Fusarium Wilt on Sweet Basil: Cause and Sources in Southeastern Spain

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This study concerned a new disease detected in 1997 in southeastern Spain – Fusarium wilt in basil (*Ocimum basilicum* L.), caused by *Fusarium oxysporum* f.sp. *basilici.* Its importance was evaluated at two locations in the Almería area, where 14% of the plants presented symptoms of the disease after 4 months of cropping. The search for sources of the disease inoculum was centered on the health of the seeds and the polypropylene trays that were reused for plant production. Analysis of four lots of seeds from Germany and Italy showed that two of them harbored *F. oxysporum* f.sp. *basilici.* This finding was confirmed by the analysis of seeds collected from diseased plants. Furthermore, analysis of three reused trays revealed the presence of the pathogen on them and it was concluded that the trays acted as the source of dispersion of the mycosis.
KEY WORDS: Basil; Fusarium wilt; *Fusarium oxysporum* f.sp. *basilici*; seed health; health of nurseries.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a plant of the Labiatae family which originated in India and spread worldwide. It is widely grown for its aromatic, ornamental, medicinal, and honey-producing characteristics (17). Nowadays it is commonly used as a culinary herb; it is rich in carbohydrates, proteins, and vitamins A and C (15).

The cultivation of sweet basil is relatively new to Almería (Spain), where the crop extends over a small area of less than 10 ha. The climate of southern Spain enables year-round export of fresh basil to other European countries. It is grown in greenhouses both on soil and in perlite throughout the year. Plants are maintained for 2 years and those that die are replaced. Thus, it is possible to find plants of different ages within the same greenhouse. Basil seedlings are produced in nurseries until transplanted to soil or perlite.

This study presents the results of the identification of one of the most severe diseases of basil, Fusarium wilt. This disease was first described in the former Soviet Union in 1957 (8) and the causal pathogen was determined in 1968 (2,4). It has been identified in numerous countries as *Fusarium oxysporum* f.sp. *basilici* (1,3,6). The typical wilt symptoms of basil are as follows: yellowing and wilting of apical tips, wilting and necrosis of leaves and petioles, stunting, black lines along the stems and petioles, vascular discoloration and necrosis of the xylem. All these symptoms begin at the apex and progress to the base of

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the plant. Vascular necrosis then extends to the roots, with the appearance of very dark spots along the stems. Young plants can die very rapidly, between 4 and 7 days after the appearance of the first symptoms (5).

The aim of the present work was to find and identify the *(a)* cause of the wilt in sweet basil and *(b)* inoculum of the pathogen sources.

MATERIALS AND METHODS

Disease assessment in commercial greenhouses The study was carried out in two greenhouses where aromatic, culinary and medicinal plants were grown, one located in the western part of Almería and the other in the eastern. In both greenhouses basil cv. 'Genovesa' was grown on soil or in perlite bags. Disease incidence was evaluated between February and May 1997 for both crops and at both locations without artificial inoculation. In the soil culture the diseased plants were evaluated in four plots of 16 m^2 each. In the case of the perlite bags substrate, evaluation was carried out in three plots of 600 transplantation holes each, with each hole containing up to eight plants. The counts of diseased or dead plants with symptoms were done fortnightly within the period indicated above. The observed progress of disease of Fusarium wilt was expressed as values of diseased or dead plants for each experiment.

Isolation and identification of *Fusarium* from basil In order to isolate the fungus from the plants with symptoms, stems were flamed with alcohol after washing them with tap water. Stem slices 0.5 cm thick were then placed on potato dextrose agar (PDA) and a selective *Fusarium* culture medium (19). A total of 58 plants from both locations were analyzed as follows: 21 from soil cultivation, 22 from soilless culture, and 15 from nursery. Identification of the fungal isolates was based on the morphology of macroconidia, microconidia and chlamydospores, following the taxonomic descriptions and the indications of Messiaen and Cassini (11) and of Nelson *et al.* (14).

Pathogenicity test and host range determination Pathogenicity tests were carried out on the basil plants. The cultivar used (Genovesa) is the most widely grown and one of the most resistant to *F. oxysporum* f.sp. *basilici* (12). Inoculum was prepared by growing the fungus in 15 ml of PDA in petri dishes (8.5 cm diam) and incubating the dishes at 25°C until the mycelium reached the edges. The contents were mixed with 100 ml distilled water to prepare the fungal suspension (10^4 cfu ml^{-1}). The inoculum suspension was applied to two true leaves of basil seedlings either by drenching the sterile substrate in which they grew or by dipping the roots and then transplanting the plants into sterile substrate. The seeds of the plants used were analyzed previously to verify that no *F. oxysporum* was present. They were pregerminated at 30°C for 24 h, and then sown in disinfested vermiculite either in 1 *l* pots or in polypropylene trays, according to the inoculation procedure. The plants were fertilized before and after inoculation with a complete nutrient solution commonly used in the greenhouse.

