Moreover, the end of this generation is well defined and easy to identify with both trapping and with forecasting models.

The starting period in apple orchards is not yet defined and the two options (initiate with first or second generation) are open, since both gave good results. It must be noted that the possibility of starting with the first generation allows coverage of the whole season, but it could be very expensive.

The intervention interval of 15 days seems to be suitable along with the rule applied: it allows avoidance of rain wash-out and is still sustainable by fruit-growers. Research on the rain-fastness and the persistence is still being done.

In conclusion, this technique looks very promising due both to its versatility and the possibility of protecting crops until harvest taking into account economic convenience in relation to the number of interventions. It must be reported that its versatility has to be managed by the aid of the extension service since the product is not an insecticide as might be mistakenly believed by fruit-growers.

Acknowledgements

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Figure 4. Mean number of adult male of codling moth catch in orchards near experimental plots and interventions made in 2008. No damage at harvest was recorded in each of three plots.
Isomate C plus dispensers as alternative means for control of codling moth, *Cydia pomonella* (L.), in apple orchards of Bulgaria

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Abstract. In the years 2006-2008, trials on the control of codling moth (CM) by mating disruption (MD) using Isomate C plus dispensers were carried out in an isolated 1-ha apple orchard in South-East Bulgaria. Dispensers were hung in the upper third of tree canopies at a density of 1000 pieces per ha before CM flights started. Dynamics of CM flights was monitored by pheromone traps installed in the trial plot and in a conventionally treated reference orchard. Fruit infestation was periodically assessed till harvest time. Hibernating population of CM was estimated in autumn by counting diapausing CM larvae in corrugated cardboard bands. In each of the years, Isomate C plus dispensers completely inhibited CM captures in pheromone traps in the trial plot. Fruit damage remained at low levels till late July and increased slightly only in August. At harvest the percentage of damaged fruits was below 1%. The hibernating population also stayed at low level. In the reference orchard, in spite of numerous chemical treatments, the final fruit damage was high (5.5-28.4%), apparently due to resistance of CM to insecticides. It has been concluded that mating disruption may serve as an alternative means for control of codling moth in Bulgarian apple orchards. Contrary to reports from other countries, this study has shown that good results from MD can be obtained even on a small-size plot, if isolated from external sources of infestation and if initial CM population is low.

Keywords: IPM, apple, codling moth, mating disruption, fruit damage, Isomate C plus dispensers

Introduction

Codling moth (CM), *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a key pest of Bulgarian pome fruit orchards. Most of recently applied insecticides have a large spectrum of action, so that they eliminate the beneficial entomo- and acarofauna, thus provoking the multiplication of other pests. Moreover, control of CM by conventional methods, in spite of numerous treatments applied, is often ineffective. In Bulgaria, the damage caused by codling moth in apple orchards has steadily increased. In 2008, in most of conventionally treated orchards in South Bulgaria fruit damage due to CM exceeded 25%. This has been apparently due to the development of resistant CM strains. Populations resistant to organophosphates and pyrethroids have been detected by testing diapausing CM larvae collected in some orchards in South Bulgaria (Charmillot et al., 2007). Therefore, the implementation of non-chemical methods for management of this pest is an urgent need. Mating disruption (MD) is one of the alternative means of control. Isomate-C dispensers, emitting synthetic CM pheromones, were released by Shin-Etsu (Japan) as early as in 1989 and then improved and successfully applied in many countries (Veronelli & Iodice 2004). Positive results obtained in trials carried out in Central-South Bulgaria were reported in a previous paper (Kutinkova et al. 2009).

Material and methods

*Trial orchard*
In the years 2006-2008, trials were carried out in a well-isolated, 1-ha apple orchard in the village Glufishevo, Sliven region, South-East Bulgaria. The orchard was established in 1999. In three consecutive years the dispensers were installed in April, before the expected start of flight of CM. They were hung in the upper third of tree canopies at a density of 1000 pieces per ha. According to the manufacturer, each dispenser is loaded with a minimum of 190 mg of the codling moth pheromone mixture.

