Development of Potato Early Dying in Response to Infection by Two Pathotypes of *Verticillium dahliae* and Co-infection by *Pratylenchus penetrans*

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ABSTRACT

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Verticillium dahliae isolates assigned to subgroups A and B of vegetative compatibility group 4 (VCG 4A and 4B) were tested for virulence to potato and ability to interact with the root-lesion nematode *Pratylenchus penetrans* in field microplot and greenhouse experiments. Microsclerotial inocula and the *Verticillium*-susceptible potato cultivar Superior were used. No differences in virulence were found between isolates from VCG 4A and 4B when plants were grown in soil infested with *V. dahliae* only. In contrast, plants grown in soil infested with *V. dahliae* isolates from VCG 4A plus *P. penetrans* exhibited higher disease severity and had lower tuber yields than plants grown in soil infested with *V. dahliae* isolates from VCG 4B plus *P. penetrans*. An increase in the total number of tubers formed per plant was found for plants infected with either VCG 4 subgroup of *V. dahliae* alone compared with control plants. This increase was due to a larger number of small (diameter <6.4 cm) and medium (diameter between 6.4 and 8.3 cm) sized tubers, while the number of large (diameter >8.3 cm) tubers was similar in all cases. Although total tuber weight from plants was similar regardless of treatment, total weight of large tubers was lower and total weight of medium and small tubers higher from plants infected by either VCG 4 subgroup. These data indicate that *V. dahliae* isolates from VCG 4A are able to interact synergistically with *P. penetrans*, while isolates from VCG 4B do not interact with *P. penetrans* or do so only weakly. Furthermore, although infection with *V. dahliae* may not necessarily result in lower total tuber weight than in controls, damage may still be significant in that total weight of marketable tubers is decreased.

Potato early dying is an important disease of potato (*Solanum tuberosum* L.) in temperate climates worldwide. Potato crops grown in severely infested fields often produce yields 30–50% lower than those grown in uninfested fields (34,35). *Verticillium dahliae* Kleb. is the primary pathogen involved in potato early dying, although various other pathogens have been associated with the disease (4,8,12–14,23,27,30,38–40). The root-lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev & Schuur.-Stekh. interacts with *V. dahliae* on potato, and co-infection results in higher disease severity and often in lower yields than would occur with fungal infection only (16,36). The interaction of *V. dahliae* and *P. penetrans* on potato has been characterized as additive or synergistic and is specific to certain *Pratylenchus* species (14,16,33,36).

Subspecific classification of *V. dahliae* isolates is difficult. Because *V. dahliae* isolates generally lack host specificity, and few host resistance genes have been found, the concepts of forma specialis and physiological races have had only limited use in

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classification of *V. dahliae* isolates (1,37). A promising alternative for subspecific classification is the use of vegetative compatibility analysis. Originally, microsclerotial color mutants were used in complementation tests to classify *V. dahliae* isolates into 16 vegetative compatibility groups (VCGs) (28). More recently, auxotrophic nitrate-nonutilizing mutants were employed to classify *V. dahliae* isolates into VCGs (10). Using this method, 230 *V. dahliae* isolates that originated from potato plants or from soil collected in potato fields were classified into only four VCGs (11). Most isolates in this collection were assigned to VCG 2 and VCG 4; VCG 4 was subdivided into subgroups A and B based on characteristics common to each subgroup but not across subgroups (9). These results were confirmed independently with a different collection of isolates (41).

In greenhouse tests, virulence of 129 Ohio isolates from a collection that had been assigned to VCG 2, VCG 4A, and VCG 4B was evaluated (11). Potato (cv. Superior) sprouts were inoculated by dipping roots into a conidial suspension. Because *V. dahliae* isolates assigned to VCG 4A caused significantly greater disease severity than isolates from VCG 4B or VCG 2, VCG 4A apparently was a separate pathotype with respect to potato (11). In a similar study in Idaho (41), virulence tests conducted with a different set of *V. dahliae* isolates also showed that those assigned to VCG 4 were more virulent on potato than isolates assigned to other VCGs. Earlier field studies in Idaho (3) demonstrated the existence of *V. dahliae* isolates with different levels of virulence on potato, and the more virulent isolates were assigned to VCG 4.

Our study had two objectives. The first was to conduct field evaluations in microplots of the virulence of *V. dahliae* isolates representing both pathotypes by using microsclerotial rather than conidial inoculum, the former being the infective propagule under natural conditions. The second objective was to test the ability of isolates representing both pathotypes to interact with the root-lesion nematode, *P. penetrans*, in development of the potato early dying syndrome.

MATERIALS AND METHODS

Preparation of *V. dahliae* inoculum and soil infestation. All isolates of *V. dahliae* used in these studies were stored in vials containing a sterile mixture of soil, perlite, and peat moss at a volume ratio of 1:1:1. Isolates were retrieved by infesting petri dishes of potato dextrose agar (PDA) with 0.3 g of this culture material colonized by *V. dahliae* and incubating the dishes for 3–5 days in the dark at 24 C.

Conidial inocula were prepared in 500-ml Erlenmeyer flasks containing 250 ml of potato dextrose broth (PDB), which were infested with five mycelial-agar plugs taken with a no. 2 cork borer from appropriate PDA dishes. Flasks were incubated on a horizontal shaker for 7 days in the dark at 24 C. Conidial suspensions were filtered through cheesecloth, and inoculum densities were determined in a hemacytometer and adjusted to 5×10^5 by addition of sterile water (sterile distilled deionized water was used throughout the study).

