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The effect of Verticillium and Fusarium wilts on the growth of four melon (*Cucumis melo* L.) cultivars

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Summary. The susceptibility of the Greek melon cv. Kokkini banana, Thraki, Peplos and Amynteou to *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melonis* was tested. Seedlings of the four cv. were inoculated by root immersion in a *Verticillium* and a *Fusarium* inoculum suspension of 10^6 spores ml^{-1} for 1 h. Disease incidence was determinated after 35 days with a disease index, calculated as the product of the 'leaf symptom index' and the 'vascular discoloration index' of each plant. In addition, certain growth characteristics: plant height, main stem diameter, above-ground fresh and dry weight and root fresh and dry weight were measured, to have a basis for determining the effect of wilt upon plant growth. The adverse effect of the Verticillium and Fusarium wilts on the plants was estimated by the regression line slope coefficient (b) between the disease index and those growth characteristics. Both fungi had a significant negative effect on all the measured characteristics irrespective of the cultivar. Cultivars Kokkini banana and Peplos were the most susceptible, cv. Amynteou and Thraki the least susceptible to both fungi. The four melon cultivars exhibited a different susceptibility to *Verticillium* and to *Fusarium* indicating that selective breeding for resistance is a practical possibility.

Key words: *Fusarium oxysporum* f. sp. *melonis*, *Verticillium dahliae*.

Introduction

Melon (*Cucumis melo* L.) is cultivated on about 270 ha in greenhouses, mainly in southern Greece, on 1700 ha in low tunnels, mainly in Thessaly, and on some 8000 ha in the open, according to data of the Ministry of Agriculture. The traditional Greek cultivars that satisfy consumer demand, Kokkini banana, Thraki, Peplos and Amynteou, are mainly cultivated in the open in northern Greece. 'Kokkini banana' has a short post-harvest life and is cultivated as a summer melon for early summer production, whereas the three other cultivars are winter melons, cultivated for late production, and with a long post-harvest life (Dogras, 1996). Increased wilt incidence caused by *Verticillium* and *Fusarium* has led to a gradual decrease in the area under melon-cultivation during the last decade due to increased plant death and yield losses. *Verticillium dahliae* Kleb. and *Fusarium oxysporum* f. sp. *melonis* Snyd. & Hans.(*Fom*) are two of the most serious diseases in the Aegean region (Evcil and Yalcin, 1977) and the main threat to melons in recent years. *Fom* attacks melon at any growth stage, even before sprouting, but mainly when the fruit

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is ripe (Mas *et al*., 1981), and causes either a slow wilt accompanied by progressive yellowing (the most common), or a sudden wilt without prior yellowing or other symptoms. In slow wilt, the leaves assume a slight violet colour and longitudinal brown necrotic streaks appear on the stems, from which gum usually oozes. The fungus sporulates in the streaks and forms pinkish sporodochia in the final stage. In the case of sudden wilting, the tips of the stem are generally first attacked and shrivel, and the wilt then progresses gradually toward the base of the plant. With both these types of wilt a light-brown discoloration of the vessels that have been attacked is evident in longitudinal sections of the stem (Cappelli *et al*., 1995). The symptoms of Verticillium wilt are similar, but milder and less dangerous than Fusarium wilt (Agrios, 1997). The chemical control of both diseases is impracticable (Talboys, 1981; Ferrari, 1998) while crop rotation appears to be difficult or impossible because the chlamydospores of *Fom* and the microsclerotia of *V. dahliae* are able to survive for a long time (Garber, 1973; Armstrong and Armstrong, 1978). A good practical solution to this problem would be the selective breeding of resistant or tolerant lines from the cultivars mentioned.

This work was undertaken in order to study the resistance of the four Greek melon cultivars to both wilt fungi after artificial inoculation of the seedlings.

