Vegetative Compatibility and Virulence of Strains of *Verticillium dahliae* from Soil and Potato Plants

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ABSTRACT

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One-hundred and eighty-seven wild-type strains of *Verticillium dahliae* were isolated from potato plants and soil from 22 potato fields in Ohio. Strains were assigned to vegetative compatibility groups (VCGs) based on pairings of complementary nitrate-nonutilizing (*nit*) mutants induced on a chlorate-containing medium. Of these strains, two were assigned to VCG 1, 53 to VCG 2, and 128 to VCG 4. The remaining four strains could not be tested for vegetative compatibility because of their inability to yield *nit* mutants. Forty-seven additional strains of *V. dahliae* isolated from potato plants were obtained from nine U.S. states and tested for vegetative compatibility. Of these strains, two were assigned to VCG 2 and 45 to VCG 4. VCG 4 of both isolate collections was further subdivided into VCG 4A and VCG 4B to accommodate differential vegetative compatibility reactions of strains from each subgroup when paired against tester strains of VCG 3. Most strains assigned to VCG 4A were weakly vegetatively compatible with tester strains of VCG 3. In contrast, all strains in VCG 4B were vegetatively incompatible with tester strains of VCG 3. Greenhouse pathogenicity tests with 129 strains of *V. dahliae* from Ohio assigned to VCGs 2, 4A, and 4B were conducted by root-dipping potato sprouts (cultivar Superior) in a suspension of 10^5 conidia per milliliter of each isolate. Areas under senescence progress curves (AUSPCs) were computed for each strain. Contrasts of mean AUSPCs for each VCG revealed that most strains in VCG 4A were significantly ($P = 0.01$) more virulent than those in VCGs 2 and 4B, which is evidence for recognition of two distinct pathotypes.

Verticillium dahliae Kleb. is an important vascular wilt pathogen of potato (*Solanum tuberosum* L.) and of many other crops worldwide. Studies in Ohio have shown that the potato early dying disease is the result of a synergistic interaction between *V. dahliae* and the root-lesion nematode *Pratylenchus penetrans* (25,37). Pathogens involved in this disease syndrome may vary regionally (36), however, and when involving *V. dahliae* and *P. penetrans*, interactions in some cases have been described as additive (24).

Although there is evidence that various species of *Pratylenchus* interact differently with *V. dahliae* in potato early dying (34), little is known about the effects of virulence differences among various strains or pathotypes of either pathogen. Current management procedures for potato early dying are often based on estimates of soil populations of *V. dahliae* prior to planting. Existing quantitative soil assay procedures for *V. dahliae* rely on the assumption that all propagules that grow on selective agar media are equally pathogenic and, therefore, that enumeration of colonies is a direct measure of inoculum potential (35). Among several factors that could affect the relationship between inoculum potential and inoculum density in field soils are the occurrence of either mixtures of pathogenic and nonpathogenic strains of *V. dahliae*, or mixtures of pathogenic strains of varying virulence capabilities (38).

Identification of subspecific groupings (i.e., formae speciales, races, or pathotypes) within *V. dahliae* has been difficult. With a few exceptions (14), host specificity is rare among strains of *V. dahliae*; host reactions, when employed (27), have not generally been useful to differentiate among strains (17,18). Physiologic races of *V. dahliae* have only been defined in tomato (*Lycopersicon esculentum*) with the *Ve* gene (1). Pathotypes have been defined only in cotton (*Gossypium hirsutum* and *G. barbadense*) (39). Schnathorst and Mathre (39) described a defoliating pathotype (T-1), which was highly virulent and caused severe defoliation on several cultivars of cotton, and a milder, nondefoliating form (SS-4), which induced little or no defoliation. Both pathotypes could also be differentiated on safflower (*Carthamus tinctorius*), snapdragon (*Antirrhinum majus*) and tomato. Other studies (4,5), however, support the existence of a continuum of virulence phenotypes among strains of *V. dahliae* on cotton, rather than the occurrence of distinct pathotypes.

Vegetative compatibility analysis has been shown to be a useful tool for examining genetic diversity among populations of plant pathogenic fungi (3,8,10,15,19,22,28-30,40), including those of *V. dahliae* (21,31,33) and *V. albo-atrum* (7). Puhalla (31) first demonstrated the existence of a vegetative compatibility system in *V. dahliae*. Strains belonging to a vegetative compatibility group (VCG) are capable of forming heterokaryons with one another, but not with strains in other VCGs. In a subsequent study, Puhalla and Hummel (33) classified 86 strains of *V. dahliae*, originating from several hosts and geographical locations, into 16 VCGs. Strains identified as defoliating and nondefoliating pathotypes on cotton consistently belonged to different VCGs. Several trends regarding strains from other hosts were also observed. For instance, of the 10 strains of *V. dahliae* isolated from potato, nine were assigned to VCGs 3 and 4. In another investigation, Corsini et al (11) tested three of these 10 strains from potato and a defoliating strain from cotton assigned to VCG 1 for pathogenicity on potato cv. Russet Burbank. They showed that the two strains of *V. dahliae* assigned to VCG 4 were more virulent to potato than the two strains in VCGs 1 and 3.