Root infection under natural field conditions was simulated in all experiments by infesting soil with microsclerotia suspended in sterile, finely sieved soil (7), except in the tests for selection of *V. dahliae* isolates. Microsclerotia were produced in petri dishes containing minimal medium (29) covered with sterile cellophane. Conidial inoculum (1 ml) of the appropriate isolate was spread aseptically across the cellophane in each dish. After incubation of the dishes for 4–5 wk in the dark at 24 C, microsclerotia were harvested by adding a few drops of sterile water onto the medium surface and detaching pieces of the cellophane with adhering microsclerotia by careful rubbing with a finger. Sterile water was added to collected cellophane pieces with microsclerotia to attain a volume of 800 ml, and the mixture was then comminuted at high speed for 30 s in an electric blender. The suspension was then rinsed with tap water through a 250-μm mesh sieve. Cellophane fragments and microsclerotia retained on the sieve were collected, comminuted again, and resieved. By this process, microsclerotia were separated from cellophane fragments and collected as a microsclerotial suspension. This suspension was then rewashed three or four times through 125- and 38-μm mesh sieves to eliminate conidia and mycelial fragments. After washing, microsclerotia collected on the 125- and 38-μm mesh sieves were rinsed into a beaker with sterile water (7).

Finely sieved, sterile soil to be used as a diluent for the microsclerotia was prepared from a potting soil mixture consisting of organic muck, silt-loam, and peat moss (5:5:2, v/v) to which were added 45.4 g of ammonium nitrate and 350 g of agricultural-grade lime (calcium carbonate) per 194-L batch. This soil mix was moistened (1.75 L of soil and 150 ml of sterile water) and then sterilized by autoclaving at 121 C for 1 h twice on each of two successive days. After sterilization, the soil mix was oven-dried for 7 days at 55 C and then sifted through three nested sieves of 1-mm, 850-μm, and 425-μm mesh. To prepare the final inoculum, approximately 300 ml of microsclerotial suspension was dripped slowly onto 3 L of the oven-dried, sifted soil mix. Each batch was mixed by hand and then placed into an electric twin-shell blender and allowed to mix thoroughly for 1 h (7).

To determine inoculum density, the resulting mixture of microsclerotia and soil was assayed by dilution plating onto petri dishes of streptomycin sulfate-alcohol agar (SAA) (24) and/or sodium polygalacturonic acid agar (SPA) (17). SPA dishes were incubated at 24 C in the dark, and SAA dishes were incubated at 24 C in either light or dark. Colonies of *V. dahliae* were counted on SAA dishes after 5–7 days and on SPA dishes after 15 days. Inoculum densities were then calculated in terms of colony-forming units per cubic centimeter (CFU/cm^3) of soil. Microsclerotial inoculum was stored in plastic bags at 5 C for up to 4 wk and at −18 C for up to 4 mo. If the storage period was longer than 4 wk, inoculum density was reassayed before use.

Infested soil used in all studies was prepared by adding appropriate amounts of microsclerotial inoculum and mixing the soil in a cement mixer for 3 min in batches of 20 or 30 L. Five 50-cm^3 subsamples were collected from each batch of infested soil, mixed together, and stored at 5 C. These were assayed 1–4 wk later to determine final inoculum densities. Ten grams of each sample were rinsed with tap water through two nested 125- and 38-μm mesh sieves. The soil residues on each sieve were distributed equally onto 10 dishes of SPA and incubated in the dark at 24 C. After 15 days, soil was washed from the agar surface, colonies of *V. dahliae* were counted, and inoculum densities were calculated.

Sieves, mixers, and all other devices used in the above procedures were rinsed with water and then disinfested with 0.05% NaOCl prior to and after each use to prevent cross-contamination among the different isolates of *V. dahliae* involved in this study.

Preparation of *P. penetrans* inoculum and soil infestation. The strain of *P. penetrans* used in these studies was originally collected from apple tree roots in Wayne County, NY (33). Nematodes were grown in monoxenic alfalfa callus cultures on yeast extract-sucrose agar and incubated at 22–24 C in the dark (31,32,36).

To prepare concentrated nematode inoculum, alfalfa callus cultures of *P. penetrans* were hand-mixed into fumigated or steam-sterilized organic muck soil (Rifle peat) 3 days before use (17,18, 36). Nematode population densities of this inoculum were determined by a 24- to 48-h Baermann pie-pan extraction method. Nematode-infested soils were prepared by mixing appropriate amounts of nematode inoculum with soil for 10 revolutions in a 28-L capacity twin-shell blender (18,36). Subsamples of infested soil were collected and assayed as before to determine actual nematode population densities.

Production of experimental potato plants. The potato cultivar Superior, which is highly susceptible to *V. dahliae*, was used in all greenhouse and field experiments. Seed tubers used in the 1990 field experiment were produced in the greenhouse from plants that originated from aseptic tissue cultures (33,36). Seed tubers used in field and greenhouse experiments in 1991 were obtained as second generation (G-2) seed from New York (Cornell University, Eiline Farm). Each seed tuber used in 1991 was tested for the presence of *Verticillium* spp. by assaying an excised sample of vascular tissue from the stem end on SPA. No seed tuber

was found to be infected with *Verticillium* spp.

Prior to use, seed tubers were stored in moist vermiculite for approximately 3 mo at 22 C until dormancy was broken. Single-eye seed pieces approximately 3 cm in diameter were cut from seed tubers with a melon-ball scoop (36) and kept under the above conditions until sprouts 0.5–1 cm in length had developed. Sprouted seed pieces then were stored at 5 C until 2–5 days before planting, when they were again warmed to 22 C.

Selection of *V. dahliae* isolates for testing. *V. dahliae* isolates used in these studies were selected from the collection assembled by Joaquim (9). These isolates had been obtained from potato plants (P) and soil (S) collected from long-term commercial potato fields in Ohio during July–October 1984. Isolates P-7, P-103, P-105, P-107, S-132, S-228, P-3, P-273, P-281, S-39, S-52, and S-260 were tested. The first six isolates belonged to VCG 4A and the second six to VCG 4B, sensu Joaquim and Rowe (11).

Selection from this group of the specific *V. dahliae* isolates used in this study was based on preliminary greenhouse virulence tests and on the ability of individual isolates to form microsclerotia on minimal medium. Single-eye potato seed pieces were kept in moist vermiculite in the greenhouse until sprouts were approximately 15 cm tall. They were then uprooted and inoculated by immersing the roots in 80–100 ml of conidial inoculum for 5 min. Roots of control plants were immersed in sterile water. Data on symptom development were collected beginning 15 days after inoculation. Each plant was evaluated weekly using the subjective rating scale described under data collection. Microsclerotial production was tested by spreading 1 ml of conidial inoculum over petri dishes of minimal medium covered with cellophane. Dishes were then incubated in the dark at 24 C, and, after 7 days, microsclerotial production of each isolate was evaluated visually.

