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Greenhouse eggplant production in France: Success and failures of grafting for controlling *Verticillium* sp.

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Abstract

After many years of utilisation of grafting on *Verticillium* wilt resistant rootstocks, mainly tomato rootstocks, growers observe new wilt like symptoms on their grafted eggplants. Accordingly, Ctifl, in close collaboration with INRA, regional extension services, and seed companies, set up a research designed to get a general picture of the various soil borne phytosanitary problems, and to identify their cause(s). The principle of the study was to monitor pathogen distribution changes in eggplant crops in the two main production regions in France. The results show the prevalence of *Verticillium* wilt and, in the majority of cases and all production areas, the simultaneous presence of a number of other bioaggressors, including fungi and nematodes. The isolates of *Verticillium* were identified at the species level (*V. dahliae*, *V. isaacii*) and characterized for their race (0 or 1) and their aggressiveness. Surprisingly, the aggressiveness of most isolates was relatively low. To our knowledge, this is the first report of *V. isaacii* in France, but its impact on production across France is unknown at this time.

1. Introduction

France is traditional area of production of eggplant (*Solanum melongena* L.) and *Verticillium* wilt is a long lasting problem for growers. Resistant cultivars are not available (Lockwood and Markarian, 1961; Alconero *and al.*, 1988; Yu *and al.*, 2015). Research on grafting started in France in the sixties (Messiaen *and al.*, 1967; Beyries, 1974). Presently, 63% of eggplant surface is grafted (Torres and Brand, 2015) and the rootstocks generally used are interspecific hybrids of tomato species; the use of *S. torvum* is less frequent.

After several years of utilization of tomato resistant cultivars and rootstocks, homozygous or heterozygous for the Ve gene, damaging wilt-like-symptoms are often observed on tomato as well as on eggplant, grafted or not. So, it was necessary to determine the cause of this re-emerging problem. The aim of this study, conducted by the Centre Technique Interprofessionnel des Fruits et Légumes (Ctifl) in association with INRA, regional experimental stations (APREL and Invenio), seed companies and growers, was to identify the major pathogen(s) causing the wilting on *Verticillium* resistant material and to determine their aggressiveness and virulence.

2. Materials and method

2.1. Sampling plant material and isolates

Plant samples were collected from French production areas on plants displaying wilt symptoms. In each location, three plants (whatever eggplant) were sampled at random and sent to Ctifl (Lanxade) for analysis. On delivery, the samples were unwrapped and rinsed with water in order to permit a detailed analysis of the root system. The number and types of samples are shown respectively in Tables 1 and 2

Provence	South-West France	Center-West
45	13	3

Table 1
Geographic distribution of the eggplants sampled from 2008 to 2015.

13 non grafted	40 grafted onto tomato rootstock	4 grafted onto <i>S. torvum</i> rootstock
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Table 2
Status of eggplants sampled.

Microbiological isolates were then collected from each plant using potato dextrose agar (PDA) medium amended with 100 mg/L⁻¹ of ampicillin in order to isolate the various pathogenic fungi potentially related to the wilting and root decay problems. Plant colonisation by pathogens was analyzed by sampling tissues from roots, collar level, grafting point and shoots. Small sections (0.5 to 1 cm) were cut with a sterilized scalpel, superficially disinfected by dipping in 0.5% sodium hypochlorite for 3 min, rinsed three times in sterile distilled water, and deposited on Petri plates, which were then incubated at 21°C in the dark for 1 week. After few days (5 to 10) actively growing fungal colonies were transferred onto new PDA plates for further purification, sporulation and identification.

2.2. Pathogenicity studies

To identify the *Verticillium dahliae* races and the aggressiveness of the isolates, bioassays were performed using several controls:

- Tomato 'Marmande verte' susceptible to *Verticillium dahliae* race 0 and 1;
- Tomato 'Marmande VR' resistant to race 0 and susceptible to race 1;
- Tomato 'IRAT L3' and 'Mel 26681 70 G' partially resistant to race 0 and susceptible to race 1;
- Eggplant 'Liu Yé Qié' susceptible to *Verticillium dahliae* race 0 and 1.

