Heterokaryosis and Vegetative Compatibility in *Leptographium wageneri*

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ABSTRACT

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Vegetative compatibility was tested among 76 isolates of *Leptographium wageneri* representing three taxonomic varieties and 14 electrophoretic phenotypes (i.e., unique combinations of isozyme patterns of 21 enzymes). Nitrate non-utilizing mutants selected on media containing the nitrate analogue chlorate were paired on nitrate media containing 200 ppm Triton X-100. The development of dense hyphal growth in the zone of confrontation between complementing phenotypes indicated compatibility. Heterokaryons were recovered from hyphal tips and conidiophores of complementing pairings but not from single conidia. Fourteen groups of vegetatively compatible isolates (VC groups) were detected among the three varieties. Each VC group contained isolates of similar electrophoretic phenotype, and most VC groups had unique geographic ranges. No complementation occurred between varieties. The relatively low number of VC groups and the correlations of VC groups with electrophoretic phenotype and geographic distributions indicate low diversity in *L. wageneri*, perhaps due to clonal selection, founder effects, and a lack of recombination.

Additional keywords: nit mutants, *Ophiostoma wageneri*.

Leptographium wageneri (Kendr.) Wingfield (syn. *Verticicladiella wageneri* Kendr.) is the cause of a unique vascular wilt of conifers, black stain root disease (10). The disease causes tree mortality in western North America and occurs in discrete infection centers by growth of the fungus for short distances through the soil between roots of adjacent trees (19,23). Infection centers are established when arthropod vectors, primarily root-feeding bark beetles in the genus *Hylastes* (Coleoptera: Scolytidae), carry sticky masses of spores from conidiophores within beetle galleries of colonized trees to uninfected trees (20).

Three host-specialized varieties of *L. wageneri* are recognized based on differences in conidiophore morphology, cultural appearance, and temperature maxima for growth (21,22). *L. w.* var. *wageneri* is pathogenic to pinyons (*Pinus edulis* and *P. monophylla*); *L. w. ponderosum* (Harrington & Cobb) Harrington & Cobb is pathogenic to hard pines (*P. contorta*, *P. ponderosa*, and *P. jeffreyi*); *L. w. pseudotsugae* Harrington & Cobb is pathogenic to Douglas-fir (*Pseudotsugae menziesii*).

In recent studies of variation in *L. wageneri* using enzyme electrophoresis, Otrosina and Cobb (31) and Zambino and Harrington (44) found that differences among varieties accounted for most of the limited genetic diversity in the species. Gene diversity (Nei's H) was 0.227 for the species and only 0.017, 0.039, and 0.040 for *L. w. wageneri, L. w. ponderosum*, and *L. w. pseudotsugae*, respectively (44). Also, only 14 combinations of putative alleles from 21 enzymes were detected among 76 isolates of this fungus. Each of these electromorph combinations or "electrophoretic phenotypes" was restricted to a single variety.

Testing for vegetative compatibility, i.e., the ability of hyphae of different strains to anastomose and produce cells containing nuclei of both strains, has been used to study population structure in a number of imperfect and ascomycete fungi (2,7–9,12,13,18, 24,25,30,33,36,39). In one method, vegetative compatibility is indicated by the development of an interaction zone with vigorous mycelial growth due to complementation between auxotrophic mutants derived from different fungus isolates paired on minimal medium. The selection of auxotrophic mutants deficient in permeases or metabolic enzymes has been facilitated by the use of toxic substrate analogues (11,14,26,42).

Cove (14) identified fast-growing sectors of *Aspergillus nidulans* (Eidam) Winter on media containing the nitrate analogue chlorate as a useful source of mutants unable to use nitrate as a nitrogen source. Three complementary classes of mutants were recovered: at the locus for nitrate reductase (E.C. 1.6.6.2), at a locus that regulates the production of the enzymes nitrate reductase and nitrite reductase (E.C. 1.6.6.4), and at any of five different loci associated with the production of the cofactor for nitrate reductase. Since the cofactor is also required for activity of xanthine dehydrogenase (E.C. 1.1.1.204), the latter mutants also have decreased ability to metabolize xanthine and hypoxanthine as nitrogen sources.

Puhalla (38) demonstrated the usefulness of nitrate non-utilizing (nit) mutants in a study of vegetative compatibility among races and formae speciales of *Fusarium oxysporum* Schlecht. Studies of vegetative compatibility using nit mutants have since been made in other ascomycetes and Fungi Imperfecti (8,11–13,18,24,26,33).

We examined vegetative compatibility between strains of *L. wageneri* as determined by complementation of nit mutants. The occurrence of vegetative compatibility groups (groups of auxotrophic strains that anastomose and complement one another) was compared with electrophoretic phenotypes to better understand the inherent population structure of this fungus.

MATERIALS AND METHODS

Isolates used. Seventy-six isolates were selected to broadly represent the hosts and geographic areas where the fungus has been reported (Table 1). Each isolate originated from a different infection center.

Media. The following media were adapted from Cove (14), Puhalla (38), and Correll et al (11). Basal medium (BM) contained 20.0 g of glucose, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.1 g of $CaCl_2$, 0.2 ml of the trace element solution

of Correll et al (11), 10.0 ml of a vitamin solution (0.1 mg of thiamine HCl, 0.075 mg of pyridoxine HCl, and 0.005 mg of biotin in 1.0 ml of 50% ethanol), and 20.0 g of agar-agar (Sigma Chemical Co., St. Louis, MO) added to 1.0 L of distilled H_2O. Complete medium (CM), hypoxanthine medium (HM), and nitrate-minimal-medium (MM) were prepared by adding 1.0 g of L-asparagine, 0.2 g of hypoxanthine, or 1.0 g of $NaNO_3$, respectively, to 1.0 L of basal medium. Nitrite medium (NM) was prepared by adding 0.1 g of $NaNO_3$ to 1.0 L of basal medium and substituting 25 g of agar noble (Difco Laboratories) for the 20 g of agar-agar. The media CMT, HMT, MMT, and NMT were CM, HM, MM, and NM to which 2 ml of 10% Triton X-100 was added to reduce radial growth rate. Weak nitrate medium contained 30.0 g of agar noble (Difco Laboratories), 5.0 g of glucose, 0.5 g of $NaNO_3$, and the vitamins and trace elements as used in NM and other media.

Nuclear staining. The number of nuclei in hyphae, hyphal tips, conidia, and developing conidiophores was determined in several isolates of *L. w. pseudotsugae*. Isolates were grown at 19 C on sterile cellophane strips placed on weak nitrate medium and MMT or on sterile slides that had been coated with these media. Most microscopic observations of hyphae were of cellophane strip cultures stained with HCl-Giemsa (3). Nuclei of conidia and immature conidiophores were best observed in materials stained with Safranin O-KOH (4).

Mutant selection and characterization. Inocula of wild-type isolates were grown at 19 C on either 1.5% malt extract agar (MEA) that contained 0.05% yeast extract (MYEA) or on CM. Mycelial plugs 9 mm in diameter were then transferred to MYEA or CM that contained 1.5% $KClO_4$ and incubated at 19 C. Fast-growing sectors that developed after 3–8 wk were subcultured to fresh plates of MYEA containing chlorate. Each mutant strain was hyphal-tipped and labeled with the wild-type designation plus a suffix indicating chlorate resistance (C1, C2, etc.).

