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

C. A. Strausbaugh, M. N. Schroth, A. R. Weinhold, and J. G. Hancock

Former postdoctoral fellow and professors, respectively, Department of Plant Pathology, University of California, Berkeley 94720.
Present address of first author: Research and Extension Center, 3793 North 3600 East, Kimberly, ID 83341.
The financial support of the USDA-ARS/State Cooperative Potato Research Program is gratefully acknowledged.
Accepted for publication 22 August 1991 (submitted for electronic processing).

ABSTRACT

Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G. 1992. Assessment of vegetative compatibility of *Verticillium dahliae* tester strains and isolates from California potatoes. Phytopathology 82:61-68.

Many strains of *Verticillium dahliae* previously considered vegetatively incompatible based on microsclerotial color mutants were found to be compatible when reassessed using nitrate-nonutilizing (*nit*) mutants. Sixteen vegetative compatibility groups (VCGs) were found previously when strains of *V. dahliae* were assayed using microsclerotial color mutants. When 26 strains representing 16 VCGs were reassessed using *nit* mutants, only four VCGs were identified. Three strains were assigned to VCG 1, 13 to VCG 2, seven to VCG 4, and one to VCG 5. The two remaining strains could not be assigned to any of the identified VCGs. Within VCG 2 and 4, subgroups were evident when percentages of similarities were compared. Strains were assigned to VCGs based only on strong compatibility reactions, because weak and incompatible reactions were unreliable. Isolates from potatoes growing in the Bakersfield region of California were only compatible with tester strains from VCG 1. Twenty-six of 30 isolates from potatoes growing in the Tulelake region of California were only compatible with strains from VCG 4. The remaining four were not strongly compatible with any tester strains, and thus may represent new VCGs. Two strains from VCG 4, TA and 277, were found to be significantly ($P = 0.05$) more virulent than strains PU, MC, 207, and PCW on the potato cultivar Netted Gem.

Potato early dying, incited by *Verticillium dahliae* Kleb. is widespread and a serious problem in some regions where intensive cultivation and high summer temperatures occur (11,30,32,38,39). Although this disease is caused primarily by *V. dahliae*, in some areas this disease has a complex etiology involving the interaction of host, environment, and a number of pathogens (3,21,26,28, 33,39). *V. dahliae* can infect a wide range of annual and perennial dicotyledonous plants as well as many ornamentals, weeds, and monocotyledonous plants (22,25,41,45).

Although there are few reports of host specialization in *V. dahliae*, it is well documented that isolates from one host can cause disease in different hosts and colonize others resulting in no apparent symptoms (5,8,36,41). However, little is known about the breadth of host range and pathotypes within a vegetative compatibility group (VCG) (36). Because strains of *V. dahliae* do not appear to have the same potential for virulence and host range (36), it will be important to identify strains more precisely to enable us to understand the epidemiology of specific Verticillium wilt diseases, estimate potential for spreading, screen for resistant cultivars, and establish control measures. Also, the geographical distribution of certain strains appears to be restricted (36). Such strains can be identified through their VCGs and thereby monitored and quarantined.

Vegetative incompatibility is widespread in many fungi and has been used to classify fungal isolates and establish the genetic diversity or subgroupings present in fungal populations (4,19, 24,27,35). Members of a VCG are considered to be genetically interactive and genetically isolated from strains in other VCGs. Establishing VCGs for strains of *V. dahliae* can be done by pairing complementary auxotrophic, nitrate-nonutilizing (*nit*) mutants (19,43). If two *nit* mutants are complementary and belong to the same VCG, hyphae from the two strains will anastomose, heterokaryons will be formed, and prototrophic growth will occur at the mycelial interface. *Nit* mutants can be recovered through a technique based on the use of chlorate, a nitrate analog, that has been very useful in studying nitrate assimilation in fungi as well as bacteria, algae, and plants (4,7,10,35). Chlorate toxicity in these organisms presumably results from the reduction of chlorate to chlorite by nitrate reductase (4). Although other modes of action may be possible, in general, chlorate-sensitive strains can reduce nitrate to nitrite but chlorate-resistant strains (e.g., *nit* mutants) cannot (4,6). The *nit* mutants are then further characterized by their ability to use various other nitrogen sources (4,7).

Puhalla (34) tested strains of *V. dahliae* for their ability to form heterokaryons through the use of microsclerotial color mutants. In a subsequent study using microsclerotial color mutants, Puhalla and Hummel (36) found 16 VCGs when 96 strains of *V. dahliae* were tested. Recently, strains previously assigned to 15 VCGs using color mutants were reassessed using *nit* mutants and only four distinct VCGs were identified (19). The results demonstrated that many strains considered to be vegetatively incompatible using microsclerotial color mutants were compatible when *nit* mutants were utilized (19).

In this investigation, strains of *V. dahliae* previously assigned to VCGs by Puhalla and Hummel (36) using microsclerotial color mutants were reassessed using *nit* mutants. These data confirm and extend research done by Joaquim and Rowe (19). The study by Joaquim and Rowe (19) established VCGs for *V. dahliae* using *nit* mutants, but did not provide a detailed analysis of the relationships between and within VCGs. By including more tester strains and doing a more comprehensive pairing of testers than the study by Joaquim and Rowe (19), we were able to perform a statistical analysis on the data. Through this analysis, we can compare and separate testers based on similarity values and thus develop a better understanding of the relatedness of tester strains. This study also establishes the vegetative compatibility and distribution of isolates of *V. dahliae* from potato plants from California. A secondary objective was to evaluate the virulence of *V. dahliae* strains from different VCGs. A preliminary report has been published (43).

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 two regions of California: the areas around Tulelake and Bakersfield. Isolates designated with a T as the first letter are from one of 10 fields sampled

in the area around Tulelake and those with a B are from one of four fields sampled in the area around Bakersfield.

