

Vegetative Compatibility and Pathogenicity of *Verticillium albo-atrum*

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ABSTRACT

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Strains of *Verticillium albo-atrum*, isolated from alfalfa, cucumber, Ceanothus, and Pelargonium, were compared for virulence on 10 different host species in greenhouse inoculation tests. The strains tested were pathogenic on cantaloupe, cotton, eggplant, potato, and Pelargonium but were not pathogenic on pepper or cucumber. Only strains recovered from alfalfa were virulent on alfalfa. All of these strains, including several recovered from potato and hop, were tested for vegetative (heterokaryon) compatibility with one another by pairing nitrate nonutilizing (*nit*) mutants. Fifteen strains recovered from and virulent on alfalfa, from throughout North America and from France, Yugoslavia, and the USSR, were vegetatively compatible with one another and, therefore, in the same vegetative compatibility group (VCG01). These data indicate that alfalfa strains of *V. albo-atrum* may represent a genetically homogeneous clonal population with a common origin that has subsequently become

distributed worldwide. Thirteen other strains of *V. albo-atrum*, recovered from several diverse hosts and geographical locations, were vegetatively compatible with one another but vegetatively incompatible with all of the alfalfa strains of *V. albo-atrum*; these strains were placed in a second VCG (VCG02). Two hop strains from the United Kingdom were in VCG02, which indicates that VCG02 may also have a worldwide distribution. Four strains recovered from hop were heterokaryon self-incompatible; that is, phenotypically distinct nitrate nonutilizing mutants derived from the same parent were unable to form a complementing heterokaryon. These four hop strains were vegetatively incompatible with two other hop strains as well as with all the other strains examined. Although strains in VCG02 were recovered from several different hosts, the three strains in this VCG that were tested could not be differentiated on the basis of virulence on 10 host species.

Additional keywords: heterokaryosis, host specificity, nitrogen metabolism, *nit* mutants.

Verticillium albo-atrum Reinke & Berth. is a destructive vascular wilt pathogen of many agricultural crops (21,25,40,41). Within the species, however, there is considerable variation among isolates of different host origin. For example, strains differ in their temperature optimum (9,25), ultraviolet light sensitivity (33), and virulence (26,28,29). There have been attempts to demonstrate host specialization among strains of *V. albo-atrum*; in particular, isolates of *V. albo-atrum* pathogenic to alfalfa appear to represent a population distinct from that of strains pathogenic to other hosts in that only isolates from alfalfa are pathogenic to alfalfa (4,9,24,25).

Verticillium wilt of alfalfa, caused by *V. albo-atrum*, was reported in Europe in 1938 (39) and in North America in 1964 (3). In the United States, Verticillium wilt was first reported in 1977 (20) and has subsequently been found in many alfalfa-growing regions throughout the northern part of North America (2). More recently, *V. albo-atrum* has been recovered from diseased alfalfa plants from several counties in California (18). In addition, *V. albo-atrum* has been identified as a pathogen of two new hosts, Ceanothus (22) and Pelargonium (A. H. McCain, unpublished) in California. The genetic and pathological (i.e., host specificity) relationship among California isolates of *V. albo-atrum* is unknown. Furthermore, the genetic and pathological relationship among isolates of *V. albo-atrum* worldwide is not well understood.

Vegetative, or heterokaryon, compatibility has been used to determine the genetic relationship among strains of both *V. albo-atrum* and *V. dahliae* (6,17,23,24,31,37,42). In such tests, auxotrophic mutants have been used almost exclusively as forcing markers to determine whether or not strains are capable of forming heterokaryons with one another. There are, however, inherent problems associated with both the recovery and use of these auxotrophic mutants. Auxotrophic mutants are often difficult to recover, require a mutagen for their induction, and frequently grow poorly or not at all on unsupplemented media. In addition, certain auxotrophs can exert a pleiotropic effect on the ability of strains to form a heterokaryon (6).

Puhalla and Hummel (36) used another class of mutants (color

mutants) to divide strains of *V. dahliae* into distinct vegetative compatibility groups (VCG) based on heterokaryon formation. They demonstrated that strains that were vegetatively compatible were much more likely to be genetically similar than strains that were vegetatively incompatible (36). In the current investigation, a different class of mutants, nitrate nonutilizing mutants (*nit* mutants), were recovered from *V. albo-atrum* and used to test strains for vegetative compatibility to determine their genetic relatedness. The advantages of using *nit* mutants over other types of mutants for vegetative compatibility tests are that they can readily be recovered, are stable, and can grow on unsupplemented media.