Microplot field studies. A microplot system was used in all field studies to contain the various isolates of *V. dahliae* and to facilitate controlled exposure of roots of each test plant to appropriate population densities of tested pathogens. Microplot field studies were conducted in 1990 and 1991 at the Ohio Agricultural Research and Development Center Muck Crops Branch, Celeryville, north central Ohio. The soil in the experimental area was Rifle peat, a well-decomposed organic soil (75% organic matter, 15% silt, 9% clay, 1% fine sand, pH 5.4). Before microplots were installed each year, experimental plot areas were fumigated with 470 kg/ha of a mixture of 67% methyl bromide and 31.8% chloropicrin (Terr-O-Gas 67, Great Lakes Chemical Co., West Lafayette, IN) (7,17,33,36). The fumigant was shank-injected into the soil at a depth of 20 cm in strips 2.1-m wide on 7 May 1990 and 9 May 1991. Soil temperatures at 20-cm application depth were approximately 13 C. As part of the fumigant application procedure, the soil was covered simultaneously with a 2.1-m-wide continuous plastic tarp. These tarps were removed 3–4 wk later, and fumigated soil was collected from the field into large plastic bags and transported to the laboratory for infestation. Unglazed clay field tiles (25-cm inside diameter, 31-cm length, 10-L capacity) were set upright to a depth of 18 cm in the center of each fumigated strip for use as single-plant microplots (17,33,36). Tiles were spaced 50-cm on center, and rows of tiles were spaced 4-m apart. Fumigated soil was mixed with *V. dahliae* and/or *P. penetrans* inoculum as described previously, transported back to the study site, and placed in appropriate microplots. One sprouted, single-eyed potato seed piece was then planted in each microplot.

In 1990, a completely randomized factorial design with 10 replications was used. The experiment included treatments of soil infested with each of the six different isolates of *V. dahliae* alone (40 CFU/cm^3 soil), the nematode *P. penetrans* alone (23 vermiforms/100 cm^3 soil), combinations of both pathogens, and a noninfested control for a total of 14 treatments and 140 microplots.

In 1991, a split-plot design was used to further limit the potential for cross-contamination among different isolates of the fungus. Fifteen replications were used for each treatment. The experiment included treatments of soil infested with each of the six different isolates of *V. dahliae* alone (40 CFU/cm^3 of soil), two inoculum densities of the nematode *P. penetrans* alone (25 and 55 vermiforms/100 cm^3 of soil), combinations of both pathogens, and a noninfested control, for a total of 21 treatments and 315 microplots. Main plots were the six different *V. dahliae* isolates, and subplots were the several inoculum densities of the nematode. Treatment blocks within the same row of tiles were separated by a 3-m space. Planting and harvest dates were 8 June and 26 September 1990 and 30 May and 18 September 1991.

Foliar symptoms of each plant were evaluated visually each week beginning on 31 July 1990 and 10 July 1991. Yield data were collected for each plant by weighing all tubers at harvest. Tubers from plants that had died by late August were harvested 1–2 wk before the remaining plants to avoid any loss of yield data due to dehydration or decay. In 1990, all plants were evaluated at harvest for the presence of *V. dahliae* by excising a 1-cm-thick section of the basal stem of each plant and aseptically placing several segments of vascular tissue from each on petri dishes of SAA. In 1991, the total number of tubers formed per plant was counted, and tubers were classified as small (diameter <6.4 cm), medium (diameter 6.4–8.3 cm), or large (diameter >8.3

TABLE 1. Monthly environmental data during field microplot studies in Celeryville, OH[a]

Month	Rainfall + irrigation (mm)		Temperature (C)					
			Average		Minimum		Maximum	
Variable	1990	1991	1990	1991	1990	1991	1990	1991
June	105.7	67.2						
1.5 m aboveground			21	22	15	7	27	35
0.3 m aboveground			22	24	16	8	30	38
5 cm below surface			20	25	17	13	22	37
15 cm below surface			20	25	18	16	21	33
July	187.7	53.0						
1.5 m aboveground			21	23	16	9	27	35
0.3 m aboveground			24	25	15	10	31	39
5 cm below surface			24	26	19	15	29	37
15 cm below surface			24	26	19	18	26	34
August	134.1	74.4						
1.5 m aboveground			21	21	15	9	26	35
0.3 m aboveground			22	24	16	10	31	40
5 cm below surface			23	...	18	18	29	35
15 cm below surface			23	...	21	18	25	30
September	85.6	109.5						
1.5 m aboveground			16	17	11	−3	22	34
0.3 m aboveground			18	19	12	−2	26	36
5 cm below surface			19	20	12	4	35	34
15 cm below surface			20	21	17	10	32	30

[a] Data obtained from a standard weather station operated by the Ohio Agricultural Research and Development Center.

cm). The number of tubers formed per plant and per size category were counted, and the total weight of tubers in each size category per plant was determined.

In both years, soil and root samples were collected from each microplot at harvest for determination of final nematode population densities and assayed as previously described. Samples collected at harvest for estimation of *P. penetrans* populations were stored at 5 C for up to 15 days until they were assayed. Air and soil temperature data and rainfall data are listed in Table 1. A fungicide (chlorothalonil) and insecticides (methamidophos, carbaryl, cyano-methyl-4-chloro-alpha benzeneacetate) were applied regularly to control early blight (caused by *Alternaria solani*) and Colorado potato beetle (*Leptinotarsa decemlineata*), respectively.