To isolate *V. dahliae* from potato plants, stems were surface sterilized for 1 min in 0.5% (v/v) NaOCl, rinsed in sterile distilled water, sliced crosswise, and transferred to water agar. Colonies with verticillately branched conidiophores formed in and around the vascular tissue in the potato stem slices. A needle was used to streak spores from these colonies onto water agar amended with 0.3 g/L of streptomycin sulfate. Germinated single spores or hyphal tips were transferred from the water agar onto potato-dextrose agar slants and cultured in the dark at 22–25 C. Monoconidial subcultures of all isolates were maintained on potato-dextrose agar or minimal agar medium (MM) at 5 C (4,35,37). Minimal agar medium was prepared as follows (per liter of distilled H_2O): sucrose, 30 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 10 mg; $NaNO_3$, 2 g; agar, 20 g; and trace element solution, 0.2 ml. The trace element solution contained (per 95 ml of distilled H_2O): citric acid, 5 g; $ZnSO_4 \cdot 7H_2O$, 5 g; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1 g; $CuSO_4 \cdot 5H_2O$, 0.25 g; $MnSO_4 \cdot H_2O$, 50 mg; H_3BO_3, 50 mg; and $NaMoO_4 \cdot 2H_2O$, 50 mg.

Generation and characterization of *nit* mutants. A procedure developed by Cove (7) and modified by Puhalla (35) 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) (4,18). After incubation for 1–2 wk at 22–25 C, chlorate-resistant sectors were subcultured onto MM, which contained nitrate as the sole nitrogen source. When placed on MM, sectors that grew as expansive colonies with thin 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 one of three nitrogen sources: nitrate, nitrite, and hypoxanthine (4,7). 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 loci that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM) (4,12,29). A total of 322 strains or isolates were characterized, although only 46 were used as testers and 53 as isolate *nit* mutants in the pairings. The characterized *nit* mutants not used were strains related to the mutants included in the pairings.

Source of fungal strains. The *nit1* mutants for strains 115, MT, HY, and TC and the NitM mutants for strains V44, 115, PG, MT, TO, PCW were produced by T. R. Joaquim and R. C. Rowe (19) and provided by Lynn Epstein (Berkeley, CA). All the other *nit* mutant testers were produced from wild-type strains previously assigned to VCGs by Puhalla and Hummel (Table 1) (36) and provided by L. Epstein. The cultures provided by L. Epstein had been stored as spore suspensions in 25% glycerol at −80 C.

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 the 9-cm-diameter petri dish on MM with four transfers of a different mutant type around it. The four mycelial transfers around the central tester were 1.5 cm from the central tester. All *nit* mutant testers, produced from strains previously assigned to VCGs by Puhalla and Hummel (36), were paired with each other and with *nit* mutants from the field isolates in all possible combinations at least twice, most four or more times. Plates were observed at weekly intervals for 4 wk after pairing. Complementation, a result of heterokaryon formation, between *nit* mutants was evident by the prototrophic growth resulting in dense aerial mycelium and/or profuse sporulation and microsclerotia formation at the mycelial interface. To preclude the possibility that paired strains were not anastomosing but simply cross-feeding extracellularly, pairings that yielded prototrophic growth were repaired and separated by a 19 mm × 42 mm × 0.2 μM polycarbonate membrane (Nucleopore Co., Pleasanton, CA) on a petri dish filled with MM. Data from the complementation tests were analyzed using Jacquard's similarity coefficient, $S_j = a/a + b + c$ (42, page 305). This coefficient does not take into account negative matches. Based on these coefficients, similarity matrixes and dendrograms were produced.

Pathogenicity tests. The pathogenicity experiments were done twice between March 1988 and July 1988. The stem half of the seed tubers (cv. Netted Gem) was discarded, and the remainder was allowed to suberize at room temperature for three days. The seed pieces were then planted in sterilized U.C. mix (peat and Colma sand; 1:1, v/v). After 2.5 wk, sprouted eyes at similar stages of growth were removed from the mother tubers, rinsed in sterile distilled water, and dipped in a spore suspension of 10^6 conidia per milliliter. Sterile distilled water was used as the control. The inoculated sprouts were replanted in 15.3-cm-diameter clay pots containing sterilized U.C. mix. The pots were incubated at 20–30 C in the greenhouse and arranged in a randomized complete block design (eight blocks). After 9 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.

Inoculum production. Inoculum for the pathogenicity tests was prepared from the following strains of *V. dahliae*: PU, 207, TA, 277, MC, and PCW (Table 1). Conidia from 3-wk-old monoconidial cultures growing on potato-dextrose agar (PDA) (Difco, Detroit, MI) slants were suspended in sterile distilled water and spread over one-fourth PDA (one-fourth strength PDA plus 7 g/L of bacto agar amended with 200 mg/L of streptomycin sulfate). These 9-cm-diameter plates were incubated in the dark at room temperature (approx. 25–27 C) for 10–14 days. The cultures from five plates were placed in 200 ml of sterile distilled water and homogenized in a blender. The conidial suspension was quantified with a hemacytometer and adjusted with sterile distilled water to yield 10^6 conidia per milliliter.

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

Strain designation[a]	Host origin	Geographical origin	VCG[b]		
			JEP	TRJ	CAS
T9	Cotton	USA (CA)	1	1	1
V44	Cotton	USA (TX)	1	1	1
138	Cotton	USA (MO)	1	1	1
PH	Pistachio	USA (CA)	2	2	2
WM	Cotton	USA (TX)	2	2	2
PU	Potato	U.K.	3	NG	NG
207	Potato	S. Australia	3	2	2
115	Cotton	Syria	3	2	2
BB	Potato	USA (ID)	4	4	4
TA	Potato	USA (ID)	4	4	4
277	Sugar beet	USA (WA)	4	NT	4
PHI	Pepper	Italy	5	NT	2
PG	Pepper	Greece	5	2	2
PJ	Pepper	Canada	5	NT	2
MT	Maple	Canada	6	4	4
CS-1	Cotton	Swaziland	7	2	2
CA	Cotton	Argentina	7	NT	2
CF	Cotton	France	8	2	2
RI	Redbud	USA (IL)	9	NT	2
CU	Catalpa	USA (IL)	10	NG	5
CW	Cherry	USA (WA)	11	4	NG
HY	Hops	USA (WA)	12	4	4
TC	Tomato	Canada	13	2	2
MC	Mum	USA (CA)	14	2	2
TO	Tomato	Canada	15	3	4
PCW	Pepper	USA (CA)	16	3	4

[a] Wild-type strains and some *nit* mutant strains were obtained from L. Epstein, Berkeley, CA, as indicated in Materials and Methods.

