

## Fusarium Wilt of Basil in Greece: Foliar Infection and Cultivar Evaluation for Resistance

D. Biris,<sup>1</sup> D.J. Vakalounakis\*,<sup>2</sup> and E. Klironomou<sup>2</sup>

Fusarium wilt of basil (*Ocimum basilicum*), caused by *Fusarium oxysporum* f.sp. *basilici*, is reported for the first time in Greece. Foliage inoculation of young plants resulted in a downward movement of the pathogen to the crown and roots and 20–30% plant mortality. Of 14 commercial basil cultivars evaluated for possible disease resistance using young plants, six out of eight large-leaved cultivars were found resistant, while all six small-leaved ones were susceptible.

KEY WORDS: *Ocimum basilicum*; *Fusarium oxysporum* f.sp. *basilici*; foliar infection; disease resistance.

### INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is a popular herb crop in several Mediterranean countries, grown commercially for essential oil extraction and for its ornamental, medicinal and culinary qualities (14). An increased interest in the so-called ‘Mediterranean cuisine’ has led to a significant increase in basil consumption worldwide (7). The name basil comes from the Greek word ‘basilicos’, which means ‘royal’ (basileus = king), and shows the Ancient Greeks’ feelings for this plant. Today, basil is grown all over Greece, mainly as an ornamental plant. Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.: Fr. f.sp. *basilici* (Dzidzariya) Armst., is a destructive disease of basil. It was first described in 1963 in Russia (4) and later reported from additional countries such as Italy, France, U.S.A., Canada, Australia, Israel and Spain (3,5,6,8,11).

In late spring 1999, severe wilt of basil cv. ‘Fin Vert Nain Compact’ occurred in a greenhouse crop in the Volos area of Thessaly, Greece. In subsequent years the disease spread to most of the greenhouse and field crops of the small-leaved cultivars in Volos as well as in Agrinio, Western Greece, and Heraklio, Crete. The first symptoms were related to water stress, *i.e.*, dull light-green coloring of the foliage, withering, shriveling and partial defoliation. Wilt symptoms, starting from the apical leaves, were initially restricted to only some branches (hemiplegia), but gradually extended to the entire plant that finally dried up. In some cases the symptoms appeared suddenly on the whole plant, which withered and dried very soon (apoplexy). Lengthwise cutting of the stem revealed a continuous brown discoloration in the vascular bundles, running from the main root up into the stem. Under high humidity conditions, the stem surface was covered by a creamy white mycelium and *Fusarium*-like conidia of the pathogen. When young plants were infected, stunting was usually evident prior to wilting.

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<sup>1</sup>Emeritus Director, N.AG.RE.F., Plant Protection Institute, 38001 Volos, Greece.

<sup>2</sup>N.AG.RE.F., Plant Protection Institute, 71003 Heraklio, Crete, Greece. \*Corresponding author [Fax: +30-2810-245858; e-mail: vakalounakis@nagref.gr].

The objectives of the present study were to: (i) examine whether this wilt disease of basil was caused by *F. oxysporum* f.sp. *basilici* (*Fob*), (ii) assess the potential of foliar inoculation to incite disease, and (iii) evaluate several commercial cultivars of basil for possible *Fusarium* wilt resistance.

## MATERIALS AND METHODS

TABLE 1. Isolates of *Fusarium oxysporum* from basil used in this study

Isolate	Geographic origin	Year of isolation	Disease incidence (%) <sup>z</sup>
<i>F. oxysporum</i> isolates from Greece			
FOB1	Alikes, Volos	2001	25
FOB3	Agria, Volos	2001	20
FOB4	Ano Volos	2001	20
FOB5	Heraklio	2001	20
FOB6	Heraklio	2001	30
FOB7	Peza, Heraklio	2001	20
FOB8	Agrinio	2001	15
<i>F. oxysporum</i> f.sp. <i>basilici</i> isolates from Italy used as reference			
FOB Rossi G.E.	Genoa	2000	
FOB Sacco G.B.	Genoa	2001	
FOB Ge1	Albenga SV	2000	

<sup>z</sup>In plots from which the samples were taken.

