Vegetative Compatibility Groups of *Verticillium dahliae* in Israel: Their Distribution and Association with Pathogenicity

Nadia Korolev, Jaacov Katan, and Talma Katan

First and third authors: Department of Plant Pathology, ARO, The Volcani Center, Bet Dagan 50250, Israel; and second author: Department of Plant Pathology and Microbiology, The Hebrew University, Rehovot 76100, Israel.

Accepted for publication 24 January 2000.

ABSTRACT

Korolev, N., Katan, J., and Katan, T. 2000. Vegetative compatibility groups of *Verticillium dahliae* in Israel: Their distribution and association with pathogenicity. *Phytopathology* 90:529-536.

A collection of 565 isolates of *Verticillium dahliae*, recovered between 1992 and 1997 from 13 host plant species and soil at 47 sites in Israel, was tested for vegetative compatibility using nitrate-nonutilizing (*nit*) mutants. Three vegetative compatibility groups (VCGs) were found and identified as VCG2A (28 isolates), VCG2B (158 isolates), and VCG4B (378 isolates) by using international reference strains. One isolate was heterokaryon self-incompatible. Of the VCG2B isolates, 92% were recovered from the northern part of Israel and 90% of VCG4B isolates were recovered from the south, with some overlap in the central region. Isolates of the minor group VCG2A were geographically scattered among the two major VCGs. Isolates of the same VCG resembled one another more than isolates from

different VCGs based on colony and microsclerotial morphology, temperature responses, and, partially, pathogenicity. Different pathotypes were defined among 60 isolates tested, using cotton (cv. Acala SJ-2) and eggplant (cv. Black Beauty) as differentials. All isolates in VCG2A and 86% of the isolates in VCG4B, irrespective of their origin, induced weak to moderate symptoms on cotton and moderate to severe symptoms on eggplant and were similar to the previously described cotton nondefoliating pathotype. In contrast, all cotton isolates in VCG2B caused severe foliar symptoms, stunting, and often death, but little or no defoliation of inoculated cotton plants. These were defined as a cotton defoliating-like pathotype and induced only weak to moderate symptoms on eggplant. We concluded that vegetative compatibility grouping of *V. dahliae* in Israel is closely associated with specific pathogenicity and other phenotypic traits.

Additional keywords: potato, tomato, Verticillium wilt.

Verticillium dahliae is an economically important pathogen causing vascular wilt on more than 160 plant species (24). In Israel, Verticillium wilt occurs in many regions, including important agricultural areas such as the Negev region in the south and Yizre'el Valley in the north. It inflicts serious losses in potato, cotton, and some vegetable crops (4,17,19,29) and occasionally occurs on ornamentals and fruit trees. In view of its broad host range and apparently low host specificity, *V. dahliae* has been considered to have little genetic diversity. However, more recent vegetative-compatibility and molecular studies suggest that there is significant genetic diversity in *V. dahliae* (23).

Vegetative compatibility refers to the ability of individual fungal strains to undergo mutual hyphal anastomosis, which results in viable heterokaryons. When a heterokaryon is established, the participating isolates are placed in the same vegetative compatibility group (VCG). In *V. dahliae*, an anamorphic fungus with no known sexual stage, hyphal anastomosis and heterokaryosis are the only means of exchanging genetic information among different strains. Thus, strains belonging to the same VCG have the potential to share a common gene pool separated from the gene pools of other VCGs. Using nitrate-nonutilizing (*nit*) mutants, three to five VCGs have been identified among *V. dahliae* strains from diverse geographic and plant sources worldwide (10–12,23,27,28). A set of isolates and *nit* mutant testers was described that represents all known VCGs (10,11,27,28). This set of reference strains was provided by R. C. Rowe (the Ohio Agricultural Research and Development Center [OARDC], Wooster).

In a previous study, 34 isolates of *V. dahliae* from six host plants in Israel were examined for vegetative compatibility (1). Two dis-

tinct VCGs were identified among 33 isolates; the status of the remaining isolate remained unclear. Using a larger number of isolates from Israel, the objectives of the current study were to (i) determine the VCGs diversity and their relatedness to internationally recognized VCGs; and (ii) reveal any association between VCG, host plant origin, regional distribution, growth characteristics, and virulence in the population.

MATERIALS AND METHODS

Isolation of *V. dahliae* from plants and soil. *V. dahliae* was isolated during 1992 to 1997 from cotton (*Gossypium hirsutum* and *G. barbadense*), tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), *Chrysanthemum* spp., potato (*Solanum tuberosum*), peanut (*Arachis hypogea*), pepper (*Capsicum annuum*), watermelon (*Citrullus lanatus*), weeds (*Astragalus* sp., *Brassica* sp., *Senecio* sp., *Urtica* sp., and *Xanthium* sp.), and soil, as previously described (15,16). One monoconidial culture was prepared from each isolate and stored on Czapek-Dox agar (CDA) at 5 to 7°C.

Generation and characterization of *nit* mutants. *nit* Mutants were generated on water agar-chlorate (WAC) medium consisting of 2% agar, 0.02% glucose, and 5% potassium chlorate (15). Mycelial plugs were placed on WAC medium at five or six points in plates (9 cm in diameter) and incubated at 24°C. Chlorate-resistant sectors, evident after 10 to 14 days, were transferred to CDA plates (5 cm in diameter). Sectors that grew on CDA as colonies with a thin, expansive mycelium were considered *nit* mutants. CDA amended with sodium nitrite (0.5 g/liter) or hypoxanthine (0.2 g/liter) was used for partial phenotyping of the *nit* mutants (7).