[b] VCG = vegetative compatibility group; JEP = VCGs determined by J. E. Puhalla and M. Hummel using microsclerotial color mutants (36); TRJ = VCGs determined by T. R. Joaquim and R. C. Rowe using *nit* mutants (19); CAS = VCGs determined in this study using *nit* mutants; NG = not assigned to a numbered VCG; NT = not tested.

RESULTS

Recovery of *nit* mutants. On MM amended with chlorate, the wild-type colonies grew slowly in a dense appressed manner with little or no aerial mycelium, while the *nit* mutants appeared as fast growing fanlike colonies exhibiting sparse appressed growth. The *nit* mutants were characterized as *nit1* and NitM based on their ability to utilize various nitrogen sources. Because the mutants were nitrate-nonutilizing, they grew in a sparse manner on BM amended with nitrate. *Nit1* mutants exhibited dense wild-type growth on BM amended with hypoxanthine, while NitM isolates grew in a sparse manner. Distinguishing *nit1* mutants from *nit3* mutants (4) was difficult, because 54% of the mutants would not grow on BM amended with nitrite. All the mutants that did grow on BM amended with nitrite, grew in a dense wild-type manner, characterizing them as *nit1* mutants. From the 140 mutants isolated from Puhalla's strains (Table 1), 49% were characterized as NitM and 51% as *nit1* (some possibly *nit3*). Although approximately half the mutants were NitM, it was not unusual to predominately isolate only one *nit* mutant phenotype from a particular strain. *Nit* mutants were not obtained from some of the isolates. From the 131 mutants obtained from the California potato isolates, only 28% were NitM, while 72% were *nit1* (some possibly *nit3*). Again, it was not unusual to isolate predominately one mutant type from a particular strain. *Nit* mutants were not obtained from some of the isolates.

Prototrophic growth responses. Pairing *nit* mutants can result in a variety of growth types at the mycelial interface, which were categorized as either strong or weak. Reactions were considered strong when the prototrophic growth, profuse sporulation, and/or abundant microsclerotial formation and/or abundant aerial mycelium occurred at the mycelial interface. When there was only growth within the agar or scattered growth on the agar surface at the mycelial interface, the reactions were considered weak. To preclude the possibility of cross-feeding and insure that the prototrophic growth at the mycelial interface was due to anastomosis and heterokaryon formation, all *nit* mutant combinations resulting in strong reactions were repaired and separated with a polycarbonate membrane. In all cases, no prototrophic growth occurred when the two mutants were separated by the membrane, but prototrophic growth did occur at the mycelial interface on the agar surface when no membrane was present. Thus, the prototrophic growth presumably results from anastomosis and heterokaryon formation and not cross-feeding at the mycelial interface.

Complementation tests. Results from pairing the NitM mutants with the *nit1* (some possibly *nit3*) mutants from Puhalla's tester strains and isolates from California potatoes in all possible combinations are presented in Tables 2 and 3. Reciprocal pairings (e.g., BB *nit1* × 277 NitM and BB NitM × 277 *nit1*) did not always result in the same degree of compatibility, therefore only the strongest compatibility reaction of the reciprocal pair was reported in Tables 2 and 3. Dendrograms (Figs. 1, 2, and 3) of the data presented in Tables 2 and 3 were constructed based on strong compatibility reactions and Jacquard's similarity coefficient (42). Figure 1 shows four VCGs are present when using *nit* mutants derived from Puhalla and Hummels's tester strains (Table 1). The designated VCGs for the strains that were reassessed using *nit* mutants are given in Table 1. Strains T9, V44, and 138 form VCG 1. A second group which is considered VCG 2, contains two subgroups. One subgroup consists of the following strains: WM, TC, CA, PH, and CS-1. The other subgroup contains strains 207, MC, 115, PJ, RI, PHI, PG, and CF. Also within the one subgroup, strains CS-1 and PH are not very closely related to strains WM, TC, and CA. VCG 4 includes strains TA, BB, 277, TO, PCW, HY, and MT. Based on Figure 1, strain MT appears to be distantly related to the other strains in VCG 4. However, when the strains in VCG 4 were compared based on their compatibility to strains isolated from California potatoes, a different pattern of similarity was evident (Fig. 2). Based on the information from these field isolate comparisons, there seems to be a stepwise trend in similarity between the isolates, with MT on one extreme and TA on the other. Thus, strain MT may

TABLE 2. Vegetative compatibility of nitrate-nonutilizing (*nit*) mutants derived from strains of *Verticillium dahliae* previously assigned to compatibility groups by Puhalla and Hummel (36)

Tester strain	Compatibility of tester strain to other testers[a]	
	Strong	Weak
T9	V44,138	...[b]
V44	T9,V44,138	...
138	T9,V44,138	...
PH	WM,CA*	...
WM	PH,CS-1*	207,115,PHI,PG,PJ
PU	...	...
207	207,115,PHI,PG,PJ,CF,RI,MC	WM,BB,CS-1,CA
115	207,115,PHI,PG,PJ,CF,RI,MC	WM,CA,CS-1*
BB	BB,TA,277,HY,TO,PCW	207,PHI,PG,PJ,MT,RI
TA	BB,TA,277	HY*,PHI,MT,RI,MC,CS-1*,CF,PCW
277	BB,TA,277,HY,TO	...
PHI	207,115,PHI,PG,PJ,CA,CF,RI,MC	WM,BB,TA,CS-1,HY
PG	207,115,PHI,PG,PJ,CA,CF,RI,MC	WM,BB,CS-1,TC
PJ	207,115,PHI,PG,PJ,CF,RI,MC	WM,BB,CS-1,CA,TC
MT	MT,HY	BB,TA,TO*
CS-1	WM*,CA,TC	207,PHI,PG,PJ,TA*,115*
CA	PH*,PHI,PG,CS-1,CF*,TC	207,115,PJ,RI
CF	207,115,PHI,PG,PJ,CA*,RI	TA
RI	207,115,PHI,PG,PJ,CF,RI,MC	BB,TA,CA
CU	CU	...
CW	...	...
HY	BB,277,MT	TA,PHI
TC	CS-1,CA	PJ,PG
MC	207,115,PHI,PG,PJ,RI	TA
TO	BB,277,TO,PCW	MT*
PCW	BB,TO,PCW	TA

[a] Tester strains were paired in all possible combinations. There was no data from the following pairings: T9 × T9; PH × PH,CF,CW,MC,PCW; CS-1 × CS-1; CA × CA,HY; CF × CF,CW,MC,PCW; CW × CW,MC,PCW; HY × HY; MC × MC,PCW. Because reciprocal pairings frequently varied, only the most compatible reaction from a reciprocal pair of *nit* mutants was recorded. Strong = abundant aerial mycelium and/or sporulation and/or microsclerotial formation at the mycelial interface. Weak = scattered thin growth and/or poor sporulation and/or poor microsclerotia formation at the mycelial interface. * = variable reactions. Source of strains mentioned in Table 1.

