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Incidence of *Verticillium* Wilt of Melon in Tunisia

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ABSTRACT

In the last years, melon culture in the eastern part of central Tunisia has been severely affected by a vascular wilt disease. Symptoms consist of wilting, chlorosis, necrosis, stunting and vascular discoloration. *Verticillium tricorpus*, *V. nigrescens* and mainly *V. dahliae* were isolated from the roots and stem tissues of affected plants. *Verticillium* wilt has caused significant damage in the early produce and late autumn melon cultures. Pathogenicity was established by dipping roots of 30-day old seedlings of melon cv. 'Ananas d'Amérique' into a suspension (10^7 conidia/ml) of each *Verticillium* species for 30 min. Disease severity was assessed through the index of leaf damage, eight weeks after inoculation. In addition, plant height, above-ground fresh and dry weight and root fresh and dry weight were measured. Only *V. dahliae* had a negative effect on all the measured characteristics. Soil from infested fields was assayed for microsclerotia on modified Nadakavukaren and Horner medium. *V. dahliae* was widely distributed in the Chott Mariem region, with a density as high as 69 CFU/g of soil. Evaluation of melon cultivars in *V. dahliae*-infested fields indicated that all were susceptible. *Verticillium* wilt has become a threat to melon production in Tunisia.

Keywords: *Cucumis melo*, melon cultivars, natural inoculum density, *Verticillium* species, virulence

INTRODUCTION

Melon (*Cucumis melo* L.) is an important vegetable crop grown commercially in Tunisia. In fact, it is considered to be the second economically most important cucurbit after watermelon and is grown year-round on 10,000 ha with a yield of over 25 tons/ha (Anonymous 2004; Jebari *et al*. 2004).

Vascular wilts are among the most serious diseases of melon culture in Tunisia (El Mahjoub 1985; El Mahjoub and Ben Khedher 1987; Jebari *et al*. 2004). The most important vascular pathogens are the soil-borne fungi *Fusarium oxysporum* f. sp. *melonis* and *Verticillium dahliae* Kleb (El Mahjoub and le Picard 1985; El Mahjoub and Ben Khedher 1987; Ayed *et al*. 2007). This last soil-borne pathogen is responsible for some of the world's major diseases affecting vegetable, field, weed, tree and ornamental crops (Skotland 1971; Schnathorst 1981; Platt and Bollen 1995; Bhat and Subbarao 1999; Rowe and Powelson 2002). In fact, yield losses as high as 30% have been documented for potato crop in the USA (Rowe *et al.* 1987) and can reach as high as 50% in *Verticillium*-susceptible cultivars in Israel (Nachmias and Krikun 1985). For tomato and cotton, yield reductions can reach respectively 70% (Nachmias *et al*. 1987) and 30% (Bolek *et al.* 2005), in many parts of the world.

V. dahliae persist in infested soils as resting structures called microsclerotia which can survive in the soil and in dried or decomposed plant material for more than 20 years (Schnathorst 1981; Melissa *et al*. 1999). This soil-borne pathogen first attacks young plant roots, colonizes the xylem and phloem cells of the vascular tissue and then flows along with water and nutrient into the stems and leaves (Garber and Houston 1966). As a result of the reduced or inhibited water and nutrient transport in chlorotic and necrotic areas, lint yield and quality components can be substantially affected (Schnathorst and Mathre 1966).

Symptoms of *Verticillium* wilt, which are easily confused with those of *Fusarium* wilt especially at harvest period, consist of chlorosis and necrosis of the leaves, defoliation, stunting and wilting. However, and especially when first appeared, symptoms of *Verticillium* wilt differed from those of *Fusariu*m wilt by a characteristic V-shaped interveinal yellowing, necrosis and dropping of leaves. In fields where these symptoms were evident, yield losses were important because of reduced size and quality of the harvested melon fruits.

In a three-year survey, we carried out in the main areas of Tunisia where cucurbits are cultivated in the field or under cover, we found out that *Verticillium* wilt is present mostly in the temperate zones and is prevalent in the coastal regions having relatively low soil temperatures, ranging from 3.2 to 45°C, such as Monastir, Mahdia and Sousse, located in the eastern part of central Tunisia. In these zones, where melon is cultivated under greenhouses, *Verticillium* wilt is most severe during cool to warm weather that coincide with the early produce and late autumn melon cultures which begin from September to June.

