European Journal of Plant Pathology **104:** 263–269, 1998.

Vegetative-compatibility groups in *Verticillium dahliae* from Israel

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Accepted 17 December 1997

Key words: cotton, potato, vegetative comptability, *Verticillium dahliae*

Abstract

Nitrate-nonutilizing (*nit*) mutants were used to determine vegetative compatibility among 34 isolates of *Verticillium dahliae* from cotton, potato, olive, eggplant, chrysanthemum and tomato from 12 sites in Israel. Based on the formation of complementary heterokaryons, 33 isolates were assigned to two vegetative- compatibility groups (VCGs): one VCG contained 15 isolates from cotton, eggplant, chrysanthemum and olive; and the other VCG contained 18 isolates from potato, olive and cotton. The status of an additional isolate from tomato, which was compatible with both VCGs, remained unclear. In a limited pathogenicity test with 10 isolates, two (from tomato and eggplant) were pathogenic on tomato, eggplant and cotton; most isolates from cotton were pathogenic on cotton and eggplant only; and one from cotton was non-pathogenic. Fewer isolates were pathogenic on tomato than on cotton or eggplant. The diversity of vegetative compatibility found in our *V. dahliae* collection is comparable to that found in studies of American populations.

Introduction

Verticillium wilt, caused by the soilborne vascular pathogen *Verticillium dahliae* Kleb., is an important disease of many crops (Pegg, 1981). In Israel, Verticillium wilt has inflicted substantial losses in a variety of crops such as potato (Krikun and Orion, 1979) and eggplant. This disease caused heavy damage to tomato, until resistant cultivars became dominant. Occasional occurrence has been recorded on fruit trees such as olive, almond, peach, mango and avocado. Since the mid 1980s, its incidence in cotton has been on the rise. In view of its broad host range and apparently low host specificity, *V. dahliae* has been considered to possess little genetic diversity. However, more recent vegetative-compatibility and molecular studies have led to the realization that genetic diversity in *V. dahliae* is more significant than previously thought (Rowe, 1995).

The identification of vegetative-compatibility groups (VCGs) has proven to be an effective approach for studying the genetic structure of anamorphic populations of soilborne wilt and root-rot pathogens such as *Fusarium oxysporum* (Katan et al., 1991; Marlatt et al., 1996) and *Verticillium* spp. (Correll et al., 1988; Joaquim and Rowe, 1991). In vegetative-compatibility analyses using nitrate-nonutilizing (*nit*) mutants, four VCGs were identified among 22 isolates of *V. dahliae* originating from 10 host-plant species in eight countries (Joaquim and Rowe, 1990). About 250 isolates from potato plants and soil in Ohio and nine other states were assigned to three of these VCGs (Joaquim and Rowe, 1990, 1991). The existence of four VCGs has essentially been confirmed (Strausbaugh et al., 1992), followed by the assignment of 29 potato isolates from California and 33 from Idaho to two and one of these VCGs, respectively (Strausbaugh, 1993; Strausbaugh et al., 1992). Three of the VCGs have been found among 36 isolates from ornamental woody plants in the USA (Chen, 1994), cotton isolates form the USA and other countries (Bell, 1994), and 28 isolates from cotton and solanaceous hosts in various countries (Daayf

et al., 1995). These findings suggest that vegetative-compatibility diversity exists in *V. dahliae*, although it is limited to a small number of VCGs (Rowe, 1995).

The few detailed studies of VCGs in *V. dahliae* have focused mainly on populations from potato fields (Joaquim and Rowe, 1990, 1991; Strausbaugh, 1993; Strausbaugh et al., 1992) and other plants (Chen, 1994; Subbarao et al., 1995) in the USA, although occasional isolates from other countries have been included in some studies (Akimov and Portenko, 1996; Bell, 1994; Daayf et al., 1995; Joaquim and Rowe, 1990; Strausbaugh et al., 1992). No similar studies have been reported on *V. dahliae* populations in the Mediterranean or Middle Eastern regions. The purpose of this study was to determine the genetic diversity in *V. dahliae* populations originating from various wilt-affected crops in Israel, using the VCG approach.