Mutants recovered early in the study were phenotyped for nitrogen-source utilization by observing growth on BM, CM, MM, HM, and NM, but it was found that media containing Triton X-100 were more effective in differentiating phenotypes. Criteria for reduced utilization of a nitrogen source were 1) slower and/or less dense growth in mutant strains than in parental wild-type isolates and/or 2) the lack of a difference in a mutant's growth on a nitrogen-source medium vs. basal medium that contained no nitrogen source. Three mutant classes were designated following suggested genetic nomenclature (43) and current usage in other plant pathogenic fungi (11,18,26): nit1 mutants had suboptimal growth only on nitrate media and were presumed to have a mutation at the gene for nitrate reductase. Nit3 mutants had suboptimal growth on both nitrate and nitrite media and were presumed to have a mutation in the gene regulating the production of nitrate reductase and nitrite reductase. NitM mutants had suboptimal growth on hypoxanthine and nitrate media and were presumed to be deficient for the enzyme cofactor of nitrate reductase.

Complementation tests. After 14 days' growth on CM, 2.0- × 2.0-mm mycelial plugs of a pair of mutant strains were placed adjacent to each other in the center of a 60-mm plate of MMT. Plates were examined after 6, 9, and 12 wk of incubation at 19 C for the presence of a broad band of dense, aerial mycelium along the line of confrontation, indicating complementation. Self-pairings of mutants and self-pairings of wild-type isolates were used as references for auxotrophic vs. prototrophic growth.

In many pairings, including all pairings with poorly defined zones of complementation, 1.5- × 1.5-mm plugs of colonized agar were transferred after six or more weeks from the line of contact between the mutants and from either side of the paired inoculum blocks, where mycelia of the mutants had not intermingled. Colonies from the transfers were examined after 3 wk of incubation at 19 C on MMT for differences in growth.

Tests for heterokaryosis. Attempts were made to recover heterokaryons (i.e., cells with nuclei of more than one genotype) from several pairings that showed good complementation. Plugs of mycelium from the growing edge of complementing colonies were transferred to weak nitrate medium. After 5–9 days at 19 C, plates were examined at ×40 under a dissecting microscope and individual hyphal tips were excised from the edge of the colony and transferred to MMT. Cultures that grew from these hyphal tips were "primary" hyphal-tip cultures. Each primary hyphal-tip culture that showed prototrophic growth on MMT was used to obtain additional "secondary" hyphal-tip cultures. Phenotypes for nitrogen utilization were determined for all cultures derived from hyphal tips or conidia after 3 wk of growth on MMT and HMT.

With starch gel electrophoresis (44, buffer system A), the electromorphs of β-glucosidase (E.C. 3.2.1.21) and esterase (E.C. 3.1.1.1) were determined for hyphal-tip cultures of some pairings. Enzymes were extracted from cultures grown for 16 days in 30 ml of MM lacking agar or liquid MM medium amended with 0.05 g/L asparagine to allow adequate growth of nitrate non-utilizing strains.

Macronematous conidiophores were produced on 2.0- × 2.0-mm plugs of colonized MMT transferred onto weak nitrate medium. After 8–10 days of incubation, plates were examined

TABLE 1. Vegetative compatibility groups and electrophoretic phenotypes of wild-type isolates of *Leptographium wageneri*

VC group	Electrophoretic phenotype[a]	Isolates[b]
L. w. wageneri		
I	A	CAS4 (ATCC 64194), CAS5, CAS7, CAS9
	B	CAS1 (ATCC 64193), CAS2, CAS3, CAS15 (ATCC 64195), COE1 (ATCC 58576) COE2, COE6, COEN, IDE1, NES1 (ATCC 64192), NES2, NES3, NES4, NME1 (ATCC 58579), UTE1
L. w. pseudotsugae		
II	H	BCD1 (ATCC 58574)
III	H	BCD11
IVa	H	BCDJ, CAD30
	G	BCH1 (ATCC 42953)
IVb	H	CAD32, ORD5
	I	ORDQ, WADU
IVc	H	CAD19, ORD4, ORDP
V	H	ORD1
VI	H	ORD2
VII	C	IDD2
VIIIa	H	CAD40, CAD56
VIIIb	H	CAD1, CAD2, CAD6, CAD18 (ATCC 64196), CAD27, CADF
IX	H	CAD22, CAD31
X	H	CAD5, CAD55
XI	H	CADX
NC[c]	H	IDD1, ORD3
	C	MOD22
	D	MOD1 (ATCC 58578)
	E	NMD1
	F	NMD2
	J	COD1 (ATCC 64191)
L. w. ponderosum		
XII	K	BCL1 (ATCC 42954), BCL2, BCW1, BCL4, MOW2
	L	BCL3
XIII	M	CAJ3, CAP3, CAP19 (ATCC 58575), CAP36, CAPC, CAPD, CAPH, CAPI, CAPW
	N	CAPY, ORL1, ORP1
XIV	M	ORMS (ATCC 58581)
NC[c]	M	IDP1 (ATCC 58577)
	N	ORH1 (ATCC 58580)
NT[d]	M	CAJ1

[a] From Zambino and Harrington (44).
[b] Culture numbers are those used in the collection of T. C. Harrington. The first two letters designate the state or province of origin. Numbers in parentheses are those of the American Type Culture Collection.
[c] Noncomplementing isolates; only one phenotype recovered.
[d] Not tested due to unusual growth characteristics.

at ×40 under the dissecting microscope. A sterile needle was used to transfer masses of conidia from individual conidiophores to plates of MMT. Phenotypes were determined for subcultures from two to six sectors and/or other selected locations at the edge of each colony. Cultures derived from single conidia were obtained by streaking a mass of conidia from an individual conidiophore onto a plate of MEA. After 2 days of incubation at 19 C, germinating conidia were individually transferred to MMT and later phenotyped on MMT and HMT.

RESULTS

Nuclear condition and anastomosis. Microscopic examination of six isolates of *L. w. pseudotsugae* indicated that cells of the hyphae and hyphal-tip cells are multinucleate (Fig. 1A and B). For example, in isolate WADU, hyphal tip cells from three hyphae had an average of 10 nuclei, and the average number of nuclei in the first seven cells of one hypha was 11 nuclei per cell, with a range of 4–17. This multinucleate condition was also observed in unbranched initials (stipes) of macronematous conidiophores (Fig. 1C).

Young conidia (i.e., terminal conidia still attached to the conidiogenous cells of mature conidiophores) were invariably uninucleate, but the binucleate condition was occasionally seen in older conidia that were either still attached to the conidiophore or that had begun to germinate in the droplet of spores adhering to the conidiophore (Fig. 1D).

Anastomoses appeared to be common but were most frequent whenever hyphae grew in a parallel orientation (Fig. 1B). Anastomoses also appeared to be more frequent in older, more heavily colonized parts of the culture and were rare in hyphal tips at the edge of the culture.

Mutant recovery and characterization of mutants. Nit mutants were obtained from all of the 76 selected isolates of *L. wageneri*. NitM mutants (cofactor mutants) of *L. w. pseudotsugae* and *L. w. ponderosum* were readily distinguished from other nit mutants by their slower growth on HMT, and in *L. w. pseudotsugae*, by the production of fewer conidiophores. However, it was difficult to detect nitM mutants of *L. w. wageneri*; these mutants had only slightly less radial growth than wild-type isolates on HMT.

Although different concentrations of nitrite were tested, suboptimal utilization was difficult to detect, apparently because of the toxicity of nitrite. In *L. w. pseudotsugae*, nit3 mutants (mutants deficient at the regulatory locus for nitrate reductase and nitrite reductase) had very thin growth on NMT, and wild-type isolates could only be distinguished by their slightly denser growth. In *L. w. ponderosum*, however, the difference was less apparent. In *L. w. wageneri*, growth of all wild-type isolates and mutant strains was poor on NMT, with less expansive growth than on the basal medium without nitrogen.