**Pathogen identification** *Fusarium* was isolated from discolored vascular bundles of the stems of seven diseased plants obtained from different locations in Greece (Volos, Agrinio and Heraklio), after surface disinfection with 95% ethyl alcohol for 5 min, plating on acidified potato dextrose agar (APDA, 4.5 ml 25% lactic acid in 1 l PDA, final pH 4.0) and incubating at 22±0.5°C in a 12-h photoperiod for 5 days. *F. oxysporum* was identified on the basis of morphological characteristics, and the *forma specialis* was confirmed using pathogenicity tests. Artificial inoculations were performed on basil (cv. Fin Vert Nain Compact), spearmint (*Mentha spicata* L.), dittany of Crete (*Origanum dictamnus* L.), cucumber (*Cucumis sativus* L., cv. 'Knossos') and tomato (*Lycopersicon esculentum* Mill., cv. 'Earlypak No. 7'), using isolates FOB3 and FOB6 obtained from diseased basil plants cv. Fin Vert Nain Compact in 2001 from the Agria (Volos) and Heraklio areas, respectively (Table 1). Reference isolate FOB Rossi G.E. of *Fob*, kindly donated by Prof. M.L. Gullino (Dept. of Plant Pathology, University of Torino, Italy) was used for comparison. Inoculum was prepared by growing each fungal isolate in a 200-ml Erlenmeyer flask that contained 100 ml of potato dextrose broth (PDB), in a rotary shaker for 7 days, at 20°C in the dark. Flasks were inoculated with 5-mm diam agar plugs (three plugs per flask) taken from the periphery of 5-day-old cultures grown at 23°C in the dark. The suspension was then filtered through a double layer of non-sterile cheesecloth to remove mycelial fragments, centrifuged at 3,000 × *g* for 10 min, and the conidial pellet resuspended in distilled water. Thirty-day-old plants (20 plants per species and isolate) were inoculated by dipping the roots in conidial suspensions of the three isolates (10<sup>6</sup> spores ml<sup>-1</sup>), transplanted to a sterile substrate ('Favorite', Ehrenborg u. Fortman, Lohne-Kroge, Germany) and incubated at room temperature for 20 days, under artificial illumination in a 12-h photoperiod, provided by Osram-type fluorescent Daylight lamps (350-750 nm), with light intensity at plant level

approximately 10,000 lux. Plants treated similarly and dipped in tap water served as control. To re-isolate the pathogen, stem sections of diseased plants were plated on APDA. The pathogenicity test was repeated once with similar results.

**Vegetative compatibility tests** The identity of the pathogen was also examined by the vegetative compatibility grouping (VCG) method (2,12,16). *nit* mutants were generated on minimal medium (MM) amended with 1.5% potassium chlorate (MMC) (12) and characterized as *nit1*, *nit3* or NitM by their growth on MM supplemented with nitrate, nitrite or hypoxanthine (2). For complementation tests, mycelial plugs (0.5-cm diam) cut from *nit* mutant cultures growing on MM were placed approximately 4 cm apart on fresh MM in petri dishes (6-cm diam) and kept at  $22\pm 0.5^{\circ}\text{C}$  in the dark. Heterokaryon formation (complementation) was first observed 5 days after inoculation and was clearly evident by the formation of a dense aerial mycelium where two vegetatively compatible *nit* mutants had met while growing. Five to 12 *nit* mutants were generated from each of the Greek isolates (FOB1, FOB3, FOB4, FOB5, FOB6, FOB7 and FOB8) and the reference isolates (FOB Rossi G.E., FOB Sacco G.B. and FOB Ge1, all kindly donated by Prof. M.L. Gullino) of *Fob* (Table 1). Two complementary NitM mutants of the Greek isolate FOB6 that formed intense heterokaryons were used as testers to determine vegetative compatibility with several randomly selected *nit1* mutants of the other isolates, including reference isolates FOB Rossi G.E., FOB Sacco G.B. and FOB Ge1. When at least one *nit* mutant from an isolate formed complementary heterokaryon with at least one tester, then its parent isolate was placed in the same VCG (16).

**Foliage inoculation of basil plants** Infection of foliage-inoculated basil plants by *Fob* was tested in two experiments.