The 16 VCGs defined by Puhalla and Hummel (33) were based on pairing reactions of complementary, ultraviolet-light-induced,

microsclerotial color mutants. Joaquim and Rowe (21), however, have shown that using different methods for assessing vegetative compatibility can lead to conflicting results. When nitrate-nonutilizing (*nit*) mutants were used to examine vegetative compatibility relationships among strains of *V. dahliae*, only four VCGs were identified among 20 strains that previously had been assigned to 14 different VCGs by the color-mutant system of Puhalla and Hummel (33). As a consequence, a revised VCG system for *V. dahliae* was proposed (21). An additional 21 strains of *V. dahliae* isolated from potato field soil and potato plants from Ohio were also tested for vegetative compatibility and were assigned to VCG 2 or 4 (21).

The objectives of this investigation were to assess VCG diversity further within a collection of isolates of *V. dahliae* recovered from naturally infected potato plants and associated infested soils from several locations in Ohio and from other potato-producing areas of the United States; to determine the range of virulence to potato cv. Superior of strains of *V. dahliae* from this collection; and to evaluate whether distinct pathotypes could be defined within this collection and whether any relationship exists between these pathotypes and their vegetative compatibility groupings. A preliminary report of this investigation has been published (20,21).

MATERIALS AND METHODS

Strains of *V. dahliae*. During July–October 1984, 187 strains of *V. dahliae* were collected from 22 fields in long-term commercial potato production in Columbiana, Portage, Sandusky, and Wayne counties, OH (Table 1). Fifteen of the 22 fields sampled were cropped to potato in 1984 and had been under an alternating potato-wheat rotation. Four fields (fields 7, 20, 22, and 25) were planted to corn and/or wheat in 1984, but had not been cropped to potato since 1982. One field (field 24) was in corn in 1984, but had been cropped to potato in 1982 and 1983. Two fields (fields 18 and 21) had been in potato monoculture for more than 10 yr. Numbers of strains collected by counties were Wayne, 87; Columbiana, 37; Portage, 55; and Sandusky, 8. Of the 187 strains, 154 were isolated directly from soil and 33 strains from stems of naturally infected potato plants. Methods for the isolation of *V. dahliae* from soil and potato plants have been published (21). A second collection composed of 47 strains of *V. dahliae* from potato originating from nine U.S. states was also evaluated (Table 2). Additional strains tested, which originated from hosts other than potato, came from the collection of Puhalla and Hummel (33), with the exception of strain 70-21 (provided by S. M. Alcorn, University of Arizona), which was isolated from pepper. All strains were stored in vials of sterile soil at 5 C.

Assignment of strains to VCGs. *Nit* mutants were used to assess vegetative compatibility relationships among strains of *V. dahliae*. Procedures for generation, phenotype identification, storage, and arrangement of *nit* mutant pairings on petri dish assays have been published (21), but will be briefly outlined here. Cornmeal agar with dextrose (Difco Laboratories, Detroit, MI) amended with potassium chlorate (15–25 g/L) was used to generate *nit* mutants from wild-type strains of *V. dahliae*. The phenotype of *nit* mutants was determined by their growth response on minimal medium agar (MM) (32) and on MM in which sodium nitrate was substituted by one of the following nitrogen sources: sodium nitrite, ammonium-tartrate, hypoxanthine, and uric acid. Assignment of *nit* mutant phenotype designations (*nit1* and NitM) corresponded to those used with *Verticillium albo-atrum* (7) and *Fusarium oxysporum* (8). *Nit1* mutants presumably arise from a mutation at the structural locus of nitrate reductase, while NitM mutants are deficient in the synthesis of a molybdenum-cofactor required for nitrate reductase and purine dehydrogenase activity (8).

A *nit* mutant derived from each of the 183 strains of *V. dahliae* from Ohio was tested against two complementary *nit* mutants (*nit1* and NitM) of tester strains of the four VCGs previously defined by Joaquim and Rowe (21). Hereafter, all VCG designations mentioned are *sensu* Joaquim and Rowe (21), unless otherwise specified. Of the 183 strains assigned to VCGs, 179 were tested simultaneously against *nit1* and NitM mutants of the following tester strains: V-44 (VCG 1); PH and/or WM and 115 and/or S-92 (VCG 2); and BB and S-39 (VCG 4). Of these same strains, 115 were tested against PCW and/or 70-21 (VCG 3). All 47 strains from the second collection of *V. dahliae* were tested for vegetative compatibility against the tester strains listed above, representing VCGs 1, 2, and 4, but only 29 of these strains were paired with tester strain 70-21 (VCG 3). All pairings were done at least twice. Plates were scored for prototrophic growth 18–24 days after inoculation. As before (21), wild-type strains were assigned to VCGs based on the reactions of their respective *nit* mutants in pairings with tester strains of each VCG. Strains were considered vegetatively compatible when prototrophic growth appeared at the mycelial interface between their respective *nit* mutants and *nit1* and/or NitM mutants of a tester strain. Among vegetatively compatible strains, however, the extent of prototrophic growth formed between their *nit* mutants often varied. *Nit* mutants of some strains complemented strongly as indicated

TABLE 1. Distribution among three vegetative compatibility groups (VCG) of strains of *Verticillium dahliae* from potato plants and soil collected in 22 potato fields in Ohio