The purpose of this investigation was to determine the genetic and pathological relationship among strains of *V. albo-atrum* from different hosts and geographical locations. Strains that originated from alfalfa, cucumber, Ceanothus, and Pelargonium were tested for their virulence on 10 different host species. Thirty-two strains from six different host species from five countries were tested for vegetative compatibility with one another. A preliminary report has been published (19).

MATERIALS AND METHODS

Strains. The 32 strains of *V. albo-atrum* used in the current study, including their host and geographical origin, are listed in Table 1. The isolates were recovered and identified by the authors or obtained from other investigators. Conidia from each strain were transferred to 3% water agar and individual uninucleate conidia isolated with a stage-mounted micromanipulator. A single germinating conidium was then transferred to cornmeal agar containing 0.02% dextrose (CM) (Difco). The resulting colony was stored on sterilized filter paper at 4 C (13). All colonies were incubated at room temperature (22–26 C).

Pathogenicity tests. The following plants were tested for their susceptibility to four strains (CA4, M3, 1598, and 749) of *V. albo-atrum*: pepper (*Capsicum annuum* L. 'Yolo Wonder'), tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'), cucumber (*Cucumis sativus* L. 'NP45'), cotton (*Gossypium hirsutum* L. 'SJ-2'), cantaloupe (*Cucumis melo* L. 'Spartan Rock'), strawberry (*Fragaria vesca* L. 'Alpine'), potato (*Solanum tuberosum* L.

'N807267-3') eggplant (*Solanum melongena* L. 'Black Beauty'), alfalfa (*Medicago sativa* L. 'Moapa 69'), and Pelargonium (*Pelargonium domesticum* Bailey 'Grand Slam'). Pathogenicity tests were conducted on 3–4-wk-old seedlings of each host with several exceptions; for Pelargonium, potato, and strawberry, rooted cuttings, tuber sprouts, or plants developed from runners were used, respectively.

Plants were inoculated as follows: Plants were removed from their pots and soil was rinsed from the root system with tap water. The lower third of the root system was cut off under water, then immersed into a spore suspension for 5 min. The spore suspension was prepared by harvesting conidia from the surface of a fully colonized 9-cm petri plate containing cornmeal agar (Difco) and adjusting the final concentration to 2×10^8 conidia per milliliter of water. Control plants were treated in a similar manner except tap water was substituted for the spore suspension. Six individual plants per host were inoculated in each pathogenicity test and each test was repeated at least once.

After inoculation, plants were replanted into 6-cm clay pots containing approximately 150 g of sterilized potting mix (50% sand, 50% peat) and thereafter maintained on a greenhouse bench. The mean maximum and minimum temperatures during the pathogenicity tests were 28 ± 6 and 20 ± 4 C, respectively. After 3–4

TABLE 1. Strains of *Verticillium albo-atrum*, host and geographical origin, and vegetative compatibility group

Strain ^a	Host origin	Geographical origin		Vegetative compatibility group ^b
		State	Country	
CA1	Alfalfa	California	USA	VCG01
CA2	Alfalfa	California	USA	VCG01
CA3	Alfalfa	California	USA	VCG01
CA4	Alfalfa	California	USA	VCG01
CA5	Alfalfa	California	USA	VCG01
CA6	Alfalfa	California	USA	VCG01
KV1	Alfalfa	Kansas	USA	VCG01
NY78	Alfalfa	New York	USA	VCG01
TRIP	Alfalfa	New York	USA	VCG01
PA1	Alfalfa	Pennsylvania	USA	VCG01
W40	Alfalfa	Washington	USA	VCG01
W11	Alfalfa	Wisconsin	USA	VCG01
Y1	Alfalfa	...	Yugoslavia	VCG01
R1	Alfalfa	...	USSR	VCG01
V312	Alfalfa	...	France	VCG01
749	Pelargonium	California	USA	VCG02
LASTF	Pelargonium	California	USA	VCG02
LAVGS	Pelargonium	California	USA	VCG02
1598	Cucumber	California	USA	VCG02
CN	Ceanothus	California	USA	VCG02
M3	Ceanothus	California	USA	VCG02
CNA	Ceanothus	California	USA	VCG02
CNB	Ceanothus	California	USA	VCG02
VAP1	Potato	Minnesota	USA	VCG02
VAP9	Potato	North Dakota	USA	VCG02
37-3	Alfalfa(?)	...	France	VCG02
619 (M50)	Hop ^c	...	UK	VCG02
1983 (M33)	Hop	...	UK	VCG02
629 (M18)	Hop	...	UK	VCG ? ^d
1776 (PV1)	Hop	...	UK	VCG ? ^d
1974 (PV3)	Hop	...	UK	VCG ? ^d
1985 (PV2)	Hop	...	UK	VCG ? ^d