In the first experiment, 30-day-old cv. Fin Verte Nain Compact basil plants about 15 cm high were planted in pots (one plant per pot) filled with sterile substrate Favorite. Three days after transplanting, plant foliage was inoculated by spraying the aboveground part of the plants to runoff with spore suspensions containing  $10^6$  spores  $\text{ml}^{-1}$  of either the FOB6 or the FOB Rossi G.E. isolate (ten plants per isolate). Plants treated similarly with tap water served as control. To avoid soil infestation during foliage inoculation, the soil surface in the pots was covered with a polyethylene sheet that was overlaid with aluminum foil. To avoid wash-off of the propagules from the foliage to the substrate, plants were irrigated *via* the bottom of the pots by filling the pot trays with water for 5 min each time. After inoculation the plants were covered with polyethylene bags for 24 h to maintain high humidity and kept at room temperature on laboratory desks for 20 days under artificial illumination as described previously. For each isolate of the pathogen ten potted plants, arranged in completely randomized design, were used. Surface-sterilized stem sections of diseased plants were plated on APDA to re-isolate the pathogen. The identity of the pathogen was confirmed by the VCG method.

In the second experiment, the foliage of five adult potted plants of cv. 'Greek' was separated into two parts with thick plastic sheets. To avoid soil infestation during aerial inoculation, the soil surface in the pots was covered with a polyethylene sheet and aluminum foil as described previously. One part of each plant was artificially inoculated with a spore suspension of the isolate FOB3 and the other part with sterile water and served as control. Each part of the inoculated plants was covered with polyethylene bags for 24 h to maintain high humidity. Plants were kept at room temperature on laboratory desks close

to the windows without additional artificial illumination for 30 days. Surface-sterilized stem, crown and root sections of plants, as well as small quantities of soil, were plated on Komada's selective medium to re-isolate the pathogen. The identity of the pathogen was confirmed by the VCG method. Both experiments with young and adult plants were repeated once.

**Evaluation of commercial basil cultivars for possible disease resistance** Seeds of various commercial basil cultivars were immersed in 0.5% sodium hypochlorite for 15 min, rinsed in sterile water twice and then transplanted into sterile substrate Favorite. Thirty-day-old plants (ten plants per cultivar) were inoculated by dipping their roots in conidial suspensions of the isolate FOB6 of *Fob* adjusted to  $10^6$  spores  $ml^{-1}$ , transplanted into sterile substrate Favorite, and then kept at room temperature for 30 days under artificial illumination as described previously. Stem sections of diseased plants were plated on APDA to re-isolate the pathogen. The whole experiment was performed three times. Disease severity (DS) was assessed on a visual scale of 0 to 2, where 0 = no symptoms, 1 = vascular discoloration in the stem, 2 = dead plants. Disease incidence (DI) was expressed as the percentage of diseased plants having a score  $\geq 1$ . Disease index was calculated as the mean disease severity and incidence on artificially inoculated plants. Disease reactions were classified into three categories: R = resistant,  $DS \leq 0.4$ ,  $DI \leq 25\%$ ; MS = moderately susceptible,  $0.5 \leq DS \leq 0.9$ ,  $26\% \leq DI \leq 50\%$ ; VS = very susceptible,  $DS \geq 1$ ,  $DI \geq 51\%$ .

## RESULTS

**Pathogen identification** Under high humidity conditions microconidia and macroconidia on sporodochia of *Fusarium* sp. were always observed on the stem lesions. The same fungus was consistently isolated from the discolored vascular bundles of the stems of the diseased plants. The morphological and cultural characteristics of the fungus either *in vivo* or *in vitro* agreed fairly well with published descriptions of *Fusarium oxysporum* Schlechtend.:Fr. (1).

TABLE 2. Percentage of dead plants and colonization (%) of young basil plants<sup>z</sup> with isolates FOB6 and FOB Rossi G.E., and of adult basil plants<sup>y</sup> with isolate FOB3 of *Fusarium oxysporum* f.sp. *basilici* (The plants were sprayed to runoff with an inoculum suspension adjusted to  $10^6$  spores  $ml^{-1}$ )

Isolate	Young plants		Adult plants					
	Dead plants (%)	Isolations from stems (%) <sup>w</sup>	Dead plants (%)	Isolations of the pathogen from organs or soil of infected plants (%)				
				Stem	Crown	Root	Soil of non-inoculated part of foliage	
FOB6	20	100	100	100	100	45	0	0
FOB Rossi G.E.	30	100	nt <sup>v</sup>	nt	nt	nt	nt	nt
Control <sup>x</sup>	0	0						

<sup>z</sup> 30-day-old plants, 20 days after foliage inoculation.