NitM mutants were the most commonly recovered mutants in *L. w. wageneri*, with five or more complementary types of nitM mutants indicated from the results of pairings (Table 2). Nit1 mutants (nitrate reductase mutants) were the most frequently recovered mutants in *L. w. ponderosum* and *L. w. pseudotsugae*. NitM mutants were rare in *L. w. ponderosum*.

Complementation and heterokaryosis. On plates of MMT, a zone of dense hyphal growth, indicating complementation, occurred in the region of contact between mutants in some pairings (Fig. 2). Complementation was more rapid and the zone of complementation more distinct on MMT than on MM or MM plus 0.25 or 0.5% sorbose. Growth inhibition caused by the Triton X-100 may have increased opportunity for contact between hyphae or increased branching. Pairings that had dialysis membrane separating the hyphae of complementing mutants did not develop the zone of dense hyphal growth, except where hyphae of both mutants had grown past the edge of this semipermeable barrier and made direct contact.

Pairings were considered positive (+) for anastomosis and complementation if a dense zone of surface and aerial hyphae developed in the area of contact between the mutants after 6 wk (Fig. 2). In positive pairings, colonies from transfers from the zone of contact were denser, fluffier, and had a faster rate of radial growth on MMT than did colonies transferred from the unmingled mutant mycelia. A pairing was labeled a "slow positive" (S+) if the zone of complementation was thin or spotty at 6 wk and/or if complementation was only evident after nine or more weeks and there was more luxuriant growth in colonies transferred from the zone of contact than in transfers from the mutants. If a barely distinguishable zone of complementation developed after nine or more weeks and there were no differences among the transfers, the pairing was labeled as a "slow negative" (S−). Pairings that lacked visible complementation at 12 wk were labeled as negative (−).

Of the hyphal tips obtained from complementing pairings within *L. w. ponderosum* or *L. w. wageneri*, 88 and 58%, respectively, failed to grow. Of the 59 primary hyphal-tip cultures obtained

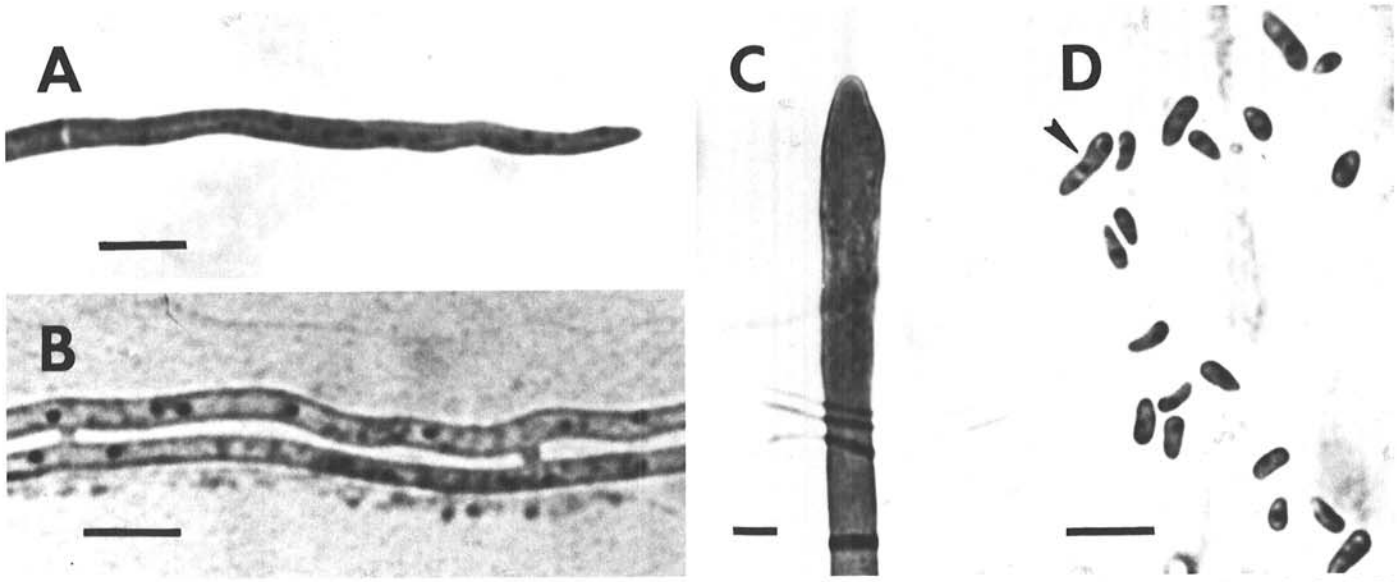

Fig. 1. Stained nuclei of *Leptographium wageneri* var. *pseudotsugae*. **A,** Multinucleate hyphal tip of isolate WADU stained with Safranin O. **B,** Multinucleate, anastomosing hyphae of isolate CAD30 stained with Giemsa. **C,** Growing tip of a multinucleate, developing conidiophore of ORD5 stained with Safranin O. **D,** Uninucleate conidia and a germinating, binucleate conidium (arrow) from the mutant pairing CAD32C1 × ORDQC1 stained with Safranin O. The bar in each photograph represents 10 μm.

from pairings of *L. w. wageneri* (CAS1C1 × CAS5C2 and CAS3C1 × CAS4C7) and the 16 primary hyphal-tip cultures obtained from pairings of *L. w. ponderosum* (BCL1C1 × BCL1C2 and BCL3C11 × BCW1C3), none was prototrophic.

Most of the transferred hyphal tips of *L. w. pseudotsugae* survived; 69% of the original hyphal-tip transfers from five pairings grew to form primary hyphal-tip cultures. The percentage of prototrophs was low in both the primary hyphal-tip cultures (0–19%) and in the derived, secondary hyphal-tip cultures (4–11%) of the five tested pairings of *L. w. pseudotsugae* (Table 3). Subcultures from the prototrophic, primary hyphal-tip cultures had various degrees of sectoring after 3 wk of growth on MMT (Fig. 3); some subcultures appeared to be totally auxotrophic.

Each of 13 prototrophic, primary hyphal-tip cultures of *L. w. pseudotsugae* (Table 3) had double-banded patterns for β-glucosidase and/or esterase. The two bands comigrated with the electromorph of each of the two wild-type isolates represented in the pairing (Fig. 4).

For each of the selected pairings (Table 3), later subcultures from the two primary hyphal-tip cultures that had the least sectoring and 29–32 secondary hyphal-tip cultures derived from the two subcultures were also characterized electrophoretically.

TABLE 2. Complementation among nitM mutants of *Leptographium wageneri* var. *wageneri*

Mutant	Phenotype[a]	CAS1C1 nitM1	CAS3C2 nitM5	CAS4C7 nitM5	CAS7C6 nitM2	CAS9C1 nitM3	CAS15C4 nitM2	COE1C1 nitM2	COE2C6 nitM2	IDE1C3 nitM2	NES2C1 nitM2	NES3C3 nitM	NME1C3 nitM2	UTE1C2 nitM5
CAS1C1	nitM1	−[b]	+	+	+	+	+	+	+	+	ND	−	+	+
CAS1C2	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS2C5	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS3C1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS4C2	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS5C1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	S+
CAS5C4	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS7C1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS9C1	nitM3	+	+	+	+	−	+	+	+	+	+	−	+	+
CAS9C8	nitM4	+	+	+	+	+	+	+	+	+	+	−	+	+
CAS15C2	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
CAS15C3	nitM2	+	+	+	S+	+	−	−	−	−	−	−	−	+
COE1C3	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
COE6C2	nitM2	+	+	+	S+	+	−	−	−	−	−	−	−	+
COENC1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
IDE1C1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
NES1C1	nitM2	+	+	+	S+	+	−	−	−	−	−	−	−	+
NES2C1	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+
NES3C1	nitM	−	−	−	−	S+	−	−	−	−	−	−	−	−
NES4C3	nitM2	+	+	+	S+	+	−	−	−	−	−	−	−	+
NME1C2	nitM2	+	+	+	S+	+	−	−	−	−	−	−	−	+
UTE1C7	nitM2	+	+	+	−	+	−	−	−	−	−	−	−	+

[a] NitM phenotypes based on growth on media containing nitrate, nitrite, or hypoxanthine. The numeric suffix 1–5 was used to designate different types of nitM mutants, as determined by differences in complementation.

