Assessment of Vegetative Compatibility and Virulence of *Verticillium dahliae* Isolates from Idaho Potatoes and Tester Strains

C. A. Strausbaugh

Department of Plant, Soil, and Entomological Sciences, Division of Plant Pathology, University of Idaho, Research and Extension Center, 3793 North 3600 East, Kimberly 83341.

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ABSTRACT

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Thirty-three wild-type strains of *Verticillium dahliae* were isolated from potato stems from 25 fields in southern Idaho. These strains were all assigned to vegetative compatibility group (VCG) 4 based on pairings of nitrate-nonutilizing mutants induced on medium containing chlorate. The Idaho strains and VCG 4 testers were divided into nine subgroups. These subgroups relate to previously designated VCG 4 subgroups as follows: 4A = 4A1, 4A2, 4A3, 4A4, 4A5, and 4A6; 4A/B = 4A/B1; and 4B = 4B1 and 4B2. Subgroup 4A contained 29 of the 33 Idaho strains. All subgroup 4A strains and most of the strains from subgroups 4A/B and 4B were pathogenic in greenhouse studies. When placed into three subgroups for analysis, the strains from subgroup 4A were significantly more virulent than those from subgroups 4A/B and 4B. However, not all individual subgroup 4A strains were significantly more virulent than strains in subgroups 4A/B and 4B. This continuum of virulence and genetic diversity among *V. dahliae* strains was evenly distributed throughout potato-growing areas in southern Idaho.

Additional keywords: pathotype.

Potato early dying (PED) is an important disease in the Pacific Northwest and other potato-producing regions of the United States. The primary cause of PED in Idaho is *Verticillium dahliae* Kleb. (7). In some potato-growing regions, PED has a complex etiology involving the interaction of host, environment, and several pathogens (3,9,17,19,20,23,28). Most strains of *V. dahliae* have a wide host range, although physiological races based on host genetics occur for the *Ve* gene in tomato (1,22). In cotton, defoliating and nondefoliating pathotypes have been described (2,29). In potatoes, pathotypes of *V. dahliae* correlate with vegetative compatibility groups (VCGs) (5,16,32).

Vegetative incompatibility is widespread in many fungi and is useful in studying population dynamics of fungi, monitoring pathogenic fungi, and classifying fungi (10). Most filamentous fungi studied to date possess a system of regulating heterokaryon formation (10). The heterokaryon formation in *Verticillium* was first reported by Hastie (12). These heterokaryotic regions are unstable and consist of a mosaic of heterokaryotic and homokaryotic regions (26). Protoplast fusion and microinjection studies with *V. dahliae* established that the hyphal wall is a major site of incompatibility (33). Incompatibility reactions also included protoplasmic death of recipient cells (33). In 1983, incompatibility reactions and microsclerotial color mutants were used to place strains of *V. dahliae* in 16 VCGs (25). Recently, these strains were reassigned to only four VCGs based on assessment with nitrate-nonutilizing (*nit*) mutants generated from media containing chlorate (15,32).

Within *V. dahliae* subgroup 4, subgroups exhibit different degrees of virulence (16). Strains in subgroup 4A are more virulent than strains from subgroups 4A/B and 4B (16). As many as 10 subgroups may exist in VCG 4 (32). This genetic diversity in *V. dahliae* may arise from genetic recombination in the parasexual cycle (13). With *Verticillium*, genetic recombination via the parasexual cycle can occur within a host plant, in traits affecting pathogenicity and specific markers (13). The presence of this genetic diversity raises questions of practical importance in terms of breeding for resistance to *V. dahliae* and trying to predict the severity of PED based on inoculum density in the soil.

Currently, PED-management recommendations in some areas of the United States are based frequently on inoculum density in the soil (16). If the virulence and frequency of propagules in the soil is variable, however, the reliability of soil inoculum density as a predictive tool is diminished. Previous work in Idaho has shown that wilt severity was affected by cultural management practices and not associated with the inoculum density of *V. dahliae* in the soil (7). In Ohio, this lack of association between inoculum density and inoculum potential maybe attributed to mixtures of pathogenic and nonpathogenic strains of *V. dahliae* (16).

The objectives of this investigation were to use the *nit* mutant system to improve our management strategies for *V. dahliae* through an evaluation of the vegetative compatibility and genetic diversity of *V. dahliae* populations isolated from potato plants in southern Idaho, to determine which VCGs or VCG subgroups exhibit the greatest virulence, and to examine the genetic relatedness of testers used to establish the VCG system for *V. dahliae* to aid in our understanding of the phylogeny of *V. dahliae* VCGs. A preliminary report has been published (31).