Against other pests, only one acaricide treatment was applied in 2006 and two aphicide treatments in 2007 and 2008 as follows: phenpyroximat against mite Panonychus ulmi Koch on August 8, 2006 and thiamethoxam against aphids on May 23, 2007 and on May 25, 2008.

**Reference orchard**

A 4.8-ha orchard located in the same area, near the city of Sliven, established in 1990 served as a conventionally treated reference. Fourteen treatments (21 active ingredients) were applied there during the 2006 and 2007 seasons, to control CM, leaf miners, leaf rollers, aphids and mites. Thirteen of them (19 active ingredients) were timed against codling moth. In 2008 seventeen treatments (24 active ingredients) were applied; fifteen of them (22 active ingredients) were supposed to have an action against CM. The insecticides used included methoxyphenozide, triflumuron, cipermethrin with chlorpyriphos-ethyl and fenitrothion in all three years of the study and additionally alpha-cipermethrin in 2006, esfenvalerate in 2007 and deltamethrin in 2008.

**Indices studied**

Monitoring of CM flights was carried out by sex trapping. Two CM pheromone traps were installed in the trial orchard – 1 triangular traps baited with a standard capsule (Pheronet OP-72-T1-01) containing 1 mg codlemone and 1 triangular trap baited with 20 mg codlemone in 2006 and 2 triangular traps baited with a standard capsule (Pheronet OP-72-T1-01) containing 1 mg codlemone in 2007 and 2008. In the reference orchard, 2 triangular traps were placed. All traps were installed prior to the beginning of flight of CM and then checked twice a week.

Fruit damage by CM was evaluated on samples of 1000 to 2000 fruits periodically during the season and on 3000 fruits before and at harvest. Sampling was always carried out in the reference orchard and in the trial plot at the same dates.

In June, 40 corrugated cardboard band traps (8 at the border and 32 inside) were attached to the tree trunks in the trial plot and in the reference orchard. They were recovered in autumn, after harvest, in order to count the hibernating population of CM.

**Elaboration of data**

Data on catches of male moths in the pheromone traps were considered as totals for each date and presented in a graphical form. Rate of fruit damage by CM was expressed as percentage of damaged fruits. Significance of differences in damage rate between the trial reference orchards was estimated by use of Chi-square tests. Significance of differences between mean values of diapausing larvae between the orchards and years was evaluated using the t-test.

**Results**

**CM flight dynamics**

In 2006, the first flight of CM in the reference orchard appeared on May 2, whereas no moths were recorded in the pheromone traps that were installed in the trial plot two weeks before. The flights of the overwintering generation reached their maximum by the fourth week of May (Fig. 1) and remained considerable till the end of June. The flights of the second generation, which overlapped the first one, started in the first week of July, reached its maximum at the end of July, then decreased till mid-August and finished on September 9. In the trial plot the sex pheromone traps have not caught any moth during the season. The traps installed in the reference orchard caught 128 moths in total.
In 2007, the first flights of codling moth in the reference orchard appeared on April 30, whereas no moths were recorded in the pheromone traps in the trial plot. The flight of the overwintering generation of CM reached its maximum in the second week of May and after the second week of June (Fig. 1). The flight of the second generation, which did not overlap the first one, started in the first week of July, reached its maximum during the second week of July, decreased in August and finished on September 14. In the trial plot, the standard sex traps have not caught any moth during the 2007 season. At the same time, the traps installed in the reference orchard caught in total 283 moths.