Vegetative compatibility grouping. Complementation between phenotypically distinct *nit* mutants was tested on CDA. Generally, each plate (5 cm in diameter) was inoculated with three mutants, 1 to 1.5 cm apart in a triangular pattern, and incubated at 24°C. Plates were scored for prototrophic growth 14 to 28 days after inocula-

Corresponding author: T. Katan; E-mail address: vpptlg@volcani.agri.gov.il

tion. Complementation was evident by the formation of a dense, aerial growth where mycelia from two mutants had met and formed a prototrophic heterokaryon. When mutants of two different strains formed a heterokaryon, their parents were assigned to the same VCG. Each pairing was performed at least twice. Twelve reference strains (10,23) were provided by R. C. Rowe (OARDC, Wooster). To compare the VCGs from Israel with the internationally recognized VCGs of *V. dahliae*, *nit* mutants generated from each of the reference strains were used as testers in complementation tests with *nit* mutants representing the local VCGs.

Effect of temperature on mycelial growth. Isolate response to temperature was determined with respect to radial growth rate on agar medium and to biomass production in liquid culture. Radial growth rate was determined at eight temperatures (10 to 35°C). Mycelial plugs were centrally inoculated on 9-cm-diameter CDA plates and incubated in triplicate at the various temperatures. The diameter of the growing colonies was recorded 7, 14, and 21 days after inoculation, and the area under the growth curve (AUGC) was calculated using a formula for area under disease progress curve (AUDPC) (5). Pigmentation and the quantity of aerial mycelium

were visually estimated. This experiment was repeated twice. To compare biomass production, 1 million conidia were added to 250-ml flasks containing 75 ml of potato dextrose broth. The liquid cultures were kept still in the dark at 21, 24, or 27°C. After 4 weeks of incubation, mycelia were harvested by filtration, lyophilized, and weighed. The experiment was run in triplicate and repeated once.

Pathogenicity tests. Pathogenicity of *V. dahliae* was determined by the root-dip method using eggplant (*Solanum melongena* cv. Black Beauty) and cotton (*Gossypium hirsutum* cv. Acala SJ-2) as differential hosts. Inoculum was prepared by macerating the contents of 14-day-old CDA cultures with 150 ml of water for 30 s using a Waring blender (Waring Products, New Hartford, CT). Seedlings at the cotyledon stage were uprooted from the substrate, and their roots were washed in tap water, trimmed, and dipped in the inoculum (1 to 5×10^6 conidia per ml) for 3 min. Noninoculated control seedlings were dipped in water. Each isolate was inoculated on 15 seedlings of each host plant. Seedlings were then transplanted (five per pot) to 700-ml pots filled with Rehovot sandy soil (3.8% clay, 0.0% silt, 96.2% sand, and 0.4% organic matter; pH 6.9), and maintained in a greenhouse at 24 to 27°C and a 12-h

TABLE 1. Isolates of *Verticillium dahliae* from Israel, listed by vegetative compatibility group (VCG), source, and pathogenicity

VCG	Isolate	Source		Pathogenicity		
		Host	Site	AUDPC (%) ^v		Pathotype ^w
				Cotton	Eggplant	
2A	chr9	Chrysanthemum	Bet Alpha	13.9 c	26.9 c	ND2
	cot150	Cotton	Gan Shmuel	19.6 c	39.5 b	ND2
	ep8	Eggplant	Kfar Azza	10.7 c	41.6 b	ND2
	ep30	Eggplant	Mishmar Ayalon	...	...	...
	ep4	Eggplant	Tamra	6.1 c	40.9 b	ND2
	ep5	Eggplant	Tamra	10.3 c	42.4 b	ND2
	pn12	Peanut	Nir Oz	...	...	...
	pt44.3G	Potato	Kerem Shalom	12.6 c	29.0 b	ND2
	7 isolates	Potato	4 sites	...	...	...
	tom24	Tomato	Ein Shemer	10.8 c	40.4 b	ND2
	11 isolates	Tomato	4 sites	...	...	...
	tom1	Tomato	Unknown	10.0 c	45.7 b	ND2
	Subtotal	28 isolates	6 hosts	14 sites		
2B	chr10	Chrysanthemum	Bet Alpha	1.6 c	0.6 c	Avirulent
	chr11	Chrysanthemum	Bet Alpha	33.8 b	66.4 a	DL2
	chr12	Chrysanthemum	Bet Alpha	9.8 c	59.7 a	ND3
	chr13	Chrysanthemum	Bet Alpha	6.1 c	70.3 a	ND3
	cot79	Cotton	Dalia	58.8 a	16.4 c	DL1
	cot17	Cotton	Ein Shemer	62.9 a	14.2 c	DL1
	cot117	Cotton	Ein Shemer	74.5 a	14.7 c	DL1
	cot135	Cotton	Ein Shemer	60.3 a	18.8 c	DL1
	cot153	Cotton	Ein Shemer	80.5 a	18.6 c	DL1
	cot202	Cotton	Gadot	...	...	...
	cot5	Cotton	Galed	55.9 a	18.1 c	DL1
	cot178	Cotton	Galed	62.3 a	20.5 b	DL1
	cot2	Cotton	Gan Shmuel	59.6 a	18.4 c	DL1
	cot19	Cotton	Gan Shmuel	56.9 a	8.3 c	DL1
	cot20	Cotton	Gan Shmuel	73.5 a	26.4 b	DL1
	cot149	Cotton	Gan Shmuel	68.4 a	22.7 b	DL1
	cot18	Cotton	Kfar Hahoreshe	71.8 a	2.7 c	DL1
	cot112	Cotton	Kfar Hahoreshe	65.4 a	11.0 c	DL1
	cot15	Cotton	Ramat David	55.9 a	18.6 c	DL1
	cot60	Cotton	Ramat David	80.1 a	15.4 c	DL1
	cot62	Cotton	Ramat David	73.4 a	23.0 b	DL1
	cot63	Cotton	Ramat David	65.9 a	16.8 c	DL1
	cot64	Cotton	Ramat David	26.0 b	6.6 c	DL1
	cot128	Cotton	Ramat David	58.0 a	25.9 b	DL1
	114 isolates	Cotton	6 sites	...	...	...