MATERIALS AND METHODS

Isolation of *V. dahliae* from potato stems. Basal stem sections of potato plants showing symptoms of Verticillium wilt were collected from commercial potato fields in three areas of southern Idaho: American Falls (10 fields in the southeast), Jerome (10 fields in the southcentral), and Parma (seven fields in the southwest). Isolates from these locations were designated AF, J, and P, respectively.

To isolate *V. dahliae* from potato plants, stems were surface-disinfested for 1 min in 0.5% (v/v) NaOCl, rinsed in sterile distilled water, sliced crosswise, and transferred to water agar (WA) (20 g/L of Bacto agar; Difco Laboratories, Detroit, MI). Colonies with verticillately branched conidiophores formed in and around the vascular tissue in the potato stem slices. A sterile needle was used to streak spores from these conidiophores onto WA amended with 0.2 g/L of streptomycin sulfate. Germinated single spores

or hyphal tips were transferred from WA onto potato-dextrose agar (PDA) (Difco) slants and cultured in the dark at 25 C. Monoconidial subcultures of all isolates were maintained on PDA or minimal agar medium (MM) at 5 C (4,24,27). MM was prepared as previously described (4).

Generation and characterization of *nit* mutants. A procedure developed by Cove (6) and modified by Puhalla (24) was adapted to recover *nit* mutants of *V. dahliae*. To obtain *nit* mutants, a mycelial transfer from a monoconidial culture was placed in the center of a 9-cm-diameter Petri dish on chlorate medium (MM amended with 25 g/L of $KClO_3$ and 1.6 g/L of L-asparagine) (4,14). After incubation for 1–2 wk at 25 C, chlorate-resistant sectors were subcultured onto MM. Sectors of mycelium on MM that grew as expansive colonies with sparse mycelial growth, no aerial mycelium, and little or no sporulation were considered *nit* mutants.

The *nit*-mutant phenotypes were determined by growing each *nit* mutant twice on basal medium (BM) (MM without nitrate, amended with nitrate or hypoxanthine) (4,6). Mutants were divided into two phenotypic classes based on mutations to the nitrate-assimilation process. These classes presumably represent a mutation at a nitrate reductase structural locus (*nit1*) and at loci that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM) (4,21). Mutants from 64 isolates were characterized, although mutants from only 33 strains were utilized in the complementation tests to keep pairings manageable.

Source of tester strains. Background information for the testers is shown in Table 1. The *nit1* mutants for strains MT and HY and the NitM mutants for strains MT, TO, and PCW were produced by T. R. Joaquim and R. C. Rowe (15) and provided by L. Epstein (University of California, Berkeley). Wild-type strains for testers 138, WM, PHI, PJ, CS-1, BB, TA, 277, TO, and CU were provided by L. Epstein; the *nit* mutants produced from them had been used in a previous study (32). The cultures provided by L. Epstein had been stored as spore suspensions in 25% glycerol at −80 C. Ten of the testers (B4B, T1B, T1F, T2E, T3B, T7C, T7G, T7Q, T8A, and T8C) were isolated from potatoes in California and characterized in a previous study (32).

Complementation tests. Strains of *V. dahliae* were tested for vegetative compatibility by pairing *nit1* and NitM mutants on MM. *nit* mutants were paired by placing a mycelial transfer in the center of a 9-cm-diameter Petri dish with four transfers of a different mutant type spaced 1.5 cm from it. All *nit*-mutant testers were paired with the isolate *nit* mutants in all possible combinations at least twice. Dishes were observed at weekly intervals for 4 wk after pairing. Complementation, a result of heterokaryon formation, between *nit* mutants was evidenced by the prototrophic growth resulting in dense aerial mycelium and/or profuse sporulation and microsclerotia formation at the mycelial interface. Data from the complementation tests were analyzed by Jacquard's similarity coefficient, $S_j = a/a + b + c$ (30). This coefficient does not take negative matches into account. Based on these coefficients, similarity matrixes and dendrograms were produced. The data also were analyzed with SAS (SAS Institute, Inc., Cary, NC) clustering methods (average linkage and centroid hierarchal) and principal components analysis.

