Genetic Relationships and Cross Pathogenicities of *Verticillium dahliae* Isolates from Cauliflower and Other Crops

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ABSTRACT

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Morphologies, genetic relationships, and host specificities of *Verticillium dahliae* isolates from artichoke, cabbage, cauliflower, cotton, pepper, potato, strawberry, tomato, and watermelon were evaluated. Temperature optima for mycelial growth were evaluated at 10, 15, 20, 25, 30, and 35°C. Depending on the isolate, temperature optimum was either 20 or 25°C. The length and width of conidia in isolates from crucifer crops were significantly greater than the dimensions of conidia in other isolates. Isolates from artichoke, cabbage, cotton, pepper, potato, strawberry, tomato, and watermelon were tested for their pathogenicity on their host of origin, as well as on cauliflower. In addition, two *V. dahliae* isolates from cauliflower were tested for their pathogenicity on all the above crops, lettuce, and other crucifer crops such as bok choi, broccoli, Brussels sprouts, napa cabbage, radish, and rapini. All isolates caused wilt on cauliflower. The cauliflower isolates caused various degrees of wilt on all crops except lettuce, but their virulence depended on the host inoculated. Cauliflower isolates were highly virulent on other crucifer crops except broccoli and Brussels sprouts, on which they were only weakly virulent. None of the isolates tested were host specific. Seventeen isolates of *V. dahliae* from noncruciferous hosts were associated with one of two vegetative compatibility groups. Twelve *V. dahliae* isolates from cruciferous crops could not be assigned to a vegetative compatibility group because they did not produce nitrate nonutilizing mutants when cultured on chlorate-containing media. This observation may reflect diploidy in the cruciferous isolates, an interpretation which was supported by conidial size and in situ measurements of conidial DNA content. Based on polymorphisms in the intergenic spacer region of the nuclear rDNA, the *V. dahliae* isolates from cauliflower were unlike isolates from other hosts.

Additional keywords: crop rotation, epidemiology, host range.

Verticillium dahliae Kleb. is a pathogen with worldwide distribution (11,17,29) causing wilt on a broad range of fruit and nut crops (2,31), legumes (25), vegetables (6,12,16,24,30), forest trees (7), and woody and herbaceous ornamentals (7,33). The pathogen is widely distributed in the agricultural soils in California, affecting such diverse crops as artichoke (*Cynara scolymus* L.), cotton (*Gossypium hirsutum* L.), pepper (*Capsicum frutescens* L.), pistachio (*Pistacia vera* L.), potato (*Solanum tuberosum* L.), strawberry (*Fragaria* × *ananassa* Duch.), tomato (*Lycopersicon esculentum* Mill.), watermelon (*Citrullus vulgaris* Schrad.), cabbage (*Brassica oleracea* L. var. *capitata* L.), and a number of other crucifer crops. In recent years, Verticillium wilt has become an important production constraint on cauliflower (*B. oleracea* L. var. *botrytis* L.) in the Salinas Valley and other production areas on the California coast (24), causing extensive losses on crops harvested between April and October in *V. dahliae*-infested fields.

The sudden, widespread appearance of the disease on cauliflower has remained enigmatic with few choices for its management. In many crops, Verticillium wilt is managed with a combination of chemical and cultural methods (2,4,5,9,15,31,35). Where economically feasible, the disease can be successfully managed by preplant soil fumigation with a mixture of methyl bromide and chloropicrin (35). For crops in which soil fumigation is not economically feasible, resistant/tolerant cultivars are available or suitable crop rotations can be practiced (3,5,9,15,31). In cauliflower, all commercially available cultivars are susceptible (24), soil fumigation is not economically viable, and information on the relative susceptibilities to *V. dahliae* from cauliflower is unavailable for the wide range of crops grown in the Salinas Valley.

V. dahliae generally lacks host specificity and, thus, formae specialis and races are seldom mentioned in the literature (32), with the exception of tomato (3). In tomatoes, based on the virulence of certain isolates on cultivars containing the *Ve* gene, race 2 has been designated (3). Classification of pathogen strains in other crops has largely depended on vegetative compatibility groupings (32). For example, in potatoes the virulence of *V. dahliae* is related to the vegetative compatibility groups (VCGs) (21). Molecular markers also have been used to define subspecific groupings in *V. dahliae* (27).

From ecological, disease management, and taxonomic perspectives, the potential pathogenic specialization, or lack thereof, within *V. dahliae* is an important consideration. For example, the choice of crops for rotation is primarily dependent on the host range of the isolates in the region. The absence of information on the relative susceptibilities of a significant number of crops grown in the Salinas Valley makes the choice of crops for rotation with cauliflower difficult. Accordingly, this study was initiated with three objectives: (i) to study the morphological and genetic rela-

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tionships among California *V. dahliae* isolates from artichoke, cabbage, cauliflower, cotton, pepper, potato, strawberry, tomato, and watermelon; (ii) to determine the pathogenicities of all isolates on cauliflower; and (iii) to determine the pathogenicities of cauliflower isolates of *V. dahliae* on the above crops, lettuce (*Lactuca sativa* L.), and other cruciferous crops such as bok choi (*B. chinensis* L.), broccoli (*B. oleracea* L. var. *botrytis* L.), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), cabbage (*B. oleracea* L. var. *capitata* L.), napa cabbage (*B. pekinensis* (Lour.) Rupr.), radish (*Raphanus sativus* L.), and rapini (*B. oleracea* L. var. *italica* Plenck.).

MATERIALS AND METHODS

Isolates and their mycelial growth at different temperatures. The 10 isolates of *V. dahliae* used in the pathogenicity tests (Table 1) and an additional 19 isolates used in the VCG tests were obtained from diseased plants. The identity of all isolates was confirmed on the basis of colony morphology and production of microsclerotia, and single spores of each isolate were transferred to petri dishes containing acidified (2.5 ml of 25% [vol/vol] lactic acid solution per liter of medium) potato-dextrose agar (APDA). The single-spore isolates were maintained on potato-dextrose agar (PDA) slants in a refrigerator (4 ± 1°C).

Growth of the 10 isolates used in the pathogenicity tests was evaluated at 10, 15, 20, 25, 30, and 35°C. Five APDA dishes were seeded centrally with a 4-mm-diameter agar plug from the advancing margins of each isolate. Cultures were incubated in the dark in a completely randomized design at each temperature. The diameter of all colonies was measured when the leading edge of the fastest growing colony at any temperature had reached the edge of the dish. The experiment was conducted three times with the assignment of an incubator to a particular temperature being random.