(continued on the next page)

^v Area under disease progress curve, expressed as percentage of the maximum possible area for a 35-day period. In each column, values followed by the same letter belong to the same cluster of mean values using the K-means clustering method. ... = Not tested.

^w ND = cotton nondefoliating, and DL = cotton defoliating-like. ND1 = cotton-ND pathotype that caused mild symptoms on eggplant, ND2 = cotton-ND pathotype that caused moderate to strong symptoms on eggplant, and ND3 = cotton-ND pathotype that caused severe symptoms on eggplant. DL1 = cotton-DL pathotype that caused mild to moderate symptoms on eggplant, and DL2 = cotton-DL pathotype that caused severe symptoms on eggplant.

^x From potato fields.

^y From cotton fields.

^z Heterokaryon self-incompatible.

photoperiod for 35 days. Mean disease severity index was assessed 2, 3, 4, and 5 weeks after inoculation on an arbitrary five-grade scale (0 = no visible symptoms, 4 = dead plant), essentially as previously described (1). A total of six greenhouse experiments was performed: 3 isolates were included in each of them as standards, and 58 additional isolates were tested one to three times. Data of experiments that did not differ significantly based on the standard isolates were combined, and AUDPCs were calculated and expressed as percentage of the maximum possible area for the 35-day periods of the experiments (2,5).

Data analysis. Biomass data for the temperature experiments were subjected to two-way analysis of variance. AUGC data of the temperature experiments were subjected to analysis of covariance, using temperature as linear and quadratic covariates. Pairs of means were compared, thereafter, by contrast *t* tests. AUDPC data of the pathogenicity experiments were subjected to the K-means clustering algorithm in order to create clusters of similar mean values. Each of the five clusters was then used to represent a pathotype, and the pathotypes were subjected to one-way analysis of variance. To compare different pathotypes, pairs of means were sub-

jected to contrast *t* tests. All tests were performed at *P* = 0.05 using the Statistical Analysis System (version 6.12 or JMP 3.2.2 software, SAS Institute Inc., Cary, NC).

RESULTS

Isolates of *V. dahliae*. A total of 565 isolates of *V. dahliae* from Israel was used in this study. Isolates were recovered from crop plants with wilt symptoms, cotton gin waste, symptomless weeds, and soil. The isolates originated from 13 host plants at 47 sites in Israel. Most of the isolates were recovered from potato (42%) and cotton (33%); the remainder came from eggplant, tomato, peanut, pepper, chrysanthemum, watermelon, weeds (five species), and soil (Table 1; Fig. 1); 173 isolates (mostly potato isolates from the Negev region) were obtained from L. Tsrur (Gilat experimental station).

Generation and characterization of *nit* mutants. WAC, a water agar-based chlorate medium developed for generating *nit* mutants from *V. dahliae* (15), was used. On WAC amended with 5% chlorate, radial growth of wild-type strains was greatly restricted, and chlorate resistance appeared as sectors growing from the inoculum

TABLE 1. (continued from the preceding page)

VCG	Isolate	Source		Pathogenicity		
		Host	Site	AUDPC (%) ^y		Pathotype ^w
				Cotton	Eggplant	
	ps1	Cotton waste	Meitav	...	...	...
	lap2	Weed ^x	Ramat David	61.7 a	16.6 c	DL1
	ep1	Eggplant	Ganey Yohanan	8.9 c	18.6 c	ND1
	ep6	Eggplant	Ganey Yohanan	9.6 c	24.1 b	ND1
	ep7	Eggplant	Ganey Yohanan	10.2 c	21.7 b	ND1
	ep2	Eggplant	Tamra	3.8 c	55.8 a	ND3
	ep3	Eggplant	Tamra	2.9 c	59.1 a	ND3
	3 isolates	Eggplant, weed	Ramat David	...	...	...
	s2	Pepper	Lahav	10.6 c	61.0 a	ND3
	7 isolates	Pepper	Lahav	...	...	...
	tom20	Tomato	Segula	...	...	...
	tom28	Tomato	Segula	5.9 c	88.3 a	ND3
Subtotal	158 isolates	6 hosts	13 sites			
4B	cot24	Cotton	Be'ery	16.4 c	36.4 b	ND2
	cot25	Cotton	Be'ery	9.3 c	34.5 b	ND2
	cot26	Cotton	Be'ery	4.7 c	31.5 b	ND2
	cot13	Cotton	Bet Kamma	13.9 c	38.4 b	ND2
	cot85	Cotton	Bet Kamma	10.6 c	43.0 b	ND2
	cot129	Cotton	Ein Hashlosa	9.4 c	35.8 b	ND2
	cot133	Cotton	Ein Hashlosa	7.8 c	46.4 b	ND2
	cot92	Cotton	Kfar Azza	11.4 c	44.8 b	ND2
	cot93	Cotton	Kfar Azza	11.5 c	49.2 b	ND2
	cot94	Cotton	Kfar Azza	13.2 c	27.3 b	ND2
	52 isolates	Cotton	5 sites	...	...	...
	cot12	Cotton	Re'im	45.2 b	43.6 b	DL2
	cot22	Cotton	Zorrah	56.3 a	52.4 a	DL2
	cot23	Cotton	Zorrah	57.5 a	61.9 a	DL2
	ep11	Eggplant	Kfar Azza	7.5 c	30.4 b	ND2
	ep12	Eggplant	Kfar Azza	9.4 c	29.5 b	ND2
	18 isolates	Eggplant	4 sites	...	...	...
	s2.1	Pepper	Lahav	8.3 c	31.7 b	ND2
	s2.2	Pepper	Lahav	5.3 c	26.3 b	ND2
	9 isolates	Peanut	Nir Oz, Re'im	...	...	...
	pn6	Peanut	Re'im	15.7 c	32.1 b	ND2
	pt15	Potato	Re'im	15.9 c	29.3 b	ND2
	pt21	Potato	Re'im	19.1 c	45.9 b	ND2
	pt26	Potato	Re'im	13.5 c	34.9 b	ND2
	232 isolates	Potato	27 sites	...	...	...
	tom5	Tomato	Ohad	8.8 c	32.7 b	ND2
	16 isolates	Tomato	3 sites	...	...	...
	wml	Watermelon	Unknown	...	...	...
	6 isolates	Weeds ^x	3 sites	...	...	...
	15 isolates	Soil ^y	Be'ery	...	...	...
	7 isolates	Soil ^x	Nir Oz	...	...	...
Subtotal	378 isolates	12 hosts	35 sites			
HSI ^z	tom2G	Tomato	Segula	...	...	...
Total	565 isolates					

