Status of soil-borne phytosanitary problems encountered in melon (*Cucumis melo*) in the main producing regions of France

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Abstract

French melon growers have found themselves confronted with melon root decay. Accordingly, Ctifl, in close collaboration with the regional research stations and INRA, set up a study designed to gain greater knowledge of the various phytosanitary problems and identify the probable cause(s). The principle of the study was to monitor pathogen distribution changes in melon. Three plots were monitored and a number of samples from other plots were also studied (19 samples in 2003, 22 in 2004, 6 in 2005, 2 in 2006 and 25 in 2007). The symptoms that developed during the years of the study were identified based on this body of data. The results show the prevalence of problems related to *Fusarium oxysporum* f. sp. *melonis* race 1-2 yellowing, often found together with other pathogenic soil fungi, which constitute aggravating factors. The isolates of *F. oxysporum* f. sp. *melonis* collected from samples appear to be more aggressive than the reference strains used in the breeding tests for resistance.

INTRODUCTION

In recent years, melon growers in the various producing regions of France have observed a rise in soil phytosanitary problems that results in plant mortality. A study on a greenhouse crop conducted by Buffière and Tausig (2004) analyzed the situation in the Provence region (Southern France) and demonstrated that both pathogens and cultivation practices have a role to play. For the other producing regions, it seemed important to identify which major pathogen(s) were causing these problems, and determine their aggressiveness. Ctifl, in close collaboration with the regional research stations (Acpel, Hortis Aquitaine, Aprel, Arelpal, Cefel and Cehm) and INRA (French National Institute for Agricultural Research), thus set up a study designed to gain greater knowledge of the various phytosanitary problems that can be broadly classified as “decay,” and determine its probable cause(s).

Earlier studies had shown the prevalence of *Fusarium oxysporum* f. sp. *melonis* even though other pathogens may be encountered, such as *Rhizoctonia solani*, *Pythium sp.*, *Didymella bryoniae* - teleomorph (*Phoma cucurbitacearum* - anamorph), *Pyrenochaeta terrestris* (*Phoma terrestris*), *Phomopsis sclerotioides*, *Macrophomina phaseolina*, *Verticillium*...

A study by Risser et al (1976) defined four races of *Fusarium oxysporum* f. sp. *melonis* based on the interaction between the major resistance genes and the pathogen
isolates. Two dominant resistance genes, Fom-1 and Fom-2, confer resistance to races 0 and 2, and to races 0 and 1, respectively. Race 1-2, for which the resistance inheritance mode is polygenic and recessive, was divided into two pathotypes: race 1-2Y, which causes a yellowing of the plants before death, and race 1-2W, which causes the plant to wilt before it dies. Race 1-2Y is the most widely one reported in France (Perchepied and Pitrat 2004).

The principle of this study was to monitor pathogen distribution changes in melon. Three plots were monitored in the main melon-producing regions. A number of so-called “additional” samples (i.e., not from the monitored plots) were also provided for study. This body of data allowed the identification of the symptoms of phytosanitary problems that developed during the years of the study, as well as the characterization of the aggressiveness of the isolated strains of *F. oxysporum* f. sp. *melonis*.

MATERIALS AND METHODS

Three producing fields (in the Quercy, Gers and Centre regions of France) were systematically sampled from five predefined experimental plots. Samples consisted of three melon plants selected at random and sent to the Ctifl research centre at Lanxade for analysis. On delivery, the samples were unwrapped and rinsed with water in order to permit a detailed analysis of the root system.

Following this procedure, the plant material was selected for collection of the microbiological isolates. In order to isolate the various pathogenic fungi potentially related to the problems of decay, four different media were used: an all-purpose medium (malt agar), the ”MAPyr” medium (Awuah and Lorbeer 1989), which isolates slow-growing species such as *Pyrenochaeta* while suppressing fast-growing fungi like *Fusarium*, the corn meal agar (CMA) medium for *Pythiaceae* and Komada’s (1975) medium, selective for *Fusarium*.

Concurrent with the monitoring of pest distribution changes in samples from these plots, additional samples were taken according to the same protocol in order to characterize the phytosanitary damage observed in the field. This involved: 19 samples in 2003, 22 in 2004, 6 in 2005, 2 in 2006 and 25 in 2007.

To identify the races of *Fusarium*, bioassays were performed using the differential genotypes shown in Table 1, in compliance with the inoculation procedures described by INRA - Avignon (Mas 1973; Molot and Mas 1975), namely, soaking the roots in a titrated solution of microconidia and macroconidia for the susceptible control sample, race 0, 1 and 2; and applying a titrated solution for race 1-2Y and 1-2W.