Greenhouse experiment. A greenhouse experiment was conducted during the summer of 1991. Plastic pots (13-cm diameter) containing 1,000 cm^3 of fumigated muck soil collected from the Celeryville experimental site were used in this study. A randomized complete block design was used to compensate for a slight temperature gradient across the greenhouse. Fifteen replications were used for each treatment. The experiment included treatments of soil infested with each of the same six different isolates of *V. dahliae* alone (40 CFU/cm^3 of soil), the nematode *P. penetrans* alone (40 vermiforms/100 cm^3 of soil), combinations of both pathogens, and a noninfested control for a total of 14 treatments and 210 pots. Inocula preparation and soil infestation were as described for microplots. Plant material was the same as that used in the 1991 microplot study. Plants were fertilized weekly with a solution of 20-20-20 (N-P_2O_5-K_2O) prepared at a concentration of 2 g/L. Insecticides were applied as needed to control sweetpotato whitefly (*Bemisia tabaci*) and western flower thrips (*Frankliniella occidentalis*). Supplemental light of 7 h during the first 4 wk of the experiment and 14 h during the remainder was provided by cool-white fluorescent bulbs suspended 1 m above the benches. Greenhouse average maximum temperatures ranged from 29 to 31 C and minimums from 20 to 22 C per month. Absolute maximum and minimum temperatures per month ranged from 33 to 44 C and from 16 to 19 C. A drip irrigation system was used to avoid potential cross-contamination among treatments due to splashing. Duration of the greenhouse experiment was approximately 3 mo.

Data collection and analysis. In all field and greenhouse studies, each plant was evaluated weekly for senescence symptoms characteristic of potato early dying. A subjective rating scale from 0 to 5 was used where 0 = no symptoms, 1 = chlorosis of leaves restricted to the lower half of the plant, 2 = chlorosis and necrosis of leaves restricted to the lower half of the plant, 3 = chlorosis of leaves on the entire plant, 4 = chlorosis and necrosis of leaves on the entire plant, and 5 = nearly dead or dead plant.

Areas under senescence progress curves (AUSPC) were calculated for each plant by using equation 8.29 in Campbell and Madden (2). Analysis of variance of AUSPC values was used to evaluate significant differences in symptom development among treatments. Linear contrasts were used to compare various groups of treatments. Tuber yield data collected from field microplot studies were analyzed by the same procedures. The general linear model of SAS (SAS Institute, Cary, NC) was used for statistical analysis of all data.

TABLE 2. Significance levels (*P*) from analysis of variance and contrasts for area under senescence progress curve (AUSPC) and total tuber yield data

	AUSPC			Field microplot yield	
	Field microplot		Greenhouse		
Source	1990	1991	1991	1990	1991
V. dahliae[a]	0.00	0.00	0.00	0.11	0.00
Block	...	0.14	0.00	...	0.02
P. penetrans[b]	0.00	0.00	0.00	0.45	0.00
V. dahliae × *P. penetrans*	0.00	0.01	0.00	0.03	0.00
Contrast[c]					
4A vs. 4B	0.52	0.00	0.41	0.75	0.68
4A vs. 4A+P_1	0.00	0.00	0.00	0.05	0.00
4A vs. 4A+P_2[d]	...	0.00	...	0.00	...
4B vs. 4B+P_1	0.91	0.01	0.74	0.51	0.29
4B vs. 4B+P_2	...	0.01	...	0.93	...
4A+P_1 vs. 4A+P_2	...	0.03	...	0.05	...
4B+P_1 vs. 4B+P_2	...	0.53	...	0.25	...
4A+P_1 vs. 4B+P_1	0.00	0.00	0.00	0.11	0.00
4A+P_2 vs. 4B+P_2	...	0.00	...	0.00	...
4A vs. control	0.46	0.00	0.71	0.11	0.67
4B vs. control	0.78	0.51	0.45	0.17	0.48
4A+P_1 vs. control	0.00	0.00	0.04	0.00	0.02
4B+P_1 vs. control	0.72	0.01	0.37	0.07	0.15
4A+P_2 vs. control	...	0.00	...	0.00	...
4B+P_2 vs. control	...	0.01	...	0.51	...
Control vs. P_1	0.33	0.07	...	0.36	...
Control vs. P_2	...	0.33	...	0.66	...
P_1 vs. P_2	...	0.41	...	0.17	...

[a]Three isolates of *Verticillium dahliae* each from vegetative compatibility groups (VCG) 4A and 4B, sensu Joaquim and Rowe (11) were compared.
[b]*Pratylenchus penetrans* vermiforms were present or absent in 1990. In 1991, nematodes were present at two initial populations or were absent.
[c]VCG 4A and VCG 4B were the subgroups of *V. dahliae* tested; P_1 = *P. penetrans* present at 23, 25, or 40 vermiforms/100 cm^3 of soil in field microplot study 1990, field microplot study 1991, and greenhouse study 1991, respectively; P_2 = *P. penetrans* present at 55 vermiforms/100 cm^3 of soil in field microplot study 1991; control plants were grown in noninfested soil. In 1990, the nematode-only treatment (P_1) was used as the control in making contrasts with yield data, because yields for the noninfested control were very low due to reasons unrelated to experimental treatments.
[d]Comparisons involving P_2 were not tested in 1990 as there was only one nematode density in that study.

RESULTS

Selection of *V. dahliae* isolates. Twelve isolates of *V. dahliae* were originally tested. Three from each subgroup of VCG 4 were selected that caused more severe disease in greenhouse virulence tests than other isolates of the same VCG 4 subgroup and also exhibited abundant microsclerotia production on minimal medium. These isolates, used in both the field and greenhouse tests, were P-7, P-105, and S-228 from VCG 4A and P-273, S-52, and S-39 from VCG 4B. The isolates selected from VCG 4A caused the greatest or nearly greatest disease development in prior virulence tests (9), whereas VCG 4B isolates S-52 and S-39 caused much less disease development in that study.