Thirty-three isolates collected from diseased plants and seedlings were inoculated (Table 1): 24 of them from plants grown in the greenhouse, and nine from seedlings grown in the nursery. Ten basil plants were inoculated with each isolate and ten were not inoculated and used as control. After inoculation, plants were maintained in a greenhouse and the disease was evaluated by counting diseased or dead plants over a period of 60 days. The pathogen was reisolated from inoculated plants using the method described above.

Five of these isolates, showing pathogenicity on basil, were used to study the parasitic specificity of *F. oxysporum*. The dipping roots technique was employed for each isolate and each species used. Ten plants per isolate were inoculated, from each of the following species: melissa (*Melissa officinalis* L), sage (*Salvia officinalis* L.), marjoram (*Origanum majorana* L), mint (*Mentha piperita* L), summer savory (*Satureja hortensis* L.) and thyme (*Thymus vulgaris* L.); ten plants of each species were not inoculated, as used as controls.

Examination of seedlings, seeds and nursery trays as possible sources of the causal agent of disease In an attempt to find the possible inoculum sources of the pathogen, 15 wilted seedlings from the nursery were washed with water, air-dried, and then analyzed using *Fusarium* selective medium and Pythiaceae medium (16). The health of four commercial basil seed lots acquired from Italy and Germany was checked. Eight hundred seeds from each lot were plated, half of them on PDA culture medium and the other half on *Fusarium* selective medium according to the Ulster method (13). These analyses were carried out also on 458 basil seeds collected from diseased plants growing in the two greenhouses. Other aspects of the nursery were considered, such as the polypropylene trays reused for seedling production of basil. The trays were washed with water and air-dried. Three of them were examined by cutting 15 pieces of 1 cm^2 each, which were placed on *Fusarium*-selective medium.

The pathogenicity of the *F. oxysporum* isolates detected was verified by the methods described above.

RESULTS

Disease assessment in commercial greenhouses Apical wilt and epinasty, external blackening of the angular stems, and yellowing of diseased plants were observed in the greenhouses without artificial inoculation.

TABLE 1. Origin of *Fusarium oxysporum* isolates inoculated and pathogenic to basil

Origin of the isolates	Number of isolates	
	inoculated	pathogenic
Diseased basil growing in		
Soil	17	12
Perlite substrate	7	1
Nursery	9	1
Commercial seeds	16	13
Seeds from diseased basil	4	2
Polypropylene trays	10	3
Total	63	32

Disease progress in plants grown in soil is shown in Figure 1A as the percentage of plants with symptoms or killed by the disease. The average of all the plots reached 14.4% of the plants. Disease incidence on crops grown in perlite (Fig. 1B) averaged 13.4% of the plants. These results prompted the investigation on the sources of the primary inoculum in the inspected greenhouses.

Isolation and identification of *F. oxysporum* The association of the symptoms observed in the commercial greenhouses with the xylem analyses of basil in the laboratory showed that *F. oxysporum* was isolated from the xylem in all cases when the plants were grown

TABLE 2. Percentage of commercial seeds of basil from which *Fusarium* spp. were obtained in culture on PDA or selective medium

Culture medium	Fusarium isolates	Percentage of seeds with *Fusarium*			
		lot 1	lot 2	lot 3	lot 4
PDA	*F. moniliforme*	-	1.25 (5)	-	-
	F. oxysporum	1 (4)[y]	-	-	-
	F. roseum[z]	-	-	-	0.25 (1)
Selective medium	*F. moniliforme*	-	1.50 (6)	-	-
	F. oxysporum	1 (4)	0.25 (1)	-	1.75 (7)
	F. equiseti	0.25 (1)	0.25 (1)	-	-
	F. roseum[z]	0.50 (2)	-	-	-

[z] *sensu* Messiaen and Cassini (11).

[y] In parentheses, number of *Fusarium* isolates.