Analysis of variance was used to evaluate the effects of experiment, temperature, isolate, and temperature × isolate interaction. Experiments were considered as replications for analysis of variance. Means and corresponding standard errors of the mean were computed for each isolate and temperature. Linear regression analysis was used to determine the relationship between temperature and colony diameter for all isolates combined, as well as for individual isolates, using the SAS procedure REG (SAS Institute, Inc., Cary, NC, Release 6.03 ed.). Regression models were evaluated by the significance of estimated parameters, the distribution of residuals, and the coefficients of determination.

Cultures of each isolate grown at the corresponding optimum temperature were flooded with 10 ml of sterile deionized water and brushed gently with a rubber spatula to dislodge the conidia. The length and width of 100 conidia from each isolate were measured using a compound microscope. Means and standard errors of the mean were computed for both the length and width of the conidia for all isolates.

Vegetative compatibility groupings. Vegetative compatibility between isolates was established by observing complementing nitrate nonutilizing mutants (*nits*) on a minimal medium containing nitrate as the sole supplementary source of nitrogen (8,20,28,32). Paired isolates which developed wild-type growth along the line of contact were judged to be vegetatively compatible; those that did not were regarded as vegetatively incompatible. For most isolates, *nits* were obtained by culturing on Puhalla's minimal medium (28) amended with 0.7 g of asparagine per liter and 3.0% potassium chlorate. For recalcitrant isolates, the medium composition was altered by changing the basal medium to either PDA or corn meal agar or by varying the concentration of chlorate and/or the type and concentration of amino acid. Isolates from each grouping we identified were paired with tester strains, supplied by C. A. Strausbaugh (University of Idaho), to determine any associations between our groupings and previously characterized groupings.

Variation in the intergenic spacer (IGS) of the nuclear rDNA repeat. Each of the isolates examined was cultured in N broth (34) with reduced micronutrients (100 ml/liter) on a rotary shaker for 1 to 3 weeks. Fungal material was collected by paper filtration (Whatman No. 1) and air-dried in petri dishes for 1 week. DNA was extracted from fungal biomass (approximately 50 mg, dry weight) according to Möller et al. (26), or by using the protocol described by Jacobson and Gordon (19). Both methods worked equally well. No RNase treatment was used. Stock samples were redissolved in 50 ml of Tris-EDTA (10 mM Tris-HCl [pH 8.0], 1 mM EDTA).

The polymerase chain reaction (PCR) was used, with primers (5′→3′) CTGAACGCCTCTAAGTCAG and AATGAGCGATTCGCAGTTTC, to amplify the IGS which separates tandem repeats of the nuclear rDNA genes. Each PCR reaction mixture (25 µl) consisted of 6.2 µl of 10% glycerol/water, 2.5 µl of 10× PCR buffer (0.5 M KCl, 0.1 M Tris-HCl [pH 8.3], 20 µM $MgCl_2$, 0.1% gelatin [wt/vol]), 2.5 µl of 10× dNTPs (2 mM each of dATP, dTTP, dCTP, and dGTP) (Promega, Madison, WI), 0.6 ml of each primer (20 µM in 50% glycerol/water), 0.1 µl of *Taq* DNA polymerase (Promega),

TABLE 1. *Verticillium dahliae* isolates, their host and geographic origin, length and width of conidia, and other characteristics

Isolate	Host	Geographic origin[v]	Conidia (µm ± SE)[w] Length	Width	Ratio	VCG[x]	IGS[y]
90-01	Cauliflower	CA	4.07 ± 0.67	1.38 ± 0.03	3.04	n.d.	D
90-02	Cauliflower	CA	4.03 ± 0.06	1.39 ± 0.03	2.97	n.d.	D
91-05	Cabbage	CA	4.21 ± 0.11	1.40 ± 0.04	3.21	n.d.	D
91-03	Chili pepper	CA	3.01 ± 0.06	1.33 ± 0.04	2.37	2	...[z]
91-04	Artichoke	CA	2.63 ± 0.09	1.24 ± 0.04	2.25	2	...
MD-01	Watermelon	CA	3.29 ± 0.08	1.38 ± 0.03	2.40	2	...
MD-04	Potato	ID	3.69 ± 0.11	1.41 ± 0.04	2.64	2×	C
MD-05	Cotton	CA	3.65 ± 0.09	1.40 ± 0.03	2.62	2	...
MD-06	Tomato	CA	3.75 ± 0.09	1.42 ± 0.03	2.65	2×	...
V1	Strawberry	CA	3.72 ± 0.11	1.35 ± 0.03	2.76	2×	B

[v] Sources were S. T. Koike = 90-01 and 90-02; T. R. Gordon = 91-03, 91-04, and 91-05; R. M. Davis = MD-01 and MD-06; W. C. Schnathorst = MD-04; J. E. DeVay = MD-05; and Driscoll = V1.

[w] Mean of measurements of 100 conidia.

[x] Isolates for which nitrate nonutilizing mutants (*nits*) were obtained were assigned either to vegetative compatibility group (VCG) 2 (32) or VCG 2×; for all others, VCG was not determined (n.d.) because *nits* could not be obtained.

[y] Five intergenic spacer (IGS) haplotypes were recognized (A to E) based on restriction fragment length polymorphisms identified with four different restriction endonucleases.

[z] Isolates not examined.

TABLE 2. Crops and the corresponding cultivars used to test the host range of *Verticillium dahliae* from cauliflower

Crop	Cultivar
Artichoke (*Cynara scolymus* L.)	Premier 5473
Bok choi (*Brassica chinensis* L.)	Joi Choi
Broccoli (*Brassica oleracea* L. var. *botrytis* L.)	Parasol
Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.)	Long Island Improved
Cabbage (*Brassica oleracea* L. var. *capitata* L.)	Grenadier
Cauliflower (*Brassica oleracea* L. var. *botrytis* L.)	White Rock
Napa cabbage (*Brassica pekinensis* (Lour.) Rupr.)	China Express
Cotton (*Gossypium hirsutum* L.)	Acala SJ-2
Lettuce (*Lactuca sativa* L.)	Salinas
Pepper (*Capsicum frutescens* L.)	Jupiter
Potato (*Solanum tuberosum* L.)	Red Lasoda
Radish (*Raphanus sativus* L.)	April Cross
Rapini (*Brassica oleracea* L. var. *italica* Plenck.)	Heirloom
Strawberry (*Fragaria* × *ananassa* Duch.)	Sequoia
Tomato (*Lycopersicon esculentum* Mill.)	Early Pak-7
Watermelon (*Citrullus vulgaris* Schrad.)	Sugar Baby

and 12.5 µl of diluted DNA extract. For preparative purposes the reaction volume was scaled up to 100 µl.