Microplot studies—Disease. Analysis of variance for AUSPC data showed that the interaction *V. dahliae* isolate × *P. penetrans* and both main effects were highly significant in both microplot experiments (Table 2). In both, significantly higher AUSPC values developed in plants grown in soil infested with *V. dahliae* isolates from VCG 4A plus *P. penetrans* than in those grown with VCG 4A isolates alone (Figs. 1 and 2; Table 2, contrasts 4A vs. 4A+P_1, and 4A vs. 4A+P_2). In 1990, no effect of the nematode was observed in plants grown in soil also infested with *V. dahliae* isolates from VCG 4B (Fig. 1; Table 2, contrast 4B vs. 4B+P_1). In 1991, soil infestation with nematodes plus *V. dahliae* isolates from either VCG 4A or 4B resulted in significantly higher AUSPC values than infestation with either isolates without nematodes (Fig. 2; Table 2, contrasts 4A vs. 4A+P_1, and 4B vs. 4B+P_1). However, plants grown in soil infested with VCG 4B isolates plus nematodes had significantly lower AUSPC values than plants grown in soil with VCG 4A isolates plus nematodes (Table 2, contrasts 4A+P_1 vs. 4B+P_1 and 4A+P_2 vs. 4B+P_2), and AUSPC values for plants grown in soil infested with VCG 4B isolates were no greater with the high inoculum density of nematodes than with the low inoculum density (Table 2, contrast 4B+P_1 vs. 4B+P_2). In comparison, AUSPC values for plants grown in soil infested with the VCG 4A isolates were greater with the high inoculum density of nematodes than with the low inoculum density (Table 2, contrast 4A+P_1 vs. 4A+P_2). Contrasts of AUSPC values for plants grown in soil infested with nematodes only versus noninfested controls (Table 2, contrasts control vs. P_1, and control vs. P_2) were not significant for both years of the study, indicating

the minor effect of the nematode alone on symptoms.

Chlorosis and/or necrosis of leaves and branches, symptoms characteristic of potato early dying, were first observed 54 days after planting in 1990 and 41 days after planting in 1991. These symptoms were often unilateral and/or interveinal. AUSPC values generally were higher in 1991 than in 1990 (Figs. 1 and 2). In 1990, AUSPC values of plants grown in soil infested with VCG 4A isolates plus nematodes were 80% higher than those of noninfested control plants (Table 2, contrast 4A+P_1 vs. control). AUSPC values of plants grown in soil infested with VCG 4B isolates plus nematodes did not differ from those of noninfested control plants (Table 2, contrast 4B+P_1 vs. control). In 1991, AUSPC values of plants grown in soil infested with VCG 4A isolates plus low or high population densities of *P. penetrans* were 76 and 93% higher, respectively, than those of noninfested control plants (Table 2, contrasts 4A+P_1 and 4A+P_2 vs. control). AUSPC values of plants grown in soil infested with VCG 4B isolates, however, were only 17 and 21% higher, respectively, than noninfested control plants (Table 2, contrasts 4B+P_1 and 4B+P_2 vs. control). In 1991, AUSPC values of plants grown in soil infested with isolate P-7 plus nematodes were lower than those of plants grown in soil infested with the other VCG 4A isolates plus nematodes but still higher than AUSPC values of plants grown in soil infested with VCG 4B isolates plus nematodes.

Some contrasts between certain groups of treatments gave contradictory results within the 2-yr study. For instance, the contrast between treatments involving plants grown in soil infested with isolates from different VCG 4 subgroups without nematodes (Table 2, contrast 4A vs. 4B) was not significant in 1990 but was highly significant in 1991. This situation probably was due to the significant interaction, which can make overall comparisons difficult to interpret. Similarly, contrasts between treatments involving plants grown in soil infested with VCG 4B isolates alone or with nematodes (Table 2, contrasts 4B vs. 4B+P_1 and 4B+P_2) were not significant for AUSPC in 1990 but were significant in 1991.

Microplot studies—Yield. Tuber yield means ranged from 750 to 1,238 g per plant in 1990 and from 831 to 3,301 g per plant in 1991 (Figs. 1 and 2). Analysis of variance of total tuber yield data showed that the interaction *V. dahliae* isolate × *P. penetrans* was highly significant in both microplot experiments, although the main factors *V. dahliae* isolate and *P. penetrans* were significant only in 1991 (Table 2). Mean tuber yields of plants grown in soil infested only with isolates of *V. dahliae* from either VCG 4A or 4B did not differ from yields of control plants in either year of the study (Table 2, contrasts 4A vs. P_1/control and 4B vs. P_1/control). However, yields of plants grown in soil infested with VCG 4A isolates plus nematode were significantly lower than yields of control plants in both years (Table 2, contrasts 4A+P_1 vs. P_1/control). This was the case in 1991 at both initial nematode population densities tested (Table 2, contrasts 4A+P_2 vs. control). In contrast, yields of plants grown in soil infested with VCG 4B isolates plus nematode did not differ from yields of control plants in either year of the study (Table 2, contrasts 4B+P_1 vs. P_1/control and 4B+P_2 vs. control). Thus the combination of root-lesion nematode and *V. dahliae* suppressed total tuber yields only in the case of VCG 4 isolates. In these comparisons, the nematode-only treatment was used instead of the noninfested control with the 1990 data because yields for the noninfested control that year were very low for reasons unrelated to experimental treatments.

In 1991, harvested tubers were separated into three size classes for further analysis. These results further clarified the effects of