[b] Indicates no reaction of that type was obtained.

not be distantly related to the other stains in VCG 4. Strain CU, VCG 5, was found to be self-compatible, but incompatible with all other strains. Based on their compatibility to field isolates, strains V44 and 138 were again considered a separate VCG (VCG 1). Tester strains PU and CW in Figure 1 and field isolates T1E, T2B, T2C, and T6G in Figure 3 were not included in these respective figures, because *nit* mutants to these strains were not compatible with the *nit* mutant testers in these respective studies. Tester strains T9 and PCW were not included in Figure 2, because neither strain was strongly compatible with any of the field isolates. Weak heterokaryotic reactions often occurred between testers from VCG 2 and 4. Testers from VCG 1 and 5 did not react weakly with testers from other VCGs. Strain PU was considered self-incompatible, because *nit1* and NitM mutants from this tester would not complement.

The strains isolated from potato plants from California formed two VCGs. All three strains isolated from potatoes in the Bakersfield region were included in VCG 1, because they were only compatible with tester strains V44 and 138. Twenty-six of 30 isolates from the Tulelake region were assigned to VCG 4, because they were only compatible with some or all of the following strains: MT, HY, TA, 277, BB, and TO (Table 3). Among the strains from the Tulelake region, a number of subgroups with varying degrees of relatedness were evident (Fig. 3).

Some *nit* mutant testers, when paired with other testers, had a tendency to produce only one type of prototrophic growth. When strains V44, PHI, PG, PJ, MT, CA, and CU were paired with other testers and strong prototrophic growth occurred, usually only aerial mycelium was abundant at the mycelial interface. Strains 138, 115, BB, TA, 277, CS-1, and RI had a tendency to sporulate profusely and form abundant microsclerotia at the mycelial interface only when pairing with other *nit* mutant testers resulted in strong prototrophic growth. When tester strains V44, HY, and MT were paired with field isolates, strong prototrophic growth usually resulted only in the formation of abundant aerial mycelium. Strains 138, BB, TA, and 277 had a tendency to sporulate profusely and form abundant microsclerotia only when strong prototrophic growth resulted from pairings with *nit* mutant field isolates.

Pathogenicity tests. Results from pathogenicity studies indicate that significant differences in virulence exist between strains from different VCGs. On the potato cultivar Netted Gem, strains TA, 277, PU, MC, 207, PCW, and the control (water) had mean disease ratings of 2.2, 1.9, 1.1, 0.9, 0.9, 0.9, and 0.6 respectively, with a Fisher's least significant difference of 0.6 ($P = 0.05$). Thus, strains TA and 277 were significantly more virulent than strains PU, MC, 207, and PCW.

DISCUSSION

Many strains of *V. dahliae* previously considered incompatible based on microsclerotial color mutants were found to be compatible when reassessed using *nit* mutants. Puhalla and Hummel (36) had previously assayed 96 strains of *V. dahliae* using microsclerotial color mutants and found 16 VCGs. When some of the strains from each of the 16 VCGs were reassessed by Joaquim and Rowe using *nit* mutants, only four VCGs were found (19). We have obtained similar results, although the data in this

TABLE 3. Vegetative compatibility of strains isolated from potato plants in California based on nitrate-nonutilizing (*nit*) mutants

Field isolates	Compatibility of isolates to tester strains[a]	
	Strong	Weak
B4A	V44,138	...[b]
B4B	V44,138	...
B4D	V44,138	...
T1A	MT,HY	BB,TO*
T1B	TA,277,MT,HY	BB,115*,PHI,RI
T1D	MT	BB,PHI,RI,CU,TC
T1E	...	MT,HY
T1F	BB,TA,277,TO?,HY	MT,PCW?
T1H	MT,HY	TA?
T2B	...	115
T2C	...	...
T2E	BB,TA?,MT	115*,277,PHI,PJ,HY
T3A	BB,MT	PHI
T3B	BB,MT	115,277,PHI,TC,HY,TA
T3D	BB,MT	115,277,PHI,RI?,TA TC,HY,PCW?
T3E	MT	BB,PHI
T6G	...	BB
T7B	BB,277	TA,PHI,MT,RI,PCW
T7C	BB,TA,277,TO,HY	PHI,MT,RI,PCW,115,PJ*
T7D	MT	BB,TO?
T7G	BB,TA,277,TO	PHI,MT,RI,HY,PCW,115
T7I	BB,TA?,277	MT,PCW
T7L	BB,TA,277,TO	MT,115
T7M	BB,TA?,277	PHI,PCW?,MT
T7N	MT	BB,TA?
T7O	MT	BB,PHI
T7Q	BB,TA,277,HY	115,PHI,PJ,MT,RI,PCW
T8A	BB,TA,277,MT	PHI,RI,PC,HY
T8C	MT	207*,115,BB,TA,PHI,PJ,HY,RI
T8E	BB,TA,277	PHI,MT,RI,PCW,TO
T9N	MT	BB,RI
T10L	MT	BB
T10P	MT	115,BB,RI

[a] Field isolates and tester strains were paired in all possible combinations. Because reciprocal pairings frequently varied, only the most compatible reaction from a reciprocal pair of *nit* mutants was recorded. If a tester is not listed as strong or weak, then there are no data for that combination. Strong = abundant aerial mycelium and/or sporulation and/or microsclerotial formation at the mycelial interface. Weak = scattered thin growth and/or poor sporulation and/or poor microsclerotial formation at the mycelial interface. * = variable reactions. ? = only tested once. Source of strains mentioned in Table 1.

[b] Indicates no reaction of that type obtained.