^a Some of the strains used in this study were provided by the following individuals: N. A. Anderson, J. H. Carder, R. G. Gilbert, W. D. Gubler, T. C. Harrington, D. Kalb, J. E. Leach, P. C. Nicot, B. W. Pennypacker, T. Roberts, and D. L. Stuteville.

^b Vegetative compatibility group was determined by pairing nitrate nonutilizing mutants from each strain with a NitM tester from strain CA1 (VCG01) and a NitM tester from strain 749 (VCG02).

^c Hop strain numbers are those given to isolates at the East Malling Research Station and were supplied by J. H. Carder. Number in parentheses is the virulence grouping (5,42).

^d These strains were heterokaryon self-incompatible; a Nit1 and a NitM mutant derived from each of these strains were unable to complement one another nor any other nitrate nonutilizing mutants with which they were paired.

wk, plants were rated for disease based on the degree of stunting, chlorotic, and/or necrotic tissue relative to the uninoculated controls.

Isolations were attempted from each of the inoculated hosts. After plants were rated for disease, several petioles from each host were removed, placed in petri plates containing 2% water agar, and incubated at room temperature. Plates were examined periodically for the presence of *V. albo-atrum*. Several of the isolates of *V. albo-atrum* that were recovered were retested in vegetative compatibility tests (see Methods below).

Generation of nitrate nonutilizing (*nit*) mutants. *Nit* mutants were used to test all of the strains of *V. albo-atrum* for vegetative, or heterokaryon, compatibility with one another. *Nit* mutants were recovered from each strain of *V. albo-atrum* with an adaptation of a technique described by Puhalla (35). Two media, cornmeal agar with 0.02% dextrose- (Difco) amended with 1.5% potassium chlorate (CMC) or minimal agar medium amended with 1.5% potassium chlorate (MMC)(11) were used to generate *nit* mutants. To obtain *nit* mutants, a mycelial transfer was placed in the center of a petri dish (6 cm in diameter) containing either CMC or MMC. Plates were incubated as previously described. Chlorate-resistant sectors were recovered from the wild-type colony after 10–20 days. These sectors were transferred to a minimal agar medium (MM), which contained nitrate as the sole nitrogen source (11,35). Sectors that grew as thin expansive colonies with no aerial mycelium were considered *nit* mutants.

The *nit* mutant phenotypes were determined by growing each *nit* mutant on a minimal agar medium amended with one of several nitrogen sources (11).

Complementation tests. Strains of *V. albo-atrum* were tested for vegetative compatibility by pairing phenotypically distinct *nit* mutants of the various strains on MM. *Nit* mutants were paired by placing a mycelial transfer of each of two *nit* mutants onto MM approximately 0.5 cm apart (Fig. 1). When two thin *nit* mutants grew together, complementation (as a result of heterokaryon formation) was evident by the development of dense aerial prototrophic growth where the two thin *nit* mutant colonies came in contact and anastomosed (11,13,35).

Heterokaryosis between phenotypically distinct *nit* mutants (see Results) was examined in more detail by: 1) placing a cellophane barrier between paired *nit* mutants to test for cross feeding (13,35), and 2) isolating individual conidia from the zone of heterokaryotic (prototrophic) growth. Conidia were isolated with a transfer loop only from the area of dense aerial mycelium. Individual conidia were isolated with a stage-mounted micromanipulator. Approximately 100 conidia were recovered from each heterokaryon examined. The *nit* mutant phenotypes of individual conidia were determined by their growth on a minimal agar medium containing either nitrate or hypoxanthine as the sole nitrogen source (11). This was done to determine the nuclear ratio of the prototrophic heterokaryons.

