

Variation in Aggressiveness of Tunisian *Verticillium dahliae* Races 1 and 2 Isolates and Response of Differential Tomato Cultivars to Verticillium Wilt

Hayfa Jabnoun-Khiareddine¹ • Mejda Daami-Remadi^{2*} • Harold W. Platt³ •
Fakher Ayed⁴ • Mohamed El Mahjoub¹

¹ Institut Supérieur Agronomique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

² Centre Régional des Recherches en Horticulture et Agriculture Biologique, 4042, Chott-Mariem, Sousse, Tunisia

³ Agriculture Canada, Research Station, P.O. Box 1210, Charlottetown, P.E.I., C1A 7M8 Canada

⁴ Centre Technique de l'Agriculture Biologique de Chott-Mariem, 4042, Chott-Mariem, Sousse, Tunisia

Corresponding author: * daami_rm@yahoo.fr

ABSTRACT

In Tunisia, *Verticillium dahliae* race 1 and race 2 isolates have been obtained from many tomato-growing regions. To assess the aggressiveness of both races, 29 race 1 and 44 race 2 Tunisian isolates were used for root dip inoculation of tomato seedling belonging to differential cultivars i.e. 'Ventura' (ve) and 'Riogrande' (Ve). Variation in aggressiveness towards both tomato cultivars was apparent among isolates of both *V. dahliae* races, via the index of leaf damage and plant stunting. All race 1 and race 2 isolates caused moderate to severe symptoms on cv. 'Ventura' and on both cultivars, respectively. All race 1 and race 2 isolates were able to cause variable degrees of stunting on the inoculated tomato plants of both cultivars, compared to the non-inoculated controls. On average, race 2 isolates were more aggressive than race 1 isolates on cv. 'Ventura'. The response of four resistant and two susceptible tomato cultivars to selected race 1 and 2 isolates was studied under controlled conditions. These cultivars exhibited varying degrees of susceptibility to Verticillium wilt (VW) ranging from moderate to high as measured by leaf damage index and plant height. When grown in a naturally *Verticillium* infested soil (49 microsclerotia/g of soil), VW incidence was 100% for all the resistant cultivars, four months post-planting. The extent of vascular discoloration occasioned by VW reached and even exceeded the half of stem height for some cultivars.

Keywords: aggressiveness classes, discoloration extent, inoculation, leaf damage, *Lycopersicon esculentum* Mill., natural infection, stunting index

INTRODUCTION

Verticillium wilt (VW), caused by the soilborne fungus *Verticillium dahliae* (Kleb.), is a widespread disease in tomato growing regions in many parts of the world (Grogan *et al.* 1979; Bender and Shoemaker 1984; Dobinson *et al.* 1996; Bhat and Subbarao 1999; Harrington and Dobinson 2000). Disease symptoms include leaf flaccidity, chlorosis and necrosis, stunting, yield loss, leaf epinasty and can, in extreme situations, lead to death of the plant (Pegg and Brady 2002). However, infection and colonization need not necessarily result in symptom development (Robb 2007).

Few strategies are available for the control of *V. dahliae* and none is totally effective (Acciarri *et al.* 2007), due to the longevity of *V. dahliae* microsclerotia in the soil, its broad host range and the inefficacy of fungicides on pathogens once they reach the xylem (Fradin and Thomma 2006). Currently, the best way to prevent VW and to maintain agronomic sustainability within a region, is the use of resistant cultivars (Fradin and Thomma 2006; Hayes *et al.* 2007).

Disease resistance has the potential to reduce crop losses with a minimum effort by growers, in an environmentally safe and cost-effective manner. In addition, resistance can be combined with other control measures to achieve optimal levels of disease management (Okie and Gardner 1982; Corsini and Pavek 1996; Atibalentja *et al.* 1997). Genotypes and varieties with varying levels of VW resistance have been described in several plant species (Arbogast *et al.* 1999; Veronese *et al.* 2003; Vallad and Subbarao 2008), but specific genes required for its resistance have been characterized only in tomato. In fact, race-

specific (vertical) resistance to VW is best exemplified by the *Ve* locus in tomato, which is composed of two closely linked genes, *Ve1* and *Ve2*, and confers resistance to race 1 isolates of *V. dahliae* (Kawchuk *et al.* 2001). Tomato plants with the *Ve* genes are able to restrict the colonization of incompatible, race 1 isolates of *V. dahliae* to the roots, allowing only limited spread to stem and foliar tissues (Tjamos and Smith 1975; Gold and Robb 1996; Heinz *et al.* 1998). This *Ve* gene that provides resistance against VW (Schaible *et al.* 1951) has been used by plant breeders for 60 years and has been introduced in most cultivated tomatoes (Fradin and Thomma 2006). However, isolates named race 2 for their ability to overcome resistance, conferred by the *Ve* gene, have been identified later worldwide and predominate in many tomato-production regions (Alexander 1962; Okie and Gardner 1982; Dobinson *et al.* 1996; Pegg and Brady 2002). The source of these race 2 isolates has never been clearly established (Vallad *et al.* 2006) and their appearance was possibly due to the spread of a new recombinant or mutant isolates or to the selection of endemic race 2 isolates, because of the widespread use of the *Ve* resistance genes in tomato production (Grogan *et al.* 1979; Tjamos 1981; Pegg and Brady 2002). These race 2 isolates decreased yields by about 10 to 50% in many parts of the world (Grogan *et al.* 1979; Bender and Shoemaker 1984; Pegg and Brady 2002).

In Tunisia, the appearance of *V. dahliae* race 2 in tomatoes poses a major threat to this crop, particularly in protected cultivation (Daami-Remadi *et al.* 2006) where no rotations are used and the absence of soil disinfection favors the build-up of soil-borne inoculum. Economic losses caused

by this pathogen can reach 50% especially on tomato cultivars severely infected by *V. dahliae* (Jabnoun-Khiareddine *et al.* 2007a). Furthermore, once symptoms have been established, it is difficult to efficiently control the disease. Durable genetic resistance to race 2, therefore, is particularly desirable as control measure (Baergen *et al.* 1993). However, to improve race 2 resistance, diverse isolates must be used, as host plant resistance is related to the aggressiveness of the isolates used for testing that resistance (Baergen *et al.* 1993). Nevertheless, no information is available on the relative aggressiveness of local isolates of *V. dahliae* race 1 and 2 obtained from many tomato growing regions in Tunisia (Jabnoun-Khiareddine *et al.* 2007a), towards tomato. Moreover, detailed information on the relative susceptibility/resistance of the most grown tomato cultivars toward *V. dahliae* races is lacking. Thus, the major objectives of the present research were to evaluate *V. dahliae* race 1 and 2 isolates for aggressiveness on two differential tomato cultivars and to evaluate the response of different cultivars, the most grown in Tunisia, toward *V. dahliae* races under controlled conditions and in a *Verticillium*-infested field

MATERIALS AND METHODS

Fungal material

A total of 77 *V. dahliae* isolates were used in this study. These isolates included 29 race 1 and 44 race 2 Tunisian isolates, previously characterized in Jabnoun-Khiareddine *et al.* (2007a), and four Canadian reference strains, kindly provided by Dr. K. Dobinson and Dr. J. Robb (Canada), including one race 1 isolate and three race 2 isolates (Dvd 2, ref 1 and ref 2).