plugs or as fanlike sectors at the colony perimeter. Only sectors that grew on CDA as expansive colonies with a thin mycelium were considered *nit* mutants. Of the examined chlorate-resistant sectors, 80 to 100% were *nit* mutants, and at least one *nit* mutant was recovered from each of the 565 *V. dahliae* isolates. A total of about 2,000 *nit* mutants was recovered and phenotypes were determined. Most mutants grew profusely (similar to wild type) on CDA with nitrite or hypoxanthine and were accordingly classified as *nit1*. Some (6%) of the *nit* mutants produced expansive thin mycelium on CDA with hypoxanthine and were classified as NitM. A few mutants grew very slowly on nitrite or developed moderately dense rather than expansive thin mycelium on this medium. Such mutants grew as expansive thin colonies on CDA and complemented NitM but not normal *nit1* mutants and were, therefore, considered *nit1*. No *nit3* mutants could be recognized.

In 564 out of the 565 isolates, complementary heterokaryons formed between different mutant phenotypes (*nit1* and NitM) derived from the same or different parental strains. One isolate was self-incompatible. Between three and five complementation groups were found among NitM mutants within each of the local VCGs.

Fig. 1. Regional distribution of vegetative compatibility groups (VCGs) of *Verticillium dahliae* in Israel.

Assignment of strains to VCGs. A group of 46 *V. dahliae* isolates from various host plants and locations was selected for initial tests. Complementary *nit* mutants (*nit1* and NitM) derived from each isolate were paired in all possible interisolate combinations (including some reciprocal pairings). Based on positive complementation reactions (Figs. 2A and 3B), three VCGs (initially designated I, II, and III) were identified among the 46 isolates tested. Complementary *nit* mutants that demonstrated the ability to form strong

Fig. 2. Complementary heterokaryon formation between nitrate-nonutilizing mutants of *Verticillium dahliae*. **A**, Strong complementation at the contact zone between mutants belonging to vegetative compatibility group (VCG) 2B; and **B**, weak complementation, in the form of black dots, at the contact zone between mutants belonging to VCG2A and VCG2B.

Fig. 3. Variation in colony growth and pigmentation on Czapek-Dox agar among vegetative compatibility groups (VCGs) of *Verticillium dahliae*. **A**, Three isolates from each VCG were grown in the dark at 24°C for 21 days (plates 9 cm in diameter). Top row: VCG2B isolates cot149, chr13, and ep1; middle row: VCG4B isolates pn10, pt45.4G, and tom5; and bottom row: VCG2A isolates cot150, chr9, and tom18. **B**, Heterokaryons of VCG2B (left) and VCG4B (right) (plates 5 cm in diameter, 14 days at 24°C).

heterokaryons with many other mutants were chosen as representative tester strains for each local VCG. The remaining 518 isolates were then assigned to these three VCGs based on the reaction of their *nit* mutants in pairings with the tester strains, and no additional VCGs were recovered. To compare the VCGs in Israel with those previously described, representative testers of the three local VCGs were paired with tester strains of the OARDC international system. Strong complementation was observed in pairings of VCG I from Israel with the international VCG2A, of VCG II with VCG2B, and of VCG III with VCG4B (Table 2). Thus, 28 isolates were assigned to VCG2A, 158 isolates to VCG2B, and 378 to VCG4B (Table 1). Partial compatibility, in the form of small dots of microsclerotia at the contact zone (Fig. 2B) was sometimes observed between testers of different VCGs (Table 2).

Regional distribution of *V. dahliae* VCGs. Of the VCG2B isolates, 92% were recovered from the northern part of Israel, and 90% of VCG4B isolates were from the south. Some overlap was observed in the central region (Fig. 1; Table 3). Isolates of the minor group VCG2A were scattered among the two major VCGs, ranging from 8% (north) to 3% (south) of the isolates tested. VCGs essentially did not correlate with host plant of origin: all crops from the south (cotton, eggplant, peanut, potato, tomato, and weeds) were infected with VCG4B and seldom with VCG2A (eggplant, peanut, potato, and tomato), whereas all crops (except tomato) from the north (cotton, eggplant, chrysanthemum, and weeds) were infected with VCG2B and sometimes with VCG2A (cotton, eggplant, and chrysanthemum). Tomato was a notable exception: unlike the VCG4B isolates from tomato in the south, which reflected the prevailing VCG in that region, only VCG2A was recovered from tomatoes in two sites in the north (Ein Shemer and Gan Shmuel), despite the fact that cotton plants from the fields surrounding these tomato fields were infected with VCG2B.

Morphology of *V. dahliae* strains. Morphological differences were evident between isolates from different VCGs. When grown on CDA, isolates of VCG2B formed black or dark gray colonies with little or no aerial mycelium (Fig. 3A). In contrast, most of the VCG4B isolates grew as colonies with dense aerial mycelium, were light gray or white with gray spots and sectors, and became dark gray only with age. The morphology of complementary heterokaryons in pairings of compatible *nit* mutants reflected the morphology of the wild-type parents: heterokaryons of VCG2B were black with abundant microsclerotia and little aerial mycelium, whereas heterokaryons of VCG4B grew slowly with more aerial mycelium and fewer, scattered microsclerotia (Fig. 3B).