RESULTS

Pathogenicity tests. Four strains of *V. albo-atrum*, CA4 (alfalfa), M3 (Ceanothus), 1598 (cucumber), and 749 (Pelargonium) were tested for pathogenicity on 10 hosts. Severe chlorotic and necrotic symptoms, typical of *Verticillium* wilt, were observed on cantaloupe, cotton, eggplant, potato, and Pelargonium when inoculated with each of the four different strains of *V. albo-atrum* (Table 2). Although no chlorotic or necrotic symptoms were observed on strawberry or tomato, both hosts were clearly stunted by all four strains relative to the controls. Pepper and cucumber appeared unaffected after inoculation with these four strains of *V. albo-atrum*.

V. albo-atrum strain 1598, originally isolated from cucumber (21) was avirulent on cultivar NP45 of *C. sativus* in the greenhouse inoculation tests (Table 2). As a result, inoculation tests were repeated on seedlings of cultivar Toska 70, the cultivar from which this strain was originally isolated. Strain 1598 was also avirulent on seedlings of this cultivar in our tests.

Unlike the other nine hosts examined, alfalfa exhibited a

differential response to the four strains. Only strain CA4, originally recovered from alfalfa, was virulent on alfalfa (Table 2).

Alfalfa and cantaloupe also were inoculated with each of three strains of *V. albo-atrum* (strains 629, 1983, and 1776) pathogenic to hop (*Humulus lupulus* L.) (5). All three strains were virulent on cantaloupe, producing chlorotic and necrotic symptoms; all three strains, however, were avirulent on alfalfa. Alfalfa plants inoculated with CA4, included in this test as a positive control, developed chlorotic and necrotic symptoms indicating that conditions were suitable for disease development.

Nit mutants. Chlorate did not greatly restrict the growth rate of any of the strains of *V. albo-atrum* examined, particularly when grown on CMC. However, chlorate-resistant sectors were evident either as slightly faster-growing sectors at the colony perimeter or as zones of dense sporulation throughout the wild-type colony. To

obtain chlorate-resistant sectors from the colony perimeter, a mycelial transfer of the sector was made to MM. A transfer loop was used to remove conidia from zones of dense sporulation; these conidia were then streaked onto MM. Frequently, a mosaic of both heavy (wild-type) and thin (*nit* mutant) growth developed from these streaks. When this occurred, a hyphal tip was taken from an area of thin growth and transferred back to MM. Only colonies that maintained a thin expansive colony morphology on MM were considered to be *nit* mutants. All *nit* mutants had a wild-type colony morphology when grown on CM or potato-dextrose agar.

Two phenotypically distinct *nit* mutants, Nit1 and NitM, were recovered from each of the strains used in this study (11). Nit1 mutants were unable to utilize nitrate but can use hypoxanthine, whereas NitM mutants cannot utilize nitrate or hypoxanthine as a nitrogen source.

In most strains of *F. oxysporum*, a *nit1* and a NitM mutant derived from the same parent are able to complement one another (11). This was also observed with most strains of *V. albo-atrum*. The complementary *nit* mutant (Nit1 and NitM) testers recovered from each strain of *V. albo-atrum* were subsequently paired in all combinations to determine which strains were vegetatively compatible with one another (Fig. 1). Twenty-eight of the 32 strains could be placed into one of two vegetative compatibility groups based on complementation reactions (Table 1). Fifteen strains of *V. albo-atrum* originating from alfalfa, including strains from France, Yugoslavia, and the USSR, were all vegetatively compatible with one another and, therefore, placed in VCG01. Thirteen other strains, isolated from various hosts and locations were vegetatively compatible with one another, but vegetatively incompatible with strains in VCG01; these strains were placed in a second VCG (VCG02) (Table 1).

One strain, 37-3 from France (Table 1), was originally recovered from alfalfa but was vegetatively incompatible with other alfalfa strains and vegetatively compatible with strains in VCG02. However, this strain was avirulent on alfalfa in our greenhouse pathogenicity tests.