Field number	County of collection	Number of strains tested	VCG				
			1	2	4A	4B	4A/B
1	Wayne	6	0	4	0	2	0
2	Wayne	7	0	0	0	5	2
3	Wayne	5	0	0	0	5	0
4	Wayne	1	0	0	0	1	0
5	Wayne	5	0	0	0	5	0
6	Wayne	1	0	1	0	0	0
7	Wayne	7	0	3	0	4	0
8	Wayne	8	0	1	0	6	1
9	Wayne	4	0	2	0	2	0
10	Wayne	9	0	1	0	8	0
11	Wayne	13	0	1	0	11	1
12	Wayne	20	2	13	0	5	0
13	Columbiana	15	0	3	1	11	0
14	Columbiana	14	0	7	0	7	0
16	Columbiana	4	0	2	0	2	0
17	Columbiana	2	0	1	0	1	0
18	Portage	25	0	1	22	2	0
20	Portage	14	0	2	7	5	0
21	Portage	9	0	6	1	2	0
22	Portage	6	0	2	0	4	0
24	Sandusky	3	0	1	1	0	1
25	Sandusky	5	0	2	0	2	1
Total		183	2	53	32	90	6

TABLE 2. Distribution among two vegetative compatibility groups (VCG) of strains of *Verticillium dahliae* isolated from potato in several states

State	Source[a]	Number of strains tested	VCG			
			2	4A	4B	4A/B
Idaho	1,2	7	0	7	0	0
Maryland	3	1	0	1	0	0
Minnesota	4	2	0	2	0	0
New York	5,6	3	0	0	1	2
North Dakota	4,7	9	0	8	0	1
Oregon	8	2	0	2	0	0
Pennsylvania	6	2	2	0	0	0
Washington	9	3	0	2	0	1
Wisconsin	10	18	0	14	4	0
Total		47	2	36	5	4

[a]Strains were provided by: 1 = J. E. Puhalla (California); 2 = L. H. Sorensen (Idaho); 3 = D. R. Fravel (Maryland); 4 = N. A. Anderson (Minnesota); 5 = A. P. Keinath (Maryland); 6 = C. Stockwell (New York); 7 = G. A. Secor (North Dakota); 8 = M. L. Powelson (Oregon); 9 = G. D. Easton (Washington); and 10 = R. C. Rowe (Ohio).

by the formation of a dense line (about 1.0–2.5 cm in width) of prototrophic growth usually consisting of aerial mycelia and/or microsclerotia. *Nit* mutants of other strains complemented only weakly by yielding a thin zone of prototrophic growth usually consisting of submerged mycelia and/or microsclerotia with little or no aerial mycelia. Strains were regarded as vegetatively incompatible when interstrain pairings with testers failed to yield prototrophic growth at the line of mycelial contact.

Pathogenicity tests. Pathogenicity tests were conducted between March 1987 and May 1988, except during the period June–September 1987, when ambient greenhouse temperatures were too high. Potato seed tubers (cv. Superior) were produced from axenically cultured potato plantlets grown to maturity in steam-disinfested soil in a greenhouse. Presprouted, single-eye hemispheres (about 2 cm diameter) cut from potato seed tubers were planted in wooden flats containing vermiculite. Flats were then placed in a greenhouse at 20–31 C, and illuminated with cool-white fluorescent bulbs on a 16-h diurnal cycle. After 3–5 weeks, 10- to 20-cm tall plants were selected for inoculations. Virulence of 145 strains of *V. dahliae* to potato was evaluated in greenhouse tests. Because of space limitations, these 145 strains were tested in two separate experiments. Experiment I consisted of 56 strains. Experiment II consisted of the remaining 89 strains plus five control strains, which varied in virulence and were selected based on virulence reactions in experiment I. Strains from Ohio tested in experiment I included 12 strains isolated from potato and 36 strains from field soil. The remaining strains isolated from potato were: BB (Idaho), WS-5 and H-5 (Wisconsin), and PU (United Kingdom). Additional strains tested in experiment I from hosts other than potato and assigned to VCGs (21) included V-44 (cotton; VCG 1), PH (pistachio; VCG 2), PG (pepper; VCG 2), and TO (tomato; VCG 3). Strains from Ohio tested in experiment II included 83 strains isolated from soil and six from potato plants. Additional strains from potato were 308 (Oregon), 318 and 319 (North Dakota), Plain-1 (Wisconsin), and TA (Idaho).

Conidial inoculum of *V. dahliae* for pathogenicity tests was prepared by adding five mycelial-agar plugs removed from the edge of 7- to 10-day-old colonies growing on potato-dextrose agar (PDA) (Difco) to 250-ml Erlenmeyer flasks containing 75 ml of potato-dextrose broth. Broth cultures were incubated for 7–10 days at 24 ± 2 C in the dark on a rotatory or horizontal shaker. One to two milliliters of this inoculum were filtered through lens paper into approximately 20 ml of sterile distilled water. The conidial concentration of each suspension was quantified with a hemacytometer by computing the average of three subsamples. Conidial suspensions were then adjusted with sterile distilled water to yield 10^5 conidia per milliliter.