Materials and methods

The experiments were carried out in 1998 and 1999 at the Agricultural Research Centre of Macedonia and Thrace in a plastic covered greenhouse. The cultivars tested were the traditional Greek melon *Cucumis melo bot. var. reticulatus*, cv. Kokkini banana (summer melon) and *C. melo bot. var. inodurus*, cv. Thraki, Peplos and Amynteou (winter melon), the latter mainly cultivated in northern Greece. Seedlings of these cultivars were artificially inoculated with *V. dahliae* and *Fom*. Isolates of *V. dahliae* from tomato and eggplant and *Fom* from melon, grown on potato dextrose agar (PDA), were used throughout. Inoculum was prepared by growing each isolate for 8 days at 20±2°C in plastic Petri dishes 5.5 cm in diameter. About 5 ml of sterile distilled water was added per dish and the colonies were scraped with a sterilized needle. The content of each dish was filtered through cheesecloth and the filtrates of each fungus were combined (an equal number of dishes for each fungus). The inoculum suspension consisted of a mixture of macroconidia from *Fusarium*, and microconidia from both fungi were adjusted to 10^6 spores ml^{-1}. Seedlings were grown up to 15 days (cotyledon stage) and uprooted. The roots were carefully washed with sterile distilled water to take off all soil remains, pruned to about 2 cm, and immersed in the inoculum suspension for 1 h. Roots of control plants were also washed, pruned to about 2 cm and immersed in sterile distilled water for the same duration. After this treatment 1,200 seedlings were transplanted to perlite in plastic boxes (60×50×10 cm) with a perforated base. Four hundred seedlings (100 per cv.) were inoculated with *V. dahliae*, over 2 years, and an equal number with *Fom*. The same number of seedlings per cv. were used as controls.

A completely randomised design with 50 plants/treatment/cultivar/year was applied. The experimental unit was each plant. The seedlings were grown at min/max temperature of 16±2°C / 34±4°C, min/max relative humidity of 33±8/92±6 from transplanting until uprooting, and were watered with the nutrient solution of Sonneveld and Straver (1989) (in g 100 l^{-1}): $Ca(NO_3)_2$, 119; KNO_3, 78.28; K_2SO_4, 19.24; $MgSO_4$, 13.75; Fe-EDTA, 0.117; H_3BO_3, 0.191; HNO_3 (67%), 29.17; H_3PO_4 (85%), 24.18; pH 6.30.

Thirty-five days after inoculation and transplanting, the external leaf symptom index (LSI) was used to determine disease development on each plant (Staffeldt, 1955; Thanassoulopoulos, 1976; Bletsos *et al*., 1997b). The LSI was scored from 1 to 4 as follows: 1) plant apparently healthy without symptoms; 2) slight chlorosis of the lower leaves, slight height decrease to 3/4 of the controls; 3) serious wilt, lower leaves dead, plants partially killed, height to half that of the controls; 4) plants practically dead. The plants were then uprooted, the stems were cut longitudinally, and the vascular discoloration index (VDI) was determined on a scale from 1 to 4: 1) root apparently healthy and white; 2) vascular discoloration in root system only; 3) brown discoloration of the vascular bundles up to the first stem knot; 4) brown discoloration in the internodes up to the top. Finally, the disease index (DI) was calculated as the product of the two above indices (LSI × VDI) and it ranged from 1 to

16. According to the DI, plants were divided into six groups: 1, plants with a product value of 1; 2, plants with a value of 2; 3, plants with values of 3 to 4; 4, plants with values of 6 to 9; 5, plants with values of 10 to 12, and 6, plants with values of 14 to 16 (product values of 5, 7, 10, 11, 13, 14 and 15 could not exist) (Bletsos *et al*., 1999a; see Table 1 for more details). The DI was chosen because no clear relationship was found between the severity of external symptoms in surviving plants and the intensity of internal vascular discoloration. This was in agreement with Armstrong and Armstrong (1978) who stated that vascular discoloration was an unreliable measure for judging susceptibility to wilt in seedling tests.

Some growth characteristics: plant height (cm) from the cotyledons to the apex, main stem diameter (mm) at the height of the cotyledons, above-ground fresh and dry weight (g) of the plants and fresh and dry weight of the roots (g) were also determined. The effect of *V. dahliae* and *Fom* on each cultivar was then determined from the correlation coefficient (r) between the DI and these growth characteristics (Fasoulas, 1964). Infection of seedlings with *V. dahliae* or *Fom* was verified by isolations in PDA from the roots and main stem of all the seedlings. The results of the 2 years were analysed jointly after confirming the homogeneity of their variance using Bartlett's test. Comparison of the means for the recorded characteristics was based on Duncan's multiple range test at 5% level of significance.

Table 1. The disease index (DI) in melon: the leaf symptom index (LSI) × the vascular discoloration index (VDI).

LSI	VDI	LSI × VDI[1]	DI
1	1	1	1
2	2	2	2
3	3	3, 4	3
4	4	6, 8, 9	4
		12	5
		16	6

[1] The numbers 5, 7, 10, 11, 13, 14 and 15 are not possible as products of columns 1 and 2.

Results

In general, all cultivars were consistently susceptible to both wilt fungi and the growth characteristics were significantly impaired by them (Table 2), as indicated by the differences between regression slopes. The reduction caused by *Verticillium* in plant height, above-ground dry weight and root fresh weight was greater on cv. Thraki, Pep-

Table 2. Regression straight line coefficient between the disease index (DI) and some growth characteristics of four melon cultivars infected with *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melonis* in 1998 and 1999.