Pathogen isolates were cultured at 20°C on potato dextrose agar (PDA) medium amended with 300 mg/l of streptomycin sulphate (Pharmadrag Production GmbH, Hamburg, Germany). Liquid cultures used for substrate inoculation were prepared on potato dextrose broth (PDB) and incubated at 20°C under continuous agitation at 150 rpm during 4 to 5 days. The spore suspension concentration used was adjusted to 10⁷ spores/ml by a Malassez cytometer. For their long term preservation, pathogen isolates were stored up to 12 months at -20°C in a 25% glycerol solution (Robb 2000).

Plant material

Two tomato cultivars, possessing or not the *Ve* gene of resistance to *V. dahliae* race 1, 'Riogrande' (*Ve*) and 'Ventura' (*ve*), respectively, were used to compare the aggressiveness of local *V. dahliae* isolates, previously characterized physiologically.

Two susceptible (cvs. '3479' and 'Sun 6200') and four resistant tomato cultivars (cvs. 'Amel', 'Bochra', 'Naya' and 'Daniella') were used to study the aggressiveness of selected *V. dahliae* race 2 and race 1 isolates in a growth chamber, under controlled conditions.

Five resistant (*Ve*) tomato cultivars (cvs. 'Chourouk', 'Cencara', 'Nazih', 'Colibri' and 'Amel') were tested under natural infection and greenhouse conditions in order to study their behavior against the predominant and the most aggressive *Verticillium* species in Tunisia, *V. dahliae* (Jabnoun-Khiareddine *et al.* 2006).

Tomato seeds were superficially disinfected by immersion in absolute ethanol for 2 min, followed by extensive rinsing in sterile distilled water. Seeds were sown in alveolus plates filled with previously sterilized peat. Seedlings were grown in a growth chamber at 24-26°C with 12-h photoperiod. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002). Experiments were performed with 3- to 4-week-old tomato plants.

Assessment of the aggressiveness of *V. dahliae* races on two differential cultivars

In this assessment, 75 *V. dahliae* isolates (29 race 1 and 44 race 2 Tunisian isolates, and two reference strains race 1 and 2) were used for inoculation of tomato plants belonging to both differential

cultivars i.e. 'Ventura' (*ve*) and 'Riogrande' (*Ve*).

Tomato seedlings (two true-leaf stage) were inoculated with a spore suspension (10⁷ spores/ml) using the root-dip method. For each cultivar, seedlings dipped in sterile distilled water only served as non-inoculated controls. All the seedlings were transplanted, immediately after inoculation, to pots (diameter 10 cm) filled with a mixture of peat and perlite (2/3: 1/3), previously sterilized at 107°C for 1 h and maintained under growth chamber conditions at 23 ± 3°C, 12-h photoperiod for eight weeks. They were watered regularly and fertilized with a nutritious solution when needed (Pharand *et al.* 2002).

The evaluation of isolates aggressiveness was assessed 60 days post inoculation (DPI) based on the records of leaf damage index (LDI) which is calculated according to 0-4 scale (Jabnoun-Khiareddine *et al.* 2007a), and plant height reduction which is estimated based on a stunting index (SI) calculated as follows:

$$SI = 1 - [\text{height of inoculated plants} / \text{height of control plants}] \times 100$$

In order to present the results, the values of the LDI and SI were grouped into classes based on 0-4 and 0-100 scales, respectively. In fact, for the leaf damage index, these values which indicate the different levels of isolate aggressiveness are grouped in classes as follows: 0.0-0.9 (weakly aggressive isolates); 1.0-1.9 (moderately aggressive isolates); 2.0-2.9 (aggressive isolates); 3.0-4.0 (highly aggressive isolates).

For the stunting index, the different classes are as follows: 0-20 (very weakly aggressive isolates), 21-40 (slightly aggressive isolates), 41-60 (moderately aggressive isolates), 61-80 (aggressive isolates) and 81-100 (highly aggressive isolates).

Assessment of cultivar behavior against *V. dahliae* races under controlled conditions

In this assessment, one local *V. dahliae* race 1 isolate (Vd45), eight local race 2 isolates (Vd2, Vd6, Vd13, Vd25, Vd32, Vd38, Vd42, and Vd78), showing important aggressiveness on the differential cultivars, as noted in the previous experiment, and three race 2 (Ref 1, Ref 2 and Dvd-2) reference strains were used for plant inoculation.

Seedlings at the two true-leaf stage, belonging to the different cultivars tested, were inoculated and grown as described above.

The evaluation of VW severity was assessed 60 DPI based on the records of plant height and the leaf damage index (LDI).

Statistical analyses were performed following a completely randomized factorial design where tomato cultivars and fungal treatments (pathogen isolates and non-inoculated control) were the fixed factors. Five replicates (plants) were used per elementary treatment and means were separated using Fisher's protected LSD test (at $P \leq 0.05$).

Assessment of cultivar behavior against *V. dahliae* under greenhouse conditions

Five tomato cultivars, the most grown in Tunisia and especially in coastal regions, were used in this experiment. The assessment of their behavior was studied in a field known with its severe VW history on the resistant tomato cv. 'Colibri' for three consecutive years. The infectious potential of this naturally infested field, located in the Higher Agronomic Institute of Chott-Mariem estate, was estimated at 49 microsclerotia/g of soil, according to the method of Nadakavukaren and Horner (1959) previously described in Jabnoun-Khiareddine *et al.* (2007b).

Seedlings, belonging to each of the tomato cultivars, were transplanted directly in the infested soil under a plastic greenhouse following six lines 80 cm apart with an inter-plant spacing of 40 cm. Plants were arranged following a randomized complete block design with 4 blocks and 5 replicates (plants) per block and per cultivar tested. Cultivars were randomly arranged into blocks. Plants were irrigated via a drip irrigation system. Fertilization and other cultural practices were the most commonly used for tomato farming in the region.

VW evaluation was done at maturity, about four months after plantation. Plants of each cultivar were examined visually for VW symptoms. In fact, the number of infected plants was noted and

the percentage of the disease incidence was calculated for each cultivar tested. All the plants were then removed from the soil and brought to the laboratory where plant height was noted and the extent of vascular discoloration was visually measured, via longitudinal stem sections. Pathogen isolations were done at different stem levels (base, middle and top) to verify the extent of fungal growth on each stem.

Statistical analyses were performed, for all parameters measured, following a randomized complete block design with 4 blocks and 5 replicates per block and per cultivar tested. Means were separated using Fisher's protected LSD test (at $P \leq 0.05$).

RESULTS

Aggressiveness of *V. dahliae* race 1 and race 2 on two differential tomato cultivars

Both *V. dahliae* races inventoried in Tunisia were tested to determine their aggressiveness on two differential cultivars 'Ventura' (ve) and 'Riogrande' (Ve), based on wilt severity (LDI) and reduction in plant height (SI).

1. Effects of *V. dahliae* race 1 and race 2 on wilt severity

All *V. dahliae* race 1 isolates tested caused moderate to severe symptoms on the susceptible tomato cultivar 'Ventura' and caused no or very mild VW symptoms when inoculated to the resistant cultivar 'Riogrande' (Table 1). In contrast, all race 2 isolates caused moderate to severe symptoms on both cultivars tested. In fact, 44.8% of race 1 isolates were moderately aggressive (1.0-1.9), 34.5% were aggressive (2.0-2.9), whereas 20.7% of the race 1 isolates were highly aggressive (3.0-4.0) when inoculated to the susceptible cultivar (Table 1) under our conditions of plant inoculation and growth. The average LDI noted on the susceptible tomato cultivar inoculated with race 1 isolates was 2.27.

