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Characterization of *Verticillium dahliae* Isolates and Wilt Epidemics of Pepper

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ABSTRACT

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Epidemics of Verticillium wilt in pepper fields of the central coast of California and isolates of *Verticillium dahliae* associated with these epidemics were characterized. The mean incidence of wilted plants per field ranged from 6.3 to 97.8% in fields with Anaheim, jalapeno, paprika, or bell peppers. In general, incidence of wilt in jalapeno and bell pepper crops was lower than in crops of other types of pepper. Inoculum density of *V. dahliae* in the surveyed pepper fields ranged from 2.7 to 66.6 microsclerotia g^{-1} dry soil, and the correlation between disease incidence and density of microsclerotia was high ($r = 0.81$, $P < 0.01$). Distribution of Verticillium wilt was aggregated in a majority of the pepper fields surveyed, but the degree of aggregation varied. Vegetative compatibility group (VCG) characterization of 67 isolates of *V. dahliae* indicated that 67% belonged to VCG 2, 22% to VCG 4, and 11% to a new group, designated VCG 6. The pathogenicity of isolates of *V. dahliae* from bell pepper and tomato plants was tested by inoculating 1-month-old bell pepper (cv. Cal Wonder) and tomato (cv. EP 7) seedlings and incubating the inoculated plants in the greenhouse. Seedlings of bell pepper were susceptible only to the isolates of *V. dahliae* from pepper, whereas seedlings of tomato were susceptible to both pepper and tomato isolates. Pepper isolates belonging to VCG 2, VCG 4, and VCG 6 were highly pathogenic to bell pepper and chili pepper. Temperatures between 15 and 25°C were optimal for mycelial growth of a majority of isolates of *V. dahliae*. Molecular characterization of pepper isolates of *V. dahliae* using a polymerase chain reaction (PCR)-based random amplified polymorphic DNA (RAPD) technique revealed minor variation among these isolates, but unique polymorphic banding patterns were observed for isolates belonging to VCG 6. Verticillium wilt of pepper is a major production constraint in the central coast of California. More aggressive isolates of *V. dahliae* may have been selected in this region as a result of intensive cropping practices.

Additional keywords: DNA fingerprinting, *nit* mutants, vascular discoloration

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a soilborne fungus that colonizes the vascular tissues of plants. The causal agent has also been described as the microsclerotial form of *V. albo-atrum* Reinke & Berth. (9,21). *V. dahliae* has a broad host range, causing vascular discoloration and wilt of many economically important crops (18). Microsclerotia produced by *V. dahliae* may survive under field conditions for up to 14 years in the absence of a host (29).

The first record of Verticillium wilt of peppers (*Capsicum annuum* L.) in the United States was in 1937 from California, when Rudolph and Snyder (20) reported that the disease caused a 20% yield loss. Since then, various levels of incidence and severity of Verticillium wilt of peppers have been reported from different parts of the world (3–5,15,27,28). In recent years, an increase in the incidence of Verticillium wilt on many types of pepper, which has reduced yields significantly, has been observed on the central coast of California (R. G. Bhat and K. V. Subbarao, *unpublished data*).

V. dahliae can infect pepper plants at any growth stage. Symptoms include yellowing and drooping of leaves on a few branches or on the entire plant (16). The edges of the leaves roll inward on infected plants, and foliar wilting ensues. The foliage of severely infected plants turns brown. Growth of pepper plants inoculated with aggressive strains of *V. dahliae* in the greenhouse, or of pepper plants infected early in the season under field conditions, is severely stunted, with small leaves that turn yellow-green. Subsequently, the dried leaves and shriveled fruits remain attached to plants that die. Brown discoloration of the vascular tissue is visible when the roots and lower stem of a wilted plant is cut longitudinally. Another important soilborne disease of pepper in California, Phytophthora root rot, causes similar foliar symptoms; however, Phytophthora root rot causes extensive browning and rotting of the root cortex, while the roots of *V. dahliae*–infected pepper plants show no external discoloration or decay.

Resistance in peppers to Verticillium wilt is not common in commercial cultivars and is difficult to identify in pepper germplasm (6,9,14,31). Chemical control of *V. dahliae* using soil fumigation with methyl bromide and chloropicrin is effective (30) but not economical for pepper production in California. Moreover, methyl bromide is being phased out due to environmental concerns, and efforts to identify chemical or other alternatives are still underway (25). Because of the longevity of microsclerotia and the broad host range of *V. dahliae*, crop rotation is usually not a feasible option for control of Verticillium wilt in many crops. Peppers are resistant to isolates of *V. dahliae* from many hosts (1), and only certain strains of *V. dahliae*, such as those from eggplant and pepper, are pathogenic to peppers (1,3,5,9,28,31). Crop rotation with broccoli, cauliflower, or lettuce may reduce levels of inoculum of pepper strains of *V. dahliae*.

Verticillium wilt occurs on the common commercial cultivars of bell and chili peppers grown in the central coast of California. Bell peppers are sweet, and thick-fleshed fruits of various colors are sold primarily as vegetables. In contrast, chili peppers are pungent or nonpungent fruits that vary in size, color, and capsaicin (measured as Scoville units that indicate the degree of hotness of pepper), and are used mainly as spices and condiments. Anaheim, jalapeno, and paprika are the main types of chili pepper grown in the central coast of California. The genetic similarity of strains of *V. dahliae* causing wilt disease on bell and chili peppers is not known.

Because Verticillium wilt is an increasing problem for pepper production in the central coast of California, it is important to document the severity of this disease in pepper crops and increase understanding of the biology, pathogenicity, and genetic relatedness of representative isolates of *V. dahliae* from pepper in this region. This study was conducted with the following objectives: (i) survey pepper crops with Verticillium wilt in the central coast of

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California to document the incidence and spatial pattern of epidemics, (ii) isolate *V. dahliae* from infected pepper plants and determine the inoculum density in fields with pepper crops, (iii) determine the genetic relatedness of isolates of *V. dahliae* obtained from different types of pepper, and (iv) evaluate the pathogenicity of isolates of *V. dahliae* from bell pepper, chili pepper, and tomato.

MATERIALS AND METHODS

Survey of the incidence of Verticillium wilt in pepper fields. In 1996 and 1997, commercial pepper fields in and around King City (Monterey County), Gilroy (Santa Clara County), and Morgan Hill (Santa Clara County) of the central coast of California were surveyed for the incidence of Verticillium wilt. Fields were chosen arbitrarily, but the number of pepper plants exhibiting characteristic symptoms of Verticillium wilt dictated this choice. Since we were assessing the levels of Verticillium wilt in different types of pepper, care was taken to include as many fields of each type of pepper as available. In each field, a single row of bell peppers per raised bed or two rows of chili peppers per raised bed had been planted by farmers, with a 1-m spacing between the centers of adjacent beds and a 15- to 30-cm spacing between plants. The size of individual fields surveyed ranged from 3 to 15 ha, and the soil type ranged from silty clay to sandy loam. Nine infected fields cropped with Anaheim, bell, jalapeno, or paprika peppers were selected for detailed assessment of the incidence of Verticillium wilt. Each field was surveyed in an 'X' pattern by walking diagonally across the field. Fifteen sites of 3 × 3 m^2 were selected along each diagonal path, and the total numbers of healthy and diseased plants in each site were recorded. In every surveyed field, soil samples (approximate volume of 200 cm^3 per site) were collected at each of the 30 sites to a depth of 15 cm to estimate the inoculum density of *V. dahliae*. Plants showing symptoms of Verticillium wilt were collected randomly from each field to confirm the causal agent of the wilt symptoms and to collect isolates of *V. dahliae*. The incidence of wilt at each site in each field was calculated as a percentage of the total number of plants within each site, and the mean incidence of wilt and standard error of the mean were computed for each field using SAS software (Version 7, SAS Institute, Cary, NC).