The reaction mixtures were overlaid with one drop of mineral oil and incubated in a Crocodile II thermo-cycler (Appligene, Pleasanton, CA), using 35 cycles of 60 s at 94°C (denaturation step), 75 s at 54°C (annealing step), and 90 s at 70°C (extension step). PCR products were separated in a 1% agarose gel and detected by UV fluorescence after ethidium bromide staining.

The restriction endonucleases (REs) *Apa*I, *Ava*II, *Cfo*I, and *Eco*RI were used for digestion of PCR products. The 10-µl reaction mixture consisted of 8.5 µl of PCR product, 1 µl of 10× RE buffer, and 0.5 µl of enzyme (except for *Apa*I, which was added in 1-µl aliquots) (all REs and buffers were from Promega). Digestions were carried out at 37°C for 2 h. Restriction fragments were separated in 2.5% agarose gels and visualized by staining with ethidium bromide. Size markers from pGEM (Promega), 100-bp and 1-kb ladders (Gibco BRL, Burlington, ON) were used to estimate the size of restriction fragments.

Pathogenicity experiments. Pathogenicity of two *V. dahliae* isolates from cauliflower was determined on hosts listed in Table 2. The isolates from other hosts were evaluated for pathogenicity on their respective hosts and on cauliflower, using the method described previously (24). Plants for inoculation were obtained by seeding into autoclaved riverbed sand in seedling trays with 200 cells. The plants were maintained on benches in a greenhouse until inoculation (23 ± 1/10 ± 2°C day/night regime).

Inoculum of all isolates was obtained by incubating cultures at the corresponding optimum temperature for 25 days. Spore suspensions of each isolate were prepared by adding 10 ml of sterile distilled water to each plate and scraping the cultures with a rubber spatula. The conidial density of each isolate was adjusted to 10^6 conidia/ml. For hosts from which a *V. dahliae* isolate was available, inoculations were made with their own isolate and the two cauliflower isolates. For all other hosts, inoculations were made only with the cauliflower isolates. Cauliflower plants were inoculated with all isolates. Two centimeters of root was trimmed from each 4-week-old plant before inoculation. Ten plants of each host were inoculated per isolate by dipping the roots in a spore suspension for 5 min and planting them individually into autoclaved soil in 12-cm-diameter pots. The roots of 10 plants of each host were trimmed, dipped in sterile water, and maintained as uninoculated controls. Thus, there were 40 plants of hosts from which an isolate was available (10 plants inoculated with its own isolate, 10 plants inoculated with each of the two cauliflower isolates, and 10 uninoculated plants), 30 plants of hosts from which an isolate was not available, and 140 cauliflower plants (10 plants inoculated from each *V. dahliae* isolate except cauliflower, 10 plants inoculated with each cauliflower isolate in both the growth chamber and greenhouse, and 10 uninoculated plants each in the growth chamber and greenhouse). Because cotton, pepper, potato, and watermelon isolates of *V. dahliae* had an optimum temperature of 25°C for growth, all inoculated and uninoculated plants from these crops, cauliflower plants uninoculated, and cauliflower plants inoculated with cauliflower, cotton, pepper, potato, tomato, and watermelon isolates, and all lettuce plants were incubated on greenhouse benches (25 ± 2/10 ± 2°C day/night regime). The isolates from cauliflower, cabbage, artichoke, and strawberry had an optimum temperature of 20°C; and, thus, all inoculated and uninoculated plants from these crops and a second set of cauliflower plants, inoculated with isolates from these crops plus the two cauliflower isolates, were incubated in the growth chamber (20 ± 1/15 ± 1°C day/night regime). The experimental design within the greenhouse and growth chamber was a factorial combination of isolate and host with 10 replications arranged in a randomized block design. The experiments were repeated once.

After 6 weeks of incubation, all plants were gently uprooted, washed free of soil, and growth data such as height, number of leaves, and dry weights of shoot and root were collected for each plant. Foliar symptom severity and root discoloration severity were rated subjectively (24). Foliar symptom severity was rated on a scale of 0 to 4 in which 0 = normal plants; 1 = 25% of leaves showing chlorosis; 2 = 50% of leaves showing chlorosis; 3 = 51 to 74% of leaves showing chlorosis; and 4 = plants with > 75% of leaves showing chlorosis. Severity of root discoloration was rated on a scale of 1 to 4 in which 1 = normal appearance; 2 = browning of < 10% of lateral roots; 3 = browning of nearly 50% of lateral roots; and 4 = extensive browning of lateral roots and reduced lateral root system. Surface-sterilized stem segments were