Race 2 isolates showed a wide range of aggressiveness on both differential cultivars ('Ventura' and 'Riogrande'). On 'Riogrande', 40% of race 2 isolates were moderately aggressive, 35.55% were aggressive, while 24.44% of race 2 isolates were highly aggressive.

On 'Ventura', 37.77% of race 2 isolates were moderately aggressive, 37.77% were aggressive while 24.44% of race 2 isolates were highly aggressive.

On both 'Riogrande' and 'Ventura', 44.44% of race 2 isolates were aggressive to highly aggressive (Table 1). The average LDI noted the susceptible and resistant tomato cultivars inoculated with race 2 isolates were 2.35 and 2.30, respectively.

2. Effects of *V. dahliae* races 1 and 2 on plant height

All the tested *V. dahliae* race 1 isolates were able to cause stunting of the inoculated tomato plants of both differential cultivars when compared to the non-inoculated controls and according to our plant inoculation and growth conditions (Table 2). However, this reduction varied depending on isolates tested. On 'Ventura', 30% of race 1 isolates were slightly aggressive (21-40), 46.66% were moderately aggressive isolates (41-60) whereas 20% were aggressive isolates (61-80).

On 'Riogrande', 43.33% of race 1 isolates were slightly aggressive, 46.66% were moderately aggressive isolates while 6.66% of race 1 isolates were aggressive (Table 2).

43.33% of race 1 isolates were slightly to moderately aggressive on both cultivars tested.

The average SI caused by race 1 isolates on 'Ventura' and 'Riogrande' were 47.28 and 40.68%, respectively.

All *V. dahliae* race 2 isolates caused stunting of both susceptible and resistant tomato cultivars with variable degree, as shown by the distribution of the isolates in the different aggressiveness classes (Table 3). On 'Ventura',

Table 1 Distribution of *V. dahliae* race 1 and race 2 isolates issued from tomato plants into different classes of aggressiveness.^a

Aggressiveness on 'Riogrande'	Aggressiveness on 'Ventura'				Total
	0.0-0.9	1.0-1.9	2.0-2.9	3.0-4.0	
0.0-0.9	0 ^b	13	11	6	30 ^c
1.0-1.9	0	10	6	2	18
2.0-2.9	0	5	8	3	16
3.0-4.0	0	2	3	6	11
Total	0	30	28	17	75
Average aggressiveness on Riogrande (race 2)					2.30
Average aggressiveness on Ventura (race 2)					2.35
Average aggressiveness on Ventura (race 1)					2.27

^a Aggressiveness estimated according to a 0-4 scale, where 0 = no symptoms on both differential cultivars, and 4 = plant death.

^b Number of isolates within each aggressiveness class.

^c Number of *V. dahliae* race 1 isolates.

Table 2 Distribution of *V. dahliae* race 1 isolates issued from tomato plants into different stunting classes.^a

Aggressiveness on 'Riogrande'	Aggressiveness on 'Ventura'					Total
	0-20	21-40	41-60	61-80	81-100	
0-20	0 ^b	1	0	0	0	1
21-40	0	6	7	0	0	13
41-60	0	2	7	4	1	14
61-80	0	0	0	2	0	2
81-100	0	0	0	0	0	0
Total	0	9	14	6	1	30
Average aggressiveness on Riogrande						40.68%
Average aggressiveness on Ventura						47.28%

^a Stunting was estimated according to a 0-100 scale, where 0 = no stunting on both differential cultivars, and 100 = plant death.

^b Number of isolates within each aggressiveness group.

Table 3 Distribution of *V. dahliae* race 2 isolates issued from tomato plants into different stunting classes.^a

Aggressiveness on 'Riogrande'	Aggressiveness on 'Ventura'					Total
	0-20	21-40	41-60	61-80	81-100	
0-20	0 ^b	0	0	0	0	0
21-40	0	17	7	0	0	24
41-60	0	5	2	1	1	9
61-80	0	0	0	1	7	8
81-100	0	0	0	2	2	4
Total	0	22	9	4	10	45
Average aggressiveness on Riogrande						48.41%
Average aggressiveness on Ventura						53.51%

^a Stunting was estimated according to a 0-100 scale, where 0 = no stunting on both differential cultivars, and 100 = plant death.

^b Number of isolates within each aggressiveness group.

48.88% of race 2 isolates were slightly aggressive, 20% were moderately aggressive whereas 8.88% were aggressive and 22.22% were highly aggressive isolates.

On 'Riogrande', 53.33% of race 2 isolates were slightly aggressive, 20% were moderately aggressive while 17.77% were aggressive and 8.88% were highly aggressive (Table 3).

26.66% of race 2 isolates were aggressive to highly aggressive on both cultivars. The average SI caused by race 2 isolates on 'Ventura' and 'Riogrande' were 53.51 and 48.41%, respectively.

Behavior of tomato cultivars against *V. dahliae* races under controlled conditions

Six tomato cultivars (4 resistant and 2 susceptible to *V. dahliae* race 1) were assessed for their behavior against some local *V. dahliae* isolates, previously characterized physiologically, compared to three Canadian reference strains.

1. Effects of *V. dahliae* races 1 and 2 on wilt severity recorded on the different cultivars

All tomato plants inoculated with *V. dahliae* race 1 and race 2 showed, 15 to 20 DPI, typical VW symptoms. However,

Table 4 Leaf damage index (LDI) recorded, 60 days post-inoculation, on tomato plants belonging to the five cultivars tested and inoculated with different *V. dahliae* race 1 and 2 isolates.

Treatments	Isolates	Tomato cultivars tested						Means ^{a*}
		'Bochra'	'Daniella'	'Amel'	'Naya'	'3479'	'Sun 6200'	
<i>V. dahliae</i> race 2 (Tunisia)	Vd32	2.00	0.73	1.20	1.07	1.95	2.15	1.52 ab
	Vd25	1.78	1.33	1.00	2.98	0.83	2.75	1.78 ab
	Vd78	1.95	1.71	0.90	1.55	1.65	2.45	1.70 ab
	Vd13	1.47	0.60	1.30	3.37	2.90	2.02	1.94 a
	Vd6	1.11	1.07	1.35	2.55	1.70	3.00	1.80 ab
	Vd38	1.45	1.27	1.80	1.32	1.55	1.47	1.48 ab
	Vd42	1.83	0.55	1.00	1.98	1.65	1.35	1.39 ab
	Vd2	2.75	1.85	1.20	1.33	2.03	2.28	1.91 a
<i>V. dahliae</i> race 2 (reference)	Ref 2	1.20	1.25	1.25	1.12	1.25	1.66	1.29 b
	Ref 1	1.33	1.75	1.50	1.77	1.90	2.40	1.77 ab
	Dvd-2	0.79	0.75	1.50	1.08	1.51	2.20	1.30 b
<i>V. dahliae</i> race 1 (Tunisia)	Vd45	0.66	0.00	0.17	0.44	1.58	1.70	0.76 c
Uninoculated control	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00 d
Means ^{b*}	-	1.41 b	0.99 c	1.09 c	1.58 b	1.58 b	1.96 a	

LSD (Cultivars x Isolates) = 0.91 at $P \leq 0.05$.^a Mean LDI per treatment (isolate or non-inoculated control) independently of tomato cultivar tested.^b Mean LDI per tomato cultivar tested independently of treatments (isolates or non-inoculated control).* For treatments (isolates and non-inoculated control) and tomato cultivars tested, values (means) affected with the same letter are not significantly different at $P \leq 0.05$.

at the end of the assay i.e. 60 DPI, all tomato plants showed leaf symptoms of variable intensity depending on the tested plant material and the fungal treatments realized (**Table 4**); a significant ($P \leq 0.05$) interaction was noted between both fixed factors. All inoculated tomato plants showed significantly greater LDI than the non-inoculated control plants which were symptomless.