Spatial pattern of Verticillium wilt in infested pepper fields. Four fields were chosen for intensive sampling to determine the spatial pattern of plants infected with Verticillium wilt. Three sites in each field were selected randomly, and 45 plants at each site in each of 16 double-rows were counted as healthy or infected with Verticillium wilt based on foliar symptoms. Occasional vacant spots in the rows were considered as missing plants. Healthy plants and infected plants were given a binary code of 0 and 1, respectively. Each site was divided into 72 quadrats arranged as a 9 × 8 grid, and in each quadrat, the incidence of Verticillium wilt on 20 plants in two double-rows was calculated as a percentage. Variance to mean ratios (*V*/*m*) and the Lloyd's Index of Patchiness (LIP) were calculated based on wilt incidence and the number of diseased plants at each site within fields. The software program DMap (J. Hao and K. V. Subbarao, *unpublished data*) was used to depict the spatial pattern of the incidence of Verticillium wilt at each site in each field. A new technique based on variance of moving window average was also employed to determine if the infected plants had an aggregated distribution pattern in the fields. A series of data sets was derived by moving a window of 1 × 1, 2 × 2, 3 × 3, and 4 × 4 quadrats, averaging the original data within each window and assigning them to new data sets at the center of the window. Based on the changes in variance in these data sets, an aggregation index was calculated for each lag distance (B. M. Wu and K. V. Subbarao, *unpublished data*). A positive index indicated a positive correlation between two quadrats, implying an aggregated distribution. A negative index indicated a negative association between two quadrats, implying a uniform distribution. A value of 0 for the index indicated a random distribution.

Isolation of *V. dahliae* and enumeration of inoculum density in soil samples. Isolations from infected pepper plants were made on the same day each field was surveyed. Sections (5 to 7 cm long) of stem tissue exhibiting vascular discoloration were rinsed thoroughly in tap water and air-dried for 5 to 10 min. The rinsed sections of discolored stem tissue were cut into pieces of approximately 0.4 × 0.4 × 0.4 cm^3. After surface-disinfesting the stem sections in 70% ethyl alcohol for 10 s, five sections for each plant sample were immersed in sterile distilled water and plated on Sorensen's NP-10 semi-selective medium (22). After a 3-week incubation period at room temperature (23 ± 2°C), colonies forming microsclerotia were confirmed as *V. dahliae* by microscopic examination, and at least five isolates from each field were single-spored and stored in vials containing potato dextrose agar (PDA) at 4°C.

The soil samples representing the 30 sites in each of the nine surveyed fields were air-dried in paper bags for a minimum of 4 weeks on a greenhouse bench. A modified Anderson sampler technique (2) using petri plates (90 mm diameter) containing NP-10 medium was used to assay the soil samples for microsclerotia. The mean number of microsclerotia g^{-1} dry soil and the standard error of the mean for each field were calculated using SAS. The relationship between the density of microsclerotia and the incidence of Verticillium wilt in each pepper field was also determined by regression analysis using SAS.

Mycelial growth of *V. dahliae* at different temperatures. To compare the optimal temperature requirements of isolates of *V. dahliae* from pepper and tomato, mycelial growth of five isolates of *V. dahliae* from pepper and four from tomato was evaluated at 10, 15, 20, 25, 30, and 35°C. The pepper isolates of *V. dahliae* were from infected plants collected during the field survey, and the tomato isolates of *V. dahliae* were obtained from Mike Davis and Tom Gordon, University of California, Davis. For each isolate and temperature combination, three plates (90 mm diameter) of PDA were seeded at the center with a 4-mm-diameter agar plug taken from the edge of an actively growing colony of each isolate. The fungal cultures were incubated in the dark at each temperature. The diameter of each colony was measured at weekly intervals for 5 weeks. The experiment was conducted twice. Analyses of variance to evaluate the effects of isolate, temperature, growth, and interaction between these variables on growth was made using the SAS GLM procedure. Since experiments were not a source of variation, means and standard errors of the means were computed over the two experiments for each isolate and temperature.

Vegetative compatibility grouping. At least four nitrate nonutilizing (*nit*) mutants from each of 67 pepper and four tomato isolates of *V. dahliae* were generated using techniques described previously (12,19). Complementation tests for each *nit* mutant were done by pairing the mutant isolate with standard NitM tester strains of *V. dahliae* belonging to VCG 1 (strain T9), VCG 2 (strain 115), VCG 3 (strain PCW), and VCG 4 (strain S39). Procedures for the complementation tests have been described (1). Isolates were assigned to VCG 1, VCG 2, VCG 3, or VCG 4 based on their complementation. Mutants of five isolates (VdCa.83, VdCa.146, VdCa.147, VdCa.148, and VdCa.149) of *V. dahliae* that did not form heterokaryons with tester strains were paired among themselves to see if they complemented each other.

Random amplified polymorphic DNA (RAPD) analysis. Nine isolates of *V. dahliae* from bell pepper, eight isolates from chili pepper, and one isolate each from eggplant, potato, and tomato were compared by RAPD analysis. The pepper isolates of *V. dahliae* were from the central coast of California, and the isolates from eggplant, potato, and tomato were from New Jersey, Oregon, and California, respectively. Total genomic DNA was extracted from each isolate of *V. dahliae* using a modification of the method described by Lee and Taylor (13). Procedures used for the polymerase chain reaction-random amplified polymorph DNA (PCR-

RAPD) assay and electrophoresis were as described by Bhat and Subbarao (1). Ten-mer nucleotide primers OPA-03, OPA-07, OPA-18, and OPB-18 (Operon Technologies, Inc., Alameda, CA) were screened with DNA of all 20 isolates of *V. dahliae*, as these primers were shown to amplify DNA of *V. dahliae* (1). Experiments were conducted to facilitate comparisons between isolates of *V. dahliae* from bell pepper and chili pepper, between isolates of *V. dahliae* from pepper and isolates from other solanaceous hosts, and between isolates of *V. dahliae* from known VCGs and an unknown VCG. Assays were conducted three times. For each primer, amplified bands were scored as present or absent.