The aggressiveness of each *Verticillium* isolate was estimated, on the basis of stem vessels browning of each control, by calculating mean values using the following formula, VD = vascular discoloration:

$$\text{Index of Aggressiveness} = I_A = \left[\frac{((VD \text{ Marmande verte}) + (VD \text{ Marmande VR} \times 2) + (VD \text{ IRAT L3} \times 3) + VD(\text{Mel 26681} \times 3))_{\text{strain } i}}{((VD \text{ Marmande verte}) + (VD \text{ Marmande VR} \times 2) + (VD \text{ IRAT L3} \times 3) + (VD \text{ Mel 26681} \times 3))_{\text{reference strain}}} \right] - 100$$

We have imputed different coefficients to the tomato and eggplant accessions, according to their level of resistance. For facilitating the comparison between strains, we imputed the level "0" to the reference *Verticillium* strain ('race 0 Toreille'). Each test consisted of 2 replications of 20 plants per control, grown in individual mini-clods, in order to avoid cross-contamination. For each plant, 3 ml of the concentration of the spore suspension adjusted to 4.10^5 conidia/mL⁻¹ was applied.

3. Results and discussion

3.1. Prevalence of soil borne diseases on eggplant

The most frequently observed soil-borne pathogen is *V. dahliae*. *V. albo-atrum* has not been isolated. In the most frequent cases, *V. dahliae* was isolated together with one or more bioaggressors, in particular *Colletotrichum coccodes*, *Pythium* sp., *Rhizoctonia solani* and *Macrophomina phaseolina*. Generally, these fungi were associated to *Meloidogyne* spp.

The situation was slightly different between the two main French production areas. In Provence, we observed more samples with *V. dahliae* alone, and only a few samples with *V. dahliae* associated with other pathogens, generally *C. coccodes*, *Pythium* sp. and *R. solani*. In the South-West area, *V. dahliae* was mostly associated with one or more fungi (Fig. 1). In the case of South-West, the species and race of *Meloidogyne* have been identified as *M. hapla* in some samples, and *M. arenaria* race B in others (Blancard *and al.*, 2007). These nematodes are not controlled by the tomato gene *Mi*.

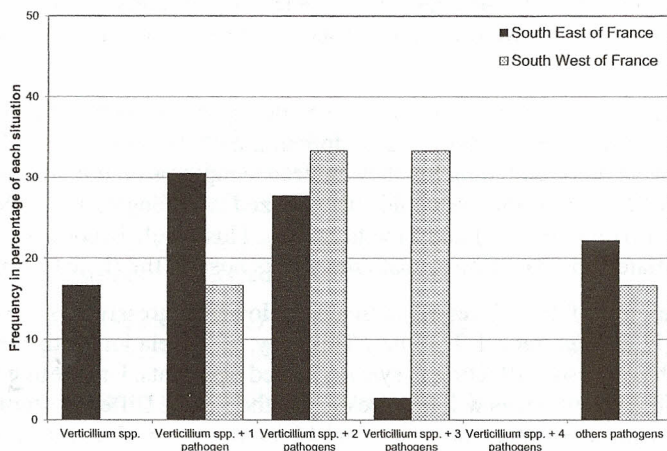


Figure 5

Relative abundance of *Verticillium* spp. and associated soil borne pathogens isolated from eggplants in two French production areas.

We did not observe any difference neither between grafted and non-grafted eggplants (Fig. 2) nor between the rootstocks used, mainly Beaufort, Maxifort and Imperador (data not shown).

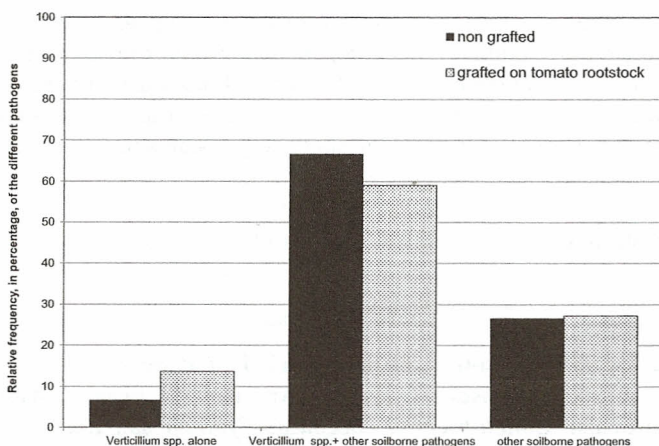


Figure 6
Interaction between grafted or non-grafted eggplants and the structure of the soil-borne pathogenic fungal complex.