In 2008, the first flights of codling moth in the reference orchard appeared on April 25, whereas no moths were recorded in the pheromone traps installed before in the trial plot. The flight of the overwintering generation of CM reached its maximum in the first week of May (Fig. 1) and continued through May and June. The flight of the second generation, which overlapped the first one, started by the end of June, reached its maximum during the second week of July, then decreased in August. A small peak appeared at the end of August. It was probably due to a partial third generation, but this was not well documented yet. The last flights were recorded on September 12. Traps installed in the reference orchard caught in total 359 moths. In the trial plot, no moths were caught during the 2008 season.

**Fruit damage by CM**

In the trial plot, where the Isomate-C plus dispensers were installed, no damage of fruits due to CM was noted till July in the years 2007 and 2008 and even till the beginning of August in 2006 (Table 1). Then fruit damage stayed at a low level, reaching finally 0.4-0.7% at harvest. In the reference orchard, located in the same regions and treated with a conventional protection programme, the first signs of damage to fruitlets were noted already in June, then the rate of damage successively progressed, reaching very high values at harvest. It is worth
noting that the final rate of damage increased in consecutive years – from 5.5% in 2006, through 14.8% in 2007 and as much as 28.4% in 2008.

Table 1. Evolution of fruit damage (% of fruits infested by CM) and number of hibernating CM larvae in corrugated paper bands in autumn, in particular years of study

<table>
<thead>
<tr>
<th>Date</th>
<th>Trial</th>
<th>Reference</th>
<th>Date</th>
<th>Trial</th>
<th>Reference</th>
<th>Date</th>
<th>Trial</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>June 1</td>
<td>0.0</td>
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<td>June 9</td>
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<td>June 8</td>
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<td>3.5</td>
<td>June 21</td>
<td>0</td>
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<tr>
<td>July 13</td>
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<td>July 5</td>
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<td>0.0</td>
<td>July 18</td>
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<td>2.6</td>
</tr>
<tr>
<td>Aug 4</td>
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<td>2.4</td>
<td>July 29</td>
<td>0.2</td>
<td>5.8</td>
<td>Aug 10</td>
<td>0.3</td>
<td>10.5</td>
</tr>
<tr>
<td>Aug 23</td>
<td>0.05</td>
<td>4.1</td>
<td>Aug 12</td>
<td>0.3</td>
<td>9.8</td>
<td>Aug 23</td>
<td>0.4</td>
<td>14.5</td>
</tr>
<tr>
<td>Sept 14</td>
<td>0.35</td>
<td>5.2</td>
<td>Sept 6</td>
<td>0.4</td>
<td>13.2</td>
<td>Sept 7</td>
<td>0.6</td>
<td>23.8</td>
</tr>
<tr>
<td>Oct 2</td>
<td>0.40</td>
<td>5.5</td>
<td>Oct 3</td>
<td>0.5</td>
<td>14.8</td>
<td>Oct 3</td>
<td>0.7</td>
<td>28.4</td>
</tr>
<tr>
<td>damage</td>
<td>0.35</td>
<td>5.2</td>
<td>damage</td>
<td>0.4</td>
<td>13.2</td>
<td>damage</td>
<td>0.6</td>
<td>23.8</td>
</tr>
<tr>
<td>at harvest</td>
<td>0.40</td>
<td>5.5</td>
<td>at harvest</td>
<td>0.5</td>
<td>14.8</td>
<td>at harvest</td>
<td>0.7</td>
<td>28.4</td>
</tr>
<tr>
<td>hibernating</td>
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<td>4.575</td>
<td>hibernating</td>
<td>0.425</td>
<td>9.025</td>
</tr>
</tbody>
</table>

Infestation rates in 2006 were significantly different between the treated plot and the reference orchard from the second check on 15 June till harvest (Chi-square tests, P < 0.001). In 2007, they were significantly different between the treated plot and the reference orchard already at the first control in June (Chi-square test, P=0.014) and, except for July 5, thereafter until harvest (Chi-square tests, P<0.001). In 2008, fruit damage rates were significantly different between the treated plot and the reference orchard already at the first control in June (Chi-square tests, P=0.002) and thereafter until harvest (Chi-square tests, P<0.001).