Pathogenicity of isolates of *V. dahliae*. Two pathogenicity experiments were conducted, and they were repeated once. In the first pathogenicity experiment, two isolates of *V. dahliae* from bell pepper (VdCa.35 and VdCa.59), two from chili pepper (VdCa.45 and VdCa.56), and four from tomato (VdLe.78, VdLe.88, VdLe.109, and VdLe.110) were inoculated onto bell pepper (cv. Early Cal Wonder) and tomato (cv. EP 7) plants. Inoculum was prepared from 1-month-old cultures, and the density of each isolate was adjusted to approximately 10^7 conidia ml^{-1}. The roots of 4-week-old plants were washed free of soil and inoculated using the root-dip technique (11). Ten plants were inoculated for each host–isolate combination. Each inoculated plant was transplanted into a 10-cm-diameter, 454-g-capacity Styrofoam cup filled with autoclaved sand. Ten bell pepper plants and 10 tomato plants with roots dipped in sterile distilled water served as noninoculated controls. Plants were arranged in a randomized complete block design and incubated on greenhouse benches (24 ± 2°C). Plants were monitored for the development of symptoms of Verticillium wilt, and the number of days between inoculation and the first observation of symptoms was recorded. Eight weeks after inoculation, the height of each bell pepper and tomato plant was measured from the soil line. The main tap root of each bell pepper and tomato plant was cut longitudinally to record the number of plants with vascular discoloration. For the bell pepper plants, the numbers of flowers and fruits were also counted. For the tomato plants, the numbers of yellowed and dead leaves were counted at the time plant height was measured. Sections of crown tissue of three plants for each host–isolate combination were surface-sterilized and placed on NP-10 medium as described above to confirm the presence of *V. dahliae*.

In the second pathogenicity experiment, five isolates of *V. dahliae* from bell pepper and seven isolates from chili pepper were inoculated onto bell pepper (cv. Early Cal Wonder) and chili pepper (cv. Sonora) plants to determine if there were pathogenic differences among isolates. The inoculation procedure used was as described above. Plant height and severity of Verticillium wilt were recorded 8 weeks after inoculation. Vascular discoloration was rated visually by cutting the crown and main root of each plant longitudinally. A scale of 0 to 5 was used to assess disease severity, in which: 0 = no vascular discoloration, 1 = 1 to 25% vascular tissue discolored, 2 = 26 to 50% vascular tissue discolored, 3 = 51 to 75% vascular tissue discolored, 4 = 76 to 100% vascular tissue discolored, and 5 = 100% of the vascular tissue discolored and wilting of the foliage observed.

Analyses of variance were conducted for each dependent variable to determine the effects of isolate, host plant, and replica-

Table 1. Incidence of Verticillium wilt and inoculum density of *Verticillium dahliae* in nine pepper fields surveyed in central coastal California

Year and location[a]	Type of pepper	Incidence of Verticillium wilt (%)[b]		Inoculum density[c] (microsclerotia g^{-1} dry soil)	
		Mean ± SE[d]	Range	Mean ± SE[d]	Range
1996					
Field 1	Anaheim	39.4 ± 5.8	3.8 – 92.3	53.7 ± 14.5	6.0 – 448.0
Field 2	Bell pepper	24.3 ± 3.6	0.0 – 73.1	9.9 ± 3.9	0.0 – 118.0
Field 3	Anaheim	28.2 ± 5.9	0.0 – 96.6	34.7 ± 2.6	8.0 – 72.0
Field 4	Bell pepper	23.4 ± 1.7	4.0 – 46.9	2.7 ± 0.6	0.0 – 10.0
1997					
Field 5	Bell pepper	9.6 ± 1.6	0.0 – 25.0	5.8 ± 0.8	0.0 – 18.0
Field 6	Jalapeno	6.3 ± 2.1	0.0 – 42.9	5.4 ± 1.7	0.0 – 52.0
Field 7	Paprika	21.2 ± 2.4	0.0 – 59.1	24.5 ± 1.9	6.0 – 52.0
Field 8	Paprika	50.8 ± 4.0	15.0 – 95.7	20.1 ± 1.8	4.0 – 44.0
Field 9	Paprika	97.8 ± 0.7	85.7 – 100.0	66.6 ± 8.0	4.0 – 184.0

[a] Commercial pepper fields with incidence of Verticillium wilt were selected randomly in the central coast of California.
[b] Each field was surveyed in an "X" pattern by walking diagonally across the field, and 15 sites of 3 × 3 m^2 were selected along each diagonal path. The total numbers of healthy and diseased plants displaying symptoms characteristic of Verticillium wilt in each site were recorded.
[c] Soil samples representing the 30 sites were analyzed for each of the nine surveyed fields for inoculum density of *V. dahliae* using a modified Anderson sampler technique (2).
[d] SE = standard error of the mean.

Table 2. Aggregation indices for the distribution of plants infected with *Verticillium dahliae* within pepper fields surveyed for Verticillium wilt in 1996 to 1997 in the central coast of California

Pepper field-site	Incidence (%)	Index values at different lags[a]				LIP[b]		*V/m*[c]	
		Lag=1	Lag=2	Lag=3	Lag=4	Incidence	Diseased plants	Incidence	Diseased plants
Field 5-1	22	0.62	0.80	–0.24	0.84	–1.85	2.17	0.37	3.54
Field 5-2	17	0.68	0.34	0.21	0.01	–2.68	2.58	0.37	3.70
Field 5-3	46	0.64	0.43	–0.10	0.36	–0.59	1.34	0.27	2.44
Field 7-1	31	0.27	0.22	0.04	0.06	–1.97	1.04	0.06	1.23
Field 7-2	38	0.21	–0.03	0.11	–0.04	–1.47	1.05	0.07	1.38
Field 7-3	27	0.05	0.05	0.03	–0.06	–2.60	0.98	0.04	0.87
Field 8-1	64	0.35	0.09	0.11	0.21	–0.52	0.96	0.02	0.47
Field 8-2	70	0.39	0.13	–0.14	–0.02	–0.39	0.97	0.03	0.65
Field 8-3	76	0.22	–0.08	–0.09	0.07	–0.29	0.96	0.02	0.38
Field 9-1	100	0.00	0.00	0.00	0.00	0.00	0.95	0.00	0.01
Field 9-2	99	–0.04	0.13	0.02	0.08	–0.01	0.95	0.00	0.01
Field 9-3	74	0.13	0.17	–0.29	0.04	–0.31	0.97	0.03	0.63

[a] The index was calculated based on variance of moving window average at different lag distances (two sample sites that fit into a $(n + 1) \times (n + 1)$ window, but not a $(n \times n)$ window, have a lag distance n). A positive value of this index indicates aggregation of infected plants or a positive association between the incidence of infected plants at two sites. The value of this index was the same regardless of whether it was calculated from the incidence of infected plants or the number of diseased plants.
[b] Lloyd's Index of Patchiness (LIP) calculated from disease incidence and number of diseased plants at each sample site.
[c] Variance to mean ratios (*V/m*) were calculated based on disease incidence and number of diseased plants at each sample site.

tion, and to examine interactions among these variables. Replications within an experiment were considered as random effects in the analysis. Means were calculated for each isolate–host combination, and comparisons of isolates within a host were made using Fisher's least significant difference (LSD) ($P \leq 0.05$) (23). Based on host response, isolates were grouped as pathogenic or nonpathogenic, and each isolate was considered aggressive on a host if it caused a disease severity rating of >4.0. All statistical analyses were performed using SAS. Results from one experiment are presented because results from the two experiments were consistent and because the experiment × isolate × host interaction was not significant.