Verticillium nigrescens, *V. tricorpus* and mainly *V. dahliae* were associated with these symptoms. Although *V. dahliae* is widely distributed in the agricultural soils of Tunisian fields; it has only been recently reported on melon (Jabnoun-Khiareddine *et al*. 2007). The objectives of this study were to identify the causal agent of the disease, to evaluate natural inoculum density in a Chott Mariem-infested field and to screen commercial melon cultivars for resistance.

MATERIALS AND METHODS

Identification of the pathogens

Melon plants showing symptoms of *Verticillium* wilt were collected over the last three years from major production areas in coastal Tunisia (Sousse, Monastir and Mahdia).

Isolations were made from roots, stems and petioles exhibiting

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vascular discoloration in order to ensure the emergence of all the pathogens that can be involved with the wilt disease. Plant parts were rinsed thoroughly in tap water and cut into 0.5 cm^2 pieces. After surface-disinfesting in sodium hypochlorite (10%) for 3 min, the plant pieces were rinsed three times in sterile distilled water and dried on sterile filter paper. Plant pieces were plated on PDA (Potato Dextrose Agar) medium with streptomycin sulphate (300 mg/l). Four pieces per plate were used for each plant tissue. Fungal cultures were incubated for two weeks at 20°C. Since problems can occur with general media such as PDA, which support growth of many microorganisms (Platt and Bollen 1995), the fungal isolates were cleaned up by subculturing successively on PDA plates, amended with streptomycin sulphate (300 mg/l), from the edge of actively growing colonies, until we have pure culture of the pathogen; single spores were then isolated. The isolates were identified as *V. dahliae*, *V. tricorpus* and *V. nigrescens* based on published descriptions (Hawksworth 1970a, 1970b; Hawksworth and Talboys 1970). In fact, *Verticillium* species can be differentiated morphologically by the types of resting structures they form in and on the surface of plant material, and on many artificial agar media, such as PDA (Gould *et al*. 2003).

Monoconidial subcultures of all *Verticillium* species were stored in 25% aqueous glycerol solution at –20°C (Robb 2002).

Pathogenicity tests

Pathogenicity of *Verticillium* species was determined by dipping roots of five 30-day old seedlings of melon cv. 'Ananas d'Amérique' in the conidial suspension (10^7 conidia ml^{-1}) of each *Verticillium* species, for 30 min (Bhat *et al*. 2003). This cultivar was chosen because it represented the most widespread cultivar in the Tunisian melon growing regions. Non-inoculated control seedlings were dipped in sterile distilled water. Seedlings were then transplanted into pots (one seedling per pot) filled with sterilized peat. Plants were arranged in a completely randomized design and incubated in glasshouse benches at 21°C-25°C. They were irrigated regularly, as needed and fertilized using a nutrient solution (N, 150 ppm; P, 50 ppm; K, 150 ppm; Ca, 150 ppm; Mg, 30 ppm; Fe, 3 ppm; Mn, 1.5 ppm; Zn, 0.2 ppm; B, 0.4 ppm; Cu, 0.1 ppm; Mo, 0.05 ppm and H2O, qsp 1l). The experiment was performed twice.

Eight weeks after inoculation, plants were monitored for the development of *Verticillium* wilt symptoms. In addition, plant height, above-ground fresh and dry weight, root fresh and dry weight and disease severity were recorded. A scale of 0 to 4 was used to assess disease severity in which: 0 = leaf of healthy aspect; 1 = epinasty or wilted leaf without chlorosis; 2 = one or several slightly chlorotic bands on the leaf; 3 = chlorotic bands over the entire surface of the leaf or chlorotic bands with a necrotic centre and 4 = complete necrosis or death of the leaf (Béye and Lafay 1985). An index of leaf damage (I.L.D.) was then calculated, 60 days post inoculation, for every plant according to the following formula:

I.L.D. = $\sum$ notes/ max

I.L.D.: Index of Leaf Damage.