**Overwintering population of CM**

In autumn 2006, i.e. after the first season of MD treatment, only 0.125 larvae per tree were found in corrugated cardboard bands in the trial orchard. At the same time in the reference orchard the overwintering population of CM reached 2.175 larvae per tree (Table 1). In the successive years (2007 and 2008) the level of overwintering population of CM in the trial plot increased slightly, stayed below the economical threshold, however. At the same time the population of diapausing larvae of CM in the reference, conventionally treated orchard increased rapidly in successive years, reaching in autumn 2008 the value more than 4 times higher than in autumn 2006.

The populations were significantly different between treated plot and reference orchard (t-test, P<0.001) as well as between the years 2006 and 2007 in the reference orchard (t-test, P<0.001). In the treated orchard there was no difference in the overwintering population between 2006 and 2007 (t-test, P=0.24). In 2008, the hybernating CM populations were significantly different between treated plot and reference orchard (t-test, P <0.001) as well as between the years 2007 and 2008 in both treated plot and reference orchard, respectively (t-test, P<0.001).
Discussion

The results obtained in this study confirm the findings of Charmillot et al. (2007) that resistance of codling moth to insecticides is a serious problem in Bulgaria. The conventional chemical control of CM is getting ineffective. Increasing intensity of CM flights as well as increased fruit damage and diapausing CM larvae population in the conventionally treated orchard – in spite of numerous chemical treatments – do indicate that the problem of resistance is aggravating.

Results obtained with mating disruption were very positive. Isomate C plus dispensers of Shin Etsu effectively inhibited flights of CM as well as fruit damage and the size of the hibernating CM population. The results obtained are in line with those reported by Gut et al. (1992), Gut and Brunner (1998), Judd et al. (1996), Barnes & Blomefield (1997), Charmillot et al. (1997), Waldner (1997) and Zingg (2001). They also confirm our previous reports (Kutinkova et al. 2009). It is worth noting that in our study the positive results with the MD method were obtained in a rather small orchard lot (1 ha). Apparently, isolation from external sources of infestation and the initial level of CM pressure are more important factors than the orchard size. It has been concluded that mating disruption may be successfully applied as alternative means for controlling codling moth in apple orchards of Bulgaria. Implementation of this method should result in reduction of the use of chemical insecticides, thus minimising environmental pollution and improving fruit quality.

Acknowledgements

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References

A field unit for automatic monitoring of insect behaviour

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Abstract: The aim of this work was the development of a field unit for automatic recording and related data analysis of insect orientation towards an attractive pheromone source. Currently the evidence of male behaviour under mating disruption is still speculative, due to the difficulty to conduct field observations which unequivocally show the operative mechanisms. This monitoring system provides behavioural data, in order to optimize the effectiveness of control strategies based on semiochemicals. Specifically, the unit records frequencies of the visits, temporal dynamics and trajectories around the attractive source. The operating principle of the unit is based on the acquisition and real-time analysis of near infrared images relative to an area of 80 x 80 cm around the source; the functioning is fully autonomous and remotely controlled via GSM network. We chose as study model the behaviour of codling moth, Cydia pomonella (L.), in an apple orchard managed with mating disruption (Isomate C Plus, 1000/ha). The operation of the unit was verified by analysing the approach of the males toward three different attractive sources: a standard monitoring lure (1 mg of E8,E10-dodecadien-1-ol), an Isomate CP Plus dispenser and two calling females. The infrared camera was placed in the middle of a field tunnel. For each trial 10 virgin, 2-3-day-old males were released. The recordings went on for 2 days, from 7.00 pm to the midnight.