Pathogenicity tests. The pathogenicity experiments were done twice between March and September 1992. The stem ends of generation 2 seed tubers (cv. Russet Burbank) were assayed for the presence of *V. dahliae* by isolation on WA amended with 0.2 g/L of streptomycin sulfate. The distal portions of the washed tubers were cut into seed pieces, suberized for 3 days, and planted in sterilized potting mix (vermiculite, sand, and peat; 2:2:1, v/v/v). After 2 wk, sprouts of similar length were removed from the mother tubers, rinsed in sterile distilled water, and dipped in a spore suspension of 10^6 conidia of *V. dahliae* per milliliter. Sterile distilled water was used for the check.

The inoculated stems were replanted in 15.3-cm-diameter black plastic pots containing sterilized potting mix. The plants were grown at 18–37 C in the greenhouse and arranged in a randomized complete block design with eight blocks. After 7 wk, plants were scored for disease severity on a scale of 0–4 in which 0 represents a healthy plant and 4 represents a completely chlorotic and/or necrotic plant.

After disease evaluations, all above-ground plant parts from four blocks were collected and individually weighed. A small segment of each stem that had been ~2.5 cm above the potting-mix surface was surface-disinfested in 0.5% (v/v) NaOCl and placed on PDA amended with 0.2 g/L of streptomycin sulfate to determine the presence of *V. dahliae*. The statistical analysis

TABLE 1. Wild-type strains of *Verticillium dahliae* from which nitrate-nonutilizing mutants were derived for vegetative compatibility analysis

Strain designation	Host origin	Geographic origin	VCG[a]
138	Cotton	Missouri	1
B4B	Potato	California	1
WM	Cotton	Texas	2
PHI	Pepper	Italy	2
PJ	Pepper	Canada	2
CS-1	Cotton	Swaziland	2
BB	Potato	Idaho	4
TA	Potato	Idaho	4
277	Sugar beet	Washington	4
TO	Tomato	Canada	4
PCW	Pepper	California	4
HY	Hops	Washington	4
T1B	Potato	California	4
T1F	Potato	California	4
T2E	Potato	California	4
T3B	Potato	California	4
T7C	Potato	California	4
T7G	Potato	California	4
T7Q	Potato	California	4
T8A	Potato	California	4
T8C	Potato	California	4
MT	Maple	Canada	4
CU	Catalpa	Illinois	5

[a] VCG = vegetative compatibility group; these groupings were established in previous studies (15,32).

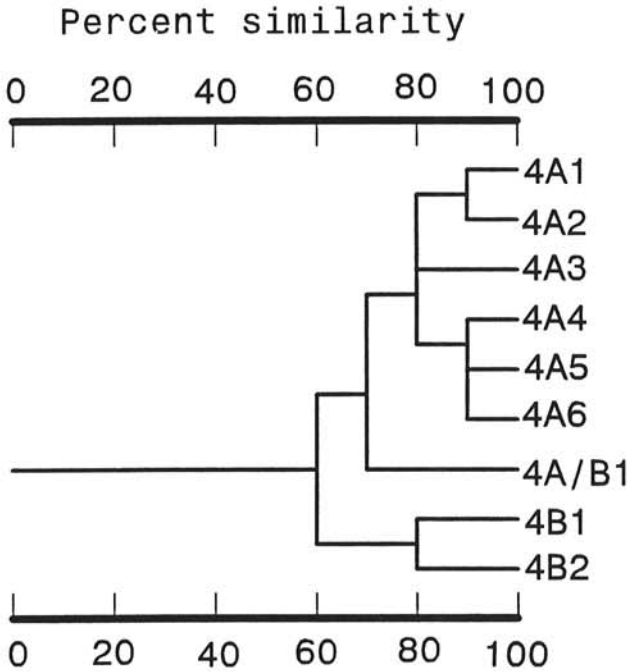

Fig. 1. A comparison of *Verticillium dahliae* isolates from Idaho potatoes based on Jacquard's similarity coefficient (30). The vegetative compatibility group 4 subgroups identified in this dendrogram include the following isolates: 4A1 = AF2-3; 4A2 = AF3-4, AF4-1, AF5-8, AF6-1, AF7-2, AF9-3, AF10-3, AF10-8, J1-4, J2-7, J4-7, J7-6, J8-5, J10-5, P4-6, P5-10, and P6-5; 4A3 = AF1-10, J5-6, J6-10, and P4-3; 4A4 = P1-3; 4A5 = AF10-4, J5-7, P3-2, P3-3, and P4-4; 4A6 = AF1-3; 4A/B1 = P2-1; 4B1 = J10-6; and 4B2 = AF10-7 and P6-3A.

of the pathogenicity data was performed with SAS.