[b] Complementation (+) was determined by the development of a dense zone of hyphal growth on Triton-X amended nitrate-minimal medium after 6 wk. Pairings with "slow complementation" (S+) developed a faint zone of complementation after 6 wk or a dense zone after 9–12 wk. Pairings with slow, appressed growth after 12 wk were labeled as negative (−). Complementation was not determined in pairings marked ND.

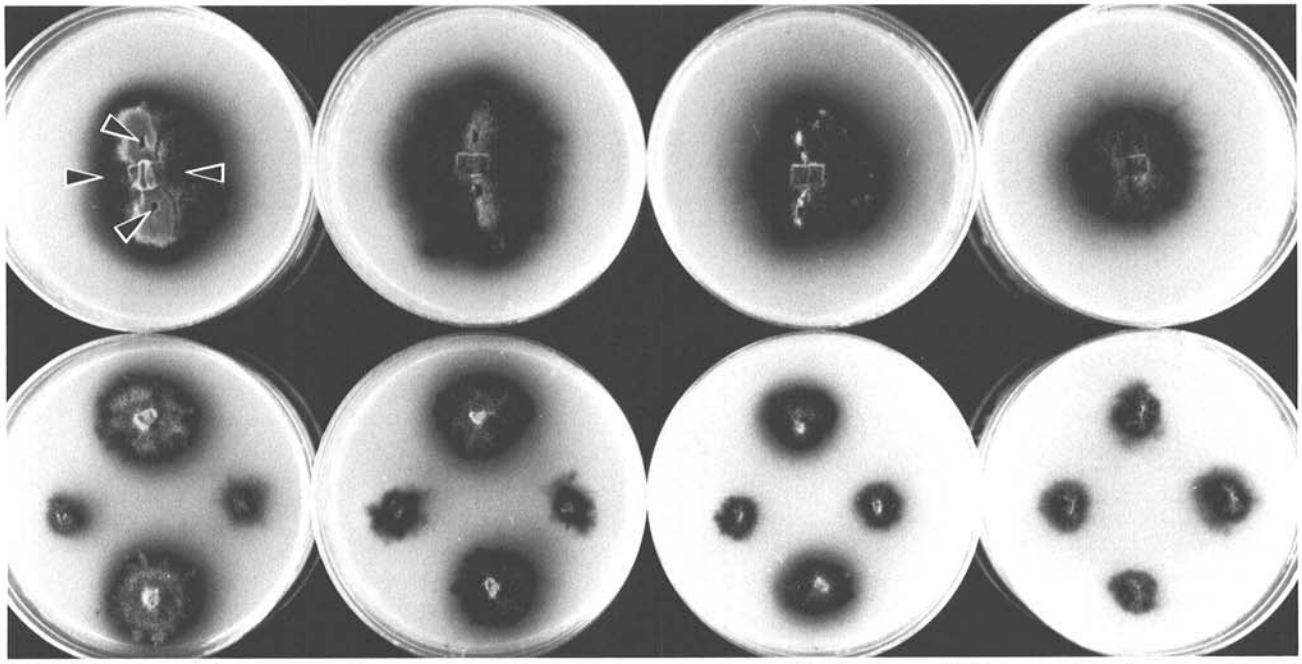

Fig. 2. Pairings between nitrate non-utilizing mutants of *L. wageneri* var. *ponderosum* (top) and mycelial transfers from the respective pairings (bottom) on minimal medium containing nitrate and Triton X-100. Arrows in upper left designate areas from which subcultures were taken. From left to right are pairings CAP19C2 × CAP36C1 and BCW1C3 × MOW2C2, both positive (+) for complementation; "slow positive" (S+) pairing CAPIC5 × ORL1C5; and negative (−) pairing BCL1C1 × CAPDC1.

Twenty-two cultures (13 subcultures and nine secondary hyphal-tip cultures) appeared to be prototrophic on MMT; the rest were auxotrophic. All strains were grown on liquid MM containing some asparagine, a medium less selective for prototrophic growth. Each of the 102 auxotrophic secondary hyphal tip cultures invariably had the electromorph of that parent mutant with the same nit phenotype (no nonparental types were recovered). In contrast to the double-banded isozyme pattern seen in each of the 13 hyphal tip cultures grown in liquid MM lacking asparagine, only 8 of the 22 prototrophic cultures had similar double-banded patterns after growth in liquid MM containing asparagine, seven had one band that was much fainter than the other, and seven had only one visible band.

In 21 of the 22 originally prototrophic cultures grown on asparagine, reisolations were made from paper wicks of enzyme extracts that had been prepared for use as electrophoresis samples and stored at −80 C. The wicks were plated onto MMT containing 200 ppm of cycloheximide to suppress growth of contaminants, and prototrophic and auxotrophic sectors that developed were transferred to MMT and HMT for phenotyping. Cultures derived from wicks of five of the prototrophic cultures yielded one or more prototrophic sectors and sectors of both auxotrophic phenotypes, 12 cultures yielded prototrophs and one of the auxotrophic phenotypes, and four yielded only one of the auxotrophic phenotypes. In most cases, mycelial extracts of cultures that had a high proportion of prototrophic sectors (or had both auxotrophic phenotypes among the sectors when the wicks were plated on MMT) had double-banded electrophoretic patterns.

Isolations from masses of conidia from single conidiophores developing on areas of complementation yielded significantly more prototrophic colonies than did isolations from hyphal tips (Table 3, $P < 0.005$ in chi-square tests of contingency for each pairing). However, 287 single-spore colonies derived from six conidiophores were all auxotrophic, even though each of the six conidiophores produced conidia of both of the two mutant types. The ratio of occurrence of the rarer vs. the more common auxotroph in these single-spore isolations from the six conidiophores ranged from 1:55 to 18:27.

Vegetative compatibility groups. Mutants derived from 19 isolates of *L. w. wageneri* appeared to be of a single VC group (Table 2). Although there was complementation between mutant CAS9C1 and each of the other tested mutants, many mutants complemented only a portion of the other mutants. All mutants of *L. w. wageneri* were of the nitM phenotype (mostly nitM2), and most of the negative pairings were probably between strains of noncomplementing phenotype, i. e., mutations at the same putative locus. However, pairings of mutants C1 and C3 of NES3 with mutants of other isolates and with each other resulted in

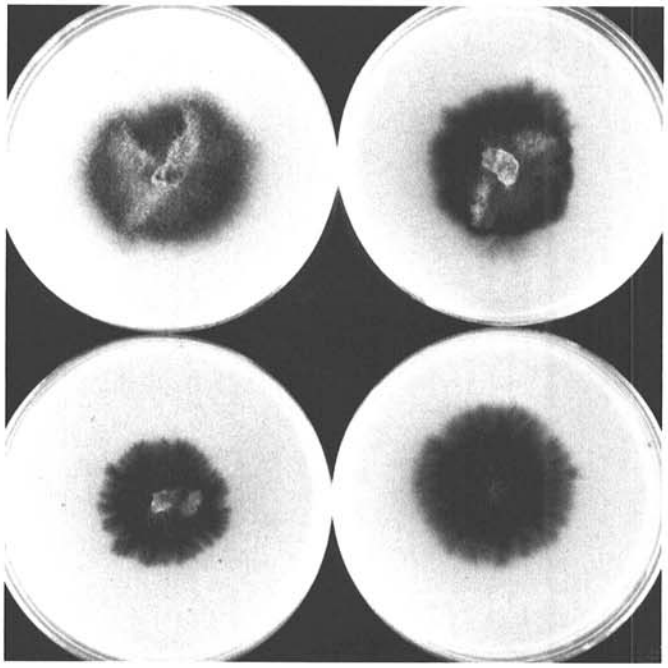

Fig. 3. Subcultures from prototrophic hyphal-tip cultures derived from pairings CAD32C1 × ORDQC1 (top left), ORD5C1 × ORDQC1 (top right), and ORD5C1 × WADUC3 (bottom, left and right) of *L. w. pseudotsugae* grown on MMT. The subcultures at top and at bottom left have auxotrophic (flat) and prototrophic (fluffy) sectors; the subculture at bottom right is lacking in prototrophic growth.