Potato plants were uprooted from vermiculite and inoculated by immersing the roots of five replicate plants per strain for 5 min in 500 ml of each conidial suspension. Inoculated plants were then transplanted into steam-disinfested, silt-loam soil in 15-cm-diameter plastic pots. Roots of control plants were immersed in sterile distilled water before transplanting. Pathogenicity tests were conducted in two or three separate greenhouse rooms (7 × 6 m) maintained under similar environmental conditions. Plants were arranged on benches in a completely randomized design. Average minimum and maximum air temperatures for all the greenhouse rooms for the 56-day period were 17.9 and 27.3 C for experiment I, and 19.3 and 27.4 C for experiment II, respectively. Both studies were repeated as experiments IA and IIA.

Disease progression during the 56-day period after inoculation was assessed visually every 7 days by the foliar senescence ratings of each plant. The following rating scale was used: 1 = no visible symptoms; 2 = slight chlorosis of the lower leaves; 3 = extensive chlorosis of the lower leaves; 4 = extensive chlorosis and some necrosis of the lower and upper leaves; 5 = severe stunting with chlorosis and necrosis of the entire plant; and 6 = dead or nearly dead plant. In both experiments, virulence of each strain was determined by computing areas under senescence progress curves (AUSPC) for foliar ratings of each plant during the 14- to 56-day period with the following formula (6):

$$\text{AUSPC} = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

in which Y_i = cumulative disease severity at the ith observation, t_i = time (days postinoculation) at the ith observation, and n = the number of observations. AUSPC values were subjected to a one-way analysis of variance (ANOVA). Fisher's protected least significant difference ($LSD_{0.05}$) values were calculated to separate AUSPC means among all strains within each experiment. Also for each experiment, contrasts were computed to separate AUSPC means between VCGs. In this instance, only the 129 strains of *V. dahliae* from potato plants and soil from Ohio assigned to a single VCG or VCG subdivision were considered in mean comparisons.

RESULTS

Isolation, identification of *nit* mutant phenotypes, and assignment of strains to VCGs. At least one *nit* mutant was readily recovered from 183 of the 187 strains of *V. dahliae* grown on chlorate-containing medium. Inability to recover *nit* mutants from the remaining four strains was because of the failure of strains to yield chlorate-resistant sectors.

Two phenotypic classes of *nit* mutants were identified among the 126 *nit* mutants characterized. Eighty-two *nit* mutants (65%) were able to utilize all nitrogen sources tested except nitrate. These mutants were designated as *nit1* mutants. Mutants in the second phenotypic class were incapable of utilizing nitrate and hypoxanthine, while retaining the ability to use the remaining nitrogen sources tested. These mutants were designated as NitM mutants and made up the remaining 44 (35%) of the total number of *nit* mutants characterized. Mutants for the pathway-specific regulatory locus (*nit3*), structural locus of nitrite reductase, and major nitrogen regulatory locus were not identified in this study.

The 183 strains of *V. dahliae* collected from Ohio and tested for vegetative compatibility were assigned as follows: 2 to VCG 1, 53 to VCG 2, and 128 to VCG 4 (Table 1). VCG 4 was further subdivided into VCG 4A and 4B because strains assigned to VCG 4 reacted differentially, either when paired against both VCG 4 tester strains (BB and S-39) or, more significantly, against VCG 3 tester strains PCW and/or 70-21. For instance, most strains whose *nit* mutants were strongly compatible with BB (VCG 4A) reacted only weakly with strain S-39 (VCG 4B) and PCW and/or 70-21 (VCG 3). Conversely, most strains whose *nit* mutants were strongly compatible with S-39 (VCG 4B) were weakly compatible with BB (VCG 4A), and incompatible with PCW and/or 70-21 (VCG 3).

A complicating factor in assigning strains to VCG subgroups was that the extent of prototrophic growth often appeared to be dependent on the *nit* mutant phenotype. For instance, some NitM mutants of strains that complemented strongly with *nit1* or *nit1* and NitM mutants of S-39 (VCG 4B) complemented strongly with *nit1* or *nit1* and NitM mutants of tester strain BB (VCG 4A). Because these strains complemented strongly with both VCG 4 tester strains, they were designated as VCG 4A/B. However, when *nit1* mutants of some of those same strains were used instead, they would complement only weakly with the NitM of tester strain BB. Exceptions were observed as some *nit1* mutants of some strains complemented strongly with the NitM mutants of tester strains BB and S-39. Additional difficulties were encountered. Some NitM mutants of strains that strongly complemented with *nit1* or *nit1* and NitM mutants of S-39 (VCG 4B) would only complement weakly with the NitM but not with the *nit1* mutant of strain BB. However, regardless of the degree of complementation with *nit* mutants of BB, all *nit* mutants of strains that were strongly compatible with S-39 were incompatible with the *nit* mutants of tester strains PCW and/or 70-21. Similar trends were also observed with *nit* mutants of strains assigned to VCG 4A. As expected, most NitM mutants of strains assigned to VCG 4A complemented, albeit weakly, with *nit1* or *nit1* and NitM mutants of tester strain S-39 (VCG 4B). However, several

nit1 mutants of strains in VCG 4A failed to yield any visible reaction with the NitM mutant of S-39 (VCG 4B).