3.2. Characterization of isolates of *V. dahliae*

The characterization of isolates of *V. dahliae* was difficult because symptoms such as yellowing and wilting on tomato and eggplant are unclear and strongly depend on environmental conditions. Vessels browning were the most discriminant symptom between susceptible and resistant controls.

Isolates are generally divided in defoliating pathotypes and non-defoliating pathotypes (Hagiwara, 1990; Usami *and al.*, 2002). The defoliating pathotypes are generally virulent. All the tested strains were non-defoliating but they induced symptoms on tomato and eggplant. Out of the 12 isolates of *V. dahliae* that we could characterized as belonging to a specific race, three belonged to race 0 (common race) and nine to race 1. This result is not surprising given the majority of the isolates originate from tomato rootstocks hosting the (race 0 resistant) gene *Ve*.

Eleven isolates out of the 12 tested presented a lower aggressiveness (Fig. 3) than the reference strain (*V. dahliae* race 1 Toreille). This may be in relation with the presence of a complex of other bioagressors affecting in synergy wilted eggplants. Unraveling the interactions between these different pathogens will be necessary in the future. Different studies have proved the existence of a positive interaction between *Meloidogyne* or *Pratylenchus* and a higher infection level by *V. dahliae*. (Bowers *and al.*, 1996 ; Scholte and s'Jacob, 1989).

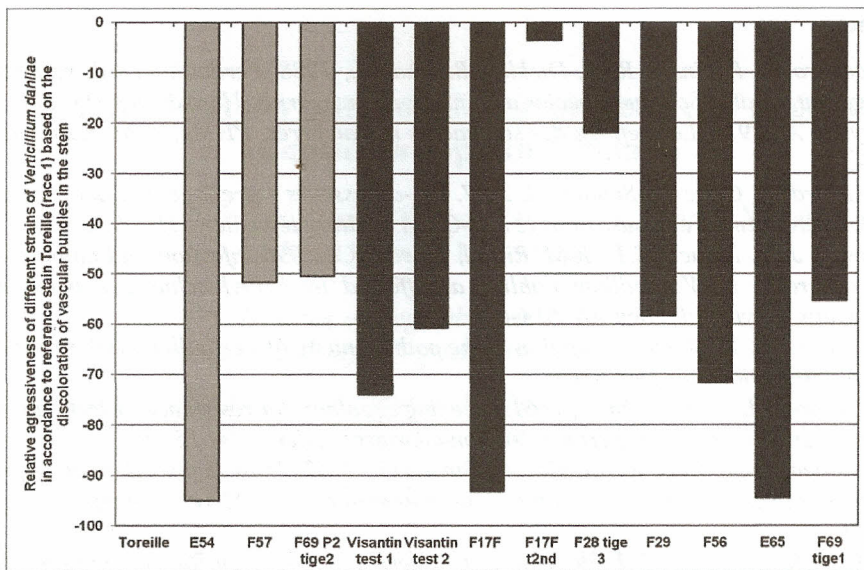


Figure 7
 Aggressiveness of the various strains of *Verticillium dahliae* sampled on eggplants in different French areas. Reference strain ('Toreille') is attributed the level 'zero' for aggressiveness.
 ■: race 1 strains. ■: race 2 strains.

4. Conclusion

After many years of using grafting on resistant rootstocks, complexes of soil-borne fungi develop in intensively cultivated soils and damage formerly resistant plants. The frequency of this phenomenon is increasing since the use of soil chemical fumigation, in particular methyl bromide, is forbidden since 2005. The most frequent situations observed are the following:

- For eggplant grafted onto tomato rootstocks, presence of race 2 of *Verticillium* that bypasses the resistance conferred by the tomato gene *Ve*,
- In addition to the presence of *Verticillium* on wilting plants, two or more additional fungi are isolated, thus revealing the development of pathogenic complexes in the soil.

Hence our future prospect is to combine the use of rootstocks or varieties resistant to a wider range of soil-borne pathogens, together with complementary control methods such as biocontrol, soil organic amendments, and crop rotation.

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