The cultivars 'Sun 6200' (*ve*) and 'Naya' (*Ve*) and at a lesser degree '3479', and 'Bochra' showed, for all physiological races pooled and according to the experiment conditions, significantly greater LDI than 'Daniella' and 'Amel'. In fact, the least LDI was recorded on 'Daniella' plants on which four *V. dahliae* race 2 isolates showed lesser aggressiveness (LDI ranging between 0.55 and 0.75) and behaved as race 1 isolates. Moreover, *V. dahliae* aggressiveness, based on LDI, was shown to be variable depending on isolates used for inoculation. In fact, isolates varied from weakly to highly aggressive when inoculated to the tested cultivars. The Tunisian race 2 isolate, Vd13, was shown to be the most aggressive for which mean LDI (for all cultivars combined) was 1.94 and the most important LDI of about 3.38 was noted on 'Naya'. Moreover, all race 2 isolates occasioned significantly more severe leaf damage than race 1 isolate on all cultivars used and according to our experiment conditions. Symptoms do not spread beyond the lower leaves in the resistant cultivar plants when inoculated with Vd45 isolate.

Furthermore, the majority of local *V. dahliae* race 2 isolates, except Vd13 and Vd2 isolates, were shown to be significantly as aggressive as the reference strains used in this study.

Verticillium isolates were all successfully re-isolated from the basal plant stems of all the cultivars tested.

2. Effects of *V. dahliae* race 1 and race 2 on plant height recorded on the different cultivars

Plant height noted at the end of the assay i.e. 60 DPI, varied depending on the tested plant material and the fungal treatments realized (**Table 5**); a significant ($P \leq 0.05$) interaction was noted between both fixed factors. All inoculated tomato plants inoculated with *V. dahliae* race 1 and 2 isolates, for all cultivars combined, showed significantly lesser height than the non-inoculated control plants, according to our plant inoculation and growth conditions.

An important reduction of plant height was also caused by the majority of *V. dahliae* race 2 isolates. Moreover, based on adverse effect on plant height compared to the non-inoculated control, the local race 2 isolate, Vd32, was shown to be the most aggressive isolate; plant height, of all tomato cultivars combined, was reduced by about 66%.

An important intra-specific variation was noted within *V. dahliae* as for height reduction on inoculated plants. In fact, contrary to the majority of race 2 isolates which occasioned

Table 5 Plant height (cm) recorded, 60 days post-inoculation, on tomato plants belonging to the five cultivars tested and inoculated with different *V. dahliae* race 1 and 2 isolates.

Treatments	Isolates	Tomato cultivars tested						Means ^{a*}
		'Bochra'	'Daniella'	'Amel'	'Naya'	'3479'	'Sun 6200'	
<i>V. dahliae</i> race 2 (Tunisia)	Vd32	10.0	15.0	6.0	11.8	13.6	7.0	10.56 e
	Vd25	14.8	10.6	7.0	13.0	15.0	8.8	12.86 bcde
	Vd78	15.4	12.6	16.0	11.9	22.2	12.4	15.08 bcd
	Vd13	15.7	6.6	8.5	7.0	19.6	11.9	11.55 de
	Vd6	15.0	8.5	14.2	8.8	12.6	11.0	11.91 de
	Vd38	16.0	10.6	8.6	13.8	19.6	16.5	13.96 bcde
	Vd42	15.2	10.2	10.1	10.7	17.8	11.0	12.50 cde
	Vd2	9.3	12.8	8.6	23.6	20.0	11.5	14.30 bcde
<i>V. dahliae</i> race 2 (reference)	Ref 2	9.6	10.0	7.0	15.4	18.4	13.7	11.96 de
	Ref 1	15.6	9.8	9.0	16.2	16.9	10.9	12.96 bcde
	Dvd-2	14.0	9.8	10.8	23.2	29.6	12.0	16.56 b
<i>V. dahliae</i> race 1 (Tunisia)	Vd45	18.0	9.0	9.2	16.1	30.6	13.0	16.05 bc
Uninoculated control	-	26.0	38.0	31.0	31.0	39.0	22.4	28.4 a
Means ^{b*}	-	15.09 b	12.00 c	11.06 c	15.04 b	21.05 a	12.83 c	

LSD (Cultivars x Isolates) = 6.25 cm at $P \leq 0.05$.^a Mean plant height per treatment (isolate or non-inoculated control) independently of tomato cultivar tested.^b Mean plant height per tomato cultivar tested independently of treatments (isolates or non-inoculated control).* For treatments (isolates and non-inoculated control) and tomato cultivars tested, values (means) affected with the same letter are not significantly different at $P \leq 0.05$.

greater height reduction (54-66%), the Tunisian isolate Vd78 and the Canadian isolate, Dvd-2, had reduced this growth parameter by 47-52% which was significantly comparable to the local race 1 isolate (Vd45) effect.

For all fungal treatments, ‘Amel’, ‘Daniella’ and ‘Sun 6200’ showed the lowest plant height compared to the other cultivars tested. However, ‘3479’ showing the higher mean plant height, for all fungal treatments, was severely affected by pathogen inoculation as height reduction by race 1 and race 2 isolates ranged between 22 and 68%, respectively compared to the non-inoculated control.

A different behavior of susceptible and resistant cultivars was noted after their inoculation with *V. dahliae* races. In fact, both susceptible cultivars tested showed significant differences as for reductions of plant height, depending on physiological races used for inoculation, compared to the non-inoculated control. In fact, for ‘Sun 6200’ and ‘3479’, the registered height reductions were 32-69% and 24-68% with *V. dahliae* race 2 isolates compared to 42 and 22% with race 1 isolate, respectively. Resistant tomato cultivars tested showed variable interaction with pathogen races used for inoculation. In fact, for ‘Bochra’, ‘Daniella’, ‘Amel’ and ‘Naya’, plant height was reduced by 39-66, 66-82, 54-81 and 24-77% with *V. dahliae* race 2 isolates compared to 31, 75, 70 and 48% with *V. dahliae* race 1 isolate (Vd45), respectively.

Moreover, for ‘Sun 6200’, the adverse effect on plant height (stunting effect) was related to the severity of leaf damage (LDI). In fact, for all fungal treatments pooled, this cultivar was most affected by pathogen inoculation as shown by the higher LDI (Table 4) and the greater stunting effect (Table 5). However, for the other cultivars tested, the plant response to inoculation was variable and no correlation was observed between these both parameters.

Behavior of tomato cultivars against *V. dahliae* under greenhouse conditions

The behavior of five tomato cultivars, the most grown in coastal regions in Tunisia, was assessed under natural conditions in a field naturally infested with *V. dahliae* race 1 and race 2.