All microstructures of VCG2B isolates were essentially larger than those of VCG4B. The very abundant, irregularly shaped microsclerotia of VCG2B isolates consisted of large cells, whereas the microsclerotia of VCG4B consisted of small cells that were more spherical and scattered (Fig. 4). VCG4B isolates produced abundant conidiophores that were often simple or with few phialide-bearing nodes, while those of VCG2B were less abundant, larger, and with more nodes. VCG2A isolates exhibited intermediate morphology between VCG2B and VCG4B (Fig. 4).

Effect of temperature on growth of *V. dahliae*. Of the isolates belonging to the three VCGs (5 isolates from VCG2A, 15 from VCG2B, and 15 from VCG4B) and originating from various hosts and locations, 35 were compared for growth rate on CDA at eight temperatures (10 to 35°C). AUGCs for a 21-day period and temperature optimum were determined and melanization of colonies was estimated. Variation was observed among *V. dahliae* isolates and VCGs with respect to temperature growth response. VCG2A and VCG4B isolates had temperature optima of 24 to 27°C, and VCG2A grew faster than VCG4B isolates at 18 to 27°C (Table 4). VCG2B isolates cumulatively showed lower and broad optimal growth temperatures, ranging from 18 to 24°C. This VCG was heterogenic: nine isolates from cotton fields in Yizre'el Valley (Galed,

TABLE 3. Host and regional source of vegetative compatibility groups (VCGs) of *Verticillium dahliae* in Israel

Source	Host	Number of isolates				
		VCG2A	VCG2B	VCG4B	HSI ^z	Total
North	Cotton	1	135	0	0	136
	Tomato	9	0	0	0	9
	Eggplant	3	4	0	0	7
	Chrysanthemum	1	4	0	0	5
	Weed	0	2	0	0	2
Center and south	Potato	8	0	235	0	243
	Cotton	0	0	65	0	65
	Tomato	4	2	17	1	24
	Eggplant	1	3	20	0	24
	Soil	0	0	22	0	22
	Peanut	1	0	10	0	11
	Pepper	0	8	2	0	10
	Weeds	0	0	6	0	6
	Watermelon	0	0	1	0	1
Total	...	28	158	378	1	565

^z Heterokaryon self-incompatible.

TABLE 2. Complementary heterokaryon formation between representative nitrate-nonutilizing (*nit*) testers from Israel and the international Ohio Agricultural Research and Development Center (OARDC) reference strains of vegetative compatibility groups (VCGs) of *Verticillium dahliae*

		NitM testers/VCG								
<i>nit1</i> Testers	VCG	T9 ^x /VCG1	PH ^x /VCG2A	ep8 ^y /VCG I	115 ^x /VCG2B	cot11 ^y /VCG II	PCW ^x /VCG3	BB ^x /VCG4A	S39 ^x /VCG4B	pt15 ^y /VCG III
T9 ^x	1	+ ^z	—	—	—	+/—	—	—	—	—
V44 ^x	1	+	—	—	—	—	—	—	—	—
442 ^x	2A	—	+	+	+/—	—	—	—	—	—
ph ^x	2A	—	+	+	—	—	—	—	—	—
ep5 ^y	I	—	+	+	—	—	—	—	—	—
tom1 ^y	I	—	+	+	+/—	—	—	—	+/—	—
115 ^x	2B	—	—	—	+	+	—	—	—	—
MC ^x	2B	—	+/—	+/—	+	+	—	—	—	—
cot64 ^y	II	+/—	—	—	+	+	—	—	—	—
chr11 ^y	II	—	—	—	+	+	—	—	—	—
70-21 ^x	3	—	—	—	—	—	+	—	—	—
PCW ^x	3	—	—	—	—	—	+	—	—	—
BB ^x	4A	—	—	—	—	+/—	+/—	+	—	—
P105 ^x	4A	—	—	—	+/—	—	—	+	—	—
P273 ^x	4B	—	—	—	—	—	—	—	+	+
S39 ^x	4B	—	—	—	—	—	—	+/—	+	+
pt24 ^y	III	—	—	—	—	—	—	+/—	+	+
pt67 ^y	III	—	—	—	—	—	—	—	+	+

^x OARDC reference strain (10).

^y Israeli tester strain.

^z + = Dense prototrophic growth, — = prototrophic growth absent or inconspicuous, and +/- = small microsclerotial dots with or without a little aerial mycelium.

Dalia, and Ramat David) grew fastest at 18 to 21°C, whereas six isolates from other sites (Ein Shemer, Kfar Hahores, and Ganey Yohanan) grew fastest at 21 to 24°C. None of the tested *V. dahliae* isolates grew at 35°C.

Higher temperatures (27 to 30°C) influenced the melanization of microsclerotia differently in the three VCGs. Melanization was completely suppressed in VCG4B strains and partially reduced in VCG2A, whereas VCG2B was, again, heterogenic. The VCG2B strains from Yizre'el Valley formed abundant black microsclerotia, with no apparent effect from the high temperatures, although the colonies became very stunted. Other VCG2B isolates formed non-melanized colonies at 27 to 30°C. A self-incompatible isolate from tomato had the same temperature responses as two tomato isolates belonging to VCG2A. Biomass production in liquid culture was compared at three temperatures (Table 5). VCG2B isolates (Yisre'el Valley) grew more slowly than the other two VCGs, and high temperature (27°C) sharply reduced their growth.