Several strains were reisolated from various hosts after the greenhouse pathogenicity tests. In all cases, a strain's original VCG identity could be demonstrated. For example, the alfalfa strain CA4 isolated from cantaloupe was still vegetatively compatible with strains in VCG01. These results point out the possible utility

TABLE 2. Pathogenicity of four strains of *Verticillium albo-atrum* on several crop hosts^a

Host	Strain (host origin)			
	CA4 (alfalfa)	M3 (Ceanothus)	1598 (cucumber)	749 (Pelargonium)
Alfalfa ^b	+ ^c	-	-	-
Cantaloupe	+	+	+	+
Cotton	+	+	+	+
Eggplant	+	+	+	+
Potato	+	+	+	+
Pelargonium	+	+	+	+
Strawberry	+/-	+/-	+/-	+/-
Tomato	+/-	+/-	+/-	+/-
Pepper	-	-	-	-
Cucumber	-	-	-	-

^aVirulence was determined in greenhouse pathogenicity tests using a root-dip inoculation technique.

^bSee Materials and Methods for plant species and cultivars used.

^cSymptoms on plants 3-4 wk after inoculation. (+) = chlorotic and/or necrotic symptoms typical of Verticillium wilt symptoms. (+/-) = no chlorotic or necrotic symptoms, but >50% of the inoculated plants were stunted relative to the water-inoculated controls. (-) = no disease symptoms or stunting visible.