RESULTS

Incidence and spatial pattern of Verticillium wilt in pepper fields. The mean incidence of Verticillium wilt ranged from 6.3 to 97.8% in the nine fields surveyed (Table 1). The incidence of Verticillium wilt also varied among sites within most fields examined. Fields with Anaheim and paprika peppers (chili pepper) had a greater incidence of Verticillium wilt in 1996 and 1997 than fields planted with bell or jalapeno peppers. On average, 47% of the chili pepper plants surveyed had Verticillium wilt, with a range of 21.2 to 97.8% incidence of infected plants. Fields planted with jalapeno and bell peppers had less than 16% incidence of wilt with a range of 6.3 to 24.3%.

The spatial pattern of plants infected with Verticillium wilt was different among

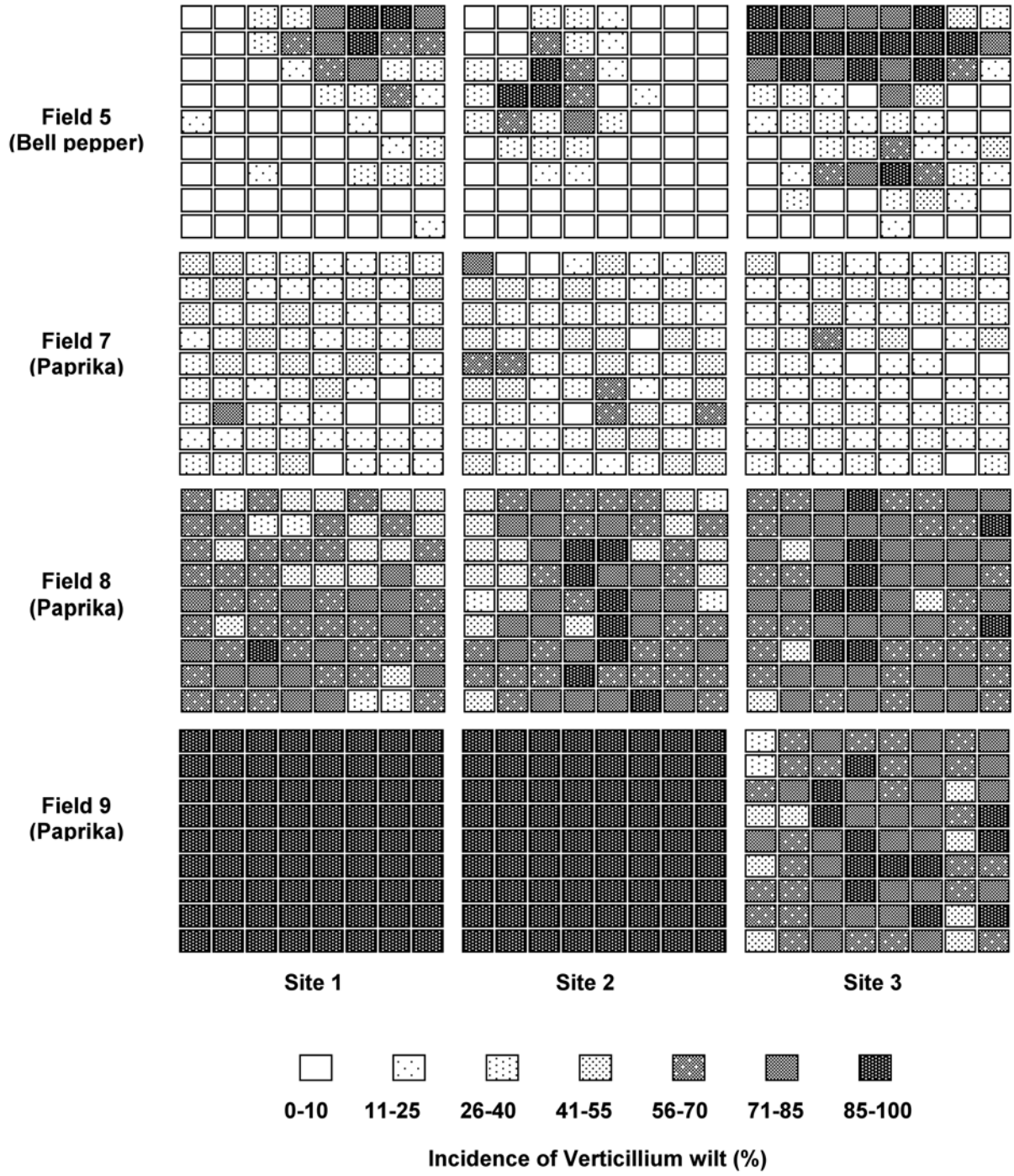

Fig. 1. Spatial pattern of incidence of Verticillium wilt at three sites in each of four pepper fields. For each site, 45 plants in each of 16 double rows were rated as healthy or infected with *Verticillium dahliae* based on symptoms. Each site was divided into 72 quadrats arranged in a 9 × 8 grid, and incidence of Verticillium wilt was rated for 20 pepper plants in two double rows in each quadrat.

fields and among sites within a field. Infected plants had an aggregated distribution in most of the fields surveyed (Table 2). The degree of aggregation was highest in field 5, where the incidence of Verticillium wilt ranged from 0 to 25% (Table 1). The incidence of Verticillium wilt was highly aggregated at all three sites in that field, and the patch size was at least 3 × 3 quadrats (lag distance 2, Table 2). Incidence of Verticillium wilt was least aggregated in fields 7 and 8. Where the incidence of wilt was close to 100%, as in sites 1 and 2 in field 9, the spatial pattern was uniform (Fig. 1 and Table 2). Neither LIP nor the *V/m* ratio detected as many aggregated spatial patterns as the index employed in this study.

Isolation of *V. dahliae* and inoculum density in pepper fields. *V. dahliae* was isolated on the NP-10 medium from all symptomatic pepper plants. Fungal colonies produced microsclerotia characteristic of *V. dahliae* within 3 weeks, and the presence of single-celled oval conidia and distinct verticillate conidiophores confirmed the isolates as *V. dahliae*.

The pepper fields surveyed had an average of 24.8 microsclerotia g^{-1} dry soil, with a range in mean inoculum density from 2.7 to 66.6 microsclerotia g^{-1} dry soil (Table 1). In general, inoculum density of *V. dahliae* was lower in fields with bell and jalapeno peppers (mean of 6 microsclerotia g^{-1} dry soil with a range in mean-inoculum density from of 2.7 to 9.9 microsclerotia g^{-1} dry soil) than in fields planted with Anaheim or paprika peppers. An average of 40 microsclerotia g^{-1} dry soil, with a range in mean inoculum density from 20.1 to 66.6 microsclerotia g^{-1} dry soil, was observed in Anaheim and paprika fields. The density of microsclerotia was variable among sites within fields and among fields surveyed (Table 1). A positive linear correlation ($r = 0.81$, $P < 0.01$) (Fig. 2) was observed between the mean density of microsclerotia and the mean incidence of Verticillium wilt.