Materials and methods

Media

Potato-dextrose agar (PDA) and *Verticillium*-selective medium (VSM) (Ausher et al., 1975) were used for isolation of *V. dahliae* from diseased plants. Puhalla's minimal medium (MM), which is a sucrose-salt medium that contains nitrate as the nitrogen source (Puhalla, 1985), was used to maintain the *V. dahliae* cultures, to recognize *nit* mutants, and for complementation (heterokaryon) tests. Chlorate media, based on MM amended with 1.5% $KClO_3$ (MMC) or cornmeal agar with glucose (0.02%) amended with 1.5–4.0% $KClO_3$ (CMC), were used to generate *nit* mutants (Correll et al., 1988). Nitrite and hypoxanthine media were used for partial phenotypic characterization of *nit* mutants as *nit1*, *nit3*, or NitM (Correll et al., 1987). Czapek-Dox agar (CDA; Biolife, Milano, Italy) was used to grow inoculum for pathogenicity tests.

Pathogen isolation

Plants showing typical Verticillium wilt symptoms, or branches of diseased olive trees, were collected from fields at various sites in Israel (Table 1). Segments (2 cm long) were taken from the stems, dipped in 70% ethanol, surface-sterilized with NaOCl (1%) for 1 min, and rinsed with sterile water. Each segment was then cut into three pieces, which were blotted and placed on PDA or VSM, and incubated in the dark for 7-14 days at 24 °C or 18 °C, respectively. Colonies of *V. dahliae* were identified visually and microscopically. One monoconidial culture was then prepared from each isolate.

Table 1. Isolates of *Verticillium dahliae*, and nitrate-nonutilizing (*nit*) mutants generated from them, listed by vegetative-compatibility group (VCG), source and isolation site

VCG	Isolate	Source	Site	Number of *nit* mutants	
				Total	NitM[1]
?[2]	tom1	Tomato	Unknown	9	2
I	cot2	Cotton	Gan-Shmuel	6	1
	cot3	Cotton	Ein-Shemer	8	2
	cot4	Cotton	Kfar-Ha'horesh	11	2
	cot5	Cotton	Galed	19	1
	cot6	Cotton	Galed	22	1
	cot8	Cotton	Galed	17	4
	cot9	Cotton	Galed	33	6
	cot10	Cotton	Galed	28	2
	ep1	Eggplant	Ganey-Yohanan	5	3
	ep1Aw[3]	ep1		3	0
	ep2	Eggplant	Tamra	27	11
	ep3	Eggplant	Tamra	7	2
	E5	Chrysanthemum	Bet-Alpha	8	3
	E6	Chrysanthemum	Bet-Alpha	10	3
	E7	Chrysanthemum	Bet-Alpha	7	3
	oli6	Olive	Karme-Yossef	8	...[4]
II	cot1	Cotton	Kfar-Azza	13	2
	pt12	Potato	Nir-Oz	19	0
	pt13	Potato	Nir-Oz	11	0
	pt14	Potato	Unknown	14	2
	pt28	Potato	Re'im	17	2
	pt29	Potato	Re'im	24	1
	pt30	Potato	Re'im	16	1
	pt31	Potato	Re'im	15	2
	pt32	Potato	Re'im	13	1
	pt33	Potato	Re'im	24	2
	pt34	Potato	Re'im	8	0
	pt35	Potato	Re'im	15	1
	pt36	Potato	Re'im	13	1
	pt37	Potato	Re'im	13	2
	oli1	Olive	Karme-Yossef	22	3
	oli2	Olive	Karme-Yossef	9	0
	oli4	Olive	Mashabey-Sadeh	8	...
	oli5	Olive	Mashabey-Sadeh	6	...
Total	34 isolates	6 hosts	12 sites	488	66

[1]The remaining mutants were *nit1*.
[2]Not assigned to a VCG.
[3]A spontaneous hyaline variant derived from ep1.
[4] Phenotype not determined.