Inoculum production. Inoculum for the pathogenicity tests was prepared from the following strains of *V. dahliae*: TA, MT, B4B, T3B, T7C, T8A, T8C, AF1-10, AF3-4, AF10-4, AF10-7, P2-1, P3-3, P6-3A, P6-5, J1-4, J5-7, J6-10, and J10-6. Conidia from 3-wk-old monoconidial cultures growing on PDA slants were suspended in sterile distilled water and spread over dilute PDA (9.75 g/L of PDA, 7 g/L of Bacto agar, and 0.2 g/L of streptomycin sulfate) in Petri dishes. These dishes were incubated in the dark at room temperature (≈25 C) for 2 wk. The cultures from two plates were washed with sterile distilled water, and the inoculum density was adjusted to 10^6 conidia per milliliter with a hemacytometer and sterile distilled water.

RESULTS

Growth responses of *nit* mutants. On chlorate medium, the wild-type colonies grew slowly in a dense, appressed mycelium with little or no aerial hyphae, whereas the *nit* mutants developed as fast-growing, fanlike colonies exhibiting sparse appressed growth. The *nit* mutants were characterized as *nit1* (possibly *nit3*) and NitM based on an ability to utilize nitrate and hypoxanthine. The mutants grew sparsely on BM amended with nitrate because they were unable to utilize nitrate. On BM amended with hypoxanthine, *nit1* mutants exhibited a dense wild-type growth, whereas NitM isolates grew in a sparse manner. Mutants from 64 Idaho potato isolates were characterized. Forty-six percent of the mutants were *nit1* (possibly *nit3*), and 54% were NitM. Although the mutants from either phenotype appeared to have approximately the same chance of being selected, it was not unusual to obtain only one phenotype from a particular isolate. *nit* mutants were not obtained from some isolates. The prototrophic growth responses were similar to those described in a previous study (32).

Complementation tests. All the Idaho isolates were included in VCG 4 because they strongly complemented at least five testers from VCG 4 and did not react strongly with testers from other VCGs (138, WM, PHI, PJ, CS-1, CU, and B4B). However, strains PHI and CU often weakly complemented the Idaho strains. Considerable variability occurred among the Idaho isolates in their ability to complement testers from VCG 4 (Fig. 1). Based on strong compatibility reactions and Jacquard's similarity coefficient (30), the Idaho isolates were separated into nine subgroups (Table 2) varying from 60 to 100% in similarity (Fig. 1). Only 4 isolates (P2-1, J10-6, AF10-7, and P6-3A) strongly complemented tester MT, and these were the most divergent of the Idaho isolates with only 60–70% similarity to the other isolates. The rest of the isolates were much more closely related (similarity ratings of 80–100%) than were the isolates that complemented tester MT. Analysis performed with SAS clustering methods and principal components also separated the Idaho strains into the same subgroups as the approach based on Jacquard's similarity coefficient (Fig. 1) (30). The subgroups found to be very similar according to Jacquard's similarity coefficient also were closely related in the other analyses.

In Figure 2, the testers that strongly complemented Idaho isolates were compared based on their reactions to the isolates and on Jacquard's similarity coefficient (30). Considerable diversity was found among the testers even though all belonged in VCG 4. Tester MT was only 10% similar to the rest of the testers, and testers TO and PCW had a similarity rating of 50%.

The same subgroups identified based on Jacquard's similarity coefficient were identified with the principal components analysis. Only eight subgroups were found in the results for the principal components analysis, however, because strain PCW (which comprised a separate subgroup in Fig. 2) was not included in this analysis due to missing data. Subgroups closely related in the similarity analysis (Fig. 2) also were closely related in the principal components analysis.

Virulence of isolates and tester strains of *V. dahliae*. The tester strains and Idaho isolates utilized in the virulence study included representative strains from subgroups identified in the vegetative compatibility analysis in this study and a previous study of California strains (32). In the virulence test, significant differences were found based on both disease ratings and top fresh weight (Table 3). In general, strains strongly compatible to tester BB and incompatible with MT were highly virulent, whereas strains strongly compatible with MT or both MT and BB were low to intermediate in virulence. When strains were combined for analysis based on predicted virulence, the group predicted to be highly virulent, subgroup 4A, was significantly more virulent than the other two subgroups, 4A/B and 4B (Table 4). Subgroups 4A/B and 4B were not significantly different from each other but were both significantly different from the check. Thus, strong compatibility to tester MT was associated with low to intermediate virulence. Strain B4B, a tester for VCG 1, was either low in virulence or nonpathogenic (Table 3). *V. dahliae* was isolated from all the treated plants but not from the checks.