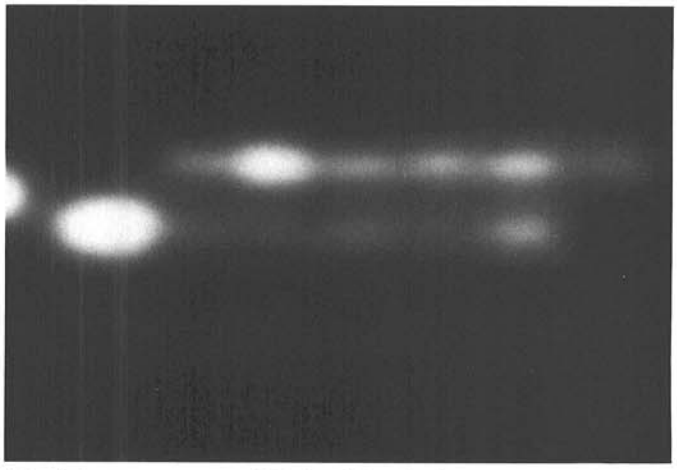

Fig. 4. Isozyme patterns of β-glucosidase for isolates and strains of *L. wageneri* var. *pseudotsugae*. Single bands at far left and far right are from wild-type isolates CAD32 and ORDQ, respectively; double bands are from prototrophic cultures obtained from single hyphal tips of the mutant pairing CAD32C1 × ORDQC1.

TABLE 3. Number of cultures having mutant (auxotrophic) vs. wild-type (prototrophic) phenotypes[a] obtained from selected pairings between nitM and nit1 mutants of *Leptographium wageneri* var. *pseudotsugae*

Pairings	Primary hyphal-tip cultures[b]			Secondary hyphal-tip cultures[b]			Cultures from single conidiophores[c]		
	Prototrophs	nitM mutants	nit1 mutants	Prototrophs	nitM mutants	nit1 mutants	Prototrophs	nitM mutants	nit1 mutants
CAD30C1:BCH1C3[d]	2	0	33	2	0	25	NT[e]	NT	NT
ORDQC1:CAD32C1	6	25	1	4	41	32	10	17	0
ORDQC1:ORD5C2	2	12	9	1	11	13	13	26	2
WADUC3:CAD32C1	0	37	0	NT	NT	NT	NT	NT	NT
WADUC3:ORD5C1	3	23	9	6	15	32	33	34	2

[a] Phenotypes determined on Triton-X amended nitrate-minimal medium (MMT) and hypoxanthine medium (HMT).
[b] For primary hyphal-tip cultures, hyphal tips were from the edge of subcultures from a mutant pairing after 5–9 days of growth on weak nitrate medium; secondary hyphal-tip cultures were similarly obtained from primary hyphal-tip cultures that had prototrophic (wild-type) growth on MMT medium.
[c] Established by transferring masses of conidia from single conidiophores that formed in subcultures from the mutant pairings after 8–10 days of growth on weak nitrate medium.
[d] For each designated pairing, the phenotypes of the mutants preceding and following the colon are nitM and nit1, respectively.
[e] Not tested.

only one slow positive pairing (Table 2), possibly indicating partial incompatibility between this and other isolates of *L. w. wageneri*.

Three VC groups were apparent among the mutants from 19 isolates of *L. w. ponderosum* (Table 4), and these three groups had unique geographic distributions (Fig. 5). One VC group (VC group XII) contained all of the isolates of the northern Rocky Mountains except an isolate from Idaho; these isolates were of two electrophoretic phenotypes (Table 1). A second large VC group (VC group XIII) contained two additional electrophoretic phenotypes (Table 1). The third VC group contained a single self-compatible isolate (ORMS). Nit mutants of two additional isolates (IDP1 and ORH1) did not complement other isolates of *L. w. ponderosum* and could not be tested for self-compatibility due to recovery of only one mutant phenotype. Another isolate (CAJ1) and its mutants could not be used due to poor growth.

For each nit1 or nitM mutant of *L. w. pseudotsugae* that did not complement any of the mutants in an initial pairing, an additional nit1 or nitM mutant previously generated from the wild-type isolate was used in a second set of pairings. In this second set, the new mutants were paired with all of the mutants used in the initial pairing, and pairings between putative VC groups or subgroups that showed limited inter-group complementation (slow positives) in the first set of pairings were repeated.

Mutants from 28 of the 35 isolates of *L. w. pseudotsugae* complemented at least one other mutant strain when paired. Ten VC groups were identified from mutants of these 28 isolates (Tables 5 and 6). Six VC groups contained a single, self-compatible isolate, two others each contained two isolates, and two VC groups (IV and VIII) each contained subgroups of two to five isolates. Subgroups were identified by partial compatibility, i.e., inconsistent or only slow positive reactions between subgroups. There was, in addition, partial compatibility (slow complementation in one of two pairings) between mutant CAD19C2 of VC group VIIIb and mutant CAD18C1 of VC group IVb. These mutants were from isolates that originated from the same geographic area, the Georgetown Divide area of the central Sierra Nevada, California.

All of the mutant strains that complemented well in this study were of electrophoretic phenotype H or one of three electrophoretic phenotypes that each differed from phenotype H for only one isozyme (Table 1). Isolates of VC subgroup IVa were of electrophoretic phenotypes G or H, and isolates of VC subgroup IVb were of electrophoretic phenotypes H or I. The VC group VII contained the self-complementing isolate IDD2 of electrophoretic phenotype C. The other VC groups were comprised of isolates of electrophoretic phenotype H.

The geographic ranges of some of the vegetative compatibility groups of *L. w. pseudotsugae* overlapped in the central Sierra Nevada and the North Coast of California. Otherwise, the VC groups were geographically isolated (Fig. 6). Isolates of VC group IV were from Vancouver Island, British Columbia, Washington, Oregon, and the north-central Sierra Nevada in California. Isolates of VC groups VIII, IX, and X were only found in California. Isolates of VC subgroup VIIIa and VC group IX were from the coastal ranges, but the six isolates of VC subgroup VIIIb were from sites in the Sierra Nevada.

Mutants selected from seven wild-type isolates of *L. w. pseudotsugae* failed to complement in pairings with nitM mutants from other isolates (Table 1). These isolates could not be tested for self-compatibility due to the recovery of only one mutant type. Five of the seven isolates differed from the electrophoretic phenotype H at from one to four enzymes. Six of the seven noncomplementing isolates were from the Rocky Mountains. Besides those shown in Figure 6 (with the designation "n") there were additional noncomplementing isolates from Colorado and New Mexico (Table 1).