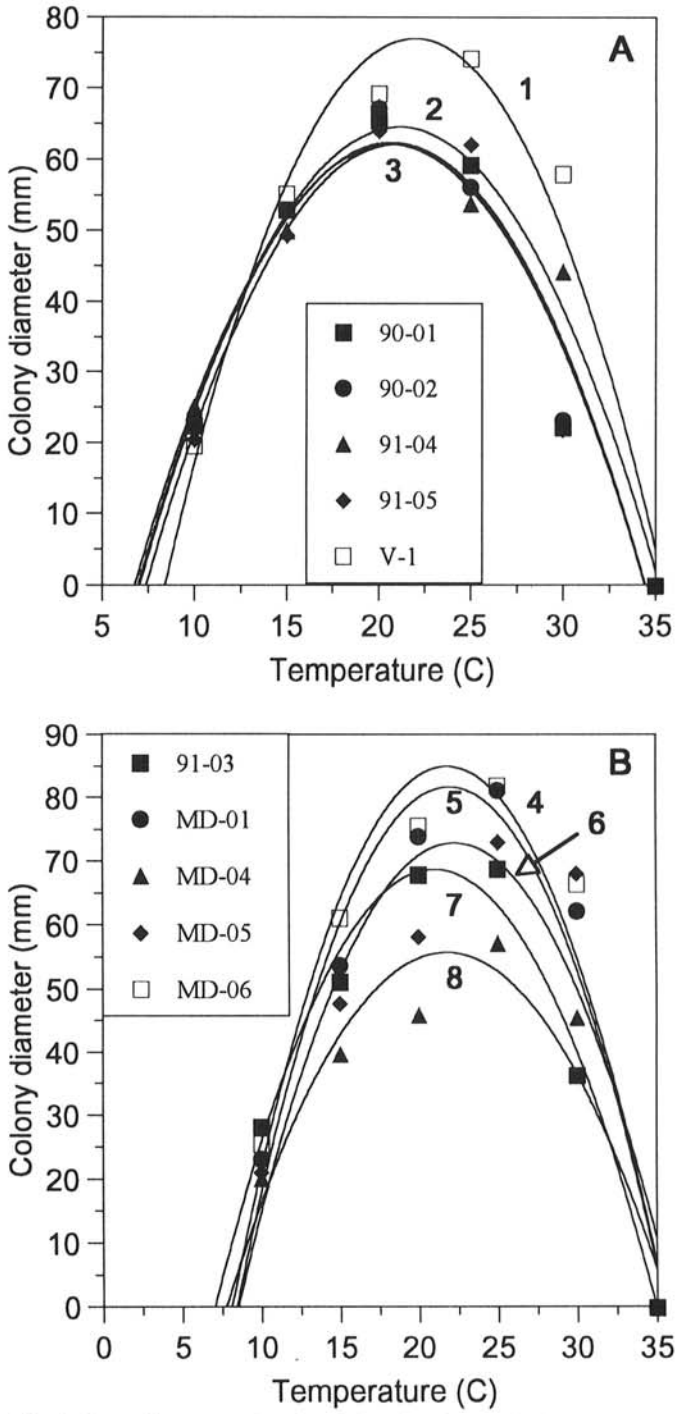

Fig. 1. Regression curves (numbered 1 to 8) along with the mean colony diameters of *Verticillium dahliae* isolates at different temperatures; **A,** from artichoke (91-04; 2 in A), cauliflower (90-01 and 90-02; 3 in A), cabbage (91-05; 3 in A), and strawberry (V-1; 1 in A); and **B,** from cotton (MD-05; 6 in B), pepper (91-03; 7 in B), potato (MD-04; 8 in B), tomato (MD-06; 4 in B), and watermelon (MD-01; 5 in B). Each point is the mean of five petri dishes each in three experiments.

placed on APDA to confirm *V. dahliae* infection. The APDA plates were incubated on laboratory benches for 3 weeks, and the number of plants yielding *V. dahliae* colonies was recorded.

Analysis of variance was conducted on each variable to determine the overall effects of experiment, isolate, host, isolate × host, and experiment × isolate × host interactions for data sets from the greenhouse and growth chamber. Experiments and replications within experiments were considered as random effects in the analysis. The error term 'experiment × isolate × host' was used to test the effects of isolates, hosts, and isolate × host interaction, and the remaining terms in the model were tested by the experimental error. Means were computed for each isolate-host combination and comparisons of isolates within a host were made using least significant difference tests ($P < 0.05$). Pathogen recovery from different hosts was expressed as percentage of the total plants inoculated.

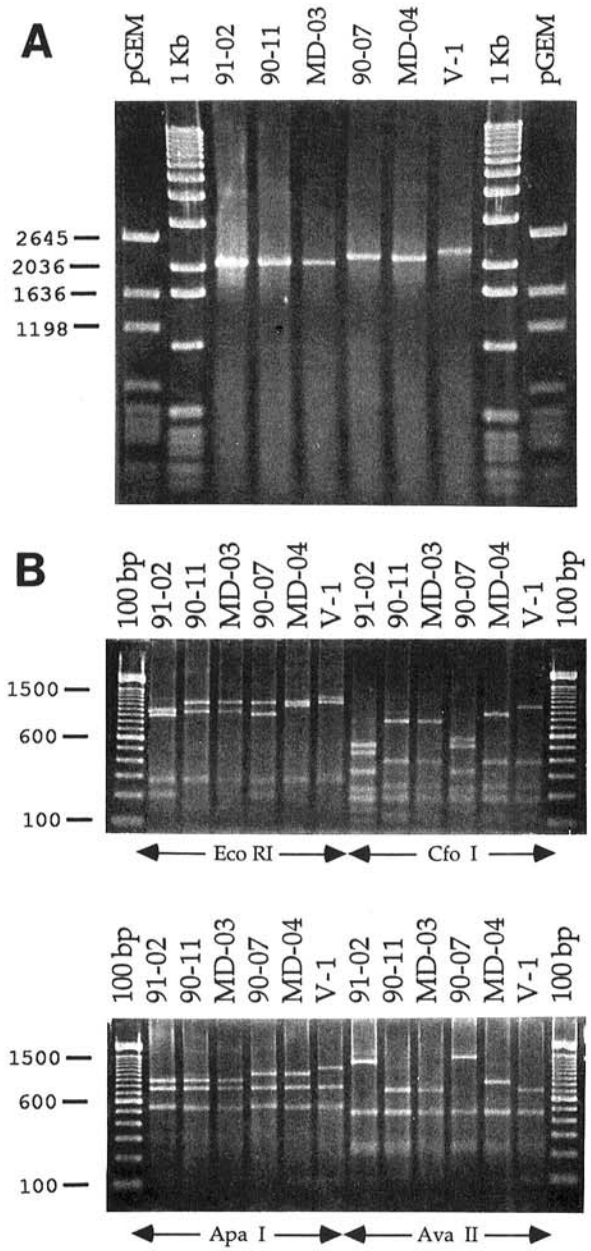

Fig. 2. A, An ethidium-stained agarose gel showing the amplified product, obtained by a polymerase chain reaction from *Verticillium dahliae* isolates, representing each of five different intergenic spacer (IGS) haplotypes. **B,** Restriction fragment length polymorphisms of isolates representing the five different IGS haplotypes which were identified among 13 isolates of *V. dahliae*. Isolate names and restriction endonucleases used are indicated on the photograph. Isolates MD-03 and 90-11 were associated with vegetative compatibility group (VCG) 2 and IGS haplotype A; isolates V-1 and MD-04 were associated with VCG 2× and IGS haplotypes B and C, respectively; isolates 91-02 and 90-07 (not assigned to a VCG) were assigned to IGS haplotypes D and E, respectively.

RESULTS

Mycelial growth at different temperatures and conidial sizes. Analysis of variance indicated that colony diameter was significantly affected by the temperature, isolates, and isolate × temperature interaction. The colony diameters of the artichoke, cauliflower, and cabbage isolates increased with increasing temperatures up to 20°C and then declined (Fig. 1A), except that the colony diameters of the cabbage isolate were not different at 20 and 25°C. The colony diameters of the strawberry isolate were maximum at 25°C and were significantly lower at other temperatures (Fig. 1A). At each temperature, the growth of the cauliflower and cabbage isolates was nearly identical. The growth response of the artichoke isolate was similar to that of isolates from the crucifer crops, but was significantly higher at 30°C. Growth of the strawberry isolate, however, was significantly higher at 20 and 25°C. Maximum growth of cotton, pepper, potato, tomato, and watermelon isolates occurred at 25°C (Fig. 1B). The colony diameters of the tomato and watermelon isolates were significantly higher at 25°C compared to all other isolates, and that of the potato isolate was significantly lower (Fig. 1B). At 35°C, there was no measurable growth of any of these isolates. The growth response of all the isolates to temperature was quadratic ($Y = -96.6 + 15.3\ T - 0.36\ T^2$; $r^2 = 0.99$ and $P = 0.0001$). However, parameter values and the coefficients of determination for individual isolates were different.