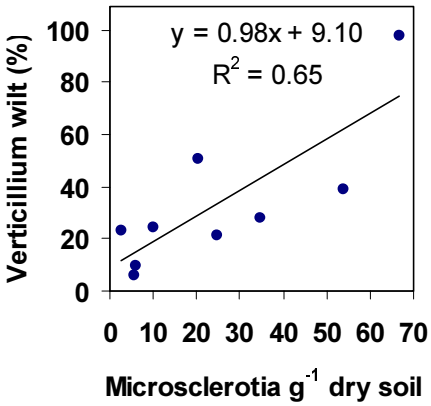

Fig. 2. Correlation of density of microsclerotia of *Verticillium dahliae* with incidence of Verticillium wilt in pepper crops in the central coast of California in 1996 and 1997.

Mycelial growth of *V. dahliae* at different temperatures. Temperature had a significant influence on the growth of isolates of *V. dahliae*. Analyses of variance indicated that the temperature of incubation, isolate of *V. dahliae*, and the interactions among these variables significantly affected the mycelial colony diameter. Growth of all the isolates of *V. dahliae* (three from bell pepper, two from chili pepper, and four from tomato) increased from 1 to 5 weeks at all temperatures except at 35°C (data not shown). None of the isolates of *V. dahliae* grew at 35°C. Mycelial growth after 4 weeks is depicted in Figure 3 for four isolates of *V. dahliae*.

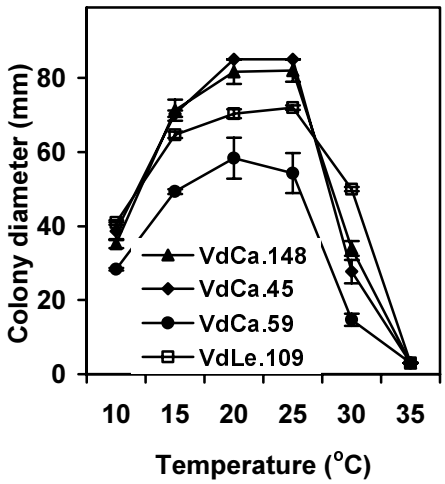

Fig. 3. Effect of temperature on mycelial growth of isolates of *Verticillium dahliae* from bell pepper (VdCa.59 and VdCa.148), chili pepper (VdCa.45), and tomato (VdLe.109). Colony diameter of each isolate was measured at weekly intervals from 1 to 5 weeks after subculturing the isolates. Results are presented for mycelial growth measured at 4 weeks.

Temperatures of 20 to 25°C were optimum for maximum growth of isolate VdCa.45 from chili pepper and VdCa.148 from bell pepper. For other isolates, maximum growth rates were observed also at 15°C. Growth of all isolates declined at 30°C, and none of the isolates grew at 35°C. Isolates of *V. dahliae* from bell pepper (VdCa.35 and VdCa.59) and one isolate from tomato (VdLe.88) were relatively slow-growing, whereas six isolates (one from bell pepper, two from chili pepper, and three from tomato) were fast-growing.

Vegetative compatibility of *V. dahliae* isolates from pepper. Sixty of the 67 isolates of *V. dahliae* from pepper plants belonged to either VCG 2 or VCG 4. Seven isolates did not form heterokaryons with any of the known VCG tester strains and could not be assigned to VCG 1 through 4. However, *nit* mutants from five of these seven isolates complemented each other by forming dense wild-type growth (Table 3), indicating that they belonged to a different VCG. These isolates were assigned to VCG 6. Of the 67 isolates of *V. dahliae* from pepper, 67% belonged to VCG 2, 22% to VCG 4, and 11% to VCG 6. Groupings of the isolates differed within pepper types. Of the isolates of *V. dahliae* from chili pepper, 81% belonged to VCG 2, 13% to VCG 4, and 6% to VCG 6. In contrast, 32% of the bell pepper isolates of *V. dahliae* belonged to VCG 2, 47% to VCG 4, and 21% to VCG 6 (Fig. 4). All four isolates from tomato belonged to VCG 4.

RAPD analysis. The four oligonucleotide primers successfully amplified DNA from all 20 isolates analyzed. Amplification of genomic DNA with primers OPA-03, OPA-07, and OPA-18 revealed polymorphisms (Fig. 5), but polymorphic banding patterns were not observed when DNA was amplified with primer OPB-18. No apparent distinction was observed among isolates of *V. dahliae* from different types of pepper (Fig. 5A). DNA banding profiles among isolates of *V. dahliae* from pepper in VCG 2, VCG 4, and VCG 6 grouped isolates according to their VCG (Fig. 5B). However, banding patterns of isolates of *V. dahliae* from pepper (VdCa.35 and VdCa.36) were distinct from the banding patterns of isolates from eggplant (VdSm.130), potato (VdSt.94), and tomato

Table 3. Complementation among *nit* mutants of pepper isolates of *Verticillium dahliae* that did not form heterokaryons with tester strains of known vegetative compatibility groups (VCGs)

	Phototropic growth of heterokaryons with *nit* mutant							
***nit* mutant**	**VdCa.146**	**VdCa.147**	**VdCa.148**	**VdCa.149**	**VCG 1**	**VCG 2**	**VCG 3**	**VCG 4**
VdCa.83	–[a]	+	–	+	–	–	–	–
VdCa.146		+	–	+	–	–	–	–
VdCa.147			+	+	–	–	–	–
VdCa.148				+	–	–	–	–
VdCa.149					–	–	–	–

[a] – = no wild-type growth at site of pairing, i.e., isolates were not compatible; + = dense, wild-type growth with or without the production of microsclerotia at site of pairing, i.e., isolates were compatible. Pepper isolates of the new VCG were designated VCG 6 because they were not compatible with isolates in VCG 1, 2, 3, or 4.

(VdLe.112) (Fig. 5C). Polymorphisms in banding patterns were observed among isolates of *V. dahliae* in the same VCG but from different hosts (Fig. 5C). Isolates of *V. dahliae* from chili pepper (VdCa.36) and tomato (VdLe.112) showed different banding patterns even though they belonged to VCG 2. Similarly, isolates of *V. dahliae* from bell pepper (VdCa.35), eggplant (VdSm.130), and potato (VdSt.94) in VCG 4 showed variations in RAPD banding profiles.

Pathogenicity of isolates of *V. dahliae*. Isolates of *V. dahliae* from pepper, but not isolates from tomato, caused vascular discoloration in all of the bell pepper plants inoculated but not isolates from tomato (Table 4). Typically, symptoms of Verticillium wilt were observed on pepper plants approximately 24 days postinoculation. Bell pepper isolates of *V. dahliae* in VCG 4 were more aggressive (judging by the magnitude of reduction in height and wilt severity) than chili pepper isolates in VCG 2 when inoculated onto bell pepper plants. Bell pepper isolates of *V. dahliae* caused severe stunting of the inoculated plants, which did not develop any flowers or fruits. The four tomato isolates of *V. dahliae* in VCG 4 did not cause wilt symptoms on bell pepper plants. Re-isolation of the tomato pathogen from the inoculated bell pepper plants was unsuccessful (Table 4).