Strains assigned to VCG 2 were also heterogeneous with the tester strain with which they would best complement. Some *nit* mutants complemented strongly with PH and/or WM and only weakly with 115 and/or S-92, while others were just the opposite. Some *nit* mutants derived from other strains were capable of complementing strongly with PH and/or WM, and 115 and/or S-92, while others only complemented with one of the two sets of strains.

Weak complementation was observed between *nit* mutants derived from strains assigned to different VCGs. This phenomenon was occasionally observed in pairings between NitM mutants of strains assigned to VCGs 1 and 2, and VCGs 2 and 4, and rarely in pairings between *nit1* and NitM mutants of strains assigned to those VCGs. Weak complementation, however, between *nit* mutants of strains assigned to VCG 4A and tester strains of VCG 3 were very common.

Distribution of strains of *V. dahliae* among VCGs varied with the fields and counties of origin (Table 1). For instance, 30 of 32 strains in VCG 4A were collected in three of four potato fields in Portage county. The remaining two strains in VCG 4A were isolated one each from Columbiana and Sandusky counties. None of the 86 strains tested from Wayne county were assigned to VCG 4A. Certain VCGs or VCG subdivisions also predominated within individual fields (Table 1). For example, among fields 11–14, 18, and 20, which were surveyed more intensively, VCG 2 was isolated more often from field 12, while VCG 4B predominated in fields 11 and 13. Twenty-two of 25 strains from field 18 were in VCG 4A; only one strain was assigned to VCG 2 and two to VCG 4B. Of the 14 strains tested from field 20, seven were assigned to VCG 4A, five to VCG 4B, and two to VCG 2. VCG 4B was the most frequently isolated group, and also the most widespread, being detected in all potato fields where two or more strains were tested for vegetative compatibility.

Forty-five of the 47 strains of *V. dahliae* from potato that originated from eight of nine states from which strains were available were assigned to VCG 4 (Table 2). Thirty-six of the 45 strains were assigned to VCG 4A, five to VCG 4B, and four to VCG 4A/B. Only the two strains from Pennsylvania were assigned to VCG 2.

Comparative virulence of strains of *V. dahliae* to potato. Significant ($P = 0.01$) treatment effects were detected in all pathogenicity experiments. Unless specified, only data obtained in experiments I and II will be discussed, because data from repeated experiments (experiments IA and IIA) were similar. In experiment I, all 52 isolates from potato plants and soil were pathogenic to potato, as their AUSPC values were significantly ($P = 0.05$) greater than the uninoculated control. Of the four strains from other hosts, only strain PH from pistachio (VCG 2) was pathogenic to potato. The other three, V-44 from cotton (VCG 1), PG from pepper (VCG 2), and TO from tomato (VCG 3), were considered nonpathogenic to potato because their AUSPC values were not significantly ($P = 0.05$) different from the uninoculated controls.

In experiment II, 10 of 11 strains from potato and 81 of 83 strains from soil from Ohio were pathogenic to potato. Because the three strains judged nonpathogenic in experiment II appeared pathogenic when retested in experiment IIA, all strains were considered pathogenic.

A continuum of virulence levels on potato, ranging from slightly to highly virulent, was observed among strains of *V. dahliae* tested in this study. Examination of mean AUSPC values for each strain in both experiments indicated that most strains allocated to VCG 4A had AUSPC means significantly ($P = 0.05$) higher than strains in VCG 2 and especially those in VCG 4B. Two strains designated as VCG 4A/B tested in each experiment had AUSPC values that did not differ significantly ($P = 0.05$) from strains assigned to VCG 4B. Some exceptions to this trend were observed across experiments. For instance, some strains within a VCG were significantly ($P = 0.05$) different from one another, and some strains in VCG 2 were as virulent as some strains in VCG 4A. This pattern of greater virulence among strains in VCG 4A was especially evident when mean AUSPC values were calculated for each VCG and contrasts were used for mean comparisons (Table 3). Collectively, strains in VCG 4A were significantly ($P = 0.01$) more virulent than strains assigned to other VCGs.

Examination of the distribution of individual strains of each VCG within selected ranges of AUSPC values illustrated that the majority of strains in VCG 4A were more virulent (Fig. 1). In experiments I and II, nine of nine and 18 of 21 strains assigned to VCG 4A, respectively, resulted in AUSPC values above 160. Only one of 15 and one of 40 strains assigned to VCG 4B in experiments I and II, respectively, had an AUSPC value >160.

TABLE 3. Comparative virulence of 129 strains of *Verticillium dahliae* isolated from soil and potato plants collected in Ohio and assigned to vegetative compatibility groups (VCG)

	Experiment I		Experiment II	
VCG	Number of strains tested	Mean AUSPC[a]	Number of strains tested	Mean AUSPC
2	21	147	23	123
4A	9	193	21	182
4B	15	135	40	124
Contrasts of AUSPC means				
VCG 2 vs VCG 4A		100.8**[b]		513.0**
VCG 2 vs VCG 4B		8.7**		0.1
VCG 4A vs VCG 4B		147.6**		592.2**

[a] AUSPC = areas under senescence progress curves were computed for each individual plant based on foliar symptom ratings during the 14- to 56-day period following inoculation. AUSPC values are the means of strains assigned to each VCG. Only strains collected in Ohio and assigned to VCGs were considered for mean comparisons. Strains assigned to VCG 4A/B were not considered in mean comparisons.