The conidia from crucifer isolates were significantly longer than from all other isolates. The width of the conidia from all the isolates was nearly identical, except those from the artichoke isolate, which were significantly narrower (Table 1). The ratio between length and width was, therefore, higher for the conidia from crucifer crop isolates compared to all other isolates (Table 1). The isolates separated into two distinct groups based on the conidial length and the ratio between length and width of the conidia. One group included isolates from only the crucifer crops and the second group included all other isolates.

Vegetative compatibility groupings. Based on the interisolate pairings of complementary *nits*, four isolates from noncruciferous hosts (MD-01, MD-05, 91-03, and 91-04) corresponded to VCG 2 (20,32). The three other isolates from noncruciferous hosts constituted a second VCG (i.e., VCG 2×); isolates associated with this VCG were not compatible with any of the tester strains provided by C. A. Strausbaugh (Table 1). Nine additional isolates obtained from noncruciferous hosts were tested and found to be associated with VCG 2 (data not shown).

The isolates from cauliflower (90-01 and 90-02) and cabbage (91-05) were not inhibited in the presence of chlorate; consequently, *nits* could not be obtained and the isolates could not be assigned to a VCG. Six additional isolates from cauliflower were cultured on chlorate-containing media and also failed to produce chlorate-resistant sectors. In the course of attempting to secure *nits* from these recalcitrant isolates, 30 different combinations of media components were tested. The tested media had chlorate concentrations ranging from 1.5 to 25% and asparagine concentrations from 0 to 1.7 g/liter.

Variation in the IGS of the rDNA repeat. Because the IGS region of the nuclear rDNA has been shown to reveal variability within a fungal species (1), we examined this region in *V. dahliae* to assess the similarity of cauliflower and noncauliflower isolates. Thirteen isolates of *V. dahliae* were examined, representing both of the VCGs identified and cauliflower and cabbage isolates, which could not be assigned to a VCG. In all cases, amplification by PCR yielded a single fragment corresponding to IGS, ranging

in size from 2,100 to 2,350 bp (Fig. 2A). Based on the restriction fragment length polymorphisms (RFLPs) identified with four different REs (Fig. 2B), 13 isolates of *V. dahliae* corresponded to five IGS haplotypes. The two isolates associated with VCG 2 had identical haplotypes (A), V-1 and MD-04, whereas VCG 2× had different IGS haplotypes (B and C, respectively). The three cauliflower isolates (90-01, 90-02, and 90-10), one from green cauliflower (91-02) and two from cabbage (91-05 and 91-06), were associated with IGS haplotype D. Three other cauliflower isolates (90-04, 90-05, and 90-07) were associated with IGS haplotype E.

Pathogenicity of *V. dahliae* isolates on crucifer crops. Analysis of variance indicated that the all variables measured were affected significantly by the isolates, hosts, and isolate × host interaction. Neither the replications within experiment nor the three-way interaction between experiment × isolate × host were significant, indicating that the results were consistent in the two experiments conducted within the greenhouse and growth chamber. The results combined over the two experiments are therefore presented. Both *V. dahliae* isolates from cauliflower were pathogenic to all other crucifer crops, but they were not equally virulent on all crops (Table 3). The cauliflower isolates were highly virulent on bok choi, napa cabbage, radish, and rapini. On these hosts, all measured variables were significantly different in inoculated plants compared with uninoculated plants (Table 3). The characteristic increase in the number of leaves caused by *V. dahliae* infection on cauliflower seedlings was not evident on the above

TABLE 3. Least square means of different variables on crucifer hosts incubated in a growth chamber following inoculations with *Verticillium dahliae* isolates, and percentage of pathogen recovery

Host	Isolate[w]	Plant height (cm)	Number of leaves	Dry weight (g)		Severity index[x]	Root discoloration[y]	Pathogen recovery (%)
				Root	Shoot			
Bok choi	90-01	15.63 a[z]	4.4 a	0.12 a	0.60 a	1.55 a	1.8 a	95
	90-02	15.98 a	4.2 a	0.12 a	0.58 a	1.60 a	1.9 a	70
	Check	17.74 b	6.3 b	0.18 b	0.75 b	0.00 b	1.0 b	0
Brussels sprouts	90-01	12.82 a	8.0 a	0.10 a	0.51 a	1.50 a	1.7 a	60
	90-02	13.01 a	7.9 a	0.10 a	0.50 a	1.30 a	1.6 a	40
	Check	13.99 b	8.1 a	0.12 a	0.53 a	0.25 b	1.3 a	0
Broccoli	90-01	17.78 a	5.1 a	0.18 a	0.67 a	0.35 a	1.4 a	50
	90-02	17.77 a	5.1 a	0.15 b	0.64 a	0.80 b	1.3 a	60
	Check	19.18 b	5.6 b	0.14 b	0.63 a	0.00 c	1.0 b	0
Cabbage	90-01	14.56 c	7.3 ab	0.09 b	0.57 ab	0.85 b	1.6 a	50
	90-02	14.86 bc	7.5 a	0.07 c	0.54 b	0.70 b	1.7 a	59
	91-05	15.24 b	7.2 ab	0.08 bc	0.57 ab	1.95 a	1.7 a	45
	Check	16.14 a	6.9 b	0.13 a	0.61 a	0.25 c	1.3 b	0
Napa cabbage	90-01	12.01 b	6.1 b	0.05 b	0.50 b	2.00 b	2.2 a	75
	90-02	12.36 b	6.2 b	0.06 b	0.48 b	2.05 b	2.3 a	80
	Check	14.60 a	7.5 a	0.11 a	0.64 a	0.15 a	1.1 b	0
Radish	90-01	18.11 b	5.7 b	0.09 b	0.60 b	2.00 a	2.5 a	70
	90-02	17.96 b	5.9 b	0.09 b	0.52 b	1.60 b	2.7 a	80
	Check	20.59 a	6.8 a	0.16 a	0.76 a	0.20 c	1.2 b	0
Rapini	90-01	20.75 b	5.3 b	0.09 b	0.52 b	2.85 a	2.4 a	75
	90-02	21.40 b	5.4 b	0.11 b	0.51 b	2.35 a	2.3 a	75
	Check	25.73 a	6.9 a	0.17 a	0.79 a	0.40 b	1.6 b	0

[w] Isolates 90-01 and 90-02 are from cauliflower, and isolate 90-05 is from cabbage.
[x] Recorded on a 0 to 4 scale, in which 0 = normal plants and 4 = > 75% of leaves showing chlorosis.
[y] Symptoms on roots recorded on a 1 to 4 scale, in which 1 = normal appearance and 4 = extensive browning and reduced root laterals.
[z] Values followed by the same letter within a host are not significantly different according to a least significant difference test ($P < 0.05$). All variables are the means of 10 plants each in two experiments.