Cydia pomonella L., Mating disruption, Pheromone, Infrared camera
Correlation between maturity of female \textit{R. cerasi}, oviposition, larval development and ripeness of cherries

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Abstract: The European cherry fruit fly \textit{Rhagoletis cerasi} L. (Diptera: Tephritidae) is the most serious pest in European cherry production. The control of the pest is difficult, especially against the background of EU wide reduction programs for broad spectrum insecticides. To find alternative control measures, the biology and behaviour of the fly must be known in more detail. One option to control the pest might be bait sprays, which have to be applied repeatedly during the main infestation period. To achieve the optimal efficacy and with respect to the costs, timing and number of applications are important questions. For this reason we investigated the correlation between the maturity of female \textit{R. cerasi} by analysing the ovary status of flies caught with yellow sticky traps, oviposition, larval development and ripeness of host fruits (varieties Sam, Van and Hedelfinger) by measuring colour, sugar content as well as solidity, recorded as pressure resistance.

The 1\textsuperscript{st} fly was trapped on May 9, whereas the 1\textsuperscript{st} ripe eggs after dissection of ovaries were found on May 13. According to the cherry variety, the first deposited eggs were detected between May 28 and June 2. During this time, the cherries turned their colour from green to yellow/orange, pressure resistance ranged between 4.0 and 6.3 kg/cm\textsuperscript{2} and sugar content between 8.4 and 13.8 °Brix. Newly deposited eggs were found until mid of July, whereas 3\textsuperscript{rd} instars occurred from mid of June until mid/end of July, depending on cherry harvest and variety.

Key words: \textit{Rhagoletis cerasi}, Tephritidae, Diptera, phenology, oviposition

Introduction

The European cherry fruit fly \textit{Rhagoletis cerasi} is the main pest in cherry orchards in Europe, causing fruit damage and yield losses (Vogt, 2002, Daniel & Wyss, 2003). In Germany, the broad spectrum insecticides lebaycid and dimethoate were registered until 2004 and 2007, respectively for conventional farming. In 2008, acetamiprid could be used with an annual exemptional permission (§ 11 PflschG), but with varying success. In organic and integrated cherry production no control at all was possible until 2006. Since 2006 ‘Spruzit Neu’ (a.i.: pyrethrines and rape seed oil; W. Neudorff GmbH KG, Emmerthal, Germany) is registered against sucking and biting insects in cherries, whereas side effects of this insecticide are used against cherry fruit fly. As its persistence is rather short, this may limit the efficacy from case to case. Hence, there is an urgent need to develop alternative strategies for all, conventional, integrated and organic cherry growers, which can also include origins of infestation, like back yard cherry trees. For environmentally sound control strategies, the biology as well as the behaviour of the pest species must be known in detail. Since the use of broad spectrum insecticides, research on biological and behavioural aspects of \textit{R. cerasi} was set in the background. Different authors worked on different biological questions in the 30’s, and the 60’s to the 80’s, repectively (Boller, 1964, Boller, 1966 a, Boller & Remund, 1983, 1987,
Fimiani, 1984, Katsoyannos et al., 1986, Skaar, 1999 etc.). Therein they tried to answer questions about phenology in form of a forecasting model, life tables or dispersal, but many aspects were not worked out. Open questions are e.g. the correlation of phenology of flies and their host fruits, development of ovaries, oviposition and fruit ripening parameters or duration of oviposition. This knowledge is especially important for more selective control strategies.

One environmentally sound control strategies could be bait sprays with natural derived insecticides. Bait sprays as a combination of food baits, mainly proteins and sugar, and low amounts of insecticides are only applied on parts of the canopy. As the known natural derived insecticides as pyrethrines and neem are not persistent for a long time, applications must be repeated for several times. To find the optimal application time and necessary replications, more information about the pest is needed.

In this study, the correlation between the phenology of flies, the development of ovaries, oviposition and ripening process of cherries were worked out. The main aim was to achieve a better understanding for e.g. bait spray application to reach a high efficacy with low side effects and to reduce costs for material and man power.