DISCUSSION

The *nit*-mutant system was successfully used to characterize isolates obtained from infected potato plants in Idaho. All the Idaho isolates were strongly compatible only with testers from VCG 4. Previous Idaho strains characterized for vegetative compatibility belonged in VCG P4 (synonymous with VCG 4) (7,25). This uniformity also was found in the Tulelake area of northern California (32). VCG 4 also was the predominant VCG found in Ohio (15,16). However, data from the Bakersfield area of California (32) and from Ohio (15,16) indicates that VCGs

TABLE 2. Differential testers that identify the nine subgroups of *Verticillium dahliae* in vegetative compatibility group (VCG) 4 and the isolates from Idaho potatoes associated with these subgroups

VCG 4 subgroups[a]	Differential tester strains[b]								Isolates[c]
	BB	277	T8A	T1B	T8C	T3B	TO	MT	
4A1	+	+	+	+	+	+	+	−	AF2-3
4A2	+	+	+	+	+	+	−	−	AF3-4, AF4-1, AF5-8, AF6-1, AF7-2, AF9-3, AF10-3, AF10-8, J1-4, J2-7, J4-7, J7-6, J8-5, J10-5, P4-6, P5-10, P6-5
4A3	+	+	+	+	−	−	−	−	AF1-10, J5-6, J6-10, P4-3
4A4	+	+	+	−	+	−	+	−	P1-3
4A5	+	+	+	−	−	−	+	−	AF10-4, J5-7, P3-2, P3-3, P4-4
4A6	+	+	+	−	−	−	−	−	AF1-3
4A/B1	+	−	−	+	+	+	−	+	P2-1
4B1	−	−	+	+	+	+	−	+	J10-6
4B2	−	−	−	+	+	+	−	+	AF10-7, P6-3A

[a] Three previously designated subgroups for VCG 4 (16) are related to these nine subgroups as follows: 4A = 4A1, 4A2, 4A3, 4A4, 4A5, and 4A6; 4A/B = 4A/B1; and 4B = 4B1 and 4B2.

[b] The tester strains were characterized in a previous study (32); + = strongly compatible and − = weakly compatible or incompatible.

[c] The first letters of the isolate name indicate their origin in Idaho: AF = American Falls, J = Jerome, and P = Parma.

1 and 2 also can be isolated from potato plants or soil in potato fields.

Nine subgroups were identified within VCG 4 through analyses of the compatibility reactions of the Idaho isolates, whereas 10 subgroups were identified in a study on VCG 4 isolates from northern California (32). However, comparing the subgroups from the two studies is difficult because different tester series were used. From the comparisons that can be made, some of the subgroups identified in the previous study are different from the nine identified in this study. In work done in Ohio, three subgroups (4A, 4A/B, and 4B) had been named in *V. dahliae* and were associated with different levels of virulence (Table 4) (16). These previously designated VCG 4 subgroups relate to the subgroups found in this study as follows: 4A = 4A1, 4A2, 4A3, 4A4, 4A5, and 4A6; 4A/B = 4A/B1; and 4B = 4B1 and 4B2. These subgroups will probably become more of a continuum instead of well-defined subgroups as larger populations of isolates are studied. Thus, the ability to distinguish nine or more subgroups and the value of naming them may be irrelevant.

Ninety percent of the Idaho isolates were placed in subgroup 4A. However, in northern California, the opposite was found because 63% of the isolates were 4A/B or 4B, and only 37% were 4A (32). In Ohio, ~50% of the strains were assigned to subgroup 4B, which resembles the California pattern. The Ohio work (16) also included 47 strains from other states, in which 80% were 4A and 11% were 4B, which resembles the pattern found in Idaho. Other differences arise when the distribution of the subgroups in the sampled regions is considered. In southern Idaho and northern California (32), the 4A, 4A/B, and 4B strains were evenly distributed throughout the sampled area on a county-wide basis; in Ohio, the strains were not evenly distributed with some counties containing only one VCG 4 subgroup (16). The