Finally, the pathogenicity of the other fungal species isolates was tested in *vitro* and *in vivo* by bioassay tests. In the *in vitro* tests, the melon plantlets were inoculated two weeks after germination by placing a 1- or 2-week old explant of mycelial culture against the upper fourth of the root. In the *in vivo* tests, the inoculum was mixed with the substrate, which was seeded with a susceptible variety of melon.

For *F. oxysporum* f. sp. *melonis* race 1-2, the aggressiveness of the isolates collected from the different samples was evaluated on various “test” varieties, which allows a characterization of the isolate aggressivity with respect to the plant material. The test varieties then serve as a reference when listing the varieties in the official French seed catalogue (CTPS): ‘Margot’ (INRA), is resistant to races 0, 1 and 2 and
susceptible to race 1-2; ‘Lunasol’ (Nunhems) is resistant to races 0, 1 and 2 and lies at the lower threshold of “partial resistance” to race 1-2; ‘Isabelle’ (INRA) is resistant to races 0, 1 and 2 and lies at the upper threshold of “partial resistance” to race 1-2; and ‘Manta’ ( Clause-Tézier) is resistant to races 0, 1 and 2 and exhibits a level of partial resistance to race 1-2 that ranks it between ‘Lunasol’ and ‘Isabelle’. The isolates were compared to the two control strains used in the screening tests for Fusarium resistance: strain Fom 1-2 TST (yellowing) and strain Fom 1-2 d’Oléon 8 (wilt). Each test consists of 2 replications of 60 plants in individual mini-clods, to avoid cross-contamination. For each plant, 3 ml of a titrated solution of 4.10^5 conidia/ml was applied.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Race 0</th>
<th>Race 1</th>
<th>Race 2</th>
<th>Race 1-2</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>No resistance gene</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Charentais T</td>
</tr>
<tr>
<td>Fom-1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>Védrantais</td>
</tr>
<tr>
<td>Fom-2</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>Charentais Fom-2</td>
</tr>
<tr>
<td>Fom-1 Fom-2</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>Margot</td>
</tr>
<tr>
<td>Fom-1 Fom-2 +</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>r</td>
<td>Isabelle</td>
</tr>
</tbody>
</table>

Table 1. Expression of resistance of a range of control varieties used for determining the race of Fusarium oxysporum f. sp. melonis.

RESULTS

Soil-borne pathogens

The investigations yielded a qualitative (rather than quantitative) vision of the phytosanitary status of melon crops in France, particularly in the Southwest region.

F. oxysporum f. sp. melonis was found to occur in the majority of the analyzed samples (from monitored or additional plots). This pathogen, widely implicated by earlier studies (Buffière and Taussig 2004), is indeed the most prevalent. This Fusarium is established in all producing regions. Regarding the F. oxysporum races encountered, all of the isolates collected between 2004 and 2006 (36) as well as 84% of the isolates collected in 2003 (34) belong to race 1-2Y (Tab. 2), thus confirming the observations of Perchepied and Pitrat (2004).

In a few cases, decay is caused by some other pathogen, namely: Macrophomina phaseolina, Didymella bryoniae, Pyrenochaeta terrestris, Phomopsis sclerotioroides or Verticillium dahliae (Tab. 3).

Plot monitoring based on five pre-defined experimental plots at three different dates gives an idea of the dynamics of pathogen development. Despite the choice of plots recognized for their history of plant mortality problems, we did not observe any visual symptoms in the monitored plot in the Gers region. This does not mean that no pathogens were present, but that they are not expressed by plant mortality. In the three situations studied, beginning with the plantlet stage, pathogens, mainly F.
*Fusarium oxysporum* f. sp. *melonis*, could be isolated. In most of the experimental plots, *F. oxysporum* f.sp. *melonis* was found together with other pathogens.

**Table 2.** Determination of *Fusarium oxysporum* f.sp. *melonis* race of different isolates from various samples.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of isolates tested</th>
<th>% of Race 1-2 yellowing</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>46</td>
<td>84 %</td>
</tr>
<tr>
<td>2004</td>
<td>29</td>
<td>100 %</td>
</tr>
<tr>
<td>2005</td>
<td>6</td>
<td>100 %</td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>100 %</td>
</tr>
</tbody>
</table>

**Table 3.** Main pathogenic fungi isolated and relative frequency of occurrence in melon from the main producing regions of France from 2003 to 2006; ■■■■■: very frequent, ■■■■: frequent, ■■■: fairly frequent, ■: infrequent.