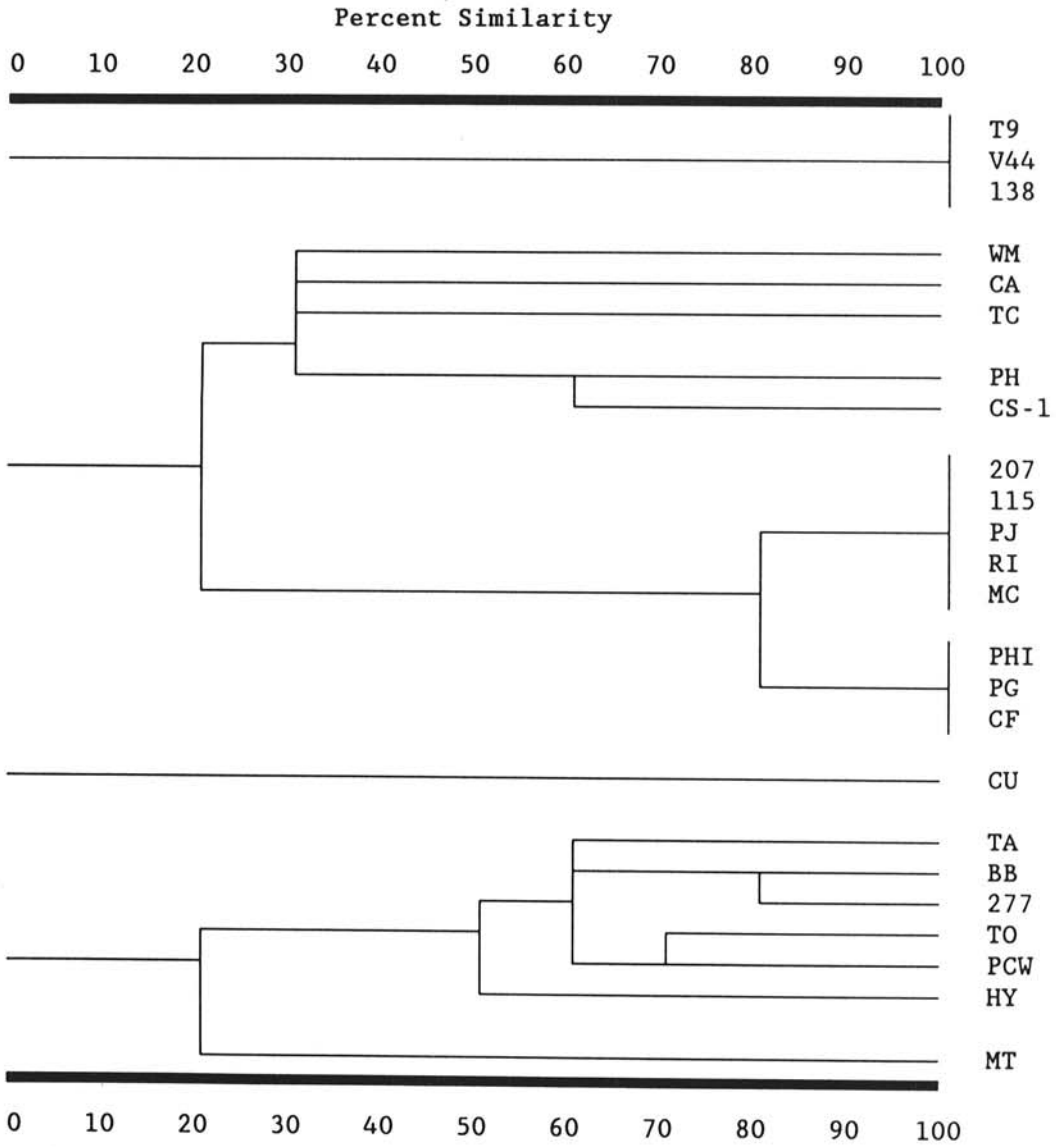

Fig. 1. Similarity of *Verticillium dahliae* tester strains (19) based on Jacquard's similarity coefficient (42).

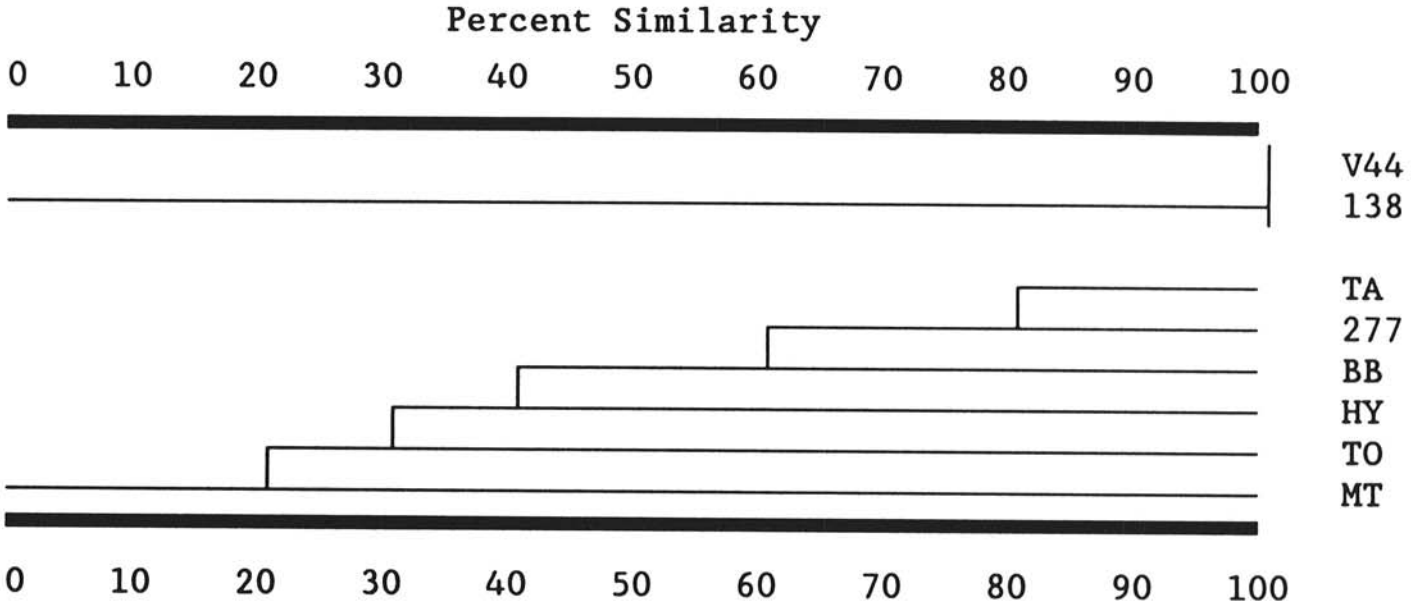

Fig. 2. Comparison of *Verticillium dahliae* tester isolates from vegetative compatibility groups 1 and 4 based on Jacquard's similarity coefficient (42).