TABLE 4. Least square means of different variables on cauliflower incubated in the greenhouse and growth chamber following inoculations with *Verticillium dahliae* isolates from cauliflower and other hosts, and percentage of pathogen recovery

Host	Isolate	Plant height (cm)	Number of leaves	Dry weight (g)		Severity index[x]	Root discoloration[y]	Pathogen recovery (%)
				Root	Shoot			
				Growth chamber				
Cauliflower	90-01 (cauliflower)	12.15 d[z]	7.0 b	0.09 b	0.75 bc	2.4 b	3.7 a	75
	90-02 (cauliflower)	13.32 bc	7.3 ab	0.06 c	0.67 c	3.0 a	3.4 a	70
	91-04 (artichoke)	13.66 b	6.8 b	0.09 b	0.80 b	3.1 a	2.4 c	85
	91-05 (cabbage)	13.05 c	8.1 a	0.08 bc	0.80 b	2.1 b	2.9 b	70
	V1 (strawberry)	13.88 b	7.1 b	0.09 b	0.81 b	2.2 b	2.9 b	70
	Check	15.16 a	5.6 c	0.11 a	0.92 a	0.2 c	1.5 d	0
				Greenhouse				
Cauliflower	90-01 (cauliflower)	13.93 cd	8.9 a	0.15 c	1.24 bc	2.1 b	2.6 b	85
	90-02 (cauliflower)	13.11 de	8.2 ab	0.12 c	1.10 c	2.7 a	3.2 a	75
	91-03 (pepper)	14.46 c	7.2 cd	0.20 b	1.73 a	1.2 d	1.9 d	30
	MD-01 (watermelon)	15.64 b	7.5 bcd	0.21 b	1.62 a	1.4 cd	1.5 e	20
	MD-04 (potato)	14.11 c	7.2 cd	0.20 b	1.11 c	1.8 c	2.3 c	65
	MD-05 (cotton)	12.54 e	7.5 bcd	0.16 bc	1.31 b	2.3 b	2.4 bc	75
	MD-06 (tomato)	13.24 de	6.8 d	0.19 b	1.14 bc	2.9 a	2.2 cd	60
	Check	17.01 a	7.9 bc	0.25 a	1.60 a	0.3 d	1.2 e	0

[x] Recorded on a 0 to 4 scale, in which 0 = normal plants and 4 = > 75% of leaves showing chlorosis.
[y] Symptoms on roots recorded on a 1 to 4 scale, in which 1 = normal appearance and 4 = extensive browning and reduced root laterals.
[z] Values followed by the same letter within a host are not significantly different according to a least significant difference test ($P < 0.05$). All variables are the means of 10 plants each in two experiments.

crucifer crops. The number of leaves was significantly lower in these crops compared with uninoculated plants. The mean severity index and root discoloration ranged from 1.6 to 2.9 and 1.8 to 2.7, respectively. The pathogen was recovered from at least 40% of the inoculated plants. On most crops, isolate 90-02 was more virulent than 90-01 (Table 3). Symptoms were not observed on uninoculated plants.

The cabbage isolate (91-05) caused wilt symptoms on cauliflower (Table 4) and on cabbage (Table 3). Similarly, both cauliflower isolates were pathogenic to cabbage. On both cabbage and cauliflower, all isolates caused an increase in the number of leaves relative to uninoculated plants, but the increase was significant only on cauliflower (Tables 3 and 4).

Both cauliflower isolates were only weakly pathogenic on broccoli and Brussels sprouts. Inoculated Brussels sprouts plants showed significant reductions only in plant height and significant increases in severity index (Table 3). In broccoli, however, significant reductions in plant height and number of leaves and significant increases in the severity index and root discoloration were observed in inoculated plants. Shoot dry weight was not significantly different from uninoculated plants, and root dry weight was significantly higher in plants inoculated with isolate 90-01 (Table 3). In both crops, the pathogen was recovered from at least 40% of the inoculated plants (Table 3).

Pathogenicity of *V. dahliae* isolates on noncruciferous crops. Both isolates from cauliflower failed to cause any visible symptoms of Verticillium wilt on lettuce. There were no significant differences in any of the measured variables between inoculated and uninoculated lettuce plants (Table 5). On all other crops, the cauliflower isolates, as well as isolates from the respective crops, caused wilt symptoms and significant reductions in the majority of the variables measured compared with uninoculated plants. The degree of virulence of cauliflower isolates was dependent upon the host inoculated. The cauliflower and pepper isolates inoculated on pepper were pathogenic, but only weakly virulent. On cotton, potato, and tomato, isolates from the respective crops caused a significantly greater reduction in plant height, number of leaves, root and shoot dry weights, and a higher severity index than isolates from cauliflower (Table 5). In the other crops, a clear virulence distinction between their own isolate and cauliflower isolates was not possible.

The reductions in plant height and root and shoot dry weights caused by the two cauliflower isolates on noncruciferous crops ranged from 8.2 to 26.0, 14.3 to 55.3, and 5.5 to 42.7%, respectively. This was in contrast to reductions in plant height and root and shoot dry weights of 18.1 to 23.0, 36.2 to 47.7, and 22.5 to 31.1%, respectively, caused by the two isolates on cauliflower (Table 5). Recovery of *V. dahliae* was almost always higher from noncruciferous crop plants inoculated with their own isolate (Table 5).

Strawberry plants inoculated with a strawberry isolate and the two cauliflower isolates did not exhibit significant reductions in plant height. However, there were significant reductions in each of the other plant growth parameters measured and significant increases in the values of the two disease variables measured compared with uninoculated plants (Table 5).