Isolates of *V. dahliae* in VCG 4 from tomato and isolates in VCG 2 from chili pepper caused wilt in 50 to 80% of the tomato plants inoculated (Table 5). However, isolates of *V. dahliae* in VCG 4 from bell pepper caused vascular discoloration in only 10 to 20% of the tomato plants inoculated. Infected tomato plants developed symptoms approximately 24 days postinoculation (Table 5). Inoculated tomato plants had significantly ($P \leq 0.05$) more dead and yellow leaves than noninoculated control plants (Table 5). In general, plants inoculated with tomato isolates of *V. dahliae* had significantly more dead and yellow leaves than plants inoculated with pepper isolates of *V. dahliae*. The height of tomato plants was not affected by inoculation with *V. dahliae*, and symptoms of foliar wilting were not evident (data not shown). *V. dahliae* was readily re-isolated from crown samples of all inoculated tomato plants (Table 5).

In the second pathogenicity test, 10 isolates of *V. dahliae*, five each from bell pepper and chili pepper plants, were highly pathogenic to both types of pepper regardless of the VCG of these isolates (Table 6). Inoculated plants were completely wilted and had developed severe vascular discoloration in the roots and stems by 8 weeks after inoculation. The mean height of inoculated plants was significantly ($P \leq 0.05$) lower than the mean height of noninoculated control plants. Isolates VdCa.44 and VdCa.158 from chili pepper did not cause wilting or severe stunting of the inoculated bell pepper plants. Isolate VdCa.158 also did not cause wilting in chili pepper.

DISCUSSION

This is the first characterization of Verticillium wilt in various types of peppers grown in the central coast of California. Because of the limited number of fields surveyed, it remains unclear if the incidence of Verticillium wilt is related to the type of pepper, although these results suggest a greater incidence of Verticillium wilt in Anaheim and paprika crops than in jalapeno and bell pepper crops. Regardless of the type of pepper, a positive linear relationship was observed between the incidence of Verticillium wilt and inoculum density in soil sampled from fields, a characteristic that is typical of monocyclic soilborne diseases. Incidence of Verticillium wilt of pepper followed aggregated patterns in the field as is typical of soilborne pathogens. An aggregated pattern may result either from secondary infection of plants by inoculum from primary foci, or in association with the distribution of pathogen propagules produced on infected plants of the previous crop. Given that this is a monocyclic disease (7), the more likely explanation for the aggregated incidence of wilt appears to be the distribution of microsclerotia in soil from the previous crop. Although the patch size of this aggregation was limited, successive pepper crops in the same field could rapidly increase the inoculum density, and the inoculum could be disseminated to other parts of the same field and to other fields, causing significant yield losses.

This study also characterized isolates of *V. dahliae* causing wilt on various types of peppers. Although a majority of the isolates of *V. dahliae* from pepper belonged to two established vegetative compatibility groups (VCG 2 and VCG 4), a few isolates were compatible only among themselves and did not belong to any established VCGs for this species. These isolates were, therefore, assigned to a new VCG, VCG 6. Isolates of *V. dahliae* from bell pepper were more aggressive when inoculated onto bell pepper plants in the greenhouse than isolates from other types of pepper. An increase in the number of pepper fields with a high incidence of Verticillium wilt, combined with the unavailability of economical control measures, poses a serious threat to pepper production in the central coast of California.

Although Verticillium wilt was a problem for pepper production in southern California from the 1930s to the 1950s (9,20), the disease was not considered a major constraint to pepper production in the central coast of California until recently. The reasons for the sudden increase in incidence of Verticillium wilt on different kinds of peppers in the central coast of California are not known, but may be associated with selection of isolates of *V. dahliae* with increased ability to infect pepper plants, or the introduction of new strains of *V. dahliae* to this region of California. Since the absence of wilt symptoms in pepper plants is not necessarily due to the inability of the pathogen to penetrate or colonize plant tissues (28), it is possible that *V. dahliae* has always been present in these fields and colonized the roots of pepper plants without noticeable reduction in

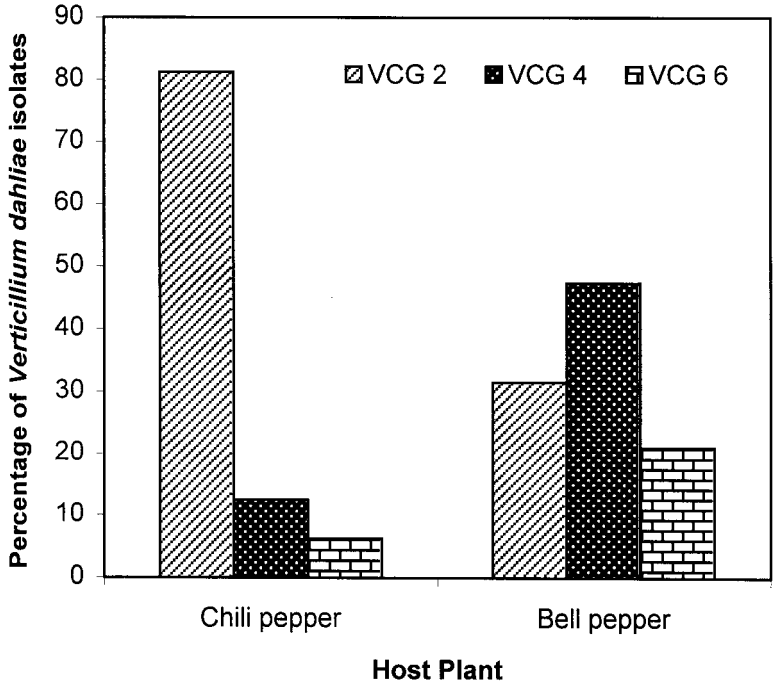

Fig. 4. Vegetative compatibility group (VCG) of isolates of *Verticillium dahliae* from bell pepper or chili pepper crops in the central coast of California.

plant vigor or yield. The intensive production of pepper in this area may have increased selection pressure on strains of *V. dahliae* that colonize and reproduce on pepper plants more effectively, resulting in an increase in inoculum levels.

Although this survey of Verticillium wilt on different types of pepper was limited, the data provide interesting clues to the nature of this disease on different types of pepper. Greater resistance to Verticillium wilt in cultivars of bell pepper relative to cultivars of chili pepper (1), lower inoculum levels in fields cropped to jalapeno and bell pepper than in fields with other types of pepper, and a positive correlation between inoculum density and incidence of Verticillium wilt may account for relatively low incidences of Verticillium wilt in bell pepper crops. Furthermore, chili peppers are harvested after the fruits have dried, and consequently, the plants remain in the field longer than bell pepper plants in which the fruits are harvested as a fresh vegetable. This difference in duration of the cropping season may contribute to a greater incidence of infected chili pepper plants compared to other types of pepper, with a corresponding increase in the density of microsclerotia of *V. dahliae* in fields planted to chili peppers.