study was analyzed differently and the results for a few of the compatibility tests differed. Strains investigated in both studies were placed in the same VCGs, except for strains TO, PCW, and CU. Joaquim and Rowe (19) placed strains TO and PCW in a separate VCG, VCG 3, even though they found strain TO was strongly compatible to BB, which is a strain included in VCG 4. In this study, strains TO and PCW were both found to be strongly compatible to strain BB. Thus, we assigned strains TO and PCW to VCG 4. Additional evidence to support this conclusion is shown in Figure 1, in which strains TO and PCW were found to be similar to other strains in VCG 4. The most likely reason the two studies differed is due to the length of time readings were taken. In this study, when strains TO and PCW were paired with BB, they showed strong compatibility approximately 21–28 days after pairing. Thus, because Joaquim and Rowe only collected data for 21 days (19), they may not have allowed enough time for strong reactions to occur with strain PCW. In this study, strain CU was considered a separate VCG, designated VCG 5 (Table 2). Strain CU was not placed in a numbered VCG by Joaquim and Rowe (19) because it was not compatible with the 11 tester strains in their study.

All the strains of *V. dahliae* considered incompatible based on the use of microsclerotial color mutants were also compatible using *nit* mutants with the exception of strain PU. Strain PU was found to be heterokaryon, self-incompatible in this study as well as in the study by Joaquim and Rowe (19).

Some variability was found between the compatibility results reported in this study and those reported by Joaquim and Rowe (19). Most of the variability concerned differences in compatibility involving pairings between strains that resulted in weakly compatible or incompatible reactions. Some variability was also observed within this study as indicated in Tables 2 and 3. Most of the variable reactions in this study involved strains in VCG 2. Also, most of the differences in compatibility reactions between

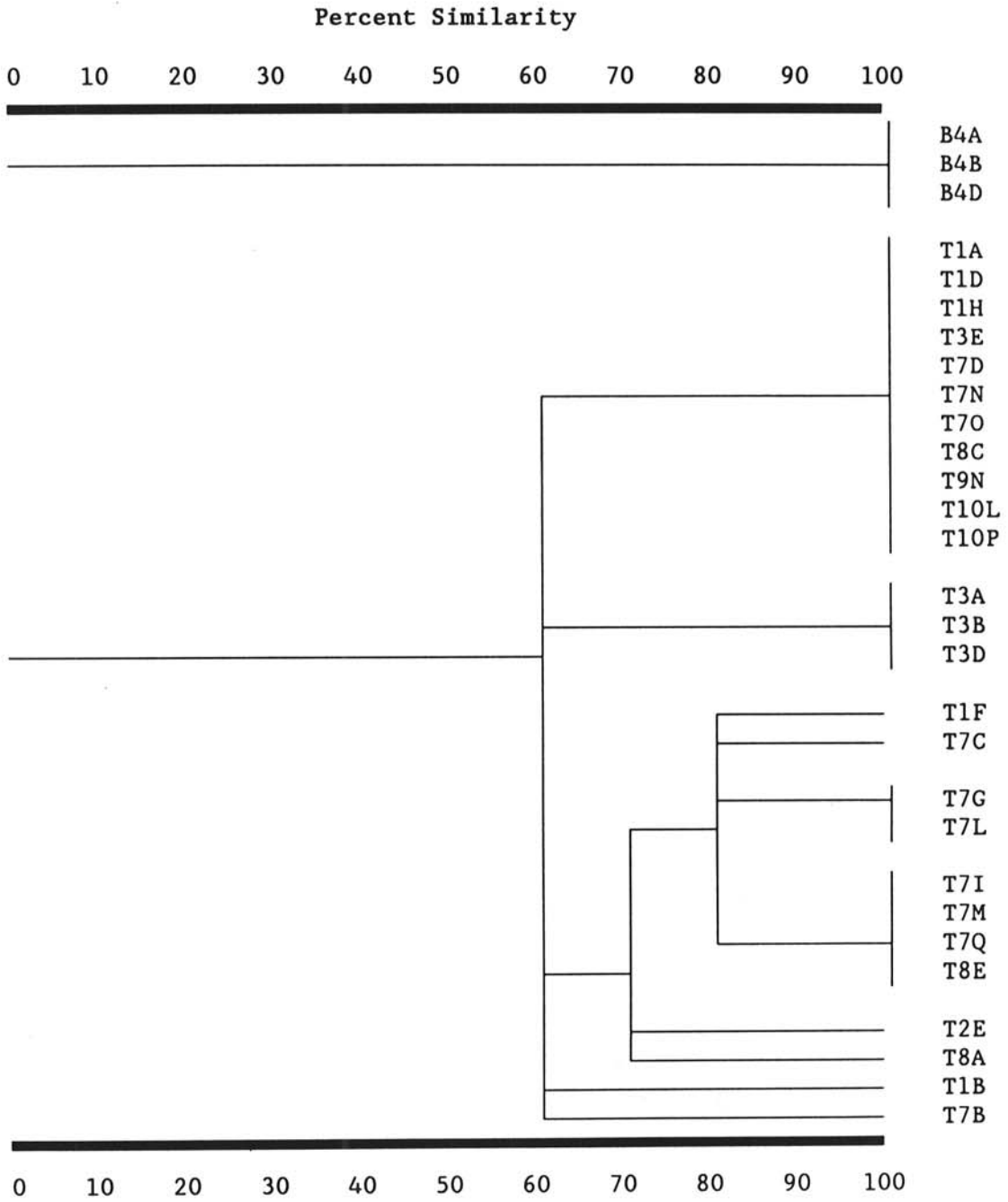

Fig. 3. Similarity of *Verticillium dahliae* isolates from California potatoes (B = from Bakersfield region and T = from Tulelake region) based on Jacquard's similarity coefficient (42).

this study and that done by Joaquim and Rowe (19) concerned incompatible or weakly compatible reactions among strains from VCG 2. The compatibility of strain BB in VCG 4 also varied between the two studies. Where compatibility reactions differed between the two studies, a consistent pattern of differences occurred. For example, the pairings PH × 115, PH × 207, and PH × CS-1 all resulted in weak compatibility reactions in the previous study (19), whereas all these crosses resulted in incompatibility in this study (Table 2). Thus, these differences between studies may only represent differences in the ability of *nit* mutants to complement one another and not differences in compatibility between strains. This points out our lack of understanding of exactly how and where these mutations occur and the influence that environmental factors such as pH (9,15,31) may have on compatibility reactions.