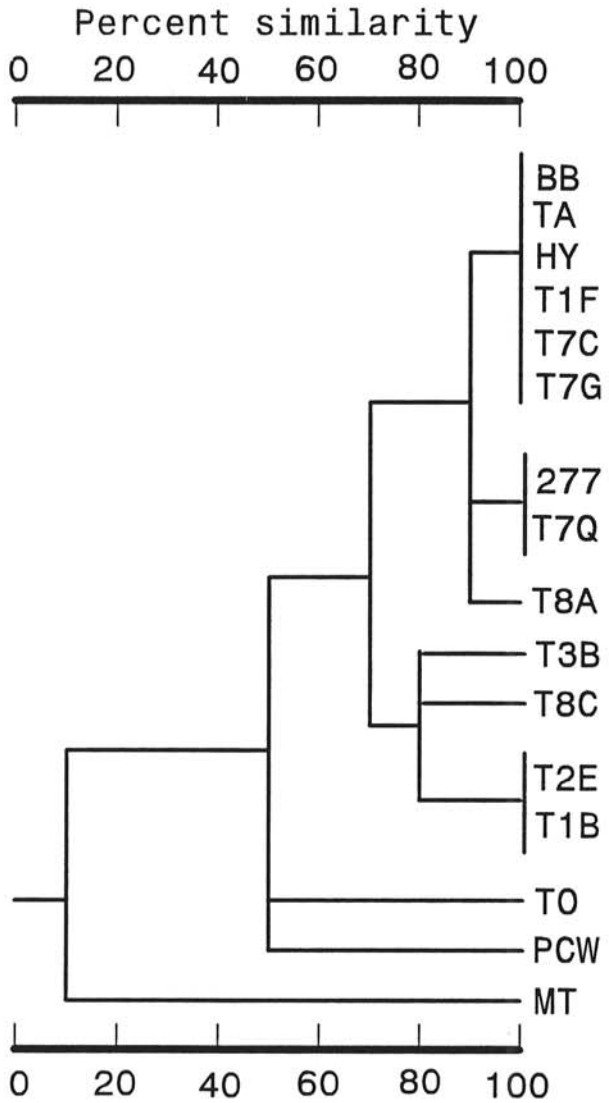

Fig. 2. Similarity of *Verticillium dahliae* tester strains for vegetative compatibility group (VCG) 4 based on Jacquard's similarity coefficient (30). Testers from the other VCGs were not included in this analysis because they were not strongly compatible with the Idaho isolates.

TABLE 3. The virulence of *Verticillium dahliae* tester strains and Idaho potato isolates as determined by disease ratings and effects on plant fresh weight

Isolates and strains	Predicted virulence[a]	Top fresh weight[b] (g)	Disease rating[c]		
			Expt 1	Expt 2	Mean
AF3-4	High	10	3.2	3.3	3.3
P3-3	High	13	3.1	2.8	2.9
J5-7	High	14	2.9	2.6	2.8
AF10-4	High	17	3.2	1.9	2.6
P6-5	High	17	2.0	2.8	2.4
AF1-10	High	15	2.0	2.4	2.2
T8A	Inter/low	25	1.5	2.4	2.0
TA	High	20	0.8	2.9	1.8
J10-6	Inter/low	24	1.4	2.0	1.7
J1-4	High	29	0.8	2.6	1.7
T7C	High	27	1.4	1.8	1.6
P2-1	Inter/low	29	1.4	1.6	1.5
J6-10	High	21	0.6	2.4	1.5
P6-3A	Inter/low	26	1.4	1.3	1.3
T3B	Inter/low	35	1.2	1.3	1.3
MT	Inter/low	33	0.9	1.6	1.2
T8C	Inter/low	26	0.9	1.4	1.2
AF10-7	Inter/low	29	0.9	1.3	1.1
B4B	Low/none	34	0.9	0.9	0.9
Check	None	42	0.4	0.4	0.4
Mean		24	1.6	2.0	1.8
LSD ($P = 0.05$)		7	0.5	0.9	

[a] Virulence was predicted from compatibility to vegetative compatibility group 4 testers BB and MT. A previous study (16) indicated that strains strongly compatible with BB and not MT were highly virulent, and strains strongly compatible with MT or both BB and MT were intermediate to low in virulence. Strain B4B belongs in VCG 1 and, thus, was not compatible with either BB or MT.