Several other factors may explain the differential incidence of Verticillium wilt

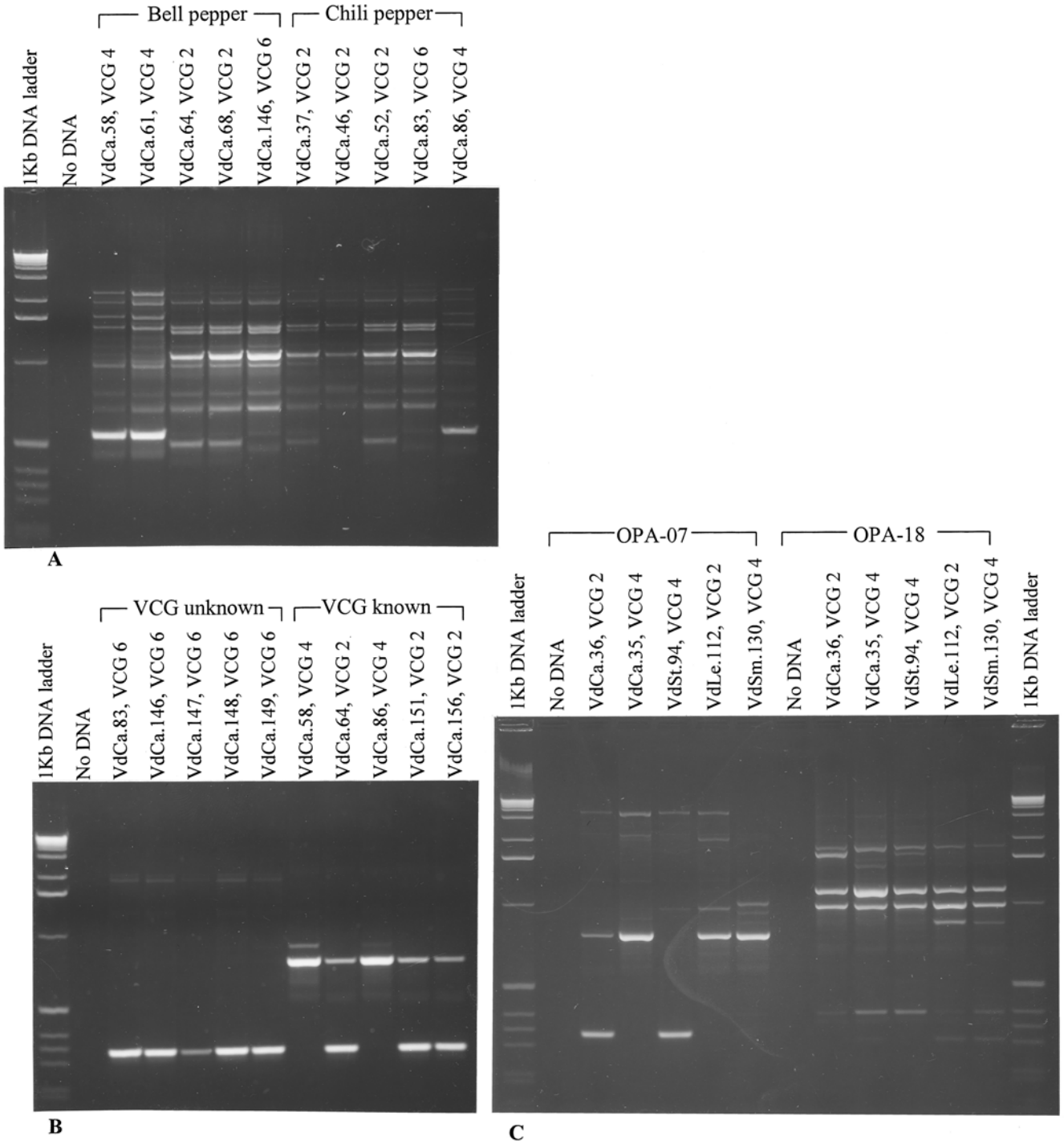

Fig. 5. Random amplified polymorphic DNA profiles of isolates of *Verticillium dahliae* using different oligonucleotide primers. **A,** DNA amplified with the primer OPA-03 for comparison of isolates of *V. dahliae* from bell pepper and chili pepper. **B,** DNA amplified with the primer OPA-07 for comparison of isolates of *V. dahliae* in established vegetative compatibility groups (VCGs) and isolates of a new VCG. **C,** DNA amplified with primers OPA-07 and OPA-18 for comparison of isolates of *V. dahliae* from the solanaceous hosts bell pepper (VdCa.35), chili pepper (VdCa.36), eggplant (VdSm.130), potato (VdSt.94), and tomato (VdLe.112). Isolate numbers and VCG designations are listed above each lane.

Table 4. Pathogenicity of pepper and tomato isolates of *Verticillium dahliae* inoculated onto bell pepper seedlings in the greenhouse

Isolate no.	VCG[a]	Plant height (mm)[b]	Vascular discoloration[c]	Days until symptoms[d]	No. of flowers[e]
VdCa.35	4	74.25	1.0	22.1	0
VdCa.45	2	129.00	1.0	25.2	6.4
VdCa.56	2	119.00	1.0	24.8	4.5
VdCa.59	4	78.50	1.0	23.4	0
VdLe.78	4	227.75	0.0	–	11.1
VdLe.88	4	230.00	0.0	–	12.8
VdLe.109	4	232.00	0.0	–	11.4
VdLe.110	4	241.00	0.0	–	12.1
Noninoculated control		234.75	0.0	–	12.9
$LSD_{(P \leq 0.05)}$		15.57	0.0	1.8	1.9

[a] Isolates of *V. dahliae* labeled with VdCa and VdLe were from pepper and tomato plants, respectively. Isolates VdCa.35 and VdCa.59 were from bell pepper plants, and VdCa.45 and VdCa.56 were from chili pepper plants. VCG = vegetative compatibility group.
[b] Plant height was measured 8 weeks after inoculation.
[c] Proportion of inoculated plants that displayed vascular discoloration typical of Verticillium wilt.
[d] Number of days from inoculation until symptoms of Verticillium wilt were observed.
[e] Total number of flowers and fruits present on plants 8 weeks after inoculation.

Table 5. Pathogenicity of pepper and tomato isolates of *Verticillium dahliae* inoculated onto tomato seedlings in the greenhouse

Isolate no.[a]	Vascular discoloration[b]	Days till symptoms observed[c]	No. of symptomatic leaves[d]
VdCa.35	0.1	24.7	5.0
VdCa.45	0.8	24.8	5.5
VdCa.56	0.8	25.1	4.3
VdCa.59	0.2	25.4	4.3
VdLe.78	0.4	21.8	5.5
VdLe.88	0.5	24.5	6.1
VdLe.109	0.8	24.5	6.9
VdLe.110	0.5	25.1	6.5
Noninoculated control	0.0	–	1.7
$LSD_{(P \leq 0.05)}$	0.3	1.9	0.9

[a] Isolates of *V. dahliae* labeled with VdCa and VdLe were from pepper and tomato, respectively. Isolates VdCa.35 and VdCa.59 were from bell pepper plants, and VdCa.45 and VdCa.56 were from chili pepper plants.
[b] Proportion of inoculated plants that developed vascular discoloration ($n = 20$).
[c] Number of days from inoculation until symptoms of Verticillium wilt were observed.
[d] Numbers of dead and yellow leaves 8 weeks after inoculation.