Two to four mutants from each major VC group of each variety that gave strong evidence of complementation in pairings within their VC group were selected for use in intervariety pairings. In these pairings, nit1, nit3, and nitM mutants were paired in various combinations. The 150 pairings were all negative, indicating a lack of vegetative compatibility among the three varieties.

DISCUSSION

Strains of *L. wageneri* can anastomose to form heterokaryons,

TABLE 4. Complementation among nitrate non-utilizing mutants of three vegetative compatibility groups (VC groups) of *Leptographium wageneri* var. *ponderosum*

Mutant	Phenotype[a]	VC group	BCL1C2 nit3 XII	BCL2C1 nit3 XII	BCL3C11 nitM XII	BCW1C3 nitM XII	CAP19C1 nit1 XIII	CAP36C1 nit3 XIII	CAPCC2 nit? XIII	CAPDC1 nit1 XIII	CAPIC5 nit? XIII	CAPWC3 nit3 XIII	ORLIC7 nit3 XIII	ORMSC2 nit3 XIV
BCL1C1	nit1	XII	+[b]	+	+	+	−	−	−	−	−	−	−	−
BCL1C2	nit3	XII	−	−	+	+	−	−	−	−	−	−	−	−
BCL3C5	nit?	XII	−	−	+	+	−	−	−	−	−	−	−	−
BCL3C11	nitM	XII	+	S+	−	+	−	−	−	−	−	−	−	−
BCL4C1	nit1	XII	S+	S+	+	+	−	−	−	−	−	−	−	−
BCW1C1	nit?	XII	−	−	+	S+	−	−	−	−	−	−	−	−
BCW1C3	nitM	XII	+	+	+	−	−	−	−	−	−	−	−	−
MOW2C2	nit?	XII	−	−	S+	S+	−	−	−	−	−	−	−	−
CAJ3C2	nit1	XIII	−	−	−	−	+	+	S+	S+	S+	+	+	−
CAP3C1	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
CAP19C2	nit1	XIII	−	−	−	−	+	+	+	+	+	S+	S+	−
CAP36C2	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
CAPDC1	nit1	XIII	−	−	−	−	−	+	−	−	−	−	−	−
CAPHC1	nit1	XIII	−	−	−	−	−	+	−	−	−	−	−	−
CAPIC3	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
CAPWC1	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
CAPYC1	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
ORL1C5	nit1	XIII	−	−	−	−	S+	−	S+	S+	S+	−	S+	−
ORLIC7	nit1	XIII	−	−	−	−	−	−	−	−	−	−	−	−
ORP1C1	nit1	XIII	−	−	−	−	−	S+	−	−	−	−	−	−
ORMSC1	nit1	XIV	−	−	−	−	−	−	−	−	−	−	−	S+
IDP1C1	nit1	NC[c]	−	−	−	−	−	−	−	−	−	−	−	−
ORH1C1	nit1	NC	−	−	−	−	−	−	−	−	−	−	−	−

[a] Phenotypes based on growth on media containing nitrate, nitrite, or hypoxanthine. Mutants designated nit? had nondefinitive growth on nitrite medium and are presumed to be either nit1 or nit3 mutants.

[b] Complementation (+) was determined by the development of a dense zone of hyphal growth on Triton-X amended nitrate-minimal medium after 6 wk. Pairings with "slow complementation" (S+) developed a faint zone of complementation after 6 wk or a dense zone after 9–12 wk. Pairings with slow, appressed growth after 12 wk were labeled as negative (−).

[c] Mutant strains that did not complement with other mutant strains.

and when auxotrophic mutants with different nitrogen metabolism phenotypes are paired, the heterokaryons may show complementation. Heterokaryons only formed between mutants of the same variety, suggesting genetic isolation between varieties. There was evidence of vegetative incompatibility within *L. w. ponderosum* and *L. w. pseudotsugae*; members of a VC group had similar or identical electrophoretic (isozyme) phenotypes.

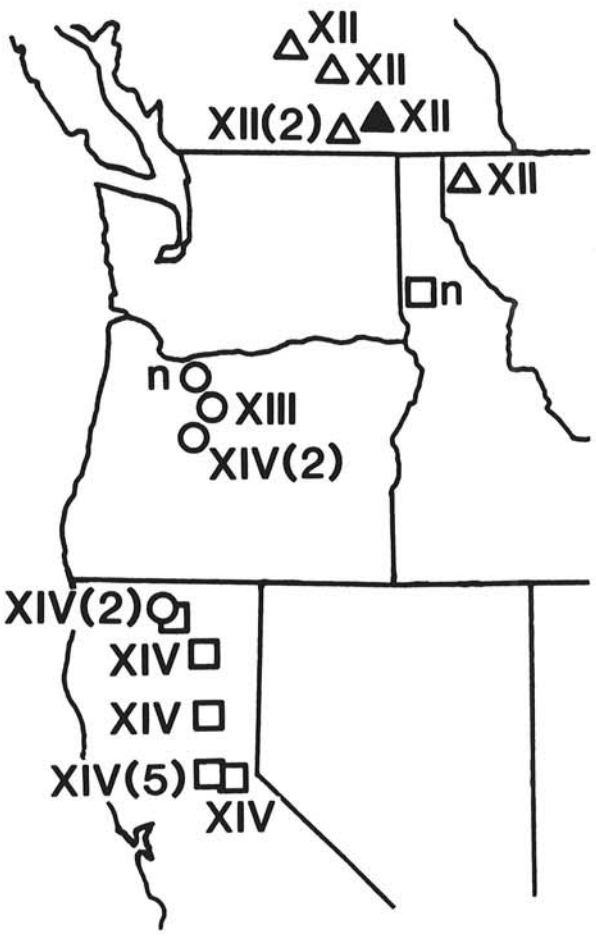

Fig. 5. Geographic distribution of VC groups XII–XIV of *L. wageneri* var. *ponderosum*. Isolates designated "n" were noncomplementing. Symbols indicate electrophoretic phenotypes as follows: open triangles = electrophoretic phenotype K, filled triangles = electrophoretic phenotype L, open squares = electrophoretic phenotype M, and open circles = electrophoretic phenotype N. Unless otherwise indicated, each symbol represents one isolate.

Five types of nitM mutants were estimated in *L. w. wageneri*, and nit1 and nit3 mutants were identified in the other two varieties. The estimate of seven mutant classes generated on chlorate media agrees with results obtained in other imperfect and ascomycetous fungi. Cove (15) obtained seven complementing classes of nitrate non-utilizing mutants in *A. nidulans*, and seven classes of mutants were recovered in *Gibberella fujikuroi* (Sawada) Ito apud Ito & Kimura (anamorph, *Fusarium moniliforme* Sheldon) (26). Similarly, in *Fusarium oxysporum*, Correll et al (11) recovered mutants of the three nitrogen metabolism phenotypes and estimated five or more classes of cofactor mutants. Four cofactor mutants were found in *Neurospora crassa* Shear & Dodge (28).

Complementation (usually slow complementation) was observed in some pairings of *L. w. ponderosum* among mutants with the nit1 phenotype. Some nit1 mutants may have been misidentified due to difficulties in determining nitrite utilization, but similar results have been reported from pairings between nit1 mutants in *Fusarium oxysporum* (11,18). As reviewed by Cove (15), nitrate reductase is composed of a number of subunits, and it is possible that nitrate reductase assembled from subunits having different defects could have at least partial enzymatic function.