1. Effects of natural infection with *V. dahliae* on tomato VW incidence

When grown in naturally infested soil, which inoculum density was estimated at 49 microsclerotia/g of soil, the five tomato cultivars tested all exhibited typical VW symptoms (Fig. 1). In fact, the first apparent symptoms generally do not occur until the beginning of fruit set and begin either with wilting and dropping, during the hottest part of the day, or with yellow spots which develop along the margins or interveinally (Fig. 1A). Leaflets may also show a characteristic V-shaped yellowing (Fig. 1A). These yellowed areas will eventually turn necrotic and wither. These symptoms, which have affected one or generally both sides of the plants, progress acropetally (Fig. 1B) and, lead at the end of the growing season, to plant death (Fig. 1C). Plants affected at an early stage, (when young) are stunted (Fig. 1B). In fact, four months post-planting, the withering of almost the whole plants occurred for all the tested resistant tomato cultivars and disease incidence was then 100% (Fig. 1C). At this stage and together with the external symptoms, an internal vascular discoloration (Fig. 1D) was clearly noted for all the cultivars.

2. Effects of natural infection with *V. dahliae* on vascular discoloration extent

The extent of vascular discoloration, estimated via longitudinal stem sections, was noted together with plant height for all the cultivars tested. Results presented in Fig. 2 shows that the extent of this vascular discoloration occasioned by VW reached and even exceeded the half of stem height, as

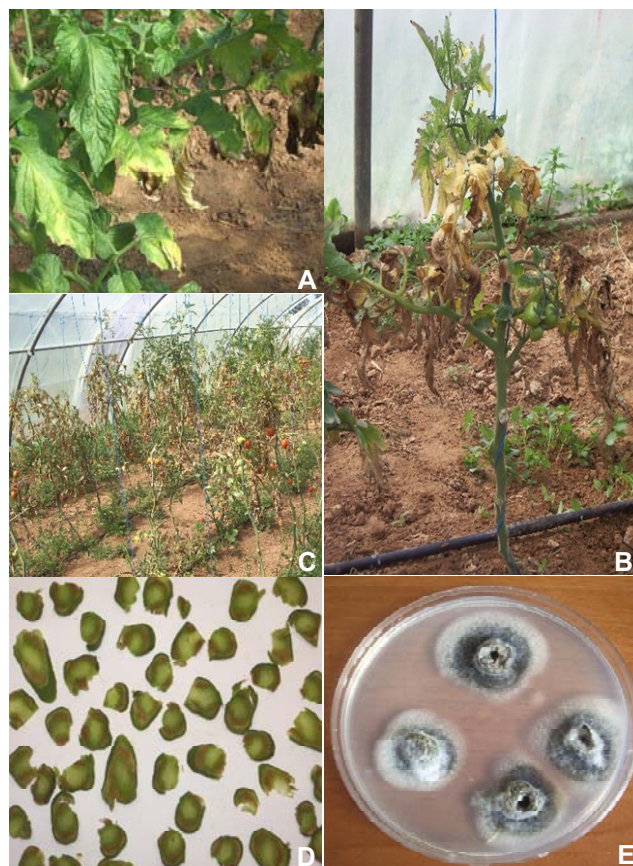


Fig. 1 Symptoms of *Verticillium* wilt in naturally infected resistant (i.e. carrying the *Ve* gene) tomato cultivar. (A) Chlorosis and characteristic V-shaped necrosis of leaves; (B) Stunting and acropetal progression of withering; (C) Whole plants with desiccated leaves; (D) Cross sections showing vascular discoloration of stem tissues; (E) Pathogen colonies developed from stem pieces on PDA after 7 days' incubation at 20°C.

for the cultivars ‘Naziha’, ‘Chourouk’, ‘Cencara’, and ‘Colibri’. Although these cultivars were resistant to *V. dahliae* race 1, an important extension of the pathogen inside vascular tissues was observed. In fact, for all the tested tomato cultivars, *V. dahliae* was successfully isolated (Fig. 1E) or microscopically confirmed at different levels from brown vascular tissues, at the base and middle. For ‘Colibri’, ‘Naziha’, ‘Amel’ and ‘Chourouk’, this pathogen was observed even at the top of the discolored tissues.

DISCUSSION

Aggressiveness of *V. dahliae* race 1 and race 2 on two differential tomato cultivars

The present study emphasis, for the first time in Tunisia, the aggressiveness of *V. dahliae* race 1 and race 2 isolates, collected from different Tunisian regions (Jabnoun-Khiareddine *et al.* 2007a), toward resistant (cv. ‘Riogrande’) and susceptible (cv. ‘Ventura’) tomato cultivars, as measured by leaf damage and plant stunting effects.

Race 1 isolates tested caused moderate to severe symptoms on the susceptible tomato cultivar ‘Ventura’. In contrast, race 2 isolates caused moderate to severe symptoms on both cultivars. On the average, race 2 isolates, caused symptoms of similar severity on both cultivars. In addition, these race 2 isolates were, on the average, more aggressive than race 1 isolates on the susceptible cultivar cv. ‘Ventura’. Similarly, race 2 isolates caused, on the average greater stunting than race 1 isolates on the cv. ‘Ventura’. Grogan *et al.* (1979) and Besri *et al.* (1984) have shown, in the contrary, that race 1 isolates were more aggressive on the tomato cultivar lacking the *Ve* gene than race 2 isolates. The

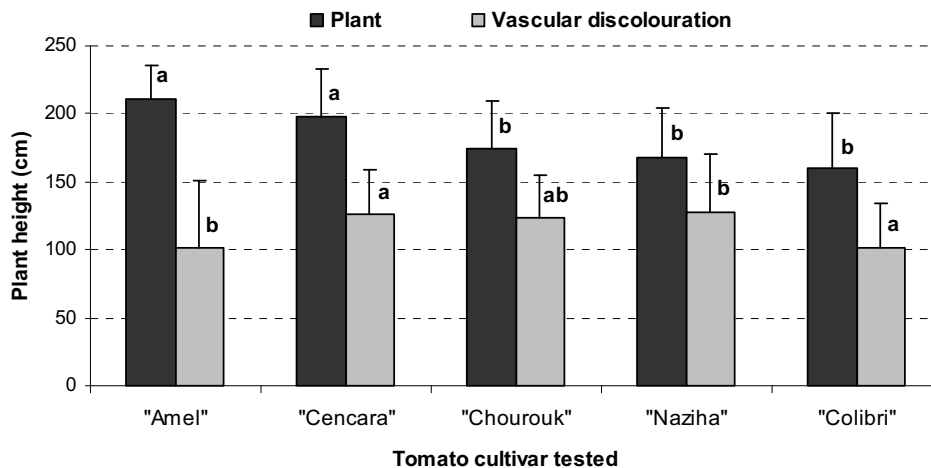


Fig. 2 Effect of *V. dahliae* on plant height and vascular discoloration extent on five tomato cultivars resistant to race 1 of the pathogen noted 120 days post-transplanting in a naturally infested soil. For plant height and vascular discoloration extent, bars with the same letter are not significantly different at $P \leq 0.05$.

former have suggested that the ability of race 2 to overcome the *Ve* gene is associated with a loss of virulence on susceptible cultivars, while Bender and Shoemaker (1984) mentioned that this difference in wilting may be due to a different response of the race 1-resistant cultivars versus the susceptible cultivars to metabolites or toxins produced by race 2 isolates or race 1 and race 2 may produce different metabolites and toxins.