Pathogenicity. Out of 61 isolates tested (Table 1), 60 were pathogenic to cotton and eggplant and 1 strain (chr10) was avirulent (or very slightly pathogenic; AUDPC <2% of the maximum possible area). The 60 pathogenic isolates varied in virulence on both host plants, as evident by comparing their AUDPC values. First, isolates that incited mild symptoms on cotton (AUDPC ≤20%) were defined as a nondefoliating (ND) pathotype. Other isolates caused severe foliar symptoms, stunting, and often death, but little or no defoliation of inoculated cotton plants (AUDPC ≥26%; for most isolates, >55%), and were defined as a defoliating-like (DL) pathotype. Second, differential aggressiveness of the isolates on eggplant was used to further define three subgroups among the ND pathotype and two subgroups within the DL pathotype. The subgroups were statistically supported by cluster analysis. Thus, isolates of

the cotton-ND pathotype that caused mild symptoms on eggplant (AUDPC ≤24%) were defined as pathotype ND1, those causing moderate to strong symptoms (AUDPC 26 to 50%) were defined as pathotype ND2, and those that caused severe symptoms (AUDPC ≥55%) were defined as ND3. Similarly, within the cotton-DL pathotype, isolates that caused mild to moderate symptoms on eggplant (AUDPC ≤26%) were defined as pathotype DL1 and those causing severe symptoms (AUDPC ≥43%) were defined as pathotype DL2. Irrespective of their host or regional origin, all VCG2A and 86% of the VCG4B isolates (19 out of 22) were of the ND2 pathotype, inducing mild wilting in cotton but moderate to strong levels of disease in eggplant (Table 1; Fig. 5). The remaining three VCG4B isolates from cotton were highly aggressive to both differential hosts (DL2 pathotype). Greater variation in pathogenicity was found in VCG2B. In this group, all the isolates from cotton (including the less virulent cot64) originating from six sites were of the DL1 pathotype, causing severe wilt in cotton and mild to moderate symptoms in eggplant. The remaining VCG2B isolates, of noncotton origin, were highly variable and included pathotypes DL2, ND1, ND3, and one avirulent isolate.

DISCUSSION

The world population of *V. dahliae* has been found to comprise three main VCGs, designated VCG1, VCG2, and VCG4 (23); each of these VCGs has been divided into two subgroups based on quantitative differences in complementation reactions among *nit* mutants (12). Three VCGs were identified among 565 isolates of *V. dahliae* from Israel. VCG2 in Israel is represented by its two known subgroups (2A and 2B). Subgroups A and B of VCG2 in Israel appear to be distinct entities, distinguished by their growth rate, morphol-

Fig. 4. Variation in microsclerotial shape and pigmentation among vegetative compatibility groups (VCGs) of *Verticillium dahliae*. From left to right: VCG2B, VCG2A, and VCG4B (bars = 50 µm, all pictures are the same magnification).

TABLE 4. Effect of temperature on radial growth of *Verticillium dahliae*

VCG ^x	No. of isolates	Mean AUGC at each temperature ^y							
		10°C	15°C	18°C	21°C	24°C	27°C	30°C	35°C
VCG2A	5	394 aD ^z	663 aB	740 aB	746 aB	878 aA	896 aA	544 aC	0
VCG2B	15	370 aD	591 aB	714 aA	766 aA	707 bA	624 bB	426 bC	0
VCG4B	15	400 aC	571 aB	604 bB	610 bB	737 bA	729 bA	445 bC	0

^x Vegetative compatibility group.

^y AUGC = area under the growth curve (Czapek-Dox agar, 3 weeks).

^z In each column, values with different lower-case letters are significantly different according to contrast *t* tests (*P* = 0.05). For each VCG (horizontal row), upper-case letters refer to differences between temperatures.

ogy, pathogenicity, and regional distribution. Interactions between complementary *nit* mutants of VCG2A and VCG2B were absent or very weak, often only forming a line of small microsclerotial dots, with very little or no aerial mycelium. No vigorous heterokaryosis has ever been described between any testers of VCG2A and VCG2B, and combining them into VCG2 is based on frequent but weak complementation between their testers (10,28). This interaction is similar to that occasionally observed between testers of different VCGs.

Most isolates from Israel belonged to VCG2B and VCG4B. These major VCGs formed two main regional populations, VCG2B in the north and VCG4B in the south, with some overlap in the center, whereas isolates of the minor group VCG2A were scattered throughout all regions among the major VCGs. In contrast, VCG2A is common in cotton fields in Spain, where no VCG2B strains have been found (14). VCGs clearly corresponded with some cultural traits and partially with pathogenicity. Using 'Acala SJ-2', two cotton pathotypes were evident: the ND pathotype caused weak to moderate symptoms, whereas the DL pathotype caused severe symptoms with little or no defoliation. The existence of cotton-defoliating (D) and -ND pathotypes in the United States and elsewhere has long been recognized (2,3,8,26). Cotton isolate 115 from Syria, which had been reported as causing intermediate-type disease on 'Acala 4-42' (25), was later assigned to VCG2B (10) and now serves as one of its reference strains. The cotton-DL isolates from the north of Israel and the "intermediate" isolate 115 from neighboring Syria may be considered members of the same geographical population. In this study, the cotton-DL pathotype in Israel was represented by VCG2B cotton isolates, along with three VCG4B cotton isolates and one VCG2B chrysanthemum isolate. Irrespective of their host or regional origin, all VCG2A, most of VCG4B, and a few noncotton VCG2B isolates belonged to the cotton-ND pathotype. Using eggplant as an additional differential host allowed further strain characterization, by which each cotton pathotype was divided into subgroups. Overall, the VCG and pathotype heterogeneity of the *V. dahliae* population in Israel coexists with distinctly localized homogeneity; occurrence of more than one VCG or pathotype in the same field was rare, except for the presence of VCG2A isolates among the major VCGs. This situation should enable regional confinement of the most dangerous pathotypes. Differential susceptibility of plant species to different pathotypes (VCGs) should be considered in resistance breeding, crop rotation, and other strategies of disease management.