<sup>y</sup> 30 days after inoculation of one part of the foliage.

<sup>x</sup> Non-inoculated plants.

<sup>w</sup> Pathogen isolated from stems of infected plants. <sup>v</sup> not tested.

In pathogenicity tests, the Greek isolates FOB3 and FOB6 as well as the reference isolate FOB Rossi G.E. of *Fob* caused typical symptoms of *Fusarium* wilt on basil plants,

but they did not cause any disease symptoms on spearmint, dittany of Crete, cucumber or tomato. In addition, the pathogen was re-isolated from all inoculated basil plants, but not from the other plant species tested.

TABLE 3. Evaluation of 30-day-old basil plants of several commercial cultivars for possible resistance 30 days after artificial inoculation with isolate FOB6 of *Fusarium oxysporum* f.sp. *basilici*

Cultivar	Type	Seed production company	Disease index <sup>z</sup>			Disease reaction <sup>y</sup>
			1st exp.	2nd exp.	3rd exp.	
Dark Opal	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.0 (0)	0.0 (0)	R
Di Toscano	Large-leaved	Geoponiko Spiti Seeds	1.0 (60)	1.9 (100)	1.9 (100)	VS
Dwarf Fine leaf	Small-leaved	Geoponiko Spiti Seeds	1.3 (92)	1.7 (100)	1.9 (100)	VS
Fine Verde Compact	Small-leaved	Geoponiko Spiti Seeds	1.8 (89)	2.0 (100)	1.9 (100)	VS
Fin Vert Nain	Small-leaved	Ets Bertrand Freres Seeds	2.0 (100)	1.8 (100)	2.0 (100)	VS
Genovese	Large-leaved	Spiros Spirou	0.5 (38)	0.7 (50)	0.4 (27)	MS
Greek	Small-leaved	Fiorel	nt <sup>x</sup>	1.8 (92)	2.0 (100)	VS
Green Fine	Small-leaved	Fitotechniki	2.0 (100)	2.0 (100)	1.8 (100)	VS
Lettuce's leaf	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.4 (24)	0.0 (0)	R
Limoncino	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.0 (0)	0.0 (0)	R
Lotto	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.3 (25)	0.2 (17)	R
Nichatos	Small-leaved	Geoponiko Spiti Seeds	2.0 (100)	1.8 (89)	2.0 (100)	VS
Red	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.0 (0)	0.1 (13)	R
Winter	Large-leaved	Geoponiko Spiti Seeds	0.0 (0)	0.0 (0)	0.0 (0)	R
Fisher's LSD ( $P=0.05$ )			0.33	0.29	0.18	

<sup>z</sup>Disease index was calculated as the mean disease severity and incidence on artificially inoculated plants. Disease severity (DS) was assessed on a visual scale of 0 to 2, where 0 = no symptoms, 1 = vascular discoloration in the stem, 2 = dead plants. Numbers in parentheses indicate disease incidence (DI), which was expressed as the percentage (%) of diseased plants having a score  $\geq 1$ .

<sup>y</sup>Disease reactions were classified into three categories: R = resistant,  $DS \leq 0.4$ ,  $DI \leq 25\%$ ; MS = moderately susceptible,  $0.5 \leq DS \leq 0.9$ ,  $26\% \leq DI \leq 50\%$ ; VS = very susceptible,  $DS \geq 1$ ,  $DI \geq 51\%$ .

<sup>x</sup>not tested.

**Vegetative compatibility tests** All six Greek isolates: FOB1, FOB3, FOB4, FOB5, FOB7 and FOB8 of the pathogen, as well as the reference isolates FOB Rossi G.E., FOB Sacco G.B. and FOB Gel of *Fob*, which belonged to VCG 0400, gave *nit* mutants which formed strong heterokaryons with the tester NitM mutants of the Greek isolate FOB6. Therefore, all seven Greek isolates of the pathogen belonged to VCG 0400 along with the reference *Fob* isolates.