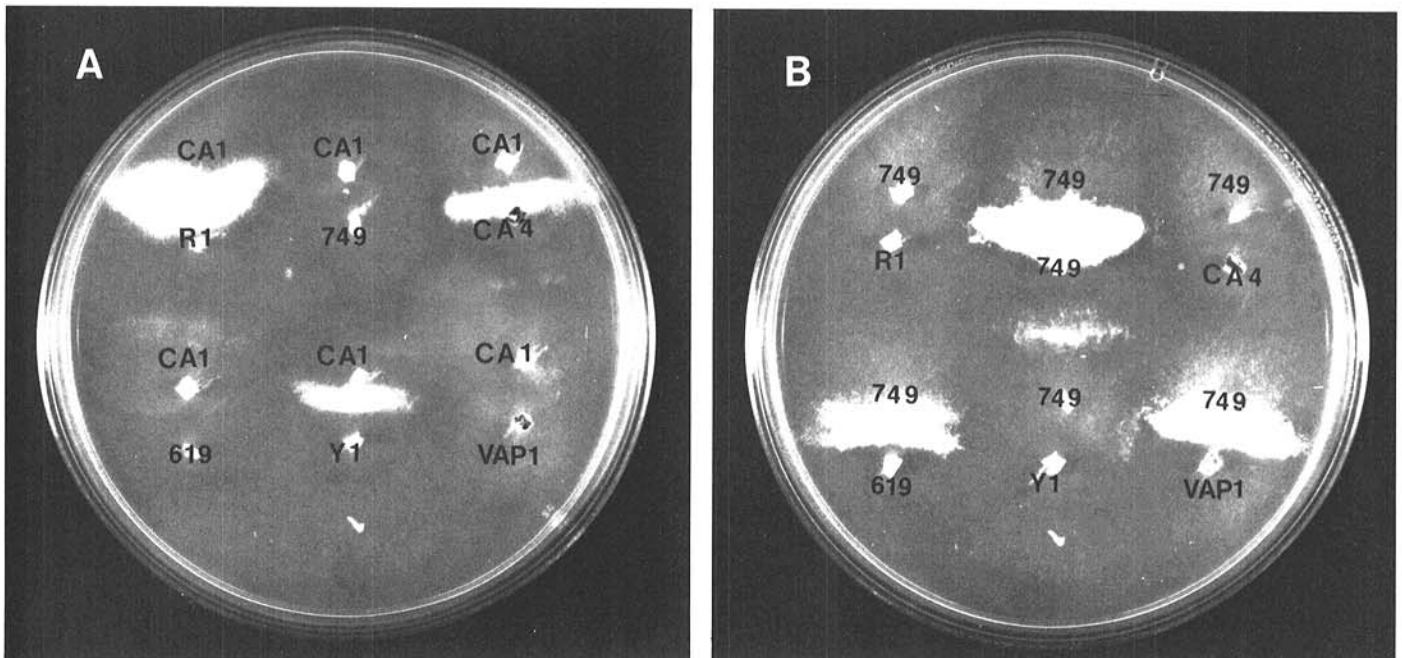


Fig. 1. Pairing of a Nit1 mutant from each of six different strains of *Verticillium albo-atrum* with A, a NitM mutant tester from strain CA1. VCG01 (note heterokaryon formation between CA1 and three strains (R1, CA4, Y1) from VCG01) and B, a NitM mutant tester from strain 749, VCG02 (note heterokaryon formation between 749 and three strains (749, VAP1, 619) from VCG02). All pairings were done on a minimal agar medium (11) containing nitrate as the sole nitrogen source and were incubated at 25 C for 10 days.

of using VCGs within *V. albo-atrum* as naturally occurring markers in both greenhouse and field inoculation experiments.

Four strains pathogenic to hop (5) were heterokaryon self-incompatible, that is, a NitI and NitM mutant derived from the same parental strain were not able to complement one another. Furthermore, none of the *nit* mutants derived from these four strains was able to complement *nit* mutants from any of the strains used in this study. As a result, the VCG identity of these four strains could not be determined.

Heterokaryosis. When separated by a cellophane barrier, prototrophic (heterokaryotic) growth did not occur between any *nit* mutants paired on MM; this indicates that the prototrophic growth observed where two thin *nit* mutant colonies came in contact on MM was not due to cross-feeding. Conidia from both of the original parental strains (i.e., NitI and NitM) were recovered from most of the heterokaryons examined. Even though only a small number of conidia from each heterokaryon were examined, the data indicate that the heterokaryons formed between *nit* mutants were very unbalanced (Table 3). In some cases, only one parental strain was recovered from a given heterokaryon. Several (<1%) of the conidia recovered from some of the heterokaryons exhibited stable prototrophic growth on MM.

DISCUSSION

For many fungi, particularly plant-pathogenic fungi, vegetative, or heterokaryon, compatibility is useful for identifying genetic diversity within a species (1, 14, 15, 32–38). Strains of a given species that are vegetatively compatible can be grouped into a VCG. In general, strains within a VCG tend to be more genetically similar than strains in different VCGs (16). For example, Puhalla and Hummel (36) divided a worldwide collection of *V. dahliae* into 16 VCGs and found that strains within a VCG were genetically homogeneous. They suggested that strains within a VCG may be similar in virulence and host range and that VCGs may actually represent genetically isolated and possibly divergent subpopulations within *V. dahliae*. In the current study, *nit* mutants were recovered from strains of *V. albo-atrum* and used to determine the vegetative compatibility relationship among these strains.

Nit mutants were recovered from each of the strains of *V. albo-atrum* used in this study. Two phenotypically distinct *nit* mutants, NitI and NitM, were recovered from each strain. NitI mutants presumably have a mutation in a nitrate reductase structural locus, whereas NitM mutants presumably have a mutation in one of several loci that code for the assembly of a molybdenum-containing cofactor that is necessary for nitrate reductase activity (11).

Two distinct VCGs were identified among the 32 strains of *V. albo-atrum* examined. The first group, VCG01, was composed only of strains recovered from and virulent on alfalfa.

TABLE 3. Phenotype determination of conidia recovered from prototrophic heterokaryons formed between vegetatively compatible strains of *Verticillium albo-atrum*^a

Heterokaryon				No. of conidia	Ratio of
Strain, <i>nit</i>	:	Strain, <i>nit</i>	:	NitI ^b : NitM ^b	NitI : NitM
CA4, NitI	:	CA1, NitM	:	59 : 0	...
CA4, NitI ^c	:	CA1, NitM	:	1 : 92	1 : 92
R1, NitI	:	CA1, NitM	:	95 : 0	...
R1, NitI	:	R1, NitM	:	3 : 102	1 : 34
749, NitI	:	749, NitM	:	88 : 2	44 : 1
VAP9, NitI	:	749, NitM	:	92 : 7	13 : 1
749, NitI	:	VAP9, NitM	:	7 : 94	1 : 13
HM50, NitI	:	749, NitM	:	57 : 0	...