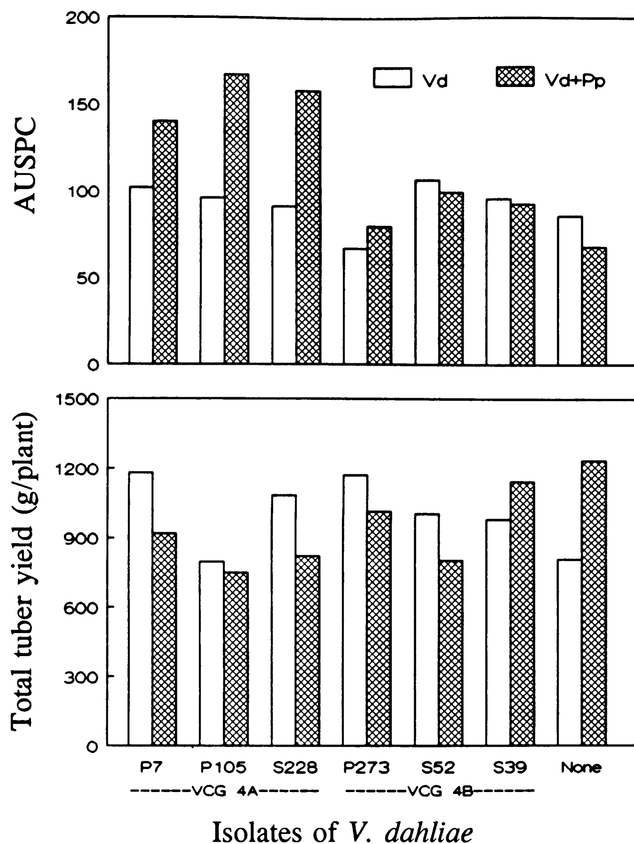

Fig. 1. Area under senescence progress curve (AUSPC) and means of total tuber yield data from 1990 potato microplot studies near Celeryville, OH. Plants were grown in soil infested with one of six isolates of *Verticillium dahliae* (Vd) or in noninfested control soil with or without *Pratylenchus penetrans* (Pp). Vegetative compatibility group designations VCG 4A and VCG 4B are sensu Joaquim and Rowe (11). Inoculum densities were *V. dahliae*, 40 microsclerotia/cm^3 of soil, and *P. penetrans*, 23 vermiforms/100 cm^3 of soil.

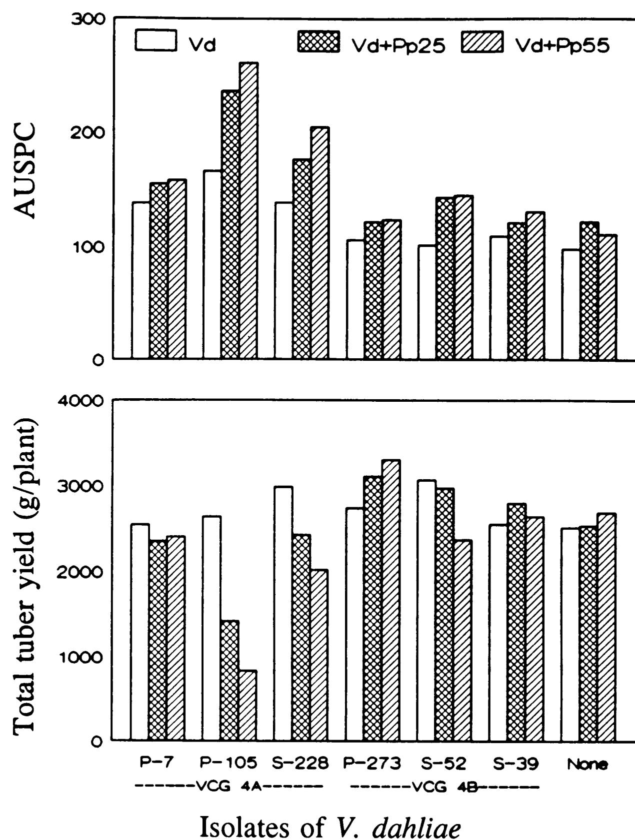

Fig. 2. Area under senescence progress curve (AUSPC) and means of total tuber yield data from 1991 potato microplot studies near Celeryville, OH. Plants were grown in soil infested with one of six isolates of *Verticillium dahliae* (Vd) or in noninfested control soil with or without *Pratylenchus penetrans* (Pp). Vegetative compatibility group designations VCG 4A and VCG 4B are sensu Joaquim and Rowe (11). Inoculum densities were *V. dahliae*, 40 microsclerotia/cm^3 of soil, and *P. penetrans*, 25 (Pp25) and 55 (Pp55) vermiforms/100 cm^3 of soil.

these pathogens on tuber yield (Table 3). Plants grown in soil infested with *V. dahliae* isolates of either VCG 4A or 4B, whether or not nematodes were present, produced 50–100% more small tubers than noninfested control plants (Table 3; Table 4 [numbers], contrasts 4A vs. control, 4B vs. control, 4A+P_1 vs. control, 4B+P_1 vs. control and 4B+P_2 vs. control). Plants grown in soil infested with VCG 4B isolates, with or without nematodes, produced the same number of large tubers as plants grown in noninfested soil (Table 4, contrasts 4B vs. control, 4B+P_1 vs. control, and 4B+P_2 vs. control). However, those grown in soil infested with VCG 4A isolates plus nematodes produced fewer large tubers than those grown in soil infested with VCG 4A isolates alone (Table 4, contrasts 4A vs. 4A+P_1 and 4A vs. 4A+P_2) or than noninfested control plants (Table 4, contrasts 4A+P_1 vs. control and 4A+P_2 vs. control). Numbers of medium-sized tubers produced were greater from plants grown in soil infested with VCG 4A or 4B isolates alone than from noninfested control plants (Table 4, contrast 4A and 4B vs. control). Fewer medium-sized tubers were produced by plants grown in soil infested with VCG 4A isolates plus nematodes than by plants grown in soil infested with VCG 4A isolates alone (Table 4, contrasts 4A vs. 4A+P_1 and 4A+P_2). This did not occur in the case of plants grown in the presence of VCG 4B isolates (Table 4, contrasts 4B vs. 4B+P_1 and 4B+P_2).

Yield data based on tuber size categories showed that weight of large tubers produced by plants grown in soil infested with VCG 4A or 4B isolates alone was 19% or 21% less, respectively, than that of noninfested control plants (Table 3; Table 4 [yield] contrasts 4A vs. control and 4B vs. control). This significant effect of either VCG 4 subgroup isolates on yield of large tubers but not on total yield (Table 3) can be attributed to the greatly increased numbers of small tubers produced by all plants grown in *Verticillium*-infested soil (Table 3, Table 4 contrasts 4A vs. control and 4B vs. control). As was observed with numbers of tubers, yields of large tubers produced by plants grown in soil infested with VCG 4A isolates and nematodes were significantly lower than those from plants grown in soil infested with VCG 4A alone (Table 4, contrasts 4A vs. 4A+P_1 and 4A vs. 4A+P_2) or from noninfested control plants (Table 4, contrasts 4A+P_1 vs. control and 4A+P_2 vs. control). Although yields of large tubers from plants grown in soil infested with VCG 4B isolates alone were significantly lower than yields from control plants, yields were not further reduced if nematodes were also present (Table 4, contrasts 4B vs. 4B+P_1 and 4B vs. 4B+P_2). A similar pattern was seen with yields of medium tubers.