Generation and characterization of nit *mutants*

Nitrate-nonutilizing (*nit*) mutants were generated essentially as described previously (Chen, 1994; Correll et al., 1988; Joaquim and Rowe, 1990, 1991). In preliminary experiments, CMC at four chlorate con-

centrations (1.5, 2.0, 2.5, 4.0%) and MMC were compared for their efficiency at yielding *nit* mutants from six *V. dahliae* isolates. In these experiments, chlorate-resistant sectors were more easily recognized on CMC than on MMC, whereas neither sectoring nor recovery of *nit* mutants was significantly affected by the chlorate concentration. Consequently, CMC with 2.0% $KClO_3$ was used to generate *nit* mutants from the remaining isolates. CMC plates (9 cm in diameter) were centrally inoculated with small mycelial agar plugs from the growing margins of *V. dahliae* isolates on MM (usually six or more plates per isolate), and incubated at 24 °C for 2–4 weeks. Densely sporulating sectors which emerged from the developing colony were subcultured by removing conidia from their surfaces, using a bacteriological loop, and streaking them on MM. Subcultures which grew as thin, expansive colonies without aerial mycelium were considered *nit* mutants. Partial phenotypic characterization of *nit* mutants was attempted by growing them on nitrite and hypoxanthine media (Correll et al., 1987). Mutants that grew sparsely on hypoxanthine were classified as NitM, whereas mutants that grew profusely on hypoxanthine were classified as *nit1*.

Complementation (heterokaryon) tests

Complementation between *nit* mutants was tested on MM in 5-cm-diameter petri plates. A mycelial plug of a NitM mutant was placed in the center of the plate, and three *nit1* mutants of the same or different isolates were placed around it, 1.0–1.5 cm away, in a triangular pattern. The plates were incubated at 24 °C for 30 days and growth was inspected at weekly intervals. Complementation was evident by the development of prototrophic growth where two mutant colonies met and formed a stable heterokaryon. The degree of complementation, measured by the intensity of prototrophic growth, was ranked as follows: strong (++), with aerial mycelium and microsclerotia; weak (+), with a zone of microsclerotia but no aerial mycelium; very weak (+−), with a zone (often uncertain) of sparse microsclerotia; and no prototrophic growth (−). Tests were repeated once. In case of uncertain or inconsistent results, additional testing was done by pairing only two mutants per plate, to avoid interference from neighboring mutants.

Pathogenicity tests

Isolates of *V. dahliae* were grown on CDA (9-cm plates) at 24 °C in the dark for 10 days. Conidia were washed off the agar surface with 30–50 ml of tap water. The inoculum concentration was determined by hemacytometer and adjusted to 10^6 conidia per ml. Seedlings of cotton (*Gossypium hirsutum* L., cv. Acala Sj-2), tomato (*Lycopersicon esculentum* Mill., cv. Rehovot 13) and eggplant (*Solanum melongena* L., cv. Classic) were used for testing pathogenicity. Seedlings were uprooted from the soil 2–4 days after emergence (7–14 days after sowing). Their roots were washed free of soil and dipped in inoculum suspension for 3 min. The roots of control seedlings were dipped in water. Seedlings were then transplanted (five per pot in three replicates) to 850-ml pots filled with Rehovot sandy soil (3.8% clay, 0.0% silt, 97.2% sand, and 0.4% organic matter, pH 6.9), and maintained in the greenhouse at 20–25 °C under natural (approximately 11–14 h) daylight. After 40 days, disease severity was assessed using the following 0-3 rating: 0= no symptoms, 1= vascular discoloration without apparent leaf symptoms, 2= vascular discoloration with leaf-wilt symptoms, and 3= dead plant. Disease severity index (DSI) was calculated using the following formula: DSI= [Σ(R x N)] x 100/H x T, where R= disease rating, N= number of plants within this rating, H= the highest rating (= 3), and T= the total number of plants counted. Thus, DSI values ranged from 0 to 100. Inoculation tests were repeated twice. Non-inoculated cotton, tomato and eggplant seedlings, maintained under the same conditions and serving as controls, showed no disease symptoms.

Results were pooled for all inoculation experiments, and experiment was regarded as a blocking factor after ascertaining that there was no isolate x experiment interaction. Analyses of variance to compare all isolates for each host were conducted according to Welch's method for unequal variances (as confirmed by the Levene test) and unequal sample sizes (Milliken and Johnson, 1989). Analysis of variance was used to establish significant interaction ($P \leq 0.05$) between host and isolate. Thereafter, one-way analysis of variance was used to compare hosts for each isolate. A simultaneous comparison of means was performed by the Bonferroni method.