$\sum$ notes: Total notes.

Max: 4 times the number of well developed leaves carried by the plant (Béye and Lafay 1985).

Re-isolations were made at the end of the experiments. Analyses of variance, associated to the test of means comparison of Newman-Keuls, were conducted to distinguish groups according to the values of the tested variables means ($P \leq 0.05$). All statistical analyses were performed using SPSS version 11 (Statistical Package for the Social Sciences).

Evaluation of natural inoculum density

The most severely affected melon field, located in Chott Mariem region, was chosen to assess its natural inoculum density. Soil core samples (2.5 cm diameter x 15 cm deep) were collected when the crop was near maturity during the months of April and June. 25 samples were taken from each greenhouse and were air dried at room temperature for approximately 3 weeks and ground briefly with a mortar and pestle. After drying, the total weight of each sample was approximately 250 g. For plating soil, a modified ethanol agar medium of Nadakavukaren and Horner (1959) was used. Water agar (20 g/l) was sterilised at 120°C for 20 min by autoclave, and cooled down at approximately 60°C. Just before pouring, 5 ml ethanol 96% and 50 mg chloro-oxytetracycline/l agar were added. From each soil sample a 20-g subsample was taken and 90 ml 0.1% water agar was added, to make a 100 ml suspension. The viscous 0.1% water agar was used to ensure a better distribution of the sample during mixing. From each soil suspension 0.5 ml was spread over each of five ethanol agar plates. The plates were incubated at 20°C in the dark. After three weeks, the soil was washed from the medium by gently rubbing off the agar surface. Colonies of *V. dahliae* were counted using a stereo microscope (Mol *et al*. 1996).

Cultivar evaluation

The influence of *Verticillium* wilt on some commercially available melon cultivars was examined in a greenhouse with naturally infested soil in the Chott Mariem region. Soil samples collected from the site prior to planting were assayed for *V. dahliae* by the method described above.

Thirty-day old seedlings of melon cultivars ('Calypso', 'Calypso HP1-06', 'Calypso HP3-06', 'Calypso HP6-06', 'HP4-06 Cantaloup', 'Galia gallicum', 'Galia HP2-06', 'Galia HP5-06') were transplanted in single row with black polyethylene mulch film under greenhouse conditions in February, 2006. Treatments were arranged in a randomized complete block design with three blocks. Each treatment consisted of ten seedlings of a single cultivar spaced 45 cm apart in rows 75 cm apart. Water was applied by drip irrigation positioned under the polyethylene mulch film. Preplant broadcast diammonium phosphate fertiliser (18-46-0) was applied at 200 kg/ha (36 kg N/ha, 41 kg P/ha). An additional 80 kg N/ha, 73 kg K/ha and 66 kg Ca/ha were applied as potassium nitrate (13-0-44) and calcium nitrate (15.5-0-0-19 Ca) through the drip irrigation system in equal portions at 12 weekly intervals starting 2 weeks after planting. Weeds between the polyethylene mulch films were eliminated manually. Plants were treated with appropriate registered insecticides as needed. At maturity, cultivars were evaluated for the percentage of diseased plants and disease severity. In addition, plant height, vascular discoloration, height and above-ground and root fresh weights were measured. In each replication, four plants were cut longitudinally and the number of plants showing vascular discoloration was recorded, and disease severity was rated on a scale of 0-2 in which 0 = no vascular discoloration, 1 ≤50% of vascular discoloration, and 2 = >50% of vascular ring showing discoloration.

Analyses of variance were performed on the data using SPSS, and mean comparisons were made using Fisher's protected LSD.

RESULTS

Symptoms

Although plants may be affected in any stage of development, the most common expression of *Verticillium* wilt symptoms, under protected cultivation, is observed after flowering set. In fact, external symptoms consisted of yellowing of older, lower leaves, chlorosis followed by typical V-shaped marginal and interveinal yellowing, necrosis and dropping of leaves which continued up the melon stem as plants matured (**Figs. 1, 2**).