In 1990, *V. dahliae* was detected in stems of potato plants grown in soil infested with microsclerotia of the fungus and in some plants grown in noninfested soil. Assays of nematode populations in soil samples taken from microplots in both years showed a four- to ninefold higher number of *P. penetrans* vermiforms recovered at harvest compared with those recovered at planting, indicating that nematodes had successfully fed and reproduced on potato roots during the growing season. In 1990, nematode populations assayed from soil samples infested with *P. penetrans* averaged 14 vermiforms/100 cm^3 soil at planting. At harvest, soils also infested with VCG 4A or 4B isolates averaged 57 vermiforms/100 cm^3 of soil compared with 76 from soils infested only with nematodes. In 1991, nematode populations assayed from soil samples infested with low and high initial population densities averaged 11 and 23 vermiforms/100 cm^3 of soil, respectively, at planting. At harvest, soils also infested with VCG 4A or 4B isolates averaged 96 and 157 vermiforms/100 cm^3 soil, respectively, compared with 54 and 171 from soils infested only with nematodes.

Greenhouse study. Symptoms of potato early dying were observed within 6 wk after the experiment was established. Initial symptoms were characteristic unilateral and/or interveinal chlorosis and progressive necrosis of leaves. At later stages, whole branches became chlorotic and/or necrotic. Many infected plants died within 8–13 wk after the experiment was established.

TABLE 3. Average number of tubers and tuber yield produced by potato (cv. Superior) plants grown in microplots containing soil infested with *Verticillium dahliae* and/or *Pratylenchus penetrans*, Celeryville, OH, 1991

	Population of *P. penetrans* vermiforms (verm.)[c] per 100 cm^3 soil					
	Average no. of tubers per plant			Average tuber yield (g/plant)		
Tuber size[a] VCG[b]	0 verm.	25 verm.	55 verm.	0 verm.	25 verm.	55 verm.
Small						
4A	16	16	12	493	494	372
4B	15	14	16	500	485	595
None	8	8	8	250	250	343
Medium						
4A	6	5	4	838	673	524
4B	6	7	6	850	1,036	773
None	4	4	5	558	612	768
Large						
4A	5	4	3	1,517	1,133	1,038
4B	5	6	5	1,473	1,529	1,550
None	6	5	6	1,866	1,558	1,869
Total						
4A	27	24	19	2,721	2,064	1,750
4B	27	27	28	2,786	2,957	2,773
None	17	18	20	2,624	2,361	2,747

[a]Small = diameter <6.4 cm, medium = diameter 6.4–8.3 cm, and large = diameter >8.3 cm.
[b]VCG = vegetative compatibility group of *V. dahliae* isolates sensu Joaquim and Rowe (11).
[c]Includes all stages except eggs.

TABLE 4. Significance levels (*P*) of contrasts for data on tuber number and yield in three size classes taken from 1991 field microplot studies

	Number of tubers per plant[b]				Yield		
Contrast[a]	Total	Large	Medium	Small	Large	Medium	Small
4A vs. 4B	0.92	0.94	0.33	0.69	0.67	0.76	0.92
4A vs. 4A+P_1	0.18	0.00	0.01	0.97	0.01	0.03	0.98
4A vs. 4A+P_2	0.00	0.00	0.01	0.07	0.03	0.01	0.15
4B vs. 4B+P_1	0.82	0.46	0.26	0.80	0.71	0.03	0.83
4B vs. 4B+P_2	0.67	0.83	0.14	0.36	0.62	0.38	0.17
4A+P_1 vs. 4A+P_2	0.03	0.09	0.79	0.06	0.74	0.31	0.15
4B+P_1 vs. 4B+P_2	0.84	0.34	0.01	0.24	0.89	0.00	0.11
4A+P_1 vs. 4B+P_1	0.14	0.00	0.00	0.49	0.01	0.00	0.89
4A+P_2 vs. 4B+P_2	0.00	0.00	0.03	0.02	0.02	0.07	0.02
4A vs. control	0.00	0.46	0.03	0.01	0.06	0.04	0.06
4B vs. control	0.00	0.49	0.01	0.02	0.03	0.06	0.05
4A+P_1 vs. control	0.04	0.00	0.68	0.01	0.00	0.53	0.06
4B+P_1 vs. control	0.00	0.87	0.00	0.03	0.05	0.00	0.07
4A+P_2 vs. control	0.59	0.00	0.82	0.15	0.00	0.81	0.59
4B+P_2 vs. control	0.00	0.40	0.07	0.00	0.06	0.17	0.01

[a]VCG 4A and VCG 4B were the subgroups of *V. dahliae* tested; P_1 = *P. penetrans* present at 25 vermiforms/100 cm^3 soil; P_2 = *P. penetrans* present at 55 verm./100 cm^3 soil; control plants were grown in noninfested soil.
[b]Small = diameter <6.4 cm; medium = diameter 6.4–8.3 cm; and large = diameter >8.3 cm.

Plants grown in soil infested with *V. dahliae* isolates from VCG 4A plus nematodes had 47% higher AUSPC values than noninfested control plants (Table 2, contrast 4A+P_1 vs. control). AUSPC values did not differ among plants grown in soils that were noninfested, infested with VCG 4B isolates, or infested with VCG 4B plus nematodes (Table 2, contrast 4B+P_1 vs. control and 4B vs. 4B+P_1). Analysis of variance and contrasts for these AUSPC data led to the same conclusions as AUSPC data from the 1990 field microplot study (Table 2).