^a Conidia were removed only from the area of robust heterokaryotic growth formed when certain nitrate nonutilizing mutants are paired on a minimal agar medium containing nitrate as the nitrogen source.

^b The NitI and NitM phenotypes are the same as those described from *Fusarium oxysporum* by Correll et al (11).

^c This NitI mutant was a different NitI mutant from the one used in the previous pairing.

Interestingly, several workers have already demonstrated that the alfalfa strain of *V. albo-atrum* differs from other strains in several biological properties (9,25,33). On the basis of virulence, Christen et al (10) have shown that alfalfa strains of *V. albo-atrum* from Europe and North America were not significantly different from one another. These findings, coupled with the known mode of dispersal of *V. albo-atrum* (7,8,27) led them to speculate that the alfalfa strain of *V. albo-atrum* was probably introduced into North America from Europe. The fact that alfalfa strains of *V. albo-atrum* from several locations throughout North America and from France, Yugoslavia, and the USSR are all vegetatively compatible with one another adds substantial support to this hypothesis. Furthermore, even though a small number of samples were examined, these data indicate that the alfalfa strain of *V. albo-atrum* may be a homogeneous clonal population with a common origin that has subsequently become distributed worldwide. Clearly, many more isolates and additional genetic markers (e.g., isozymes, restriction fragment length polymorphisms) need to be examined to validate such a hypothesis.

A second VCG, VCG02, was represented by strains from several diverse hosts from rather different geographical locations. For example, strains of *V. albo-atrum* recovered in California from cucumber (21) and Pelargonium from commercial greenhouses were vegetatively compatible with strains recovered from *Ceanothus* (22), an understory shrub in forests along the northern coast of the state. These strains were also vegetatively compatible with two strains recovered from potato in Minnesota and North Dakota. Two of the six hop strains of *V. albo-atrum* from the United Kingdom were also vegetatively compatible with strains in VCG02; these data indicate that, like the alfalfa strains in VCG01, VCG02 may also have a widespread distribution. The fact that three of these strains (from three different hosts, Table 2) were identical in greenhouse virulence tests may reflect additional similarities among strains in this VCG. However, the nature of greenhouse virulence tests could mask subtle differences in virulence that may exist between these strains. More isolates and additional criteria will be required to determine whether this VCG is actually composed of genetically homogeneous strains.

In light of the degree of VCG diversity that has been observed in other vascular wilt fungi (14,35,36), it was surprising that all of the nonalfalfa strains examined were vegetatively compatible with one another. Although our sample was small, it did include strains from five different hosts and several locations. It seems likely, however, that more VCGs will be identified if more strains are examined.

The four hop strains examined exhibit a phenomenon that has previously been designated heterokaryon self-incompatibility (11,12,30), which occurs when two phenotypically distinct *nit* mutants (NitI and NitM) derived from the same parent are unable to complement one another. In these four strains, heterokaryon self-incompatibility was associated with the inability of hyphae of wild-type strains to anastomose. This phenomenon has also been observed in other fungi (12,30). Several *nit* mutants from each of these four strains also were unable to complement any other *nit* mutants used in this study. These strains may be analogous to the nonreacting strains of *V. dahliae* described by Puhalla (36). Work is in progress to determine the frequency of the heterokaryon self-incompatible phenotype among mild and progressive (5,6) strains from hop.

Conclusions drawn concerning vegetative compatibility among strains of a fungal species may differ depending on the techniques employed. For example, all six hop strains were considered to be vegetatively compatible with one another in a study that employed more traditional auxotrophic mutants to force heterokaryons (6). In our study, however, four of these same six hop strains were clearly vegetatively incompatible with the other two hop strains. The *nit* mutants used in this study can grow, albeit thinly, on a minimal medium with nitrate as the sole source of nitrogen; complementation occurs where two phenotypically distinct *nit* mutant colonies actually grow together and the mycelia anastomose. Auxotrophic mutants, on the other hand, frequently are unable to grow on unsupplemented media and, therefore, are

mixed in co-culture and scored for prototrophic growth to determine vegetative compatibility. Which method more closely reflects actual vegetative incompatibility barriers in fungi is open to speculation. However, it is apparent that caution should be exercised when concluding strains of a species are vegetatively compatible based solely on complementation reactions with certain auxotrophic mutants (6,15).

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