Material and Methods

Laboratory: To provide an age scale of flies according to their ovary development, 800 pupae were used. These pupae were obtained from field-collected infested cherries in 2007, kept under room conditions (20 to 25 °C) for two months and transferred into the cold room (3 to 5 °C) for approximately six to eight months. For post-diapause development, pupae were brought to a climate chamber (25 ± 0.5 °C / 18 ± 0.5 °C, RH 65 ± 5 %, photo period light : dark 16:8 h, 4 to 6 klux). Adults were sorted according to their hatching date to provide a defined age and kept in BioQuip cages (30 x 30 x 30 cm plastic cages; “Bug Dorm-1”, BioQuip Products, Rancho Dominguez, CA, USA). Aside from the availability of water, flies were fed with sucrose and brewer’s yeast (Diana Bierhefe, Spezialfuttermittel M. Diekmann, Versmold-Bockhorst, Germany), ratio 4:1 by weight as dry food. Ovaries of female flies of a defined age (N=10 for age classes of 1 to 30 days) were dissected and classified according to their ovary development: 1 = ± no visible development, 2 = unequal vesicles, 3 = ripe eggs.

Field: Adult flies were trapped with yellow sticky Rebell® traps (Andermatt Biocontrol AG, Grossdietwil, CH) from May 5 to August 29 in 2008 at the cherry orchard in Dossenheim and transferred (N = 10 ♀ at maximum according to the number of trapped flies) into 70 % EtOH three times a week. These flies were dissected and classified the same way as the laboratory flies. Traps were placed in the southern part of the trees at an approximate height of 2 m in three different cultivars with N = 5 in each case (Sam, Van and Hedelfinger). Parallel to the trapping of flies, cherries were harvested (N = 50) and controlled for eggs using a microscope (magnification: 10x) and different larval stages while opening the cherries (N = 100) and putting them into salt water (10 %) for at least 1 hour. Larvae could then be collected from the water surface and approximately classified according to their length into 1st, 2nd and 3rd instars. Another 10 cherries per cultivar were harvested for measuring their colour, using a RAL® Design Colour Fan (RAL Dr. Inst. f. Gütesicherung u. Kennzeichnung e.V., Sankt Augustin, Germany), their sugar content [°Brix] using a Hand Held °Brix Refractometer (RHB-32 ATC, 0-32 °Brix) as well as their solidity recorded as pressure resistance [kg/cm²] with a Digital Penetrometer PCE-PTR 200 (PCE Group, Meschede, Germany).

Results and Discussion

Laboratory: The ovary dissection of the laboratory flies showed that females are one to four days old in stage 1 (± no visible development), four to nine days in stage 2 (unequal vesicles)
and 10 days and older with ripe eggs (stage 3) at the defined temperature in the climate chamber. A preoviposition period of approximately 10 days was already shown in different studies (Wiesmann, 1933, Boller, 1966 b). The main reason of dissecting flies of the defined age was to provide an age scale for the flies trapped in the field. **Field:** To ease the demonstration of the results, different cherry cultivars were combined. The main results are listed in Table 1.