[b] Numbers followed by (**) denote significant F test at $P = 0.01$.

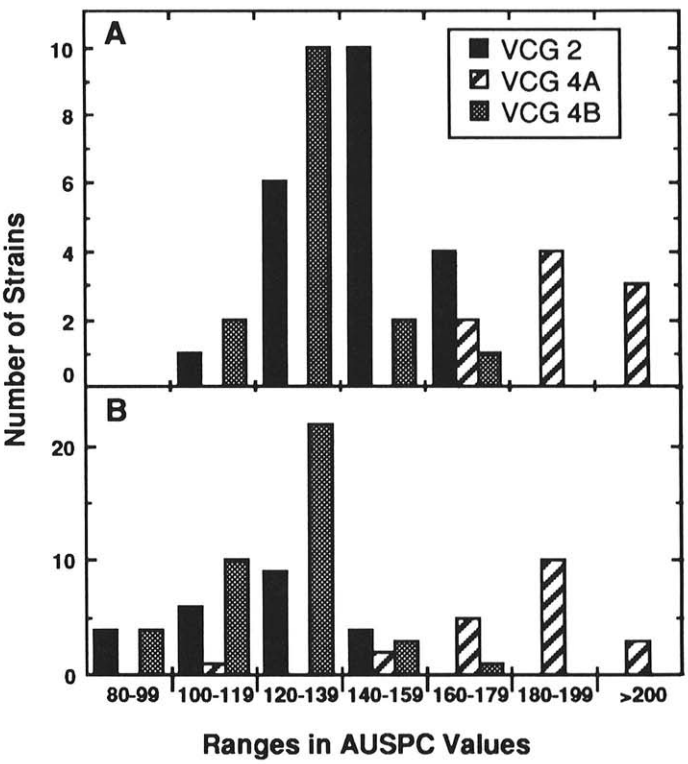

Fig. 1. Distribution of strains of *Verticillium dahliae* collected in Ohio and assigned to vegetative compatibility groups (VCGs) among ranges of AUSPC (areas under senescence progress curve) values for strains tested for pathogenicity to potato cultivar Superior. Plants were root-dipped in a suspension of 10^5 conidia per milliliter. Data for 45 and 84 strains are illustrated in **A** (experiment I) and **B** (experiment II), respectively.

Of strains assigned to VCG 2, only four of 21 in experiment I and none of 23 in experiment II had AUSPC values >160.

Pathogenicity tests with a limited number of strains in VCG 4A from other geographical locations suggested that this pattern of greater virulence to potato among strains in VCG 4A is not confined to strains from Ohio only (Table 4). With the exception of strain 308 (Oregon), all strains had AUSPC values significantly ($P = 0.05$) greater than the mean AUSPC values of strains from Ohio in VCGs 2 and 4B, in at least one of the two experiments. Finally, strain PU (United Kingdom), a heterokaryon, self-incompatible strain, (21) was virulent to potato, but its AUSPC values of 125 and 144 in experiments I and IA, respectively, were not significantly different ($P = 0.05$) from the mean AUSPC values of strains from Ohio in VCGs 2 and 4B in either experiment (Table 4).

DISCUSSION

The *nit* mutant system of VCG analysis was successfully used in this study to identify VCGs among a large isolate collection of *V. dahliae*. Data from our study suggest that populations of *V. dahliae* in commercial potato fields in Ohio have limited VCG diversity. Only three VCGs were detected among 183 strains tested. With the exception of two strains from soil assigned to VCG 1, the remaining strains from Ohio, isolated from either potato plants or soil, were in VCG 2 or 4. There was an uneven distribution of strains among these two VCGs. Seventy percent of the strains tested were assigned to VCG 4. Detection of only three VCGs among a moderately large isolate collection was surprising, considering the number of VCGs frequently encountered in many other fungi (3,9,15,28–30). A limited diversity of VCGs was also reported in a study involving *V. albo-atrum* (7). In this case, only two VCGs were found among 32 strains isolated from four different hosts and collected from diverse geographical locations. Limited VCG diversity in our study was not only confined to populations of *V. dahliae* from Ohio. Only VCGs 2 and 4 were identified among 47 strains isolated from potato plants from several diverse potato-producing areas of the United States. The vast majority (45 strains) were assigned to VCG 4. Considering results obtained with both isolate collections, strains of *V. dahliae* from potato or soil cropped to potato appear to be primarily confined to VCGs 2 and 4.

TABLE 4. Comparison between areas under senescence progress curves (AUSPC) means of strains from Ohio assigned to vegetative compatibility groups (VCG) 2, 4A, and 4B, and strains assigned to VCG 4A from several states

	Mean AUSPC[a]			
	Experiments			
Strain/Source	I	IA	II	IIA
Strains from Ohio[b]				
VCG 2	147	155	123	130
VCG 4A	193	212	182	183
VCG 4B	135	134	124	128
Strains in VCG 4A				
WS-5 (Wisconsin)	158	195	···	···
H-5 (Wisconsin)	177	197	···	···
BB (Idaho)	160	186	···	···
TA (Idaho)	···	···	174	200
318 (N. Dakota)	···	···	193	185
319 (N. Dakota)	···	···	194	200
308 (Oregon)	···	···	124	142
Plain-1 (Wisconsin)	···	···	155	154
LSD[c] $P = 0.05$	32	24	24	25

[a] AUSPC means were calculated for each individual plant based on foliar symptoms initiated 14 days after inoculation.