As well as race 2, the Tunisian race 1 isolates were able to cause variable stunting effects on both the resistant and susceptible cultivars 'Riogrande' and 'Ventura'. In contrast, other researchers have mentioned that race 1 isolates caused slight or no stunting on the resistant cultivar (Bender and Shoemaker 1984; O'Garro and Clarkson 1988). Thus, Tunisian race 1 isolates could not be distinguished from race 2 isolates, on the basis of the stunting level only. In this sense, O'Garro *et al.* (1988) pointed out that in cv. 'Roma VF', the *Ve* gene appears to limit but not exclude vascular colonization by race 1 isolates, a factor that perhaps contributes most to the long-term survival of race 1 isolates.

When we consider the distribution of the Tunisian isolates into the aggressiveness classes (Tables 1-3), we clearly notice that individual isolates within each race have a wide range of aggressiveness on both susceptible and resistant cultivars. Apparently, each of the two tomato races of *V. dahliae* is comprised of many strains (groups of isolates), with different degrees of aggressiveness on cultivars with or without the *Ve* gene (Grogan *et al.* 1979). In fact, regardless of the race, this continuum of aggressiveness has been observed for isolates of *V. dahliae* pathogenic on tomato, potato, cotton, and lettuce (Grogan *et al.* 1979; Asworth 1983; Bender and Shoemaker 1984; Besri *et al.* 1984; Joaquim and Rowe 1991; Vallad *et al.* 2006), suggesting that the genetic control of virulence may differ from that controlling race specificity (Grogan *et al.* 1979; Pegg and Brady 2002; Vallad *et al.* 2006). In fact, it has been suggested that despite their common ability to cause disease on tomato cultivars carrying the *Ve* resistance gene, race 2 isolates from geographically separate regions and even from the same locality, may be genetically distinct (Tjamos 1981; O'Garro and Clarkson 1988; Baergen *et al.* 1993; Dobinson *et al.* 1998). In the same way, Dobinson *et al.* (1998) suggested that the characterization of race 2 isolates genetically different may reveal specific physiological differences with respect to virulence, host range, cultivar specificity and interactions with other pathogenic microorganisms; information that would be directly relevant to the management of tomato VW. For example, our data suggest that it may be useful to use diverse race 2 isolates, from the different aggressiveness classes, when screening tomato varieties for potential resistance/tolerance to VW caused by *V. dahliae* race 2.

Behavior of tomato cultivars against *V. dahliae* races under controlled conditions

In this study, root dip method is used for plant inoculation as it is known to be consistent and reliable method (Atibalentja *et al.* 1997) and as it did not affect the interaction between host cultivars and VW (Steventon *et al.* 2002; Debode *et al.* 2005). Moreover, two criteria were used to evaluate the resistance of selected tomato cultivars to VW, leaf damage and plant height, whereas the vascular discoloration in the roots and the stem base was not sufficiently pronounced to be used as disease evaluation criterion. In this sense, Baergen *et al.* (1993) have mentioned that an evaluation that considers foliar symptoms together with plant stunting may accurately indicate potential host-plant resistance.

In addition, the behavior of the six tomato cultivars tested was studied at a temperature of $23 \pm 3^\circ\text{C}$ which is favorable for *V. dahliae*, as temperature can affect the level of resistance of different plant hosts to VW (Bolek *et al.* 2005).

According to plant inoculation and growth conditions used in this study, all of the race 1 resistant tomato cultivars tested ('Naya', 'Amel', 'Bochra' and 'Daniella') were found to be susceptible to race 2 isolates of *V. dahliae*. Although these cultivars differ in their susceptibility, none has shown levels of resistance to all race 2 isolates, comparable to those conferred by *Ve* gene. Similar results have been signaled by Okie and Gardener (1982) and Baergen *et al.* (1993) when screening tomato germplasm resistance to race 2 and by Hayes *et al.* (2007) when testing lettuce genotypes to *V. dahliae* race 2.

Significant differences exist among tomato cultivars tested and *V. dahliae* isolates as shown by the comparison of mean values for the measured parameters. In fact, an important intra-specific variation was noted within *V. dahliae* as for leaf symptoms and height reduction on inoculated plants, in comparison to the non-inoculated control.

Plant resistance depends, in fact, on the aggressiveness of the tested isolates (Beye and Lafay 1988). In this sense, Baergen *et al.* (1993) suggested the use of diverse isolates when screening for improved race 2 resistance and mentioned that the genetic diversity in the host and pathogen and the environmental conditions favoring the pathogen likely contributed to the different genotype x isolate interactions. In the same way, Cirulli *et al.* (2008) emphasize the importance of using different *V. dahliae* pathotypes in screening for resistant olive germplasm, as these may induce different resistance/susceptibility reactions.

Tomato cultivars, tested in our study, did not fall into discrete categories of resistant or susceptible, but may be ranged from the most to the least susceptible. Similar results were shown by Baergen *et al.* (1993) when testing the

resistance of eight genotypes to four isolates of *V. dahliae* race 2. In addition, resistant cultivars responded differently to the inoculation with race 2 isolates as for the leaf symptoms and stunting effect. As an example, we can notice that 'Daniella' plants which exhibited the least leaf symptoms (mean LDI of about 0.99), have shown a reduction in their height ranging from 66 to 82%, when inoculated with race 2 isolates compared to 75% with race 1 isolate. However, 'Naya' plants which have exhibited, for all physiological races combined and according to the experiment conditions, significantly greater LDI than that of the other cultivars, showed a reduction in plant height ranging from 24 to 77% with *V. dahliae* race 2 isolates compared to 48% with *V. dahliae* race 1 isolate. Similar results were signaled by Beye and Lafay (1985) who mentioned, in their study, that no tomato variety exhibited optimal behavior for all *Verticillium* pathogenic effects and that differences between varieties could be either quantitative or qualitative. They also suggested that no particular effect of VW express globally all the genes which govern general resistance of tomato to disease. Baergen *et al.* (1993) reported a similar reaction with the genotype 'Philippino 2' which was more resistant than the other seven genotypes tested because of the very subtle foliar symptoms exhibited, but in terms of dry weight reduction; its performance was consistently significantly worse than almost each other genotype.

The present results highlight the different aptitudes that exhibited our Tunisian isolates in inducing various manifestations of Verticillium disease on tomato cultivars which themselves exhibit different behaviors towards the pathogen.

Behavior of tomato cultivars against *V. dahliae* under greenhouse conditions

In order to evaluate the response of five tomato cultivars to VW, in naturally infested soil, foliar symptoms together with extent of vascular discoloration were noted. In fact, foliar symptoms alone are not sufficient to evaluate cultivar resistance and aggressiveness levels, especially since these symptoms can be induced by both abiotic and biotic stress (Uppal *et al.* 2007; Alkher *et al.* 2009; Daami-Remadi *et al.* 2009). In addition, when the vascular discoloration is confirmed by pure pathogen isolation, it surely reflects the importance of pathogen proliferation in the stem tissue and consequently the degree of plant resistance to this pathogen.

In the case of our study, all together plants of the five cultivars tested, the most grown in Tunisia, have shown typical *Verticillium* external symptoms including chlorosis, necrosis and stunting. These symptoms are very important and reached the totality of the plants which became prematurely desiccated. However, it is known that in resistant plants, the above symptoms are milder and usually restricted to the roots, lower stem and leaves (Robb 2007).