Table 6. Pathogenicity of pepper isolates of *Verticillium dahliae* inoculated onto bell pepper and chili pepper plants in the greenhouse 8 weeks after inoculation

		Bell pepper		Chili pepper	
Isolate no.	VCG[a]	Plant height (mm)	Disease severity[b]	Plant height (mm)	Disease severity[b]
VdCa.38	6	134	5.0	108	5.0
VdCa.44	2	223	4.2	110	5.0
VdCa.47	2	157	5.0	116	5.0
VdCa.68	2	167	5.0	110	5.0
VdCa.83	6	192	5.0	137	5.0
VdCa.86	4	109	5.0	74	5.0
VdCa.146	6	120	5.0	87	5.0
VdCa.147	6	119	5.0	85	5.0
VdCa.148	6	142	5.0	104	5.0
VdCa.149	6	147	5.0	128	4.8
VdCa.158	2	215	3.8	153	4.0
VdCa.162	6	152	4.8	105	5.0
Noninoculated control		263	0.0	250	0.0
$LSD_{(P \leq 0.05)}$		30	0.3	27	0.2

[a] Isolates of *V. dahliae* labeled with VdCa.68 and VdCa.146 to VdCa.149 were from bell pepper plants, and all other isolates of *V. dahliae* were from chili pepper plants. VCG = vegetative compatibility group.
[b] Severity of Verticillium wilt was measured on a scale of 0 to 5, where 0 = no vascular discoloration observed, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, 4 = 76 to 100%, and 5 = 100% of the vascular tissue discolored, with foliar wilting also observed.

between bell pepper and chili pepper crops. A majority of the isolates of *V. dahliae* obtained from chili pepper in this study belonged to VCG 2, and a majority of the isolates of *V. dahliae* infecting many other crops in the central coast of California belong to VCG 2 (1; R. G. Bhat and K. V. Subbarao, *unpublished data*). Therefore, inoculum levels of *V. dahliae* in the fields evaluated may have increased in association with crops other than peppers. The spatial distribution of infected plants in some bell pepper fields was aggregated relative to the more uniform distribution of infected plants in the chili pepper fields evaluated, even though inoculum levels in bell pepper fields were relatively uniformly distributed. This suggests that a proportion of the total inoculum of *V. dahliae* is capable of infecting bell pepper plants, and the aggregated nature of this inoculum means the pathogen population only increases at the foci of infection.

Vegetative compatibility grouping and RAPD analysis of the isolates of *V. dahliae* obtained from pepper plants indicated that there are three groups of *V. dahliae* infecting peppers in the central coast of California. The predominant isolates infecting chili pepper belonged to VCG 2, whereas isolates infecting bell pepper were more equally distributed among VCG 2, VCG 4, and the new VCG 6. Genetic differences among isolates of *V. dahliae* from bell pepper and chili pepper were not obvious from the VCG and RAPD analyses employed in this study. There were distinct genetic variations among pepper isolates of *V. dahliae* in each of the three VCGs, indicating that vegetative incompatibility separated these isolates. Isolates of *V. dahliae* in VCG 6 may be relatively new to this area because no such isolates have been found in other studies of *V. dahliae* in this region (1; R. G. Bhat and K. V. Subbarao, *unpublished data*).

The isolates of *V. dahliae* obtained from pepper plants that were incompatible with the standard tester strains for VCG 1, 2, 3, and 4 were designated tentatively to VCG 6. A tester strain of *V. dahliae* for VCG 5 was not available for complementation tests in this study, as only one isolate of *V. dahliae* has been designated to VCG 5, an isolate from a *Catalpa* species in Illinois (24). It is unlikely that the isolates of *V. dahliae* designated to VCG 6 in this study are the same as VCG 5 because of the distinct geographical areas and hosts from which isolates belonging to these two groups are described. Furthermore, isolates of *V. dahliae* from pepper with the new VCG phenotype did not belong to VCG 2A, VCG 2B, VCG 4A, or VCG 4B since the *nit* mutants of these isolates did not undergo weak or strong anastomosis with tester isolates of VCG 2 (strain 115), VCG 3 (strain PCW), and VCG 4 (strain S39) (8,24). Additional studies are needed

to characterize the origins of the pepper isolates of *V. dahliae* belonging to VCG 6.

Isolates of *V. dahliae* from three solanaceous hosts, eggplant, potato, and tomato, differed with respect to RAPD banding profiles. Isolates belonging to the same VCG but obtained from different hosts were different at the molecular level, indicating that these isolates did not have the same clonal lineage. This is in contrast to the report by Koike et al. (10), who suggested that RAPD banding patterns correspond to the pathogenicity groups of *V. dahliae*. Isolates of *V. dahliae* from eggplant, pepper, potato, and tomato, which belonged to the same VCG, were from different geographical locations (1). It is, therefore, important to restrict the spread of *V. dahliae* from one location to another because of the risk of genetic recombination among isolates of the same VCG that may produce new strains of *V. dahliae* with increased virulence.

In this study, only isolates of *V. dahliae* from pepper were able to cause severe symptoms of Verticillium wilt when inoculated onto pepper plants in the greenhouse. Tomato isolates of *V. dahliae* could not be re-isolated from the crown tissue of inoculated bell pepper plants, suggesting that bell pepper plants are unlikely to be symptomless carriers of tomato isolates of *V. dahliae*. These results support previous reports that isolates of *V. dahliae* from various host plants, except those from eggplant, were unable to cause Verticillium wilt on peppers (1,5,9,26,28). However, tomato plants were susceptible to pepper isolates of *V. dahliae*, and *V. dahliae* was successfully re-isolated from crowns of tomato plants inoculated with pepper isolates. Our results support other reports of the ability of pepper isolates of *V. dahliae* to cause Verticillium wilt in eggplant, potato, and tomato (1,21,28).

Isolates of *V. dahliae* from pepper plants grew well at 15, 20, and 25°C. The optimum temperature for robust growth of peppers is about 25°C (17). Growth of pepper plants is not only reduced at temperature below 25°C, but pepper plants are also rapidly colonized by *V. dahliae* at low temperatures (9). Kendrik and Middleton (9) concluded that the severity of Verticillium wilt is closely associated with growth of the pathogen. Verticillium wilt has become a major problem for production of many cool-season vegetable crops in the central coast of California, because the cool climate prevalent during most of the growing season favors growth and reproduction of *V. dahliae*. Perhaps Verticillium wilt of pepper can be managed partially by altering planting dates so that less favorable temperatures restrict infection of plants by *V. dahliae*.

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