[b] AUSPC data for strains from Ohio in experiments I and IA are the means of 21, 9, and 15 strains in VCGs 2, 4A, and 4B, respectively. In experiments II and IIA, AUSPC data are the means of 23, 21, and 40 strains in VCGs 2, 4A, and 4B, respectively.

[c] LSD = Fisher's protected LSD ($P = 0.05$) for mean comparisons within a column.

Because of the differential reactivity among strains in VCG 4 towards tester strains S-39 and BB, and tester strains of VCG 3, we opted for the subdivision of VCG 4. This system resembles that described by Nash and Stambaugh (26) for *Cryphonectria parasitica* from oak, in which some strains within a VCG behaved differently when paired against tester strains of other VCGs, although the criterion for assessing vegetative compatibility in their study differed from the method employed here. Assignment of strains to a VCG subdivision in *V. dahliae* is problematic, however, because in part it is dependent on the extent of prototrophic growth produced at the mycelial interface of two complementary *nit* mutant pairs, a rather subjective characteristic (21). Because the intensity of prototrophic growth in some pairing combinations is dependent on strains and *nit* mutant phenotypes, difficulties were expected. In keeping our VCG system for *V. dahliae* as simple as possible, we avoided the subdivision of VCG 2, even though some strains had a tendency to strongly complement some tester strains, while only reacting weakly with others.

Weak heterokaryons between complementary *nit* mutants, especially when produced between strains assigned to different VCGs, could be attributable to "transitory" heterokaryosis. In this situation, strains are vegetatively incompatible, but the rate at which the cytoplasmic-killing reaction of the anastomosed cells proceeds is not rapid enough to halt the synthesis of some functional nitrate reductase. As a consequence, heterokaryons are formed that are stable long enough for some functional nitrate reductase to be synthesized, but not long enough for production of large quantities of the enzyme required to yield extensive prototrophic growth. The formation of heterokaryons between otherwise vegetatively incompatible strains could result from allele differences at one or a few loci that control vegetative compatibility (2). Typas (42) has suggested that, at least in some strains, cytoplasmic-killing reactions identical to those described in other fungi (2,29) are responsible for some of the incompatibility reactions in *V. dahliae*.

Greenhouse pathogenicity tests on potato provided further evidence for the subdivision of VCG 4, and evidence for recognition of two pathotypes among naturally occurring populations of *V. dahliae* from Ohio potato field soils. Strains of the more virulent pathotype are most likely to be found in VCG 4A, while strains in VCGs 2 and 4B are most likely to be of the less virulent pathotype. The more virulent pathotype could be differentiated in this study by root-dip inoculation of potato cv. Superior at 10^5 conidia per milliliter. Usually, plants exhibited extensive stunting, chlorosis, and necrosis in both lower and upper leaves, which generally remained attached to the stem after death. Most plants were dead or nearly dead 42 days after inoculation. In contrast, most potato plants inoculated with strains in VCG 4B showed milder symptoms, usually restricted to chlorosis in the lower and upper leaves. Stunting and death of plants were rarely observed in inoculations with these strains. Inoculations with strains in VCG 2 resulted in mildly virulent reactions similar to those with VCG 4B, although some strains in VCG 2 were as virulent as strains in VCG 4A. Because of the limited number of strains tested that were originally isolated from potato plants, it was not possible to determine whether differences in virulence exist between strains isolated from potato plants and those from soil.

The pathogenicity experiments reported in this investigation are in agreement with a study reported by Corsini et al (11) in which two strains from potato assigned to VCG 4 (*sensu* Puhalla and Hummel; presently VCG 4A, *sensu* Joaquim and Rowe) were significantly more virulent than two other strains assigned to VCGs 1 and 3 (*sensu* Puhalla and Hummel). Further study of these pathotypes under field conditions is necessary to validate whether these differences in virulence among pathotypes are important and result in differential effects on potato yield. Information also is needed on whether these differences in virulence persist when other potato cultivars are considered. It is also unknown what effect environmental conditions may play on symptom severity, which may interfere with identification of these two pathotypes in greenhouse or field pathogenicity tests.

In cotton, virulence reactions of some strains considered to be of the most virulent pathotype (defoliating strain) could be rendered to a milder one depending on environmental conditions or the cultivar used (5).

All 25 strains that had been recovered from potato stems were pathogenic to potato in at least one of two pathogenicity experiments in this study. Of more significance, however, is the finding that all 116 strains isolated directly from soils taken from Ohio potato fields also were pathogenic to potato in at least one of two pathogenicity experiments. This corroborates earlier findings in Idaho by Davis and Everson (13) in which all soil strains of *V. dahliae* tested were pathogenic to potato. Thus, it seems that lack of correlation between inoculum density and inoculum potential in potato fields most likely cannot be attributed to the occurrence of mixtures of pathogenic and nonpathogenic strains of *V. dahliae*.