TABLE 5. Least square means of different variables on crops incubated in the greenhouse and growth chamber following inoculations with *Verticillium dahliae* isolates from that crop and from cauliflower, and percentage of pathogen recovery

Host	Isolate	Plant height (cm)	Number of leaves	Dry weight (g)		Severity index[x]	Root discoloration[y]	Pathogen recovery (%)
				Root	Shoot			
				Greenhouse				
Cotton	90-01	19.98 b[z]	3.1 b	0.02 bc	0.20 b	2.25 b	2.4 c	65
	90-02	16.66 c	3.3 ab	0.03 b	0.18 b	2.50 b	2.8 b	60
	MD-05	14.57 d	0.5 c	0.01 c	0.10 c	3.95 a	3.9 a	85
	Check	22.50 a	3.7 a	0.05 a	0.31 a	1.00 c	1.6 d	0
Lettuce	90-01	14.51 b	7.6 b	0.67 a	1.88 a	0.00 a	1.13 a	0
	90-02	15.08 a	8.4 a	0.68 a	2.02 a	0.00 a	1.13 a	0
	Check	14.71 ab	7.9 ab	0.79 a	2.21 a	0.00 a	1.63 b	0
Pepper	90-01	20.24 b	8.5 b	0.10 b	0.29 b	0.25 ab	1.0 b	25
	90-02	21.08 ab	8.6 b	0.10 b	0.30 b	0.45 a	1.1 ab	25
	91-03	20.31 b	9.3 a	0.12 a	0.30 b	0.25 ab	1.2 a	20
	Check	21.70 a	8.6 b	0.14 ab	0.35 a	0.00 b	1.0 b	0
Potato	90-01	24.69 b	5.4 b	0.15 b	0.56 b	2.25 b	2.5 b	35
	90-02	24.12 b	6.0 a	0.15 b	0.55 b	1.00 d	1.9 d	25
	MD-04	13.84 c	3.3 c	0.13 c	0.30 c	3.10 a	3.3 a	65
	Check	28.07 a	5.5 ab	0.20 a	0.67 a	1.60 c	2.2 c	0
Tomato	90-01	29.43 b	7.2 ab	0.22 b	0.96 b	1.10 c	2.2 a	35
	90-02	28.90 b	7.4 a	0.23 b	0.97 b	1.60 b	1.6 b	40
	MD-06	26.96 c	5.7 c	0.23 b	0.83 c	2.35 a	1.7 b	50
	Check	31.33 a	6.9 b	0.29 a	1.12 a	0.00 d	1.0 c	0
Watermelon	90-01	14.59 b	4.9 a	0.03 b	0.23 b	1.45 b	2.4 a	40
	90-02	14.59 b	5.0 a	0.04 b	0.17 c	1.90 b	2.3 a	50
	MD-01	15.87 ab	3.1 b	0.03 b	0.24 b	2.70 a	2.3 a	65
	Check	16.88 a	4.9 a	0.06 a	0.29 a	0.10 c	1.3 b	0
				Growth chamber				
Artichoke	90-01	21.95 b[z]	5.1 a	0.24 ab	0.78 ab	2.00 a	1.9 a	55
	90-02	21.39 b	4.6 b	0.19 b	0.71 b	1.80 a	2.0 a	60
	91-04	19.83 c	4.3 b	0.20 b	0.70 b	2.35 a	2.0 a	90
	Check	23.91 a	4.7 ab	0.28 a	0.82 a	0.65 b	1.2 b	0
Strawberry	90-01	26.04 a	4.8 b	0.89 b	2.09 b	1.95 b	2.5 a	60
	90-02	24.69 a	5.6 a	1.08 ab	2.00 b	1.50 c	2.0 b	65
	V1	25.87 a	4.8 b	0.94 b	2.02 b	2.50 a	2.6 a	80
	Check	26.38 a	5.9 a	1.30 a	2.37 a	1.30 c	1.8 b	0

[x] Recorded on a 0 to 4 scale, in which 0 = normal plants and 4 = > 75% of leaves showing chlorosis.

[y] Symptoms on roots recorded on a 1 to 4 scale, in which 1 = normal appearance and 4 = extensive browning and reduced root laterals.

[z] Values followed by the same letter within a host are not significantly different according to a least significant difference test ($P < 0.05$). All variables are the means of 10 plants each in two experiments.

Pathogenicity of *V. dahliae* isolates on cauliflower. Cauliflower isolates, as well as isolates from other crops, were pathogenic to cauliflower in the greenhouse and growth chamber tests (Table 4). Isolates differed, however, in the severity of symptoms they induced on cauliflower. Isolates from cotton (MD-05), cauliflower (90-02), and tomato (MD-06) were the most virulent on cauliflower, and pepper (91-04) and watermelon (MD-01) isolates the least virulent. The pathogen was recovered from at least 65% of the inoculated plants in the growth chamber and from at least 60% of all inoculated plants, except those inoculated with pepper and watermelon isolates in the greenhouse. Uninoculated cauliflower plants grew significantly better in the greenhouse than in the growth chamber (Table 4). The reactions of all isolates evaluated in this study on different crops have been summarized in Fig. 3.

DISCUSSION

The results of this study suggest that host specificity was not common in *V. dahliae* isolates, although a given isolate may have exhibited differential virulence against different hosts. Isolates from all other crops included in this study caused Verticillium wilt on cauliflower and vice versa. The cauliflower isolates were more virulent on all cruciferous crops, except broccoli and Brussels sprouts, than on noncruciferous crops, and certain isolates from other crops were as virulent as the cauliflower isolates on cauliflower.

In a limited host range study of *V. dahliae* isolates from Brussels sprouts, Isaac (16) concluded that they are not pathogenic to several crop species including broccoli, cauliflower, and strawberry, and suggested that *V. dahliae* from Brussels sprouts is a distinct strain. Recent evaluation (6) of the pathogenicity of *V. dahliae* isolates from horseradish on several crops showed that they are pathogenic to China aster, eggplant, potato, and sunflower, and nonpathogenic to pepper, tomato, and watermelon. Brussels sprouts plants inoculated with the horseradish isolate do not show external symptoms of Verticillium wilt, but show vascular discoloration in both roots and shoots (6). In our study, although we did not have an isolate from Brussels sprouts, isolates from cabbage and cauliflower caused Verticillium wilt on both hosts. Similarly, the strawberry isolate caused Verticillium wilt on cauliflower plants and vice versa. Thus, host specificity was not observed, even in isolates from within the cruciferous crops. Both isolates from cauliflower reduced plant height in inoculated broccoli and Brussel sprouts plants, and they exhibited a significantly higher severity index than uninoculated plants. While the isolates from cauliflower were certainly pathogenic on both Brussels sprouts and broccoli, they were weakly virulent. Nevertheless, in commercial broccoli fields in the Salinas Valley, Verticillium wilt has never been observed even when broccoli is planted in soils rich in *V. dahliae* propagules (24).

V. dahliae isolates from certain noncruciferous crops were as virulent on cauliflower as cauliflower isolates. The two cauliflower isolates also were virulent on noncruciferous crops. Such lack of host specificity and pathogen specialization in *V. dahliae* is well documented in most crops (21,32), except tomato (3). While the *V. dahliae* isolates from noncruciferous crops were pathogenic to cauliflower and vice versa, there were differences in their virulence. Pathogens require unique genetic information to colonize plant tissues and establish parasitic relationships. But their virulence and host ranges are determined by a complex of factors such as enzymes, toxins, and other plant metabolites (23). The factor responsible for the wide host range in *V. dahliae* remains to be determined.