Cultivars	Regression straight line coefficient[a]					
	Plant height (cm)	Main stem diameter (mm)	Above-ground weight (g)		Root weight (g)	
			fresh	dry	fresh	dry
Verticillium dahliae						
Kokkini banana	-1.457 b	-0.408 b	-1.174 d	-0.091 b	-0.125 b	-0.028 a
Thraki	-3.885 ab	-0.706 a	-2.966 b	-0.316 a	-0.374 a	-0.095 a
Peplos	-5.035 a	-0.539 ab	-3.320 a	-0.335 a	-0.482 a	-0.077 a
Amynteou	-3.759 ab	-0.474 b	-2.056 c	-0.214 ab	-0.327 a	-0.050 a
F. oxysporum f. sp. *melonis*						
Kokkini banana	-2.143 c	-0.843 a	-1.706 c	-0.144 b	-0.256 b	-0.036 a
Thraki	-2.936 b	-0.350 b	-2.547 b	-0.257 ab	-0.269 b	-0.067 a
Peplos	-5.908 a	-0.811 a	-3.578 a	-0.339 a	-0.607 a	-0.092 a
Amynteou	-2.331 c	-0.266 b	-1.164 d	-0.109 b	-0.208 b	-0.032 a

[a] The plot regression statistical method of the MSTAT-C program was used.
[b] In each column the numbers with the same letter are not significantly different at $P \leq 0.05$ according to the t-test.

los and Amynteou (winter melon) than on cv. Kokkini banana (summer melon) (Table 2). All the growth characteristics of the four cultivars were more strongly affected by *Fusarium* than by *Verticillium* (Table 2). Plant height was the most strongly affected in all cultivars, while root fresh and dry weight was the least affected by both fungi. However, cv. Peplos was the most susceptible to *Fusarium* while the cv. Amynteou the most resistant (Table 2). The means of all growth characteristics of the cultivars were more strongly reduced by *Fom* than by *V. dahliae*. In particular, both fungi were more destructive in the cv. Kokkini banana and Peplos, while *Fom* was less destructive in cv. Amynteou (Table 2). The means of all the growth characteristics in all cultivars were significantly affected by both fungi, the most destructive being *Fom* (Table 3). All the characteristics of the cv. Kokkini banana and Peplos (except root fresh weight of 'Kokkini banana' and main stem diameter of 'Pep-

Table 3. Effect of *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melonis* on mean plant height, main stem diameter, above-ground fresh and dry weight and root fresh and dry weight of four melon cultivars in 1998 and 1999.

Cultivar/Growth characteristics	Treatments			CV %[a b]
	V. dahliae	*F. oxysporum*	Control	
'Kokkini banana'				
Plant height (cm)	7.08 b	3.52 c	13.03 a	48.99
Main stem diameter (mm)	5.09 b	3.3 c	6.49 a	31.19
Above ground fresh weight (g)	5.71 b	3.06 c	10.04 a	56.35
Above ground dry weight (g)	0.76 b	0.44 c	1.05 a	79.25
Root fresh weight (g)	1.58 a	0.81 b	1.81 a	51.17
Root dry weight (g)	0.26 b	0.17 c	0.35 a	32.51
'Amynteou'				
Plant height (cm)	16.5 b	14.90 b	22.35 a	39.82
Main stem diameter (mm)	5.34 ab	4.98 b	5.85 a	23.39
Above ground fresh weight (g)	8.51 b	8.72 b	11.59 a	47.3
Above ground dry weight (g)	1.07 b	1.05 b	1.31 a	46.76
Root fresh weight (g)	1.49 b	1.41 b	2.05 a	51.47
Root dry weight (g)	0.28 b	0.29 b	0.39 a	41.7
'Thraki'				
Plant height (cm)	15.23 b	14.5 b	21.87 a	36.75
Main stem diameter (mm)	5.41 a	4.48 b	5.53 a	29.06
Above ground fresh weight (g)	10.1 b	9.45 b	13.95 a	60.94
Above ground dry weight (g)	1.22 b	1.09 b	2.05 a	56.39
Root fresh weight (g)	1.74 ab	1.58 b	1.67 a	55.51
Root dry weight (g)	0.39 b	0.34 b	0.54 a	46.78
'Peplos'				
Plant height (cm)	18.88 b	9.07 c	29.19 a	34.28
Main stem diameter (mm)	5.25 a	4 b	5.95 a	35.10
Above ground fresh weight (g)	10.93 b	5.53 c	16.51 a	54.63
Above ground dry weight (g)	1.23 b	0.81 c	1.83 a	45.24
Root fresh weight (g)	2.07 b	0.92 c	3.09 a	62.92
Root dry weight (g)	0.42 b	0.26 c	0.57 a	42.85

[a] CV, Coefficient variation.
[b] In each column the numbers with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.
[c] See Table 2.