VCG4 is common in *Verticillium* wilt-affected potato fields in North America, where it consists of two or more subgroups apparently varying in pathogenicity (3,11,12,20,27). *V. dahliae* is known to have been introduced with infected potato seed tubers from Europe into the Negev region (southern Israel), where it became established in many fields with expanding potato cultivation (4,17, 21). The presence in Israel of only subgroup VCG4B probably manifests a founder effect (18). VCG2, but not VCG4, has been reported from western and central Asia and China (3,8,10,22,25, 30), suggesting that VCG2 might be native (endemic) to Asia. Situ-

ated since ancient times at the crossroads of the world's human civilizations, the Mediterranean Basin is infested with all known VCGs of *V. dahliae*: VCG1, VCG2A, and VCG4B have been found in Spain (14); VCG2, VCG3, and VCG4 have been reported from Greece (9); and now we show that VCG2A, VCG2B, and VCG4B are present in Israel.

Selective pressure exerted by the main crops in Israel apparently enhanced the establishment of two main homogeneous populations of *V. dahliae*: the DL1 pathotype of VCG2B in cotton fields in the north and the ND2 pathotype of VCG4B in potato fields (with cotton as part of the crop rotation) in the south. Noncotton or nonpotato hosts from distinct, local foci on the periphery of these regional populations provide additional, less-common pathotypes. Thus, VCG2B isolates of the ND3 pathotype were recovered from chrysanthemum and eggplant at two sites in the north and from pepper and tomato at two sites in the south. *V. dahliae* isolates recovered in 1992 from eggplant in Ganey Yohanan (the central region) belonged to VCG2B and were of the ND1 pathotype, whereas isolates recovered from the same site and host in 1997 belonged to VCG4B and were of the ND2 pathotype, demonstrating an apparent northbound spread of the VCG4B population. The less-common VCG2B pathotypes (DL2, ND1, and ND3) may represent the survivors of an ancient *V. dahliae* population that inhabited the region prior to the large-scale cultivation of cotton and potato. The highly aggressive DL2 pathotype includes a VCG2B isolate from chrysanthemum (north) and VCG4B isolates from cotton at two sites. The latter are the only examples of heterogeneity within VCG4B, which is predominantly of the ND2 pathotype.

Higher temperatures (27 to 30°C) suppressed microsclerotial melanization in VCG4B isolates commonly associated with potato, but did not suppress this process in VCG2B isolates originating from cotton. Microsclerotial melanization is an important survival factor. Potato is a temperate-climate crop, while cotton is a hot-climate crop, and the differential temperature response of the two VCGs apparently reflects an ecological adaptation of the pathogen to various environments.

So far, vegetative compatibility has been studied in five *Verticillium* spp. Genetic diversity in populations of the strongly pathogenic species *V. albo-atrum* and *V. dahliae* has been found to be low: two VCGs were detected among 28 isolates of *V. albo-atrum* from Europe and America (6), and three to five VCGs among several hundred *V. dahliae* isolates from numerous hosts and countries (12,23). In contrast, VCG diversity in the weak plant pathogens or soil saprophytes *V. nigrescens* and *V. tricorpus* appears to

TABLE 5. Effect of temperature on biomass production in liquid cultures of *Verticillium dahliae*

VCG ^x	No. of isolates	Weight (g per flask) at each temperature ^y		
		21°C	24°C	27°C
VCG2A	2	0.43 aA ^z	0.50 aA	0.33 aB
VCG2B	5	0.38 aA	0.36 bA	0.16 bB
VCG4B	5	0.37 aA	0.42 abA	0.32 aA

^x Vegetative compatibility group.

^y Mean dry weight of mycelium. Isolates were grown in potato dextrose broth for 4 weeks.

^z In each column, values with different lower-case letters are significantly different according to contrast *t* tests (*P* = 0.05). For each VCG (horizontal row), upper-case letters refer to differences between temperatures.

Fig. 5. Pathotypes of *Verticillium dahliae* defined by disease severity in cotton and eggplant. AUDPC = area under disease progress curve. DL1 and DL2 = subgroups within the cotton defoliating-like (DL) pathotype; ND1, ND2, and ND3 = subgroups within the cotton-nondefoliating (ND) pathotype. For each test plant, columns designated by the same letter are not significantly different according to contrast *t* tests at *P* = 0.05.

be higher: 9 VCGs were found among 26 isolates of *V. tricornis* and 11 VCGs among 19 isolates of *V. nigrescens*, recovered from plants and soil in several locations in Israel (16). The entomopathogen *V. lecanii* seems to differ from both the strong and weak plant pathogens in exhibiting a high proportion of self-incompatibility and a high frequency of incompatibility between self-compatible strains (13).

This study demonstrates that different VCGs have distinct pathogenicity, macroscopic and microscopic morphology, and temperature-dependent growth rates. This finding indicates that strains belonging to the same VCG share common traits, possibly reflecting a common history. Thus, the study of VCGs in *Verticillium* spp. can suggest relatedness among certain strains within the pathogen population, shedding light on their evolutionary relationship and, often, predicting their pathogenic potential.

ACKNOWLEDGMENTS

This research was supported, in part, by a fellowship (for N. Korolev) from the Israeli Ministry of Immigration, by the United States-Israel Binational Agricultural Research and Development Fund (BARD Project US-2659-95), by the research fund of the Chief Scientist of Israel Ministry of Agriculture, and by the Israel Cotton Production and Marketing Board. Contribution 508/99 from the ARO Institute of Plant Protection. We thank R. C. Rowe for providing the international reference strains of the *Verticillium dahliae* VCGs; L. Tsrer for isolates of *V. dahliae*; G. Fishler and Y. Peretz for long-term cooperation; and E. Dubitzki, G. Elal, A. Gamliel, A. Grinstein, D. Levy, Y. Sachs, D. Sadeh, and T. Sando for assistance in collecting diseased plants.