DISCUSSION

The key finding in this study was that isolates of *V. dahliae* from VCG 4A were more virulent to potato than those from VCG 4B when roots of these plants were infected simultaneously with *P. penetrans*. When plants were grown in soil infested only with *Verticillium*, AUSPC values did not differ among plants infected with VCG 4A or 4B isolates or control plants except in the 1991 field microplot study. In that case, AUSPC values of plants infected with isolates of VCG 4A were greater than those of plants infected with VCG 4B isolates or of control plants, but AUSPC values were greater yet when roots of these plants were also infected with nematodes. The differential interaction that occurred with *P. penetrans* and isolates of *V. dahliae* from VCGs 4A and 4B revealed significant differences in virulence between these two subgroups that were not apparent when plants were grown in soil infested only with *V. dahliae* isolates. Interaction between *V. dahliae* and *P. penetrans* on potato is well documented (16,17,36), but this study is the first report that *V. dahliae* isolates from VCG 4A can interact strongly with *P. penetrans* while those from VCG 4B interact weakly or not at all. This interaction was synergistic rather than additive because AUSPC values of plants grown in soil infested only with nematodes did not differ from plants grown in noninfested soil.

Effects of this synergism on tuber yield were less pronounced than on symptom development. The only yield effect that was observed in plants grown in soil infested only with *V. dahliae* was related to tuber size. In the 1991 microplot study, plants grown in the presence of either VCG 4 subgroup produced a higher number of medium and small tubers than did plants in noninfested soil. Although numbers of large tubers produced by these plants did not differ from those produced by control plants, total weights of large tubers were lower. Thus *Verticillium*-infected plants apparently set more tubers than healthy plants, but most of these remained small and diverted phytosynthates from the larger tubers.

Tuber yields from plants grown in soil infested both with VCG 4A isolates and nematodes were lower than yields from plants grown with VCG 4B isolates and nematodes, plants grown with isolates of either *V. dahliae* subgroup alone, or control plants. In the 1991 microplot study, plants grown in soil infested with VCG 4A isolates and nematodes produced fewer medium and large tubers than those grown with VCG 4A isolates alone. Total weights of tubers in these size classes produced by plants grown with both pathogens were considerably lower. This did not occur, however, in the case of VCG 4B isolates. Thus, *V. dahliae* isolates from different subgroups of VCG 4 reacted differently in the presence of *P. penetrans*, with effects on both the total yield of tubers produced by infected plants and the size distribution of these tubers. This did not occur in a similar study with *V. dahliae* and *P. penetrans* on cv. Russet Burbank (16). In that case, no differences were found in the total number of tubers formed per plant among treatments involving all possible combinations of two population densities of *V. dahliae* and *P. penetrans*, as well as treatments with each pathogen alone. Only one isolate of *V. dahliae* of unknown VCG was used in that study. This may have affected the results and points out the importance of defining the VCG of *V. dahliae* isolates used in studies of virulence and nematode interactions.

High virulence to potato associated with *V. dahliae* isolates from VCG 4 (sensu Joaquim and Rowe, 11) has been reported previously. In greenhouse studies, isolate BB from Puhalla's collection was more virulent to cv. Russet Burbank than isolate 207 from Nicot's collection (3,9). Isolate BB was assigned later to VCG 4A and isolate 207 to VCG 4B (10). In other greenhouse studies, isolate TA from Nicot's collection was more virulent to cv. Netted Gem (syn. Russet Burbank) than isolate 207 from the same source and more virulent than isolates MC, PU, and PCW from Puhalla's collection (41). Isolate TA has been assigned to VCG 4A, isolates 207 and MC to VCG 2, and isolate PCW to VCG 3 (10).

Our results differ somewhat from earlier greenhouse studies in which differences in virulence were detected between VCG 4A and 4B isolates in the absence of nematodes in plants that had been inoculated by dipping roots in conidial suspensions (11). Differences in results between these two studies are probably due to the different inoculation procedures and types of plant material used. In the earlier study (11), uprooted potato sprouts were inoculated by dipping the root systems into conidial suspensions of different *V. dahliae* isolates and then replanting them. Growing plants in soil infested with microsclerotia, as was done in this study, facilitated a more natural infection process. This technique avoided wounding of roots, which presumably occurred when sprouts were uprooted and then dipped in conidial suspensions, and avoided rapid invasion of the fungus into the xylem followed by upstream dispersion of conidia throughout the vascular system of the host, which results from that type of inoculation procedure. Thus virulence differences between isolates of the two VCG subgroups were less pronounced in this study than in our earlier one (11) and became apparent only with the additional stress of nematode infection.

The interaction of *P. penetrans* with *V. dahliae* probably is due to physiological effects on the host brought about by nematode feeding rather than by root wounds facilitating entrance of the fungus into the vascular system (5,19–22). The high virulence of VCG 4A isolates to potato was clearly demonstrated when the fungi were introduced directly into the vascular system (11). When infection proceeds naturally, these virulence differences may be expressed only when host defenses are altered by co-infection with *P. penetrans* or plants are stressed in other ways.

This study was based on a limited number of isolates of *V. dahliae* from each of the VCG 4 subgroups, and further study is needed to confirm that our findings are generally applicable. If consistent differences are found to exist between VCG 4A and 4B isolates of *V. dahliae* with regard to virulence to potato and ability to interact with *P. penetrans*, it will affect management strategies for potato early dying. The geographic distribution of VCG 4A and 4B strains in commercial potato production areas should be investigated. If *V. dahliae* isolates from VCG 4A are found to be the strains predominantly involved in the potato early dying syndrome, VCG 4A strains should be used when potato germ plasm is screened for resistance to *V. dahliae* in breeding programs aimed at development of new *Verticillium*-resistant potato cultivars. If there are potato production areas that presently are not infested with VCG 4A strains, steps might be taken to prevent introduction of these virulent strains. Studies evaluating pathogenicity and virulence of these strains on other crops may be useful to devise potential control strategies aimed at managing fungal populations through crop rotation.

It is likely that mixtures of both VCG 4 subgroups exist in many fields (11). This may be important when data on population densities of *V. dahliae* microsclerotia obtained from soil assays are used in yield-loss predictive models (6,42). Inconsistencies among economic threshold values reported for *V. dahliae* microsclerotia in soil (6,15,25,26,42) may be due to variability in proportions of *V. dahliae* strains within fields used in various studies. Information on the proportion of VCG 4A isolates within specific soil populations of *V. dahliae* may help clarify these inconsistencies and lead to improved accuracy in evaluations of potential losses due to potato early dying.

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