TABLE 3. Number of pathogenic *Fusarium oxysporum* isolates on basil from commercial seeds

Seed lot #	Number of isolates	
	Inoculated	Reproducing the disease
1	8	7
2	1	0
4	7	6

in soil, whereas for the diseased materials obtained from plants grown in perlite, fungal isolation fluctuated between 33% and 40%. The association between symptoms and the (positive) isolation of *F. oxysporum* reached 93% when the xylem analyses were carried out on nursery seedlings. *F. oxysporum* was identified by morphology of macroconidia, microconidia and chlamydospores, following the taxonomy indicated in the methods described above.

Pathogenicity tests and host range of *F. oxysporum* isolates The artificial inoculation of 33 isolates of *F. oxysporum* obtained from diseased plants from the sampled greenhouses, including the nursery, revealed that 14 of them (42.4%) caused the disease syndrome on basil (Table 1). Of the 14, 36.4% had been obtained from diseased plants grown in soil, 3% from those grown in perlite, and 3% from the nursery. The first symptoms appeared 12 days after inoculation, showing the same syndrome described for commercial greenhouses plants, and more than 50% of the plants died within 60 days after inoculation. Fusarium wilt was closely associated with the number of inoculated isolates collected from symptomatic plants. Of 17 inoculated isolates from plants grown in soil, 12 reproduced the disease on basil under test conditions. The pathogen was reisolated from diseased plants, following Koch's postulates for all of them.

Results of the artificial inoculations to verify the parasitic specificity of five *F. oxysporum* isolates which were pathogenic on basil, showed that none of them caused any symptoms in other inoculated plant species: sage, marjoram, thyme, melissa, mint, or summer savory. The results indicated that symptoms of the disease on basil were caused by the fungus *F. oxysporum* f.sp. *basilici.*

Possible sources of disease contamination The investigations to find the inoculum sources of *F. oxysporum* f.sp. *basilici* in greenhouses and nurseries were focused on two aspects: the health of the seeds and the health status of the polypropylene trays for transplanting which were reused in nurseries.