Moreover, the extent of vascular discoloration was very important comparatively to plant height and was not restricted to roots but reached more than half of the plant. In addition, the presence of *V. dahliae* at different levels (base, middle, and top of stems) from the discolored tissues was noted in all the plants of the tested cultivars. However, resistance is generally considered to be a state in which pathogen growth in the xylem is very limited and few, if any symptoms occur (Robb 2007). Thus, as the vascular discoloration extent in all the tested resistant cultivars, together with the foliar symptoms is very important, these cultivars behaved as susceptible ones, although they carry the resistance *Ve* gene. In fact, resistance conferred by the *Ve* gene in tomato, restricted the colonization of incompatible, race 1 isolates of *V. dahliae* to the roots, allowing only limited spread to stem and foliar tissues (Tjamos and Smith 1975; Gold *et al.* 1996). The results presented here show clearly the breakdown of the *Ve* resistance present in the tested tomato cultivars and highlight the severity of VW caused by race 2 isolates present in the field soil. In fact, since 1960, Alexander mentioned that the failure of the tomato varieties which possess *Ve* gene to express resis-

tance under 1960 field conditions in Ohio and in greenhouse experiments may be due either to the presence of different physiological races of the pathogen or to different levels of resistance (Alexander 1962).

In fact, under our experiment field conditions, it is possible that many factors have contributed to the breakdown of resistance conferred by the *Ve* gene including the presence of race 2 isolates, favorable temperatures and high inoculum density.

In the field chosen for experiment, the inoculum density was estimated to 49 microsclerotia/g of soil. This inoculum was sufficient to cause a 100% wilt on all the tested tomato cultivars. In fact, on tomato, inoculum density of 4.5 microsclerotia/g of soil was sufficient to cause 100% of infection (Grogan *et al.* 1979).

Moreover, the type and the intensity of symptoms are, to some extent, environmentally dependent (Pegg and Brady 2002). In fact, factors which can influence symptom development include, among others, temperature. Indeed, our experiment took place from December to March. The lower temperatures during this period which favored the pathogen possibly were responsible for intensifying disease symptoms. In the same way, Besri and Zroui (1983) have mentioned that under plastic tunnels, temperature is the limiting factor for tomato wilt, 90% occurring between November and April.

Given the complexity of possible factors, it is entirely possible that each pathosystem is to some extent unique, there being many means to common end (Robb 2007), which is in our case, susceptibility. Race 2 isolates that overcome *Ve* resistance now predominate in many tomato-production regions in Tunisia and in the world (Pegg and Brady 2002; Jabnoun-Khiareddine *et al.* 2007a). Consequently, the usefulness of *Ve*-mediated resistance has been limited (Hayes *et al.* 2007). Until nowadays, a high level of resistance to *V. dahliae* race 2, comparable to that conferred by the *Ve* gene, are still absent. In fact, Gordon *et al.* (2006) mentioned that resistance conferred by a major gene is often completely effective when initially deployed but may later be compromised by the occurrence of a new pathogenic race. In contrast, polygenic resistance though typically more difficult to incorporate into elite germplasm, may ultimately prove to be more durable (Gordon *et al.* 2006).

In conclusion, the present work is the first to report the wide range of aggressiveness that exhibited local isolates of *V. dahliae* race 1 and 2 towards the susceptible 'Ventura' and the resistant 'Riogrande' cultivars, as measured by leaf damage and stunting indexes. Further studies are needed to better characterize Tunisian *V. dahliae* races, as for their host range, cultivar specificity and interactions with other pathogenic microorganisms, as well as their genotypic variation.

All the resistant tomato cultivars tested under controlled conditions are susceptible to race 2 isolates of *V. dahliae* as measured by leaf damage and plant height; however whether these evaluations accurately predict resistance in the field is unknown. Moreover, the breakdown of the *Ve* resistance gene in tomato cultivars grown under Tunisian field conditions was shown by the importance of the foliar symptoms and the extent of vascular discoloration; however, to what extent do these symptoms affect fruit yield is unknown. Further field trials are needed to evaluate, under Tunisian conditions, the effect of local race 2 isolates on yield reductions of the most grown tomato cultivars.

Moreover, findings from our results are still important as Verticillium wilt continues to threaten tomato production in Tunisia and highlights the urgent need of breeding tomato genotypes with useful levels of resistance especially under Tunisian conditions or Mediterranean, in general, which are conducive to disease expression.

ACKNOWLEDGEMENTS

We are grateful to Dr K. Dobinson and Dr J. Robb (Canada) for supplying the Canadian race 1 and 2 of *V. dahliae* isolates. We

thank the head of the Regional Center of Research in Horticulture and Organic Agriculture (CRRHAB) and the Higher Agronomic Institute of Chott-Mariem (ISA-CM) for financial contribution, and Mrs. Mouna Gueddess-Chahed and Mr. Aymen Youssef for technical assistance.