Results

Isolates of V. dahliae

The 34 isolates used in this study originated from six host plants at 12 sites in Israel. Most of the isolates were obtained from potato and cotton (13 and 9, respectively) whereas the remainder came from olive, chrysanthemum, eggplant and tomato (Table 1). When the isolates were grown on MM or CDA, three morphological types emerged with respect to pigmentation: black isolates, with very dark cultures due to quick melanization of microsclerotia at early stages of colony development, and sparse aerial mycelium; gray isolates, with dense hyaline aerial mycelium and slow melanization of microsclerotia; and white (albino) isolates, which appeared as occasional sectors from the previous types (e.g. isolate ep1Aw), or as monoconidial segregants from originally mixed isolates. During prolonged incubation, the colonies of gray isolates became darker while those of white isolates remained hyaline. White isolates did not revert to the wild type but retained the *V. dahliae* morphology of verticillate conidiophores and conidia.

Generation and characterization of nit *mutants*

The isolates varied in both the yield of *nit* mutants and the proportions of the different phenotypic classes among these mutants. A total of 488 mutants was obtained, ranging from 3-33 mutants per isolate. Out of 466 mutants phenotyped, 66 (14%) were classified as NitM (Table 1) and the remainder as *nit1*. No *nit3* phenotype, characterized by thin growth on nitrite medium, could be recognized, in agreement with previous reports on *V. dahliae* (Chen, 1994; Joaquim and Rowe, 1991; Strausbaugh et al., 1992).

Vegetative compatibility

Different mutants of each isolate were first paired among themselves to determine self-compatibility. Complementary (NitM and *nit1*) mutants of several isolates were then paired in various inter-isolate combinations, and where the pairings resulted in strong complementary heterokaryotic growth the parent isolates were assigned to the same VCG. Based on their ability to form strong heterokaryons with many *nit* mutants, seven NitM mutants were chosen as tester strains. These testers, which represented seven isolates originating from four host plants, were paired with *nit1* mutants of the 34 isolates of the collection to determine vegetative compatibility.

According to the formation of strong complementary heterokaryons, 33 of the isolates could clearly be grouped into two VCGs tentatively designated VCG I and VCG II (Table 2). VCG I comprised 15 isolates (eight from cotton, three from eggplant, three from chrysanthemum, and one from olive; ep1Aw, a hyaline variant from ep1 that retained the parental vegetative-compatibility characteristics, was not considered an independent isolate), and its tester strains were cot3/P1, cot4/K1 and ep1/A5. VCG II comprised 18 isolates (13 from potato, four from olive, and one from cotton) and its tester strains were cot1/C6, pt32/A3, pt31/D3 and oli1/D3. Different mutants of the same isolates usually showed similar reactions (positive or negative with respect to prototrophic growth and microsclerotial formation), although variations were sometimes observed in the intensity of complementation. Such variation did not affect the VCG assignment, which was based on the strongest reaction observed. Heterokaryon morphology often reflected the morphology of the parent isolates: mutants of black isolates tended to form dark heterokaryons with little aerial mycelium; whereas mutants of gray isolates tended to form whitish heterokaryons, consisting of dense aerial mycelium, with slow microsclerotial melanization and gradual darkening during prolonged incubation. The interaction between *nit* mutants of one VCG and testers of the other was usually negative, although very weak or uncertain complementation was observed in 6-11% of such combinations (Table 2).

Using the seven testers of the two VCGs, isolate tom1 from tomato could not be unequivocally assigned to either VCG, because its *nit1* mutants showed positive complementation reactions with two NitM testers of VCG I as well as two of VCG II (Table 2). In an attempt to clarify this situation, the interaction between four *nit* mutants of tom1 (three *nit1* and one NitM) and complementary mutants of 20 isolates from the two VCGs was further assessed in 54 *nit1* $\times$ NitM combinations (Table 3). The three *nit1* mutants of tom1 showed positive reactions (strong-to-weak prototrophic growth) with NitM mutants from four out of 12 VCG I isolates and three out of eight VCG II isolates. In the reciprocal pairings, the NitM mutant of tom1 showed complementation (very weak to weak) only with *nit1* mutants of VCG I isolates. Since the ambiguity concerning isolate tom1, which seems to bridge between the two VCGs, was not resolved, it has not been assigned to a VCG.