As disease progressed, the oldest leaves desiccated. At maturity, infected plants were generally stunted and foliage exhibited wilting during warmer daytime temperatures (**Fig. 2**). Cross and longitudinal sections of roots and stems revealed dark discoloration of the vascular tissue (**Fig. 1**) which extended from the base of the stem upward (O'Brien 1983; Koike *et al*. 1994; Elmer 2000).

Isolation and identification of *Verticillium* species

During the surveys, epinasty, chlorosis and necrosis on the leaf, stunting and light to dark brown vascular discoloration in the roots, collar and stem of diseased plants were observed in the growing season. Typical cultures of *Verticillium* were isolated from representative diseased plants in

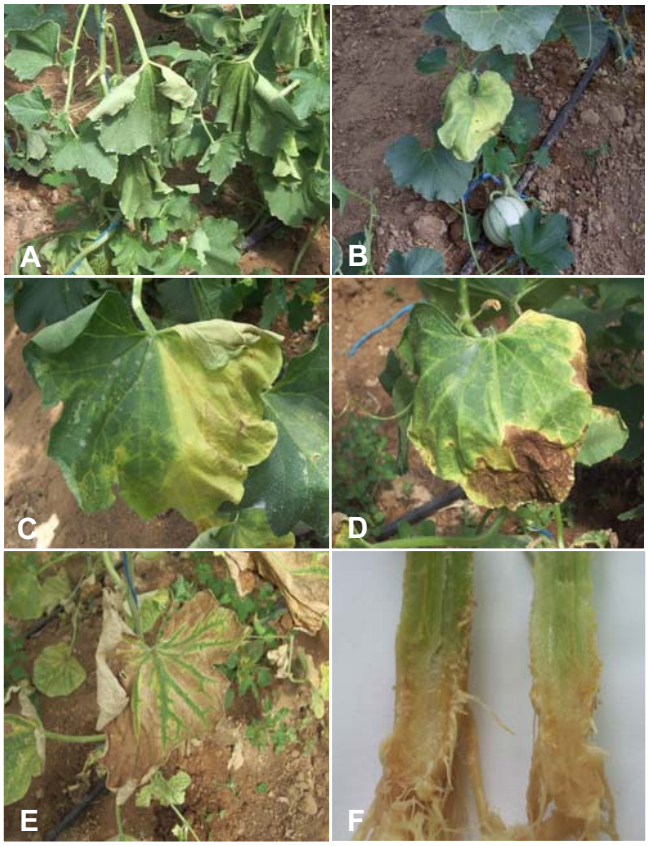

Fig. 1 *Verticillium* wilt symptoms on melon leaves: wilted leaves (**A**), chlorotic leaf (**B**), hemiplegic yellowing (**C**), V-shaped marginal necrosis (**D**), intervenal necrosis (E), discoloration of the vascular tissue (**F**).

the different surveyed regions.

Verticillium isolates were identified based on their morphological and cultural characteristics (**Fig. 3**). All *Verticillium* isolates produced characteristic verticilliate conidiophores. *Verticillium* species were differentiated morphologically by the types of resting structures they formed on PDA medium (**Fig. 3**). Three species of *Verticillium* were identified: *V. dahliae*, *V. tricorpus* and *V. nigrescens*. In fact, *V. dahliae* forms microsclerotia which are clusters of thick-walled heavily melanized cells which separate as discrete bodies from the parent mycelium; *V. tricorpus* forms microsclerotia, dark mycelium and chlamydospores, while *V. nigrescens* forms only chlamydospores. On PDA, the microsclerotia of *V. tricorpus* are large and irregularly shaped, whereas *V. dahliae* forms smaller and oval to elongate microsclerotia which are sharply differentiated from the hyaline mycelium and hyaline conidiophores. Moreover, *V. tricorpus* often causes a yellow discoloration of the PDA medium up to 1-2 weeks.

V. dahliae was consistently isolated from symptomatic melon roots and stems while the two other species were isolated from roots only.