<table>
<thead>
<tr>
<th>Year</th>
<th><em>F. oxysporum</em> f.sp. <em>melonis</em></th>
<th><em>Macrophomina phaseolina</em></th>
<th><em>Pythium</em> sp.</th>
<th><em>Rhizoctonia solani</em></th>
<th><em>Didymella bryoniae</em></th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>■■■■■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>2004</td>
<td>■■■■■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>2005</td>
<td>■■■■■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>2006</td>
<td>■■■■■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
<tr>
<td>2007</td>
<td>■■■■■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
<td>■</td>
</tr>
</tbody>
</table>

**Pathogenicity of the soil-borne fungi encountered**

The study of the pathogenicity of fungi other than *F. oxysporum* encountered through *in vivo* tests on young plantlets indicated that a number of them may cause necrotic lesions and lead to plant mortality. This is notably the case for certain strains of *Pythium* sp., *Didymella bryoniae* and *Pyrenochaeta terrestris* (Fig. 1).

The results on very young plantlets were confirmed by another test performed on young plants (Fig. 2) the mortality rate can reach 90 % for some isolates. It clearly appears that although the cause of plant mortality is most frequently related to *F. oxysporum*, the other pathogenic fungi encountered constitute an aggravating factor, but their exact role remains to be determined. These findings corroborate those of Buffière and Taussig (2004).

Regarding the condition of the root systems, many of the samples analyzed showed a significant deterioration of the roots, especially when they were taken at the “harvest” stage of the crop.

Moreover, in many of the samples, the root system was poorly established, especially in transplanted melon plants. The poor rooting quality is evidenced by the limited number of root shoots, which may be unidirectional or knotted. This poor rooting may be a factor leading to the fungal disease subsequently encountered in the crops.
The analysis of some strains of *F. oxysporum* f. sp. *melonis* race 1-2Y suggests aggressiveness considerably greater than the reference strains (Fom 1-2 TST, the reference yellowing strain and Fom 1-2 d'Oléon 8, the reference wilt strain) used for screening tests (Fig. 3).

![Pathogenicity Index](image)

**Figure 1.** *In vitro* pathogenicity on plantlets of various isolates of fungi representative of the mycelial flora isolated from melon root in 2003 and 2004, expressed by the pathogenicity index (green: *Pythium* sp.; orange: *Pyrenochaeta terrestris* and blue: *Didymella bryoniae*).

**DISCUSSION AND CONCLUSION**

This five-year investigation has highlighted several key aspects. *F. oxysporum* f.sp. *melonis* race 1-2Y is the most widespread pathogenic fungus established in the field in the various melon-producing regions of France. It may be considered as the primary cause of soil phytosanitary problems of melon in France. This result corroborates the findings from earlier studies. The *F. oxysporum* f.sp. *melonis* isolates appear to be more aggressive than the reference strains used in varietal screening tests, raising a number of questions, namely: Have these more aggressive strains of *F. oxysporum* emerged under selection pressure associated with growers’ use of partial resistant varieties? How representative of adult plant behavior in field cultivation conditions is a test performed on young plantlets? Further, what behavior can be expected from a plant material that is nominally more resistant, such as BIZ (Herman and Perl-Treves 2007) than our resistance reference, Isabelle? Finally, how does this aggressiveness affect the behavior of so-called “resistant” varieties and rootstock produced from *Cucumis melo*?
Figure 2. *In vivo* pathogenicity on young plants of various isolates of fungi representative of the mycelial flora isolated from melon root in 2003 and 2004, evaluated and expressed in terms of plant mortality rate (green: *Macrophomina phaseolina*; purple: *Pythium*).

Other pathogens such as *Macrophomina phaseolina*, *Phomopsis sclerotioides* and *Verticillium dahliae* have also been encountered and are causes of plant mortality. In many cases, *F. oxysporum* f.sp. *melonis* occurs together with other pathogens that are truly virulent, such as *Pythium* spp., *Didymella bryoniae*, *Pyrenochaeta terrestris* and even *Macrophomina phaseolina*. Although the precise role of these pathogens has not been clearly established, they can aggravate the observed decay.

The fungal analyses performed on some 41 samples received from non-monitored plots did not reveal any presence of *Monosporascus cannonballus*, despite conditions leading to its expression in 2003 and 2006. This fungus, against which there is currently no effective means of protection, has not yet been reported in France, but is found in Spain and causes the death of plants.

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Figure 3. Aggressiveness of various strains of Fusarium oxysporum f. sp. melonis race 1-2 coming from different French areas related to the reference strains used in the screening tests.

Literature cited
Gordon TR, Okamoto D, Jacobson DJ (1989) Colonization on muskmelon and non susceptible crops by Fusarium oxysporum f. sp. melonis and other species of Fusarium. Phytopathology 79: 1095-1100
Molot PM, Mas P (1975) Influence de la température sur la croissance mycélienne et le pouvoir pathogène des quatre races physiologiques de Fusarium oxysporum f. sp. melonis. Ann Phytopathol 7: 115-121