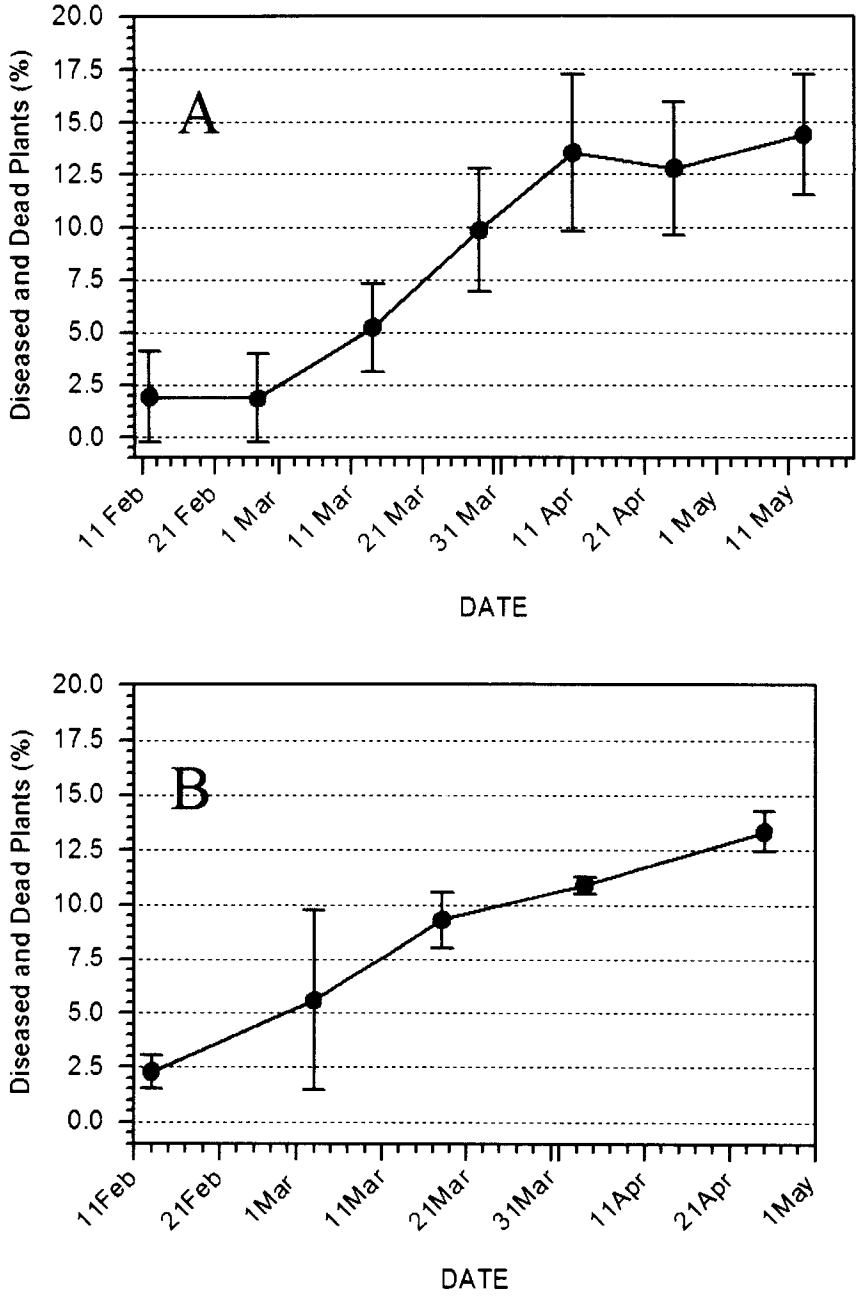

Fig. 1. Progress of Fusarium wilt disease on basil grown under commercial greenhouse conditions. A: In soil. Mean percentage of diseased or dead plants in four plots of 16 m^2. B: In perlite. Mean percentage of diseased or dead plants in three plots with up to 1000 basil plants in each plot. Vertical bars indicate standard deviation of the mean.

The results of the health tests in PDA and *Fusarium*-selective medium from four lots originating in Germany and Italy are shown in detail in Table 2. The fungus *F. oxysporum* was detected in the selective medium in three of the lots. Table 3 summarizes the results of the pathogenicity tests after inoculation of basil with 16 isolates obtained from the 3200 seeds analyzed on culture medium. At least two lots of seeds were infected by *F. oxysporum* f.sp. *basilici.*

In the analysis of 458 seeds collected from diseased basil plants, *F. oxysporum* was isolated on a selective medium in 0.9% of the seeds. Pathogenicity tests carried out on basil with four isolates revealed that half of them were *F. oxysporum* f.sp. *basilici,* reproducing the symptoms on basil but not in the other plant species (Table 1).

Fifteen samples from each of three trays were analyzed; four, three and five isolates, respectively, were found. Thirty per cent of the *F. oxysporum* isolates obtained from the trays were pathogenic on basil, reproducing the disease symptoms on cv. Genovesa (Table 1).

DISCUSSION

The disease syndrome found on basil grown in commercial greenhouses was similar to that described previously in the literature on Fusarium wilt by various authors: Mercier and Pionnat (10), Tamietti and Matta (18), Elmer *et al.* (2), Gamliel *et al.* (4), Garibaldi *et al.* (5), Keinath (7). The symptoms differ from those of other Fusarium wilt types in the external blackening of stems and leaves, to such an extent that identification by symptoms can lead to confusion with other pathogens such as *Botrytis cinerea* and *Sclerotinia sclerotiorum*, at least under our working conditions.

The evolution of the disease in the investigated greenhouses was rapid, comparable to that reported by others, *e.g.* Keinath (7). Our results also show the small difference between the two cropping systems (soil or perlite). This fact is not devoid of importance, as better control of the disease had been sought by soilless cultivation. Finally, these findings prompted the investigation into the sources of the primary inoculum in the inspected greenhouses.

The results suggested that seeds were a source of the pathogen in greenhouses and nurseries. This had been shown previously by Martini and Gullino (9) and Gamliel *et al.* (4), who worked with seeds gathered from diseased plants.

The percentage of *F. oxysporum* detected on selective medium, from seeds collected from diseased basil plants, can be considered low (0.9%). Therefore, the fact that half of the isolates were able to reproduce the symptoms on basil and not on the other herbs emphasizes the possibility of contamination of commercial seeds. Certainly, the percentage of infected seeds seems small in relation to the extent of Fusarium wilt found in the greenhouses.

The high level of disease in the commercial greenhouses can not be explained only by the pathogenicity of *F. oxysporum* f.sp. *basilici* isolates originating in basil seed. An explanation for the extent of Fusarium wilt might be found in the reuse of alveolar polypropylene trays in the nursery for the production of basil. Thirty per cent of the *F. oxysporum* isolates detected on the trays were pathogenic on basil. Therefore, the spread of the disease could be caused by the transfer from nursery trays to soil or perlite bags, and by the use of non-disinfested trays.

In the absence of regulations regarding basil seeds certification in the European Union,

we recommend the use of clean disinfested containers for seedling and plant production. This should be very helpful to prevent the spread of Fusarium wilt in basil crops.

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