LITERATURE CITED

- Bao, J. R., Katan, J., Shabi, E., and Katan, T. 1998. Vegetative-compatibility groups in *Verticillium dahliae* from Israel. *Eur. J. Plant Pathol.* 104: 263-269.
- Bejarano-Alcazar, J., Melero-Vera, J. M., Blanco-Lopez, M. A., and Jimenez-Diaz, R. M. 1995. Influence of inoculum density of defoliating and nondefoliating pathotypes of *Verticillium dahliae* on epidemics of verticillium wilt of cotton in Southern Spain. *Phytopathology* 85:1474-1481.
- Bell, A. A. 1994. Mechanisms of disease resistance in *Gossypium* species and variation in *Verticillium dahliae*. Pages 225-235 in: *Proc. World Cotton Res. Conf.* 1. G. A. Constable and N. W. Forrester, eds. CSIRO, Melbourne, Australia.
- Ben-Yephet, Y., and Szmulewicz, Y. 1985. Inoculum levels of *Verticillium dahliae* in the soils of the hot semi-arid region of Israel. *Phytoparasitica* 13:193-200.
- Campbell, C. L., and Madden, L. V. 1990. *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, Inc., New York.
- Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. *Phytopathology* 78:1017-1021.
- Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77:1640-1646.
- Daayf, F., Nicole, M., and Geiger, J. P. 1995. Differentiation of *Verticillium dahliae* populations on the basis of vegetative compatibility and pathogenicity on cotton. *Eur. J. Plant Pathol.* 101:69-79.
- Elena, K., and Paplomatas, E. J. 1998. Vegetative compatibility groups within *Verticillium dahliae* isolates from different hosts in Greece. *Plant Pathol.* 47:635-640.
- Joaquim, T. R., and Rowe, R. C. 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology* 80:1160-1166.
- Joaquim, T. R., and Rowe, R. C. 1991. Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. *Phytopathology* 81:552-558.
- Katan, T. 2000. Vegetative compatibility in populations of *Verticillium*—An overview. Pages 77-94 in: *Advances in Verticillium Research and Disease Management*. E. Tjamos, R. C. Rowe, J. B. Heale, and D. Fravel, eds. The American Phytopathological Society, St. Paul, MN.
- Korolev, N., and Gindin, G. 1999. Vegetative compatibility in the entomopathogen *Verticillium lecanii*. *Mycol. Res.* 103:833-840.
- Korolev, N., Jimenez-Diaz, R., Katan, J., Perez-Artes, E., Garcia-Pedrajas, M., Bejarano-Alcazar, J., Rodriguez-Jurado, D., and Katan, T. 2000. Comparative study of vegetative compatibility and pathogenicity among cotton isolates of *Verticillium dahliae* from Spain and Israel. Pages 117-119 in: *Advances in Verticillium Research and Disease Management*. E. Tjamos, R. C. Rowe, J. B. Heale, and D. Fravel, eds. The American Phytopathological Society, St. Paul, MN.
- Korolev, N., and Katan, T. 1997. Improved medium for selecting nitrate nonutilizing (*nit*) mutants of *Verticillium dahliae*. *Phytopathology* 87: 1067-1070.
- Korolev, N., and Katan, T. 1999. Vegetative compatibility grouping in *Verticillium nigrescens* and *V. tricornis*. *Mycol. Res.* 103:65-76.
- Krikun, J., and Orion, D. 1979. Verticillium wilt of potato: Importance and control. *Phytoparasitica* 7:107-116.
- McDonald, B. 1997. The population genetics of fungi: Tools and techniques. *Phytopathology* 87:448-453.
- Nachmias, A., Kaufman, Z., Livescu, L., Tsrer, L., Meiri, A., and Caligari, P. D. S. 1993. Effects of salinity and its interaction with disease incidence on potato grown in hot climates. *Phytoparasitica* 21:245-255.
- Omer, M. A., and Rowe, R. C. 1998. Virulence of two *Verticillium dahliae* pathotypes isolated from potato seed tubers. (Abstr.) *Phytopathology* 88 (suppl.):S69.
- Pegg, G. F. 1974. Verticillium diseases. *Rev. Plant Pathol.* 53:157-182.
- Portenko, L. G., and Akimov, G. I. 1997. Vegetative compatibility among *Verticillium dahliae* populations of cotton-growing regions of the Middle Asia. (Abstr.) Page 93 in: *Proc. 7th Int. Verticillium Symp.*
- Rowe, R. C. 1995. Recent progress in understanding relationships between *Verticillium* species and subspecific groups. *Phytoparasitica* 23: 31-38.
- Schnathorst, W. C. 1981. Life cycle and epidemiology of *Verticillium*. Pages 113-144 in: *Fungal Wilt Diseases of Plants*. M. E. Mace, A. A. Bell, and C. H. Beckman, eds. Academic Press, New York.
- Schnathorst, W. C., and Fogle, D. 1976. Comparative virulence and other characteristics of *Verticillium dahliae* isolates from cotton plants in the United States, USSR, Syria and Iran. Pages 22-23 in: *Proc. Beltwide Cotton Prod. Res. Conf. Cotton Disease Council*, Memphis, TN.
- Schnathorst, W. C., and Mathre, D. E. 1966. Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. *Phytopathology* 56:1155-1161.
- Strausbaugh, C. A. 1993. Assessment of vegetative compatibility and virulence of *Verticillium dahliae* isolates from Idaho potatoes and tester strains. *Phytopathology* 83:1253-1258.
- Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G. 1992. Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. *Phytopathology* 82: 61-68.
- Tsrer, L., Erlich, O., Amitai, S., and Hazanovsky, M. 1998. Verticillium wilt of paprika caused by a highly virulent isolate of *Verticillium dahliae*. *Plant Dis.* 82:437-439.
- Xia, Z., Achar, P. N., and Gu, B. 1998. Vegetative compatibility grouping of *Verticillium dahliae* from cotton in mainland China. *Eur. J. Plant Pathol.* 104:871-876.