Studies of anastomosis and heterokaryosis in other species of imperfect and ascomycete fungi reveal several ways in which heterokaryons can be maintained. In some species (e.g., *Aspergillus nidulans* [34], *Neurospora crassa* [32], and *Penicillium chrysogenum* Thom [35]), nuclei of both strains migrate and divide in the hyphae of the paired strains. Heterokaryons can become established in multinucleate hyphal tips, thus the heterokaryon can be maintained by further hyphal growth without recurrent anastomosis. In other species (e.g., *Verticillium dahliae* Klebahn [40], *Magnaporthe grisea* (Herbert) Barr [16], *Ophiostoma ulmi* (Buisman) Nannf. [9], and *Fusarium oxysporum* [37]) recurrent anastomosis is required, apparently because nuclei do not migrate from the cell in which anastomosis has occurred. Stable heterokaryons do not develop.

Hyphal tip cells of *L. wageneri* were invariably multinucleate in the examined isolates, and the presence of enzyme electromorphs and nitrogen utilization phenotypes of both parents in some cultures derived from hyphal tips demonstrated that some hyphal tips are heterokaryotic. There were, however, indications that frequent anastomosis is important in maintaining heterokaryosis. There was only a low number of prototrophs in hyphal tip cultures derived from prototrophic colonies from complementing pairings of *L. w. pseudotsugae*, and no prototrophic

TABLE 5. Complementation among nitrate non-utilizing mutants of vegetative compatibility groups (VC groups) II–VII of *Leptographium wageneri* var. *pseudotsugae*

Mutant	Phenotype[a]	VC group	BCD1C1 nitM II	BCD11C3 nitM III	CAD30C1 nitM IVa	WADUC3 nitM IVb	ORDQC1 nitM IVb	ORDPC3 nitM IVc	CAD19C2 nitM IVc	ORD1C1 nitM V	ORD2C4 nitM VI	IDD2C8 nitM VII
BCD1C2	nit1	II	+/−[b]	−	−	−	−	−	−	−	−	−
BCD11C1	nit1	III	−	S+	−	−	−	−	−	−	−	−
BCD11C2	nit1	III	−	+	−	−	−	−	−	−	−	−
BCH1C3	nit1	IVa	−	−	+	−	−	−	−	−	−	−
BCDJC1	nit1	IVa	−	−	+	−	S−/+	−	−	−	−	−
CAD30C2	nit1	IVa	−	−	+	−	S+/+	−/S+	−	−	−	−
WADUC2	nit1	IVb	−	−	−	+	+	−	−	−	−	−
ORD5C1	nit1	IVb	−	−	−	+	+	−	−	−	−	−
ORDQC2	nit1	IVb	−	−	−	+	+	−	−	−	−	−
CAD32C1	nit1	IVb	−	−	−/S−	+	+	−	−	−	−	−
ORDPC1	nit1	IVc	−	−	−	−	S+/−	+	+	−	−	−
ORD4C1	nit1	IVc	−	−	−	−	−	+	+	−	−	−
CAD19C3	nit1	IVc	−	−	−	−	−	+	+	−	−	−
ORD1C3	nit1	V	−	−	−	−	−	−	−	+	−	−
ORD2C2	nit1	VI	−	−	−	−	−	−	−	−	+	−

[a] Phenotypes based on growth on media containing nitrate, nitrite, or hypoxanthine.

[b] Complementation (+) was determined by the development of a dense zone of hyphal growth on Triton-X amended nitrate-minimal medium after 6 wk. Pairings with "slow complementation" (S+) developed a faint zone of complementation after 6 wk or a dense zone after 9–12 wk. A pairing with slow complementation in which transfers from the zone of complementation resembled mutant growth was labeled S−. Pairings with slow, appressed growth after 12 wk were labeled as negative (−). Slashes separate different results of two sets of pairings.

hyphal tips were recovered from pairings of *L. w. wageneri* or *L. w. ponderosum*. Also, there was frequent sectoring of heterokaryotic, prototrophic cultures to auxotrophy, particularly when the selection pressure was lessened by the addition of asparagine. Thus it would appear that *L. wageneri* is capable of having multinucleate, heterokaryotic hyphal tips, but that most hyphal tips become homokaryotic.

As with hyphal tips, stipe cells of developing conidiophores are multinucleate and can be heterokaryotic. Although conidia are uninucleate, both mutant types were identified among conidia of individual macronematous conidiophores, and the recovery of prototrophs in isolations from masses of conidia from single conidiophores was higher than from hyphal tips. There may be several explanations for the latter finding. First, stipe cells may have a greater number of nuclei and would be more likely to be heterokaryotic than would the individual cells of the hyphal tips. Also, more nuclei would be transferred from a conidial mass than would be transferred from a hyphal tip, thus increasing the chances of transferring a rare genotype. Alternatively, if heterokaryons are unstable and anastomosis is important in maintaining heterokaryosis, anastomoses are most abundant in the older portions of cultures where conidiophores form, whereas hyphal tips are from the advancing margin of subcultures where anastomosis is rare. The weak nitrate medium used for obtaining hyphal tips may also have affected the recovery of prototrophs, as this medium promotes thin, fast growth. Random loss of one or the other type of nucleus could easily occur under these growth conditions.

Low diversity in *L. wageneri* was suggested by low isozyme diversity within varieties (31,44), uniformity in electrophoretic phenotypes within geographic regions (44), and the occurrence of relatively few vegetative compatibility groups. In other ascomycetous fungi, vegetative compatibility is determined by 3 to 10 bi- or multi-factorial loci (1,5,6,9,25,29), and isolates must be homoallelic at most or all of these loci for anastomosis to occur. If a similar basis for compatibility exists in *L. wageneri*, it would be expected that differences in the numbers of VC groups in the three varieties would correspond with relative differences in the amount of electrophoretic variation. As expected, *L. w. wageneri*, which had the lowest electrophoretic variation, had the fewest VC groups; *L. w. ponderosum* was intermediate in both respects; and *L. w. pseudotsugae* had the greatest electrophoretic variation and the highest number of VC groups.

There were fewer VC groups than electrophoretic types detected in *L. w. wageneri* and *L. w. ponderosum*. However, in *L. w. pseudotsugae*, vegetative compatibility detected more variation than did enzyme electrophoresis; isolates of the most common electrophoretic phenotype (phenotype H) were found in nine VC groups. The subgroups in VC groups IV and VIII of *L. w. pseudotsugae* may have differences at one or several VC loci. Also, many of the noncomplementing isolates of *L. w. pseudotsugae* represented unique electrophoretic phenotypes and may

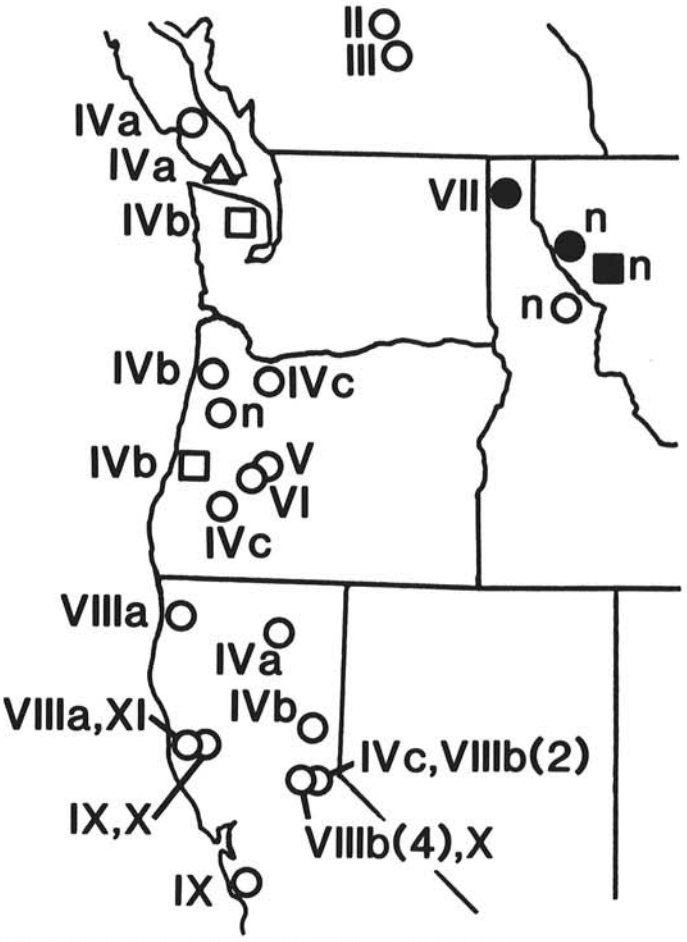

Fig. 6. Geographic distribution of VC groups II–XI of *L. wageneri* var. *pseudotsugae*. Isolates designated "n" were noncomplementing. Symbols indicate electrophoretic phenotypes as follows: filled circles = electrophoretic phenotype C, filled squares = electrophoretic phenotype D, open triangles = electrophoretic phenotype G, open circles = electrophoretic phenotype H, and open squares = electrophoretic phenotype I. Unless otherwise indicated, each symbol represents one isolate.