In the Salinas Valley, even though many of the noncruciferous crops are commercially grown and commonly affected by Verticillium wilt, cauliflower had not been reported as a host until recently (24). There are no known cultural shifts that could explain the sudden appearance of the disease on cauliflower. Indeed, a varietal shift from the open-pollinated, highly variable cultivars to the more uniform hybrid cultivars did occur in the last 10 years, but all available cultivars are susceptible to Verticillium wilt (24). Regarding the shifts in the pathogen itself, the *V. dahliae* isolates from both cruciferous and noncruciferous crops had temperature optima at either 20 or 25°C and, thus, the groups could not be distinguished on that basis. However, the conidia from pathogen isolates from cruciferous crops were significantly larger than those from noncruciferous crops, which might indicate the latter are diploid (18) as others have reported for certain crucifer strains of *V. dahliae* (14). Karapapa et al. (22) suggested that the diploid strain of *V. dahliae* associated with cruciferous crops be recognized as a separate species.

The failure of cauliflower strains to be inhibited by chlorate is also suggestive of diploidy. Chlorate resistance is generally attributed to a mutation affecting the enzymatic reduction of nitrate (and chlorate, which is processed by the same pathway). In haploid fungi, such mutations directly affect the phenotype, which is both resistant to chlorate and unable to utilize nitrate. In contrast, a comparable mutation in a diploid would be recessive, leaving the phenotype unaffected. Thus, diploidy in the cauliflower isolates would explain our inability to obtain *nits* from *V. dahliae* isolates originating on this host. Also consistent with diploidy is an approximately two-fold higher DNA content of cruciferous as compared to noncruciferous isolates, based on in situ DNA microfluorometry of conidial nuclei (P. Bonello and T. R. Gordon, *unpublished data*).

Because we could not associate cauliflower isolates with a VCG on the basis of heterokaryon formation between complementing *nits*, we examined polymorphisms in IGS to determine if there was any similarity between the cauliflower isolates and any of the identified *V. dahliae* VCGs. No such affinity was apparent. However, given that the only two VCG 2× isolates examined had different IGS haplotypes, it remains possible that, if more isolates were sampled, the IGS haplotype(s) associated with cauliflower isolates would also be associated with isolates of an identified VCG.

The distinctiveness of the cauliflower strains would be consistent with the recent establishment of a new strain in the Salinas Valley (24). That we identified two different IGS haplotypes among the cauliflower isolates suggests that this population is not homogeneous and, thus, may not be directly traceable to a single

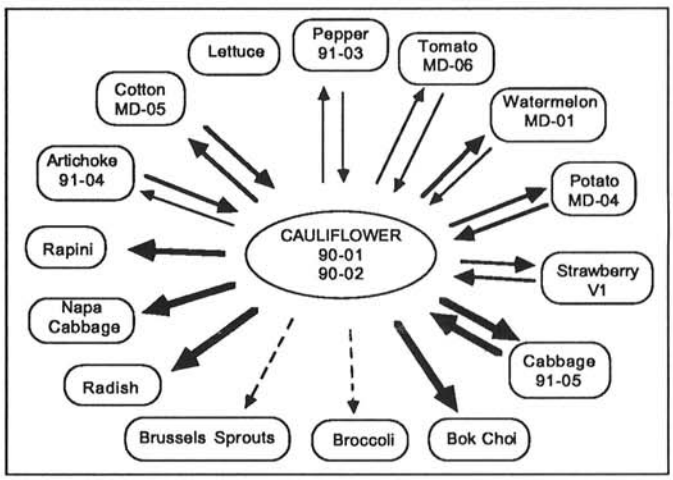

Fig. 3. Summary of the pathogenicity tests of different *Verticillium dahliae* isolates on different hosts. Arrows pointing both ways indicate that the isolate originating from each was tested on the other. Arrows in one direction means that only cauliflower isolates were inoculated. No arrow means the crop is not a host of *V. dahliae*. The thickness of arrows indicates the degree of virulence of the corresponding isolate; the thicker the arrow, the greater is the virulence. Dashed arrows indicate that the isolates were weakly pathogenic on the crops.

recent introduction. However, the IGS haplotypes may differ only in length. Changes leading to length differences occur commonly in the IGS, and length variants may even occur within the same individual (13). Thus, our observation did not necessarily reflect significant genetic heterogeneity among the cauliflower isolates.

Although the sudden high incidence of Verticillium wilt on cauliflower may reflect the activity of a new strain, it does not mean that isolates comprising the well-established population of *V. dahliae* in coastal California are incapable of causing disease on cauliflower or other cruciferous crops. Our host range tests clearly showed that isolates from noncruciferous hosts (e.g., cotton and tomato) could induce symptoms on cauliflower. Also, two isolates from cruciferous crops (cabbage and napa cabbage) were associated with VCG 2 and, thus, presumably were of relatively long residence in coastal California. The important differences may be in virulence, with the newly introduced strain(s) having a greater ability to induce damage on mature plants in the field.

The lack of host specificity in *V. dahliae* isolates can have a significant impact on Verticillium wilt management in different crops by limiting the choice of crops for rotation. Apparently, *V. dahliae* propagules from the crops included in this study could contribute inoculum for cauliflower and vice versa. Crop rotation has been the primary means of managing Verticillium wilt in crops in which soil fumigation is either not economically feasible or host resistance is unavailable (4,5,9,10,15). However, Huisman and Ashworth (15) concluded that the value of short-term rotations in reducing the number of propagules is limited because the numbers of microsclerotia after rotation usually are above the threshold level at which most plants get infected. In the Salinas Valley, cauliflower is rotated regularly with lettuce. In our study, *V. dahliae* isolates were not pathogenic to lettuce and, thus, lettuce crops were not likely to increase the numbers of propagules in soil. The nonappearance of Verticillium wilt on broccoli in commercial fields with very high numbers of *V. dahliae* microsclerotia suggests that broccoli is somehow immune to the disease. In our study, the root and shoot dry weights of broccoli plants inoculated with both isolates from cauliflower were not significantly different from uninoculated plants. This result suggests that rotations of broccoli with cauliflower may be a viable means of reducing the number of microsclerotia in the soil and managing the disease in commercial fields. Research is currently underway to study the effects of broccoli rotation on the dynamics of *V. dahliae* microsclerotia and Verticillium wilt incidence in cauliflower.

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