Pathogenicity of *Verticillium dahliae*, *V. tricorpus* and *V. nigrescens* on melon

Pathogenicity of all *Verticillium* isolates (two isolates of *V. dahliae*, one isolate of *V. tricorpus* and one isolate of *V. nigrescens*) on melon cv. 'Ananas d'Amérique' was determined by the root dip method. The same inoculum concentration and root dipping period was used for the three *Verticillium* species. The criteria used to assess pathogenicity were: leaf damage, plant height, above-ground fresh and dry weight and root fresh and dry weight (**Table 1**). Each species was tested in two experiments with similar results.

Typical *Verticillium* wilt symptoms were observed on melon plants inoculated with both *V. dahliae* isolates. These symptoms consist of a general decline of plant growth, chlorosis of the leaves, stunting followed by wilting (**Fig. 4**).

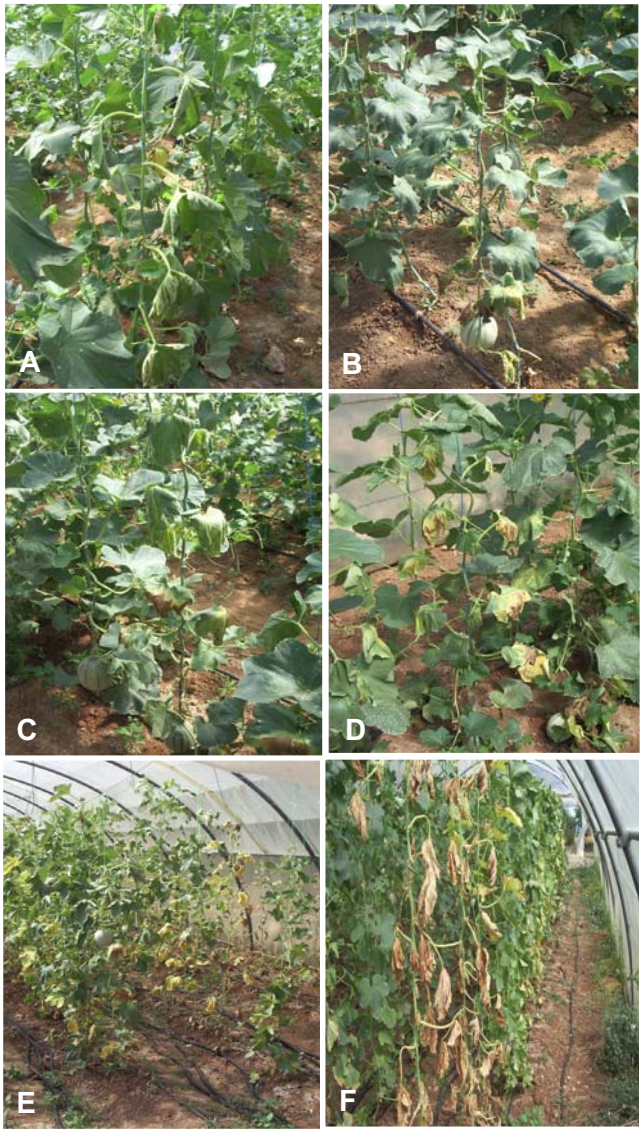

Fig. 2 Progression of *Verticillium* wilt symptoms on melon plants naturally infected by *V. dahliae*. Plant wilting (**A**), yellowing of older, lower leaves (**B**), leaf drop (**C**), marginal necrosis on the leaves (**D**), yellowing of the whole plant (**E**), withering of the whole plant (**F**).

These symptoms were first observed from 15 to 30 days post inoculation. However, the two other species of *Verticillium* did not exhibit any external symptom even after eight weeks. Only a vascular root discoloration of *V. nigrescens* and *V. tricorpus* isolates was observed compared to root and stem discoloration in plants inoculated with *V. dahliae*.

A significant difference ($P \leq 0.05$) between *Verticillium* isolates was noted. *V. dahliae* isolates were more aggressive (judging by the magnitude of disease severity, reduction in height, above-ground fresh and dry weight and root fresh and dry weight) than *V. nigrescens* and *V. tricorpus* isolates when inoculated to melon plants (**Table 1**), compared to the non-inoculated control plants which remained symptomless.