TABLE 6. Complementation among nitrate non-utilizing mutants of vegetative compatibility groups (VC groups) VIII–XI of *Leptographium wageneri* var. *pseudotsugae*

Mutant	Phenotype[a]	VC group	CAD40C4 nitM VIIIa	CAD56C3 nitM VIIIa	CAD6C4 nitM VIIIb	CADFC1 nitM VIIIb	CAD31C18 nitM IX	CAD5C3 nitM X	CAD55C5 nitM X	CADXC2 nitM XI	CADXC5 nitM XI
CAD40C1	nit1	VIIIa	+/S+[b]	+	S+/+	S+	−	−	−	−	−
CAD56C1	nit1	VIIIa	+	+/S+	−?/+	−/S−	−	−	−	−	−
CAD6C1	nit1	VIIIb	−	−	+	+	−	−	−	−	−
CADFC3	nit1	VIIIb	S+	−	−	+	−	−	−	−	−
CAD1C2	nit1	VIIIb	S+	S+	+	+	−	−	−	−	−
CAD2C1	nit1	VIIIb	−	S+	+	+	−	−	−	−	−
CAD27C1	nit1	VIIIb	S+	S+	+	+	−	−	−	−	−
CAD18C1	nit1	VIIIb	−/S+	−	+	+	−	−	−	−	−
CAD22C1	nit1	IX	−	−	−	−	+	−	−	−	−
CAD22C3	nit1	IX	−	−	−	−	+	−	−	−	−
CAD31C3	nit1	IX	−	−	−	−	+	−	−	−	−
CAD31C4	nit1	IX	−	−	−	−	+	−	−	−	−
CAD55C7	nit1	X	−	−	−	−	−	+	+	−	−
CADXC1	nit1	XI	−	−	−	−	−	−	−	S+/−	+
CADXC3	nit1	XI	−	−	−	−	−	−	−	+	+

[a] Phenotypes based on growth on media containing nitrate, nitrite, or hypoxanthine.

[b] Complementation (+) was determined by the development of a dense zone of hyphal growth on Triton-X amended nitrate-minimal medium after 6 wk. Pairings with "slow complementation" (S+) developed a faint zone of complementation after 6 wk or a dense zone after 9–12 wk. Pairings with slow, appressed growth after 12 wk were labeled as negative (−). Slashes separate different results of two sets of pairings.

represent additional VC groups. The lack of complementation by some mutant strains (e.g., NES3 of *L. w. wageneri*) could indicate inability to anastomose and form heterokaryons, as has been reported in *F. oxysporum* (13,24) and *Gibberella fujikuroi* (12).

The isozyme variation detected within VC groups or subgroups was minor and limited to a single isozyme difference, usually in the most variable of the enzymes assayed (44). Jacobson and Gordon (24) and Elmer and Stevens (18) have similarly suggested that the pathogenic differences within VC groups of *F. oxysporum* f. sp. *melonis* and *F. o. asparagi*, respectively, may represent only minor genetic differences. A mixture of isolates pathogenic to different hosts is also found in some VC groups of *Verticillium dahliae* (39). In contrast, culture morphology and pathogenicity traits are homogeneous within a VC group of *F. o. apii* (13) and some VC groups of *F. o. cubense* (33). Bosland and Williams (8) found correspondence between VC groups and electrophoretic phenotypes of *F. o. conglutinans*, though more than two races were found in one VC group.

The results of vegetative compatibility testing and enzyme electrophoresis suggest that reproduction in *L. wageneri* is largely or solely asexual, although a teleomorph (*Ophiostoma wageneri* (Goheen & Cobb) Harrington) has been described (20). The limited number of VC groups in *L. wageneri* contrasts sharply with the extensive diversity found in the related Ascomycete, *Ophiostoma ulmi* (9). In an extensive study of vegetative compatibility in the races and isolates of *O. ulmi*, Brasier (9) reported that in one "endemic" population (of the nonaggressive strain), nearly every isolate was a member of a different VC group. Similarly high numbers of VC groups are present in local, sexual populations of *Neurospora crassa* (30) and North American populations of *Cryphonectria parasitica* (Murrill) M. E. Barr (2). Sexual recombination among numerous compatibility loci accounts for the predominance of isolates with differences in compatibility. Anagnostakis (1) has recovered 106 combinations of VC alleles in just one cross between strains of *C. parasitica*.

In contrast, in *Leucostoma kunzei* (Fr.) Munk ex Kern (cause of Cytospora canker of spruce) (36) and *C. parasitica* in Europe (27) perithecia are infrequently produced and all isolates from a given group of trees may be in the same VC group. Relatively few VC groups are also found in strictly asexual fungi (7,8,13, 24,33,39). The number of VC groups would tend to be low in a species that lacks an effective method of generating new allelic combinations.

Asexuality, founder effects, and clonal selection could explain the establishment and maintenance of homogeneity within local populations of *L. wageneri*. Crow and Kimura (17) have suggested that in organisms with strictly asexual reproduction, the genome, rather than the allele, is the unit of selection. Isolates of a rare VC group may be unable to compete effectively for substrate beyond the site of introduction (41), whereas isolates of a predominant VC group could continue to spread throughout the root system and to adjacent trees. The localized occurrence of VC groups of *Cryptostroma corticale* (Ellis & Everhart) Gregory & Waller (an asexual pathogen of sycamores) (7), of some of the 16 VC groups of *V. dahliae* (39), and of some of the VC groups of *F. o. cubense* (33) suggests clonal and frequency-dependent selection (selection against rare VC groups) as a potential model for explaining population structure of some asexual plant pathogens.

The presence of isolates of differing electrophoretic phenotypes within some VC groups of *L. wageneri*, the recovery of mixed conidia from heterokaryotic conidiophores, and the recovery (albeit rare) of heterokaryotic hyphal tips suggest that heterokaryosis could play a limited role in maintaining diversity within VC groups or in generating new genotypes through parasexual recombination. The lack of recombinants among the 109 hyphal tip cultures tested does not rule out the possibility of parasexual recombination. Also, no attempt was made to characterize prototrophs as heterokaryons vs. diploid strains.

In spite of a capacity for heterokaryosis, variation in *L. wageneri* appears to be extremely limited. The relatively low number of VC groups per variety, the geographic distribution of isolates in each VC group, and the correlation between electrophoretic phenotype and VC group found in *L. wageneri* are indications that this fungus is essentially asexual. Genetic diversity may be further constrained by the lack of inter-variety heterokaryosis and the limits on intra-variety heterokaryosis imposed by vegetative incompatibility. Clonal selection and founder effects are suggested as important factors in determining population structure in this and other asexual fungi.

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