**Foliage inoculation of basil plants** Disease incidence in foliage-inoculated young basil plants was 20–30% and infected plants died about 12 days after inoculation (Table 2). Isolation of the pathogen from infected plants was 100%. In adult plants disease incidence was 100% and all the inoculated aboveground parts of the plants died about one month

after inoculation (Table 2). At that time the non-inoculated aboveground parts had been defoliated partly, but they still remained alive; however, they died 10 days later. *Fob* the identity of which was confirmed by VCG tests, was isolated from the stem and crown of all five plants and the roots of three plants, but not from the soil (Table 2). The repetitions of both young and adult plant experiments gave similar results.

**Evaluation of commercial basil cultivars for possible disease resistance** There was considerable variability in *Fusarium* wilt resistance among the 14 commercial cultivars tested (Table 3). Most (6/8) large-leaved cultivars – Dark Opal, Lettuce’s leaf, Limoncino, Lotto, Red and Winter – were resistant, while all six small-leaved cvs. Dwarf Fine Leaf, Fine Verde, Fin Vert Nain Compact, Greek, Green Fine and Nichatos were very susceptible.

## DISCUSSION

The morphological and cultural characteristics of the wilt pathogen, its pathological specificity on basil and the VCG tests confirmed it as *Fusarium oxysporum* Schlechtend.:Fr. f.sp. *basilici* (Dzidzariya) Armst. (4). To the best of our knowledge, this is the first record of this fungus in Greece.

In the present study seven *Fob* isolates, obtained from diseased basil plants from various locations in Greece, were assigned to the single VCG 0400, described previously as encompassing all the tested isolates from Italy, Israel and the United States (9). This suggests that all these isolates belong to the same clonal lineage, presumably originating from a common progenitor. Although geographically separated, Greece has close agricultural contacts with most countries where *Fob* had previously been recorded (e.g. Israel and Italy) and that could allow frequent redistribution of genetically similar isolates. Therefore, the pathogen could have been introduced into Greece from any of these countries, possibly with contaminated basil seeds or organic substrates. The hypothesis that contaminated basil seeds or organic substrates could spread a clonal population of *Fob* internationally strongly supports the feasibility and reliability of a diagnostic tool based on the VCG method.

Foliage-inoculated basil plants with culture-derived conidia of *Fob* were infected through direct penetration by the pathogen, which then spread downward from the leaves and stem toward the crown and roots. This may be considered simulation of the situation where airborne propagules disseminate the pathogen from one aboveground part of the plant to another, as has been shown by other researchers (5,13). This may increase the chance for a disease outbreak and intensify the polycyclic nature of the pathogen. Soilborne pathogens that could behave in addition as foliar pathogens require different management for disease control. Therefore, soil disinfestation before planting may not be sufficient to manage *Fusarium* wilt of basil and an integrated approach (viz., sanitation, soil disinfestation, use of tolerant cultivars, reduction of relative humidity in greenhouses by heating or ventilation, spraying of the foliage with protectant fungicides) should be followed before, during and after planting (5,7,10,13).

The reactions of several basil cultivars to inoculation with *Fob* showed that all small-leaved cultivars were susceptible, whereas most large-leaved cultivars, like Lettuce’s leaf, Lotto and Winter, were resistant. This is in agreement with our observations on commercial crops in Greece, where all local large-leaved populations of basil are resistant. Although Genovese seems to be less susceptible than for example, all small-leaved cultivars, in practice the Genovese basil crops in Italy used for sauce (pesto) production and/or

fresh consumption are severely damaged by the pathogen (anonymous reviewer, personal communication). In a field evaluation in the United States, all basil cultivars examined, some of which were large-leaved, were susceptible to *Fusarium* wilt (15). This suggests that either other races of *Fob* exist in Greece and possibly in other countries, or our results are an artifact of the artificial inoculation method used. It may also be due to the fact that the local large-leaved populations of basil in Greece happen to be more or less resistant to the races of the pathogen present in the country. Therefore, the large-leaved basil cultivars found resistant in our experiments using young potted plants should be evaluated in the field to verify their probable resistance.

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