Unlike *V. tricorpus* and *V. nigrescens*, both isolates of *V. dahliae* significantly ($P \leq 0.05$) reduced plant height and fresh and dry weights of roots and shoots and significantly increase symptom severity compared to non-inoculated controls. Reduction in plant height and fresh weight of shoots and roots ranged from 71.73 to 75.48%, 81.99 to 88.22% and 50.63 to 68.35%, respectively; the reduction in dry weight of shoots and roots ranged from 83.33 to 86.11% and from 25 to 60%, respectively.

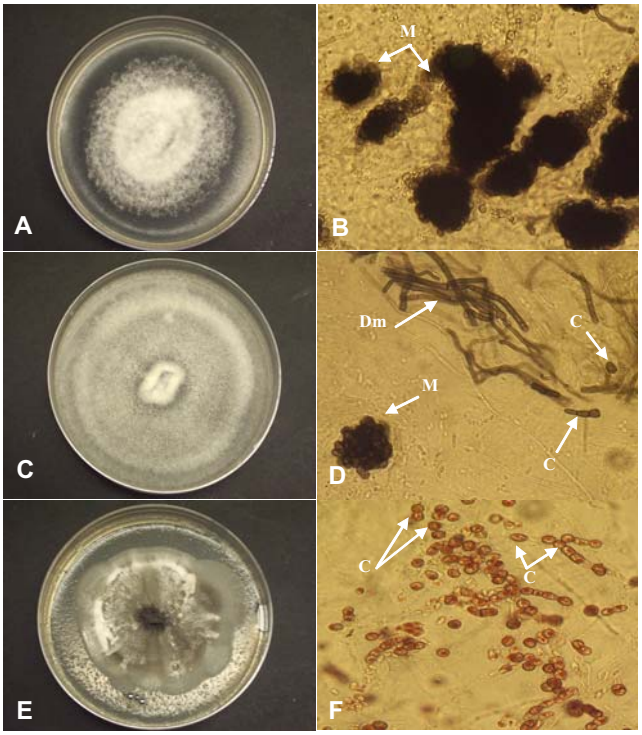

Fig. 3 Morphology of *Verticillium* species on PDA medium and their resting structures. Colony of *V. dahliae* (**A**) and its microsclerotia (M) (**B**); Colony of *V. tricorpus* (**C**) and its three resting structures: Dark mycelium (Dm), microsclerotia (M) and chlamydospores (C) (**D**); Colony of *V. nigrescens* (**E**) and its chlamydospores (C) (**F**).

Fig. 4 Comparison between a healthy melon plant (A), cv. 'Ananas d'Amérique', and an inoculated plant by *V. dahliae* isolate (B) 45 days after inoculation.

All *Verticillium* isolates were readily re-isolated from inoculated melon plants.

Evaluation of natural inoculum density

A severely affected field in Chott Mariem region was chosen to assess its inoculum density. We found out that in this field, 69 microsclerotia of *V. dahliae* per gram of soil were associated with 100% wilt of melon. Occasional colonies of *V. tricorpus* were observed in the water agar plates and were distinguished from *V. dahliae* by larger sized microsclerotia, spatial arrangement of microsclerotia and yellow pigment surrounding the colony (Koike *et al*. 1994).

Cultivar evaluation

All commercially available melon cultivars included in the experiments were susceptible to *V. dahliae*. *Verticillium* wilt affected all the measured characteristics significantly, independent of the melon cultivar (**Table 2**). The negative effect on cv. 'Galia HP2-06' was more intense for all characteristics than the effect on the other cultivars (**Table 2**). The vascular discoloration showed most clearly the susceptibility of melon cultivars to *V. dahliae*. In fact, this discoloration reached the top of the plant, for the eight tested melon cultivars. Furthermore, *V. dahliae* was re-isolated from the discoloured vascular tissues of these plants.

DISCUSSION

Verticillium wilt is a widespread disease in solanaceous crops (tomato, potato, eggplant) in Tunisia (Jabnoun-Khiareddine *et al*. 2005a, 2005b; Daami-Remadi *et al*. 2006; Jabnoun-Khiareddine *et al*. 2006). It has been only recently reported on melon (Jabnoun-Khiareddine *et al*. 2007) given that it was often confused with *Fusarium* wilt and no work has been established on its presence in Tunisia.