Reciprocal crosses were important in assigning strains to VCGs, especially when establishing a strain as a distinct VCG. Reciprocal crosses can be made only if *nit1* and NitM mutants are obtained from each strain, thereby making it possible to make two-way pairs of strains. If paired, *nit* mutants from two strains gave strong compatibility reactions, we felt confident that these strains were vegetatively compatible. Thus, doing reciprocal crosses under these circumstances was not important. However, if two strains were paired and only weak compatibility or incompatibility resulted, reciprocal crosses were necessary. Reciprocal crosses may indicate that the two strains are considerably more compatible.

The VCGs in *V. dahliae* were found to be stable. Once a strain is established as part of a VCG based on strong compatibility reactions, that strain was never found to mutate in a way that would allow it to strongly complement other testers and fit into a different VCG. However, some strains appeared to have a tendency to readily lose there ability to complement some testers when experiments were repeated a number of times. Some strains also have a tendency to revert back to the wild-type. This variability in *V. dahliae* strains may result from mutations or the repair of mutant genes. Others believe that some process other than background mutation is responsible for these chlorate-resistant mutants (24). It has been proposed that these naturally occurring mutations may be the result of transposable elements (23). Transposon movement has been associated with high mutation frequency in a number of eukaryotic organisms (23,29). It would also appear that certain loci are more frequently mutated, because only *nit1* and NitM mutants were predominantly isolated from certain strains. A similar pattern has been found in other studies (23,24).

Some of the testers used in the compatibility tests frequently produced only a specific type of prototrophic growth, aerial mycelium, or abundant sporulation and microsclerotial formation, at the mycelial interface. These results may be due to a number of nonallelic genes, some cytoplasmic and some nuclear, controlling the development of darkly pigmented structures (44). The presence or absence of resting structures per se is at least partially controlled by a cytoplasmic factor (44).

A distinct difference in the distribution of *V. dahliae* VCGs was found on potatoes in California. Strains from the Bakersfield region were included in VCG 1, whereas those strains from the Tulelake region were included in VCG 4. Several strains from Tulelake, T1E, T2B, T2C, and T6G, were not strongly compatible with the testers and may represent new VCGs. Further testing needs to be done before these strains could be considered new VCGs. This is the first report of a VCG 1 strain being isolated from a potato plant. The virulence of strains from VCG 1 on potato is not known. The strains from VCG 4 are considered the potato pathotype (5), although there is some diversity in virulence among the strains within VCG 4 in this study and that by Joaquium and Rowe (20). Other data has shown that not all strains of *V. dahliae* have the same potential for virulence and host range (1,2,13,14,16,17,34,36,40). Thus, there is a need for more testing of the virulence and host range of the strains included in the various VCGs not only for potatoes but for other crops as well.

The data presented confirm that the method used to establish VCGs for strains of *V. dahliae* can dramatically affect the number of VCGs obtained from the same strains. Many of the strains of *V. dahliae* assigned to VCGs based on microsclerotial color mutants (36) were found to be compatible using *nit* mutants. Through the use of *nit* mutants and strong compatibility reactions in complementation tests, strains of *V. dahliae* can be reliably assigned to VCGs. By more precisely identifying strains using VCGs, and establishing their potential for host range and virulence, researchers will be able to develop a better understanding of the epidemiology of Verticillium wilt diseases, the potential for spreading of strains, and the strains for which resistant cultivars and controls are needed.