Table 2. Complementation between *nit1* mutants from 34 isolates (vertical axis) and seven NitM mutants (horizontal axis) used as tester strains of *Verticillium dahliae*

VCG[1]	Isolate	NitM tester strains (isolate/mutant)						
		VCG I			VCG II			
	(*nit 1)*	cot3/P1	cot4/K1	ep1/A5	cot1/C6	pt32/A3	pt31/D3	oli1/D3
?[2]	tom1	+/+–[3]	++/+	+–	–	–	++	++
I	cot2	++	++	++	–	–	+–	–
	cot3	++	++	++	–	–	–	+–/–
	cot4	++	++	++	–	–	–	–
	cot5	++	++	++	–	–	–	–
	cot6	++	++	++	–	–	–	–
	cot8	++	++	++	–	–	–	–
	cot9	++	++	++	–	–	–	+–/–
	cot10	++	++	++	–	–	+–/–	+–/–
	ep1	++	++	++	–	–	–	–
	ep1Aw	+	++	++	–	–	–	–
	ep2	++	++	++	–	–	–	–
	ep3	+–	++	++	–	–	–	–
	E5	++	++	++	–	–	+–/–	+–/–
	E6	++	++	++	–	–	–	–
	E7	++	++	++	–	–	–	–
	oli6	++	++	++	–	–	–	–
II	cot1	–	–	–	++	++	++	++
	pt12	–	–	–	++	++	++	++
	pt13	–	–	–	++	++	++	++
	pt14	–	–	–	++	++	++	++
	pt28	–	–	–	++	++	++	++
	pt29	–	–	–	++	++	++	++
	pt30	–	–	–	++	++	++	++
	pt31	–	–	–	++	++	++	++
	pt32	–	–	–	++	++	++	++
	pt33	–	–	–	++	++	++	++
	pt34	–	–	–	++	++	++	++
	pt35	–	–	–	++	++	++	++
	pt36	–	–	–	++	++	++	++
	pt37	+–/–	–	–	++	++	++	++
	oli1	+–/–	+–/–	–	++	++	++	++
	oli2	–	–	–	++	++	++	++
	oli4	–	–	–	++	++	++	++
	oli5	–	–	–	++	++	++	++

[1]Vegetative-compatibility group.
[2]Not assigned to a VCG.
[3]Intensity of prototrophic growth of the heterokaryon: ++ = strong, with aerial mycelium and microsclerotia; + = weak, with a zone of microsclerotia but no aerial mycelium; +– = very weak, often uncertain, with a sparse microsclerotial zone; – = no growth. Reactions separated by a slash indicate variation among mutants or experiments.

Pathogenicity of V. dahliae *isolates*

The pathogenicity of 10 isolates, originating from three hosts and representing the two VCGs, was compared by inoculating each isolate on the three hosts. One isolate (cot9) was not pathogenic at all (Table 4). All the remaining isolates were pathogenic, at various levels of aggressiveness, to cotton and eggplant, irrespective of their host of origin. Only two isolates, originating from tomato and eggplant, were also pathogenic to tomato,

Table 3. Complementation in NitM × *nit1* pairings between mutants of isolate tom1 and 20 isolates representing two VCGs[1] of *Verticillium dahliae*

VCG	Isolate	Mutants		tom1 mutants			
		NitM	*nit1*	*nit1*			NitM
				A1	B6	D1	D30
I	cot2	R20	R18	–[2]	–	–	–
	cot3	P1	P9	+–	–	+	+–
	cot4	K1		+–	–	–	–
	cot6	F12	F9	++	++	...[3]	+–
	cot9	E7	E3	–	–	...	–
	cot10		B1	...	...	...	+
	ep1	A5	A1	–	–	...	–
	ep2	B40	B7	–	–	...	+–
	ep3	F30	F21	–	–	...	+–
	E5	C12		+	+	...	...
	E6	C36	C17	+	+	...	+–
	E7	B17		+	+	...	...
II	cot1	C6	C10	–	–	–	–
	pt14	A4		–	–	...	...
	pt28		A3	...	...	...	–
	pt32		B1	...	...	...	–
	pt33		B3	...	...	...	–
	pt36	A1	A2	+	+	...	–
	pt37	D1	C1	++	+–	++	–
	oli1	D3	D2	+–	+–	+	–

[1]Vegetative-compatibility groups.
[2] See footnote 3 in Table 2.
[3] Not tested, or complementary mutants not available.