[b] Data for the two experiments were not significantly different ($P = 0.05$) and, thus, were combined for analysis.

[c] Disease ratings for experiments 1 (Expt 1) and 2 (Expt 2) were based on a scale of 0–4 in which 0 represents a healthy plant and 4 represents a completely chlorotic and/or necrotic plant.

TABLE 4. Comparison of disease ratings and effects on plant fresh weight of strains and isolates of *Verticillium dahliae* that were grouped based on predicted virulence[a]

VCG4 subgroups	Predicted virulence[b]	Top fresh weight (g)	Disease rating[c]
4A	High	18	2.3
4A/B	Intermediate/low	30	1.6
4B	Intermediate/low	28	1.3
Check	None	42	0.4
LSD ($P = 0.05$)		6	0.5

[a] Data from the two experiments were combined for analysis because the experiments were not significantly different ($P = 0.05$). VCG = vegetative compatability group.

[b] Virulence was predicted from compatibility to testers BB and MT. A previous study (16) indicated that strains strongly compatible with BB and not MT were highly virulent, whereas strains strongly compatible with MT or both MT and BB were intermediate to low in virulence. Data from B4B was not included in this analysis because it belongs in vegetative compatibility group 1 and, thus, was not compatible with either BB or MT.

[c] Disease ratings were based on a scale of 0–4 in which 0 represents a healthy plant and 4 represents a completely chlorotic and/or necrotic plant.

distributions found in these studies may have been influenced by the sample sizes. The sampling in this study probably would have identified the common strains in southern Idaho. To identify subgroups or strains present in low frequencies, the sampling intensity would have to be increased.

The testers utilized in this study represent the full range of genetic diversity known for *V. dahliae* based on previous work (32). Genetic diversity ranged from 10 to 100% similarity in this study, which was similar to the 20–80% established previously (32). In both studies, tester MT, the tester used to identify the 4B subgroup, was only 10–20% similar to the other testers. Tester BB, which was used to identify 4A isolates, was just the opposite in its similarity to several other strains. Similar relationships between MT and BB were evident in the principal components analysis.

The strains included in subgroup 4A were collectively more virulent than strains in subgroups 4A/B or 4B. However, individual strains from these three VCG 4 subgroups were not always significantly different from one another. There was a gradient from the highly virulent subroup 4A strains to the less virulent subgroups 4A/B and 4B strains. This gradient should have been expected because the Idaho strains included in this study were representative of all nine subgroups identified in VCG 4. Previous studies also indicated that subgroup 4A strains were more virulent than subgroup 4B strains (5,16). The work done in Ohio (16) also indicated that subgroup 4A strains were significantly more virulent than strains from subgroup 4B, even though individual strains from different subgroups were not always significantly different. A similar continuum of virulence has been shown with *V. dahliae* in cotton (2) and tomato (11).

The recovery of phenotypic classes of *nit* mutants on chlorate-containing media has led to similar results in two studies. In this study, 46% of the mutants recovered were *nit1* (possibly *nit3*), and 54% were NitM, whereas in a previous study (32), 51% of the mutants were *nit1* (possibly *nit3*) and 49% were NitM. However, some strains yielded only one mutant phenotype. The reason *nit1* mutants could possibly be *nit3* mutants is the difficulty in getting the isolates to grow on nitrite-amended media, which is used to distinguish these phenotypes. The *nit1* mutants are unlikely to be *nit3* mutants, however, because workers in two previous studies (16,32) were unable to isolate *nit3* mutants from *V. dahliae* with the chlorate system. Other studies have shown that the frequency of recovery of phenotypic classes of *nit* mutants on chlorate-containing media can be influenced by the N source used (6,18).

The results of this and other studies (15,16,32) indicate a lack of diversity in VCGs in potato-growing regions in the United States. However, within VCG 4, numerous subgroups have been identified. The gradient in similarity between these subgroups corresponded with the continuum in virulence. The variability in virulence among *V. dahliae* strains in this study and the Ohio study (16) raises questions about the usefulness of predicting the severity of Verticillium wilt based on soil inoculum density. Currently, genetic diversity does not appear to be of immediate concern in breeding for disease resistance because the high degree of field resistance to *V. dahliae* developed by the breeding program at Aberdeen, ID, has remained stable in many locations over many years (5,8). However, if a population shift to a more virulent strain occurs, the *nit*-mutant system and the information presented here should be useful in identifying the new populations and utilizing them in screening for resistance.

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