Verticillium wilt is presently widely distributed in the coastal regions and has become a significant threat to melon production.

Verticillium wilt symptoms on melon, under protected cultivation, are seldom seen prior to flowering set due to the physiological stress induced by flowering that elicits the onset of symptoms on previously infected plants (Koike *et al*. 1994). The symptoms are most severe on the early produce and late autumn melon cultures which matures during January through June. The summer crop which matures during June through September does not exhibit *Verticillium* wilt symptoms even when grown in fields known to be infested with *V. dahliae*. Thus, the physiological stress induced by both the onset of flowering and the moderate temperatures during autumn and spring may accentuate the severity of the disease. In fact, temperatures during autumn and spring in coastal Tunisia are in the range of 9° to 18°C.

In our inoculation experiments, one seedling per pot was used to ovoid the alteration of plant vigour and to assess the effect of the pathogen in the favourable plant conditions. In fact, the effect of plant density on disease is not well understood in populations of a single host plant genotype and has been studied even less in mixtures of host genotypes (Garrett and Mundt 2000). Symptom development and growth reductions caused by *V. dahliae* were the most severe compared with *V. tricorpus* and *V. nigrescens* that did not exhibit any *Verticillium* symptoms. These reductions may be due to a more serious effect on the root system, as shown by the difference in root fresh and dry weights of the infected plants compared to the control. In fact, pathogenicity factors have been identified as protein-lipopolysaccharide complexes and small peptides released by the fungus that are able to evoke host leaf chlorosis and necrosis and root inhibition (Nachmias *et al*. 1987, 1990). This root inhibition differed from an isolate to another and is clearly shown in the root dry weight reductions in our experiment. Melon plants infected by *V. dahliae* were chlorotic, severely stunted and died prematurely. In a recent study, Jabnoun-Khiareddine *et al*. (unpublished data) proved that isolates of *V. dahliae* from melon were able to severely attack resistant tomato plants.

Although *V. tricorpus* and *V. nigrescens* are considered soil saprotrophs, they proved mildly pathogenic to tomato, potato, eggplant, antirrhinum and other plants (Isaac 1967; Pegg 1974; Pegg and Brady 2002; Robb 2002; Barbara and Clewes 2003). In fact, in previous works, Jabnoun-Khiareddine *et al*. (2005a) showed that Tunisian *V. tricorpus* isolates obtained from tomato were able to attack tomato, eggplant and potato, causing wilt and stunting on the inoculated plants. In the present study, none of *V. tricorpus* and *V. nigrescens* isolates obtained from infected melon

Table 1 Mean effect of *V. dahliae*, *V. tricorpus* and *V. nigrescens* on disease severity (ILD), plant height, above-ground fresh and dry weight and root fresh and dry weight on melon plants.[1]

Verticillium isolates	ILD	Height (cm)	Root fresh weight (g)	Above-ground ground fresh weight (g)	Root dry weight (g)	Above-ground dry weight (g)
V. dahliae 1	2.091 b	8.82 a	0.25 a	1.04 a	0.016 a	0.10 a
V. dahliae 2	2.58 b	11.3 a	0.39 ab	1.59 a	0.03 ab	0.12 a
V. tricorpus	0 a	39.54 b	0.80 b	8.87 b	0.04 b	0.71 b
V. nigrescens	0 a	40.1 b	0.79 b	8.73 b	0.04 b	0.72 b
Control	0 a	39.98 b	0.79 b	8.83 b	0.04 b	0.72 b

[1] Disease severity (ILD), plant height, above-ground fresh and dry weight and root fresh and dry weight were calculated 60 days post-inoculation (cv. 'Ananas d'Amérique'; 21<T<25°C)

Numbers in the same column followed by the same letter (a, b, c) are not significantly different ($P \leq 0.05$)

Table 2 Mean effect of *V. dahliae* on disease incidence, severity, plant height, above-ground fresh and root weight and vascular discoloration on eight melon cultivars under greenhouse conditions.