REFERENCES

- Acciarri N, Rotino GL, Tamietti G, Valentino D, Voltattorni S, Sabatini E (2007) Molecular markers for *Ve1* and *Ve2* *Verticillium* resistance genes from Italian tomato germplasm. *Plant Breeding* **126**, 617-621
- Alexander LJ (1962) Susceptibility of certain *Verticillium*-resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* **52**, 998-1000
- Alkher H, El Hadrami A, Rashid KY, Adam LR, Daayf F (2009) Cross-pathogenicity of *Verticillium dahliae* between potato and sunflower. *European Journal of Plant Pathology* **124**, 505-519
- Arbogast M, Powelson ML, Cappaert MR, Watrud LS (1999) Response of six potato cultivars to amount of applied water and *Verticillium dahliae*. *Phytopathology* **89**, 782-788
- Ashworth LJ Jr. (1983) Aggressiveness of random isolates of *Verticillium dahliae* from cotton and the quantitative relationship of internal inoculum to defoliation. *Phytopathology* **73**, 1292-1295
- Atibalentja N, Eastburn DM (1997) Evaluation of inoculation methods for screening horseradish cultivars for resistance to *Verticillium dahliae*. *Plant Disease* **81**, 356-362
- Baergen KD, Hewitt JD, St. Clair DA (1993) Resistance of tomato genotypes to four isolates of *Verticillium dahliae* race 2. *HortScience* **28**, 833-836
- Bender CG, Shoemaker PB (1984) Prevalence of *Verticillium* wilt of tomato and virulence of *Verticillium dahliae* race 1 and race 2 isolates in Western North Carolina. *Plant Disease* **68**, 305-309
- Besri M, Zrouri M (1983) Température et manifestation des trachéomycoses de la tomate cultivée en plein air et sous tunnel plastique au Maroc. *Bulletin OEPP* **13**, 127-131
- Besri M, Zrouri M, Beye I (1984) Appartenance raciale et pathogénie compare de quelques isolats de *Verticillium dahliae* (Kleb.) obtenus à partir de tomate résistante au Maroc. *Phytopathologische Zeitschrift* **109**, 289-294
- Beye I, Lafay JF (1985) Etude de critères de sélection pour une résistance générale à la Verticilliose chez la tomate. *Agronomie* **5**, 305-311
- Bhat RG, Subbarao KW (1999) Host range specificity in *Verticillium dahliae*. *Phytopathology* **89**, 1218-1225
- Bolek Y, Bell AA, El Zik KM, Thaxton PM, Magill CW (2005) Reaction of cotton cultivars and F₂ population to stem inoculation with *Verticillium dahliae*. *Journal of Phytopathology* **153**, 269-273
- Cirulli M, Colella C, D'Amico M, Amenduni M, Bubici G (2008) Comparison of screening methods for the evaluation of olive resistance to *Verticillium dahliae* Kleb. *Journal of Plant Pathology* **90**, 7-14
- Corsini D, Pavek JJ (1996) Agronomic performance of potato germplasm selected for high resistance to *Verticillium* wilt. *American Potato Journal* **73**, 249-260
- Daami-Remadi M, Ben Oun H, Souissi A, Mansour M, Jabnoun-Khiareddine H, Nasraoui B (2009) Effects of saline irrigation water on *Verticillium* wilt severity and tomato growth. *Plant Stress* **3**, 40-48
- Daami-Remadi M, Jabnoun-Khiareddine H, Barbara DJ, Ayed F, El Mahjoub M (2006) First report of *Verticillium dahliae* race 2 in Tunisia. *Plant Pathology* **55**, 816
- Debode J, Declercq B, Hôte M (2005) Identification of cauliflower cultivars that differ in susceptibility to *Verticillium longisporium* using different inoculation methods. *Journal of Phytopathology* **153**, 257-263
- Dobinson KF, Patterson NA, White GJ, Grant S (1998) DNA fingerprinting and vegetative compatibility analysis indicate multiple origins for *Verticillium dahliae* race 2 tomato isolates from Ontario, Canada. *Mycological Research* **102**, 1089-1095
- Dobinson KF, Tenuta GK, Lazarovits G (1996) Occurrence of race 2 of *Verticillium dahliae* in processing tomato fields in southwestern Ontario. *Canadian Journal of Plant Pathology* **18**, 55-58
- Fradin EF, Thomma BPHJ (2006) Physiology and molecular aspects of *Verticillium* wilt diseases caused by *V. dahliae* and *V. albo-atrum*. *Molecular Plant Pathology* **7**, 71-86
- Gold J, Lee B, Robb J (1996) Colonization of tomatoes by *Verticillium dahliae*: determinative phase II. *Canadian Journal of Botany* **74**, 1279-1288
- Gordon TR, Kirkpatrick SC, Hansen J, Shaw DV (2006) Response of strawberry genotypes to inoculation with isolates of *Verticillium dahliae* differing in host origin. *Plant Pathology* **55**, 766-769
- Grogan RG, Ioannou N, Schneider RW, Sall MA, Kimble KA (1979) *Verticillium* wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. *Phytopathology* **69**, 1176-1180
- Harrington MA, Dobinson KF (2000) Influences of cropping practices on *Verticillium dahliae* populations in commercial processing tomato fields in Ontario. *Phytopathology* **90**, 1011-1017
- Hayes RJ, Vallad GE, Qin QM, Grube RC, Subbarao KV (2007) Variation for resistance to *Verticillium* wilt in lettuce (*Lactuca sativa* L.). *Plant Disease* **91**, 439-445
- Heinz R, Lee SW, Saparno A, Nazar RN, Robb J (1998) Cyclical systemic colonization in *Verticillium*-infected tomato. *Physiological and Molecular Plant Pathology* **52**, 385-396
- Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, El Mahjoub M (2007a) Incidence and distribution of *Verticillium dahliae* races infecting tomato in Tunisia. *Tunisian Journal of Plant Protection* **2**, 63-70
- Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, Jebari H, El Mahjoub M (2007b) Incidence of *Verticillium* wilt of melon in Tunisia. *The African Journal of Plant Science and Biotechnology* **1**, 10-15
- Jabnoun-Khiareddine H, Daami-Remadi M, Hibar K, Ayed F, El Mahjoub M (2006) Pathogenicity of Tunisian isolates of three *Verticillium* species on tomato and eggplant. *Plant Pathology Journal* **5**, 199-207
- Joaquim TR, Rowe RC (1991) Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology* **81**, 552-558
- Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, van Rooijen G, Waterer DR, Robertson A, Kokko E, Byers R, Howard RJ, Fischer R, Prüfer D (2001) Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proceedings of the National Academy of Sciences USA* **98**, 6511-6515
- McKeen CD, Thorpe HP (1981) *Verticillium* wilt of potato in southwestern Ontario and survival of *Verticillium albo-atrum* and *V. dahliae* in field soil. *Canadian Journal of Plant Pathology* **3**, 40-46
- Nadakavukaren MJ, Horner CE (1959) An alcohol agar medium selective for determining *Verticillium* microsclerotia in soil. *Phytopathology* **49**, 527-528
- O'Garro LW, Clarkson JM (1988). Pathogenicity of race-1 and race-2 tomato wilt isolates of *Verticillium dahliae* from different geographical origins. *Journal of Phytopathology* **123**, 297-303
- Okie WR, Gardner RG (1982) Screening tomato seedlings for resistance to *Verticillium dahliae* race 1 and 2. *Plant Disease* **66**, 34-37
- Pegg GF, Brady BL (2002) *Verticillium Wilts*, CABI Publishing, New York, 552 pp
- Pharand B, Carisse O, Benhamou N (2002) Cytological aspects of compost-mediated induced resistance against *Fusarium* crown and root rot in tomato. *Phytopathology* **92**, 424-438
- Robb J (2007) *Verticillium* tolerance: resistance, susceptibility, or mutualism? *Canadian Journal of Botany* **85**, 903-910
- Schaible L, Cannon OS, Waddoups V (1951) Inheritance of resistance to *Verticillium* wilt in a tomato cross. *Phytopathology* **41**, 986-990
- Steventon LA, Fahleson J, Hu Q, Dixelius C (2002) Identification of the causal agent of *Verticillium* wilt of winter oilseed rape in Sweden, *V. longisporium*. *Mycological Research* **106**, 570-578
- Talboys PW (1970) Variation in the virulence of *V. dahliae* from strawberry. *Annals of Applied Biology* **66**, 43-49
- Tjamos EC (1981) Virulence of *Verticillium dahliae* and *V. albo-atrum* isolates in tomato seedlings in relation to their host of origin and the applied cropping system. *Phytopathology* **71**, 98-100
- Tjamos EC, Smith IM (1975) The expression of resistance to *Verticillium albo-atrum* in monogenically resistant tomato varieties. *Physiological Plant Pathology* **6**, 215-222
- Uppal AK, El Hadrami A, Adam LR, Tenuta M, Daayf F (2007) Pathogenic variability of *Verticillium dahliae* isolates from potato fields in Manitoba and screening of bacteria for their biocontrol. *Canadian Journal of Plant Pathology* **29**, 141-152
- Vallad GE, Subbarao KV (2008) Colonization of resistant and susceptible lettuce cultivars by a green fluorescent protein-tagged isolate of *Verticillium dahliae*. *Phytopathology* **98**, 871-885
- Vallad GE, Qin QM, Grube RC, Hayes RJ, Subbarao KV (2006) Characterization of race-specific interaction among isolates of *Verticillium dahliae* in lettuce. *Plant Disease* **89**, 317-324
- Veronese P, Narasimhan ML, Stevenson RA, Zhu JK, Weller SC, Subbarao KV, Bressan RA (2003) Identification of a locus controlling *Verticillium* disease symptom response in *Arabidopsis thaliana*. *Plant Journal* **35**, 574-587