Table 4. Pathogenicity to cotton, tomato and eggplant of *Verticillium dahliae* isolates originating from these host plants

VCG[1]	Isolate[2]	Disease severity index[3]		
		Cotton	Eggplant	Tomato
?[4]	tom1	37.7 cB[5]	68.3 aA	49.2 aB
I	cot2	86.0 aA	31.5 bcB	0.8 bC
	cot3	92.2 aA	18.5 cB	0.0
	cot4	96.6 aA	50.0 abcA	NT[6]
	cot5	88.3 aA	36.2 bcB	3.2 bC
	cot6	96.6 aA	50.0 abcA	NT
	cot8	93.3 a	NT	0.0
	cot9	0.0	0.0	0.0
	ep1	44.2 abcA	54.8 abA	30.5 aA
II	cot1	59.3 bA	61.3 abA	6.7 bB

[1]Vegetative-compatibility group.
[2] Prefix of isolate name indicates host of origin: tom = tomato, cot = cotton, ep = eggplant.
[3]On a scale of 0-100 (see Materials and methods).
[4]Not assigned to a VCG.
[5]In each column, values with different lower-case letters are significantly different according to pairwise t - tests with the Bonferroni correction ($P = 0.05$). For each isolate (horizontal line), upper-case letters refer to differences between hosts.
[6]Not tested.

and thus pathogenic to the three hosts. All pathogenic cotton isolates belonging to VCG I were highly aggressive to cotton (DSI = 86–97), less aggressive to eggplant (DSI = 18–50), and non-pathogenic to tomato (DSI = 0–3). Although several isolates induced severe symptoms on cotton, they did not appear to be of the defoliating type (Bell, 1994; Schnathorst and Mathre, 1966). An overall comparison of DSI by the Kruskall-Wallis nonparametric test, followed by the Wilcoxon test for each pair of hosts, showed significant differences between the three hosts; cotton being the most susceptible and tomato the least ($P < 0.0001$ in each case).

Discussion

Two distinct VCGs were identified among 33 isolates of *V. dahliae* from Israel. Isolates belonging to VCG I originated from cotton, olive, eggplant and chrysanthemum, whereas isolates belonging to VCG II originated from potato, cotton and olive. Thus, with respect to the host of origin, each of these two VCGs contained isolates from at least three different host plants, cotton and olive being represented in both. The status of an additional, single isolate from tomato was not clear since it seemed to be vegetatively compatible with isolates from both VCGs I and II. Such overlap, or cross-VCG compatibility, has occasionally been noticed previously (Joaquim and Rowe, 1990; Rowe, 1995). Additional isolates from tomato need to be tested to examine whether they constitute a specific subpopulation of *V. dahliae*. Incidence of *V. dahliae* on tomato has become rare due to wide use of cultivars possessing the *Ve* gene for resistance.

All pathogenic isolates tested, regardless of their host of origin, were pathogenic to cotton and eggplant, but only isolates which originated from tomato and eggplant were pathogenic to tomato. Eggplant is considered susceptible to isolates of *V. dahliae* from different sources (Horiuchi et al., 1990; Resende et al., 1994), and our results agree with this concept. Results from other studies concerning the specificity of *V. dahliae* isolates to tomato have been inconclusive: in some cases high specificity has been suggested (Horiuchi et al., 1990), while in others this has not

been the case (Ligoxigakis and Vakalounakis, 1994; Tjamos, 1981). In another study, *V. dahliae* strains from cotton manifested differential virulence to tomato (Schnathorst and Mathre, 1966), whereas none of our cotton isolates was pathogenic to tomato.