Melon varieties	Height (cm)	Vascular dicoloration (cm)	Root fresh weight (g)	Above-ground fresh weight (g)	Incidence (%)	Severity
Calypso	239.58 b	239.58 b	219.58 c	1.84 a	96.25 abc	1 a
Calypso HP1-06	232.92 b	166.67 b	1.68 a	81.92 bc	100 a	2 a
Calypso HP3-06	220.58 b	143.58 b	3.50 b	102.83 abc	100 a	2 a
Calypso HP6-06	252.58 a	193.08 a	1.84 a	91.33 a	100 a	2 a
HP4-06 Cantaloup	243.75 b	191.67 b	2.21 a	146.83 a	100 a	2 a
Galia gallicum	200.42 b	172.08 b	1.94 a	124.83 ab	100 a	2 a
Galia HP2-06	160.00 b	103.75 bc	1.67 a	53.33 abc	100 a	2 a
Galia HP5-06	216.33 b	166.17 bc	1.67 a	63.08 c	100 a	2 a

Numbers in the same column followed by the same letter (a, b, c) are not significantly different ($P \leq 0.05$)

plants incited either leaf symptoms or stunting of inoculated melon seedlings. Colonisation of plants was mainly restricted to roots. Since pathogenicity tests did not exceed 60 days, however, the possibility that disease development may take a whole season has not been excluded (Korolev and Katan 1999).

At present, growers avoid the disease by growing alternate crops in problem fields during autumn and winter or by planting summer melon crops in these fields. All commercially available melon cultivars are susceptible to *Verticillium* wilt as reported by Bletsos and Thanassoulopoulos (2000) for the Greek melon cultivars.

An extensive breeding programme has been in existence (Pegg and Brady 2002) for melon cultivars resistant to the major pathogen *F. oxysporum* f. sp. *melonis* and for watermelon resistant to *F. oxysporum* f. sp. *niveum*. The limited work on *Verticillium* resistance has had for the most part to consider linked resistance to *Fusarium* in addition to other diseases. Many *Fusarium*-resistant cultivars are wholly susceptible to *V. dahliae*. Zink and Gubler (1986, 1987) developed cv. 'U.C. PMR-45', a three-parent compound cultivar with *Verticillium* resistance combined with single-gene resistance to race 1 of *Fusarium* and race 1 of *Sphaerotheca fuliginea*.

The genetics of *Verticillium* resistance, unlike those of *Fusarium*, have not been described. Isolates and strains of *V. dahliae* from many vegetables, cotton, soft fruit and ornamentals are capable of causing wilt in melon (Pegg and Brady 2002). In Tunisian coastal regions, tomato, pepper, eggplant and potato are grown extensively in addition to melon (Jabnoun-Khiareddine 2004). Furthermore, many of the cultural practices contributed to the rapid dissemination of the pathogen. In addition, the seed-borne nature of the fungus and its contribution to the rapid spread of the disease should not be discounted. Another factor may influence the rapid spread of *Verticillium* wilt of melon is the limited crop rotation practiced in the vegetable-growing areas of coastal Tunisia.

Vigouroux (1971) pointed out that isolates from a region of permanent monoculture are similar and all display high virulence against this particular crop (preferential host) but generally a weak virulence against other species which they can, nevertheless, infect (occasional host). This high virulence may be due to the permanent monoculture and the short rotation with susceptible hosts that may have increased selection pressure on strains of *Verticillium* that colonize and reproduce on those plants more effectively, resulting in an increase of inoculum levels causing significant yield losses (Vigouroux 1971; Tjamos 1981; Bhat *et al*. 2003). In our study, we have chosen the most severely affected melon field, located in Chott Mariem region, to assess its natural inoculum density. In this field, where a 100% wilt in melon plants was observed, the density of microsclerotia per gram of soil was 69. However, the soil inoculum levels of *V. dahliae* did not predict disease incidence. In fact, Grogan *et al*. (1996) reported essentially 100% incidence of disease in race-1 resistant tomatoes grown in soils containing as few as 5.7 microsclerotia/g. Similarly, Koike *et al*. (1994) pointed out that as few as 5 microsclerotia/g of soil were associated with wilt in nearly 100% of the cauliflower plants. Therefore the choice of a rotation crop should be made with extreme caution.

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