LITERATURE CITED

1. Alexander, L. J. 1962. Susceptibility of certain Verticillium-resistant tomato varieties to an Ohio isolate of the pathogen. Phytopathology 52:998-1000.
2. Ashworth, L. J., Jr. 1983. Aggressiveness of random and selected isolates of *Verticillium dahliae* from cotton and the quantitative relationship of internal inoculum to defoliation. Phytopathology 73:1292-1295.
3. Corbett, D. C., and Hyde, G. A. 1971. Interactions between *Heterodera rostochiensis* Woll. and *Verticillium dahliae* Kleb. on potatoes and the effect of CCC on both. Ann. Appl. Biol. 68:71-80.
4. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
5. Corsini, D. L., Davis, J. R., and Pavek, J. J. 1985. Stability of resistance of potato to strains of *Verticillium dahliae* from different vegetative compatibility groups. Plant Dis. 69:980-982.
6. Cove, D. J. 1976. Chlorate toxicity in *Aspergillus nidulans*: Studies of mutants altered in nitrate assimilation. Mol. Gen. Genet. 146:147-159.
7. Cove, D. J. 1976. Chlorate toxicity in *Aspergillus nidulans*: The selection and characterisation of chlorate resistant mutants. Heredity 36:191-203.
8. DeVay, J. E., and Pullman, G. S. 1984. Epidemiology and ecology of diseases caused by *Verticillium* species, with emphasis on Verticillium wilt of cotton. Phytopathol. Mediterr. 23:95-108.
9. Duncan, D. R., and Himelick, E. B. 1986. Inhibition of conidial production of *Verticillium dahliae* with ammonium sulfate. Phytopathology 76:788-792.
10. Dunn-Coleman, N. S., Smarrelli, J., Jr., and Garrett, R. H. 1984. Nitrate assimilation in eukaryotic cells. Int. Rev. Cytol. 92:1-50.
11. Francl, L. J., Madden, L. V., Rowe, R. C., and Riedel, R. M. 1990. Correlation of growing season environmental variables and the effect of early dying on potato yield. Phytopathology 80:425-432.
12. Garrett, R. H., and Amy, N. K. 1978. Nitrate assimilation in fungi. Adv. Microb. Physiol. 18:1-65.
13. Grogan, R. G., Ioannou, N., Schneider, R. W., Sall, M. A., and Kimble, K. A. 1979. Verticillium wilt on resistant tomato cultivars in California: Virulence of isolates from plants and soil and relationship of inoculum density to disease incidence. Phytopathology 69:1176-1180.
14. Horner, C. E. 1954. Pathogenicity of *Verticillium* isolates to peppermint. Phytopathology 44:239-242.
15. Isaac, I. 1949. A comparative study of pathogenic isolates of *Verticillium*. Trans. Br. Mycol. Soc. 32:137-157.
16. Isaac, I. 1957. Verticillium wilt of brussels sprout. Ann. Appl. Biol. 45:276-283.
17. Isaac, I., and Keyworth, W. G. 1948. Verticillium wilt of the hop. III. A study of the pathogenicity of isolates from fluctuating and progressive outbreaks. Ann. Appl. Biol. 35:243-248.
18. Joaquim, T. R., and Rowe, R. C. 1989. Differentiation of tomato races 1 and 2 of *Verticillium dahliae* using vegetative compatibility analysis. Rep. Tomato Genet. Coop. 39:18-19.
19. Joaquim, T. R., and Rowe, R. C. 1990. Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. Phytopathology 80:1160-1166.
20. Joaquim, T. R., and Rowe, R. C. 1991. Vegetative compatibility and virulence of strains of *Verticillium dahliae* from soil and potato plants. Phytopathology 81:552-558.
21. Joaquim, T. R., Smith, V. L., and Rowe, R. C. 1988. Seasonal variation and effects of wheat rotation on populations of *Verticillium dahliae* Kleb. in Ohio potato field soils. Am. Potato J. 65:439-447.
22. Johnson, W. M., Johnson, E. K., and Brinkerhoff, L. A. 1980. Symptomatology and formation of microsclerotia in weeds inoculated

with *Verticillium dahliae* from cotton. Phytopathology 70:31-35.
23. Klittich, C. J. R., Correll, J. C., and Leslie, J. F. 1988. Inheritance of sectoring frequency in *Fusarium moniliforme* (*Gibberella fujikuroi*). Exp. Mycol. 12:289-294.
24. Klittich, C. J. R., and Leslie, J. F. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118:417-423.
25. Lacy, M. L., and Horner, C. E. 1966. Behavior of *Verticillium dahliae* in the rhizosphere and on roots of plants susceptible, resistant, and immune to wilt. Phytopathology 56:427-430.
26. MacGuidwin, A. E., and Rouse, D. I. 1990. Effect of *Meloidogyne hapla*, alone and in combination with subthreshold populations of *Verticillium dahliae*, on disease symptomology and yield of potato. Phytopathology 80:482-486.
27. Manicom, B. Q., Bar-Joseph, M., Kotze, J. M., and Becker, M. M. 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f. sp. *dianthi*. Phytopathology 80:336-339.
28. Martin, M. J., Riedel, R. M., and Rowe, R. C. 1982. *Verticillium dahliae* and *Pratylenchus penetrans*: Interactions in the early dying complex of potato in Ohio. Phytopathology 72:640-644.
29. Marzluf, G. A. 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. 45:437-461.
30. Nachmias, A., and Krikun, J. 1985. Verticillium wilt of potato in Israel. Am. Potato J. 62:201-205.
31. Pateman, J. H., and Kingborn, J. R. 1976. Nitrogen metabolism in the filamentous fungi. Pages 159-237 in: The Filamentous Fungi. J. E. Smith and D. R. Berry, eds. John Wiley & Sons, New York.
32. Pegg, G. F. 1974. *Verticillium* diseases. Rev. Plant Pathol. 53:157-182.
33. Powelson, M. L. 1985. Potato early dying in the Pacific Northwest caused by *Erwinia carotovora* pv. *carotovora* and *E. carotovora* pv. *atroseptica*. Am. Potato J. 62:173-176.
34. Puhalla, J. E. 1979. Classification of isolates of *Verticillium dahliae* based on heterokaryon incompatibility. Phytopathology 69:1186-1189.
35. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
36. Puhalla, J. E., and Hummel, M. 1983. Vegetative compatibility groups within *Verticillium dahliae*. Phytopathology 73:1305-1308.
37. Puhalla, J. E., and Spieth, P. T. 1983. Heterokaryosis in *Fusarium moniliforme*. Exp. Mycol. 7:328-335.
38. Rowe, R. C., Davis, J. R., Powelson, M. L., and Rouse, D. I. 1987. Potato early dying: Causal agents and management strategies. Plant Dis. 71:482-489.
39. Rowe, R. C., Riedel, R. M., and Martin, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology 75:412-418.
40. Schnathorst, W. C., and Mathre, D. E. 1966. Host range and differentiation of a severe form of *Verticillium albo-atrum* in cotton. Phytopathology 56:1155-1161.
41. Smith, I. M., Dunez, J., Lelliot, R. A., Phillips, D. H., and Archer, S. A., eds. 1988. *Verticillium dahliae* Kleb. and *Verticillium albo-atrum* Reinke and Berthold. Pages 299-302 in: European Handbook of Plant Diseases. Blackwell Scientific Publications, Boston. 589 pp.
42. Sneath, P. H. A. 1962. The construction of taxonomic groups. Pages 289-332 in: Microbial Classification. Cambridge University Press, Cambridge.
43. Strausbaugh, C. A., Schroth, M. N., Weinhold, A. R., and Hancock, J. G. 1990. Reassessment of vegetative compatibility groups of *Verticillium dahliae* and the compatibility of isolates from California potatoes. (Abstr.) Phytopathology 80:965-966.
44. Typas, M. A., and Heale, J. B. 1978. Heterozygous diploid analysis via the parasexual cycle and a cytoplasmic pattern of inheritance in *Verticillium* spp. Genet. Res. Camb. 31:131-144.
45. Williams, G. E. 1966. Host range and symptomatology of *Verticillium dahliae* in economic, weed, and native plants in interior British Columbia. Can. J. Plant Sci. 46:661-669.