Although greenhouse pathogenicity tests conducted in this study support the assumption that most soil isolates are pathogenic to potato, substantial differences in virulence do exist among isolates from potato field soils in Ohio. Because of the existence of virulence differences among populations of *V. dahliae*, the relationship between inoculum density and disease severity or yield loss may vary among fields, depending on the prevalence of specific pathotypes within the local population. For example, if levels of soilborne inoculum in fields 13 and 18 (Table 1) were identical and environmental and cultural conditions were uniform, correlations between inoculum density and disease severity or yield loss may vary among fields in view of the different distribution of pathotypes. This may have significant bearing on epidemiological studies such as those aimed at predicting potato yield loss as a function of preplant soilborne inoculum levels. For instance, Francl et al (16) has shown that 15 microsclerotia per cubic centimeter of soil are necessary to incur a 10% yield loss. This relationship would have to be adjusted to take into account the virulence of the various pathotypes within a population in a given field. Each of these pathotypes may also interact differently with various *Pratylenchus* spp. In addition to the epidemiological implications, the awareness of pathotypes, as pointed out by Corsini et al (11), is also important in breeding programs in which it is crucial that prospective *Verticillium*-resistant potato lines be exposed to the most virulent pathotype for proper evaluation.

In this study, the distribution of the most virulent pathotype of *V. dahliae* on potato was limited primarily to two fields in Portage county. Twenty-nine of the 32 strains identified as VCG 4A were recovered from fields 18 and 20. Both of these fields are under the same ownership and are approximately 1 km apart. At the time of sampling, field 18 had been in potato monoculture for more than 10 yr. Movement of inoculum from one field to another via agricultural equipment may explain why this pathotype predominated in these two fields with different cultural backgrounds. However, why these strains are primarily confined to these two fields is more difficult to explain. Introduction of this strain into one of the fields followed by an increase in population in field 18 because of potato monoculture may be one explanation. This pathotype was undetected in Wayne county, and only recovered once each in Columbiana and Sandusky counties. Failure of detection, however, does not necessarily imply absence. These strains may exist at low population levels, escaping detection because of the relatively small sample size obtained from each field. Nevertheless, the highly virulent pathotype of *V. dahliae* appears not to be the predominant population in most potato field soils in Ohio. Reasons for such an imbalance in geographical distribution of pathotypes in Ohio remains unclear, but environmental, biological, and/or cultural conditions may be responsible. It is interesting to note that strains of VCG 4A were rarely found in fields that were managed with crop rotation. Although research is needed in this area, crop rotation could be preventing or at least delaying the buildup of the most virulent strains in Ohio potato fields.

Phenotypic characterization of 126 *nit* mutants by their growth response on various nitrogen sources revealed only two phenotypic classes, i.e., *nit1* and NitM. *Nit* mutants for the pathway-specific regulatory locus (*nit3* mutants), frequently recovered from wild-type strains of *F. oxysporum* (8) and *F. moniliforme* (23) but rarely from *Neurospora crassa* (41) when using chlorate-containing media, were not isolated in this study.

Several studies have shown that the frequency of recovery of each phenotypic class of *nit* mutants on chlorate-containing media can be influenced by the nitrogen source used (12,23). For instance, in *Aspergillus nidulans*, *nit3* mutants were never recovered when the chlorate-containing medium was supplemented with either glutamate or arginine (12). However, the frequency of isolation of *nit3* mutants averaged 20 and 64% when asparagine or uric acid, respectively, were used as the nitrogen sources (12). Similar findings were reported for *F. moniliforme* (23) in which chlorate medium amended with either glutamate or uric acid yielded 8 and 47% of *nit3* mutants, respectively. It is unclear why *nit3* mutants were not isolated in our study. Although the effect of nitrogen sources in differential selection of *nit* mutants in *V. dahliae* is unknown, it is unlikely that the chlorate-containing semisynthetic medium employed in our study promotes only the recovery of *nit1* and NitM mutants, to the absolute exclusion of *nit3* mutants.

The failure to identify *nit3* mutants in this study may have stemmed from the inability of the medium to distinguish them from *nit1* mutants. Because complementation was observed occasionally in pairings between *nit1* mutants derived from strains assigned to the same VCG, it is conceivable that some of these *nit* mutant pairing combinations may have been between *nit1* and *nit3* mutants, instead. However, in *F. oxysporum*, complementation between *nit1* mutants, and between *nit1* and *nit3* mutants has been observed (8,15). In light of this, it remains unclear whether the complementation that we observed between *nit1* mutants was the result of either one or both of those *nit* mutant pairing combinations.

In general, vegetative compatibility analysis of strains of *V. dahliae* in Ohio potato fields, as well as a limited number of strains from other potato-producing areas of the United States indicated a lack of VCG diversity within this pathogen. Pathogenicity tests revealed a continuum of virulence among strains assigned to different VCGs; however, significant differences in virulence were observed. Strains allocated to VCG 4A were usually more virulent than strains of VCGs 2 and 4B. If the relationship between virulence and VCG on potato persists across other geographical areas, then this technique, in spite of being labor-intensive, may accelerate the identification and management of the most virulent pathotype of *V. dahliae*.

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