Table 4. Percentages of diseased plants in four melon cultivars infected with *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *melonis* in each disease index category (1-6) in 1998 and 1999.

Cultivar	Disease index						$\overline{X}$
	1	2	3	4	5	6	
V. dahliae							
Kokkini banana	13 a[a]	14 a	9 b	32 a	5 a	27 a	3.78 a
Thraki	23 a	23 a	10 b	17 b	6 a	21 ab	3.13 ab
Peplos	23 a	22 a	21 a	27 ab	4 a	3 c	2.76 b
Amynteou	21 a	12 a	15 ab	33 a	11 a	8 bc	3.25 ab
F. oxysporum f. sp. *melonis*							
Kokkini banana	9 a	11 a	4 b	17 b	6 b	53 a	4.59 a
Thraki	19 a	7 a	20 a	26 b	11 b	17 ab	3.54 ab
Peplos	11 a	7 a	9 b	28 ab	37 a	8 c	3.97 ab
Amynteou	16 a	14 a	18 ab	32 a	9 b	11 bc	3.37 b

[a] In each column the numbers with the same letter are not significantly different at $P \leq 0.05$ according to the t-test.

los') were significantly reduced by the two fungi, but *Fom* was the most destructive. The loss of growth characteristics of the other two cultivars fell in between (Table 3).

The four cultivars exhibited varying degrees of susceptibility to the two wilts. The most susceptible was 'Kokkini banana', with 32 and 59% "practically dead" plants (DI=5 and 6), from *Verticillium* and *Fusarium* respectively. The DI on this cv. (3.78 with *Verticillium*, 4.59 with *Fusarium*), was also greater than on the other cultivars. In contrast, the cv. most resistant to *Verticillium* was Peplos, with 7% "practically dead" plants, while cv. Amynteou showed most resistance to *Fusarium*, with 20% "practically dead" plants. In these more resistant cultivars, infected plants generally developed mild symptoms, as indicated by the DI of 2.76 for *Verticillium* and 3.37 for *Fusarium*, respectively (Table 4).

Discussion

The root dipping technique was used in the pathogenicity tests because it is quick and reliability is satisfactory (Yuko, 1974). The reduction in growth characteristics was significant and differed among cultivars. These findings were consistent with those reported by Bletsos *et al*. (1999b) for the same cultivars grown on peat moss and inoculated with *V. dahliae* and *Fom*. The greater reduction in plant height, main stem diameter and above-ground fresh and dry weight of winter melon compared with summer melon could be due to greater damage to the root system, as indicated by the lower root fresh weight of seedlings infected with *Verticillium* and *Fusarium*, as compared with the controls (Table 3).

The significant reduction in growth characteristics caused by *Verticillium* and *Fusarium* in all cultivars (compared with the controls) indicated that all cultivars were more or less susceptible to the fungi (Table 2, 3). Similar results were reported, also in melon, by Zink *et al*. (1983), and for *Verticillium* in Greek eggplant cultivars by Bletsos *et al*. (1997a).

Fusarium was more destructive than *Verticillium* in all cultivars (Table 3). Thus the percentage of "seriously diseased" plants (DI=5 and 6) ranged from 20 to 59% with *Fusarium* and from 7 to 32% with *Verticillium*, by contrast the percentage of "healthy" and "slightly diseased" plants (DI=1 and 2) after inoculation with *Fusarium* ranged from 20 to 30%, while in plants inoculated with *Verticillium* it ranged from 27 to 46% (Table 4).

The study found that the cultivars Kokkini banana, Thraki, Peplos and Amynteou vary in their susceptibility to both pathogens. This susceptibility may have been due to the high concentration of the inoculum (10^6 spores ml^{-1}) used (similar to that used by Zink *et al*., 1983), and to the longer time of

root immersion (1 h) in the inoculum (Zink *et al*., [1983] dipped the roots only for 1 min). This may have prevented detection of low levels of resistance. Lastly, the finding that the four cultivars exhibited varying degrees of susceptibility, and the high coefficient variation (CV%) values in all the measured characteristics (Table 3) should encourage breeders to select for melons less susceptible or even tolerant to *Verticillium* and *Fusarium* (Fasoula and Fasoula, 1997). This view is supported by the relatively high percentage of melons that remained healthy (DI=1) when infected with *V. dahliae* (13-21%) and with *Fom* (9-19%) (Table 4).

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