First flies were trapped in the cherry cultivar Hedelfinger on May 9 and the last fly was trapped in the same cultivar on August 8. After dissection of trapped flies, ripe eggs were found from May 13 until the end of the trapping period in August. According to the results of the laboratory flies, cherry fruit flies must have been emerged at least six days earlier at the orchard than the first trap catch. Following, it is unlikely to trap the first flies in an orchard with yellow traps and control strategies have to be adapted to this fact. In contrast to mature flies in the field, eggs were found in the cherries 19 days past the first trap catch of a female fly, on May 28. This can either mean, that there are flies with ripe eggs in the field but the cherries are not attractive enough, not in the appropriate stage for oviposition or the sample size of 50 cherries per cultivar was not big enough to reach a high probability to find the first eggs. Newly deposited eggs were found in the cherries until July 11, although ripe fruits were available until mid/end of July and flies with ripe eggs were detected until August, as mentioned before. The oviposition into ripe cherries will probably result in a loss of a part of the offspring. But the conclusion of Levinson & Haisch (1984), that *R. cerasi* has a marked preference for oviposition into unripe fruits, can not be confirmed completely. Beside an oviposition peak at the end of May until mid of June in all cultivars, when cherries have been still in their ripening process, the cultivar Hedelfinger, showed a second oviposition peak into ripe dark red or black fruits at the end of June/beginning of July. Accordingly, comparing the colour of cherries with the newly deposited eggs, a specific colour does not seem to be the key factor for oviposition. First eggs were found, when cherries started to turn their colour from green to yellow at the end of May 2008 and last eggs were found in highly ripe cherries of a dark red to black colour mid of July 2008. This corresponds to some extend with studies of e.g. Haisch & Levinson (1980) and Levinson & Haisch (1982), that increasing ovopisition takes place in spheres with a higher contrast to the background and not the colour itself. Also Boller (1969) and Prokopy (1969) showed for *R. cerasi*, that dark coloured small spheres are preferred over those of lighter colours. Additionally, Katsoyannos (1979) highlighted that hue discrimination may also be involved in the selection of oviposition site. Beside these findings for oviposition, the preference of yellow colour in small to medium size panels or X-shaped rectangles must be kept in mind (Boller, 1969, Prokopy, 1969, Prokopy & Boller, 1971).

3rd instar larvae, which could leave the host fruits for pupation occurred in cherries from June 11, when the cherries were reddish to dark red until July 16 in very ripe fruits. Following, immigration of larve into the soil was a process of more than four weeks in Dossenheim in 2008.

The sugar content of cherries during oviposition and larval development revealed increasing values from 6.2 °Brix at minimum in the cultivar Sam at the end of May to 22.9 °Brix in the cultivar Hedelfinger June 14. Newly deposited eggs were found with values between 8.4 in the cultivar Sam and the mentioned maximum value in Hedelfinger. Hence, newly deposited eggs as well as larvae of different stages occurred in the cherries during a strongly varying sugar content. It means that the cherry fruit fly larvae have a wide tolerance of sugar content in the host fruits and sugar content in its range in ripening cherries does not to be an important factor for neither oviposition nor larval development.

Measuring the solidity of cherries was not possible before May 28 for Van and Sam, respectively and for Hedelfinger before June 2. The cherries have been to hard for the measurement device. Following, the first measurement of solidity corresponds with the first eggs. The highest solidity (6.3 kg/cm²) was found in the cultivar Van at the end of May.
Before this date no eggs were found. It decreased permanently during the ripening process depending on the cultivar around one or below 1 kg/cm². The solidity of cherries seem to be more significant for initialization of oviposition than the other mentioned features. But, to find a definite solidity threshold and a real correlation between solidity and oviposition, laboratory trials as a choice test should be undertaken.

The results implicate, that depending on the mode of action of alternative control strategies (prevention of maturation or killing of flies), these strategies have to be applied at different periods in fly phenology. For some control measures, a control immediately after the first trap catches is useful or it won’t be enough to control the pest at the beginning of the oviposition period when the cherries turn their colour from green to yellow or reddish. In the case of an incomplete harvest of cherries the oviposition in almost ripe or ripe cherries must be kept in mind to prevent the development of a new generation in the following season.

Table 1. Overview about main phonological parameters of *R. cerasi* in correlation to the host fruit ripening process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
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<tr>
<td>trapped ♀</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ripe eggs after dissection of ♀</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>deposited eggs in cherries</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3rd instars in cherries</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>colour of cherries</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>sugar content [°Brix]</td>
<td>6.2</td>
<td>22.9</td>
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<td></td>
</tr>
<tr>
<td>solidity [kg/cm²]</td>
<td>6.3</td>
<td>0.6</td>
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References


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173.