Although isolate cot9 was vegetatively compatible with testers of VCG I, it was not pathogenic on any of the hosts tested. This isolate may have lost its pathogenicity upon cultivation, or may represent a non-pathogenic fraction of unknown size that exists in the *V. dahliae* population. The level of VCG diversity among the 34 isolates from Israel is comparable to that found among populations of *V. dahliae* in American potato fields and other collections of strains (Rowe, 1995). More isolates need to be tested to better estimate the spectrum of VCG diversity, and reveal any association between VCG, host plant and virulence that may exist in the local population.

Acknowledgements

This research was supported in part by the Cotton Production and Marketing Board (Israel); and by a Fellowship (JRB) from MASHAV, Center for International Cooperation, Ministry of Foreign Affairs (Israel). We thank Dr. Hillary Voet for assistance with statistical analyses. Contribution from the ARO, no. 2255-E, 1997 series.

References

Akimov GI and Portenko LG (1996) Studying vegetative compatibility in *Verticillium dahliae* Kleb. with non-nitrate-utilizing mutants. Genetika 32: 211–217 (in Russian)

Ausher R, Katan J and Ovadia S (1975) An improved selective medium for the isolation of *Verticillium dahliae*. Phytoparasitica 3: 133–137

Bell AA (1994) Mechanisms of disease resistance in *Gossypium* species and variation in *Verticillium dahliae*. In: Constable GA and Forrester NW (eds), Proceedings of the World Cotton Research Conference - 1 (pp 225-235) CSIRO, Melbourne, Australia

Chen W (1994) Vegetative compatibility groups of *Verticillium dahliae* from ornamental woody plants. Phytopathology 84: 214–219

Correll JC, Gordon TR and McCain AH (1988) Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. Phytopathology 78: 1017–1021

Correll JC, Klittich CJR and Leslie JF (1987) Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77: 1640–1646

Daayf F, Nicole M and Geiger JP (1995) Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. European Journal of Plant Pathology 101: 69–79

Horiuchi S, Hagiwara H and Takeuchi S (1990) Host specificity of isolates of *Verticillium dahliae* towards cruciferous and solanaceous plants. In: Hornby D (ed), Biological Control of Soil-borne Plant Pathogens (pp 285–298) CABI, Wallingford, UK

Joaquim TR and Rowe RC (1990) Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. Phytopathology 80: 1160–1166

Joaquim TR and Rowe RC (1991) Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. Phytopathology 81: 552–558

Katan T, Zamir D, Sarfatti M and Katan J (1991) Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Phytopathology 81: 255–262

Krikum J and Orion D (1979) *Verticillium* wilt of potato: importance and control. Phytoparasitica 7: 107–116

Ligoxigakis EK and Vakalounakis DJ (1994) The incidence and distribution of races of *Verticillium dahliae* in Crete. Plant Pathology 43: 755–758

Marlatt ML, Correll JC, Kaufmann P and Cooper PE (1996) Two genetically distinct populations of *Fusarium oxysporum* f.sp. *lycopersici* race 3 in the United States. Plant Disease 80: 1336–1342

Milliken JA and Johnson DE (1984) Analysis of Messy Data. Van Nostrand Reinhold Co, New York, USA. 473 pp.

Pegg GF (1981) The impact of Verticillium diseases in agriculture. In: Cirulli M (ed) Pathobiology of *Verticillium* Species (pp 82–98) Mediterranean Phytopathological Union, Bari, Italy

Puhalla JE (1985) Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Canadian Journal of Botany 63: 179–183

Resende MLV, Flood J and Cooper RM (1994) Host specialization of *Verticillium dahliae*, with emphasis on isolates from cocoa (*Theobroma cacao*). Plant Pathology 43: 104–111

Rowe RC (1995) Recent progress in understanding relationships between *Verticillium* species and subspecific groups. Phytoparasitica 23: 31–38

Schnathorst WC and Mathre DE (1966) Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. Phytopathology 56: 1155–1161

Strausbaugh CA (1993) Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. Phytopathology 83: 1253–1258

Strausbaugh CA, Schroth MN, Weinhold AR and Hancock JG (1992) Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. Phytopathology 82: 61–68

Subbarao KV, Chassot A, Gordon TR, Hubbard JC, Bonello P, Mullin R, Okamoto D, Davis RM and Koike ST (1995) Genetic relationships and cross pathogenicities of *Verticillium dahliae* isolates from cauliflower and other crops. Phytopathology 85: 1105–1112

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