Vegetative Compatibility Groups in *Leucocytospora kunzei*

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ABSTRACT

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Vegetative compatibility groups (v-c groups) in *Leucocytospora kunzei* were demonstrated by pairing isolates on potato-dextrose agar and observing the reaction along the line of contact of the expanding colonies. By this method, 487 isolates of *L. kunzei*, from 196 cankers on 121 trees, were examined. The isolates were obtained primarily from infected *Picea* spp. located in urban sites in Michigan and in one forest site in Colorado. Thirty-six single v-c groups and eight multi-merge groups in *L. kunzei* were identified. In 152 of 154 cases, multiple isolates recovered from a given canker were in the same v-c group. Within a tree or among closely spaced trees, a single or a few v-c groups predominated. Some v-c groups contained isolates from different species of *Picea* or from distant sites. Vegetative compatibility groups do not segregate during conidiogenesis in *L. kunzei*.

Additional key words: Cytospora canker, *Cytospora kunzei*.

Cytospora canker of Colorado blue spruce (*Picea pungens* Engelm.) is an important and common disease of this popular and widely planted landscape ornamental in the northeastern and midwestern United States (7). Although generally not fatal, the loss of branches due to Cytospora canker dramatically reduces the aesthetic value of these large, prominent specimen trees. Cytospora canker of spruce and other conifers is caused by *Leucostoma kunzei* (Fr.) Munk ex Kern (syn. *Valsa kunzei* Fr.) (4,6,12), which is most frequently seen in its anamorphic form, *Leucocytospora kunzei* (Sacc.) Urban (syn. *Cytospora kunzei* Sacc.) (6). No cultural or chemical methods are available for the control of this disease.

A potential biological control strategy for Cytospora canker of spruce would make use of viruses or viruslike agents capable of debilitating *L. kunzei*. This strategy is similar in concept to the hypovirulence system for limiting canker expansion and tree mortality caused by *Cryphonectria parasitica* (Murr.) Barr to American chestnut (*Castanea dentata* (Marsh.) Borkh.) (2,3,8–10). The transmission of viruses or viruslike agents between fungal isolates requires hyphal anastomosis and exchange of cytoplasmic materials (5,9). The inherent ability of two fungal isolates to fuse and exchange nuclear or cytoplasmic material is called vegetative compatibility (1,5,9). Vegetative incompatibility can limit virus transmission and, hence, the effectiveness of this biological control strategy (3,8,9).

The occurrence of stable groups of compatible and incompatible isolates within a fungal species has been demonstrated for several fungi (1,2,5,10,11). The objective of this research was to establish if vegetative compatibility groups (v-c groups) occur in *L. kunzei*.

MATERIALS AND METHODS

Isolation and culturing of *L. kunzei*. Sections of cankered branches 0.5–1.0 m in length were collected from spruces and other conifers in several Michigan cities and from trees in the Pike National Forest in Colorado. Except for the forest trees in Colorado, cankers were collected from specimen trees in residential areas and cemeteries. Records of the host and its location were maintained for each sampled canker. The collections were made from 1983 through 1985. Cankered branches were brought into the laboratory and isolations were made either from infected host tissue, from conidial cirrhi, or from a combination of the two.

For tissue isolations, portions of the cankered branch were surface disinfested by wiping with 95% ethanol and flaming. The bark was aseptically removed. Pieces of the underlying discolored cambial/cortical tissues were excised and placed in petri plates containing Difco potato-dextrose agar (PDA). Several points along the length of the cankered branch section were sampled. When possible, isolations were made from tissues at the interface of healthy and diseased areas. The plates were incubated under ambient laboratory lighting and temperature (20–24 C) conditions. The resulting fungal colonies were examined and sorted. Isolates with appressed, felty colonies and which produced pycnidia with small allantoid conidia characteristic of *L. kunzei* were transferred and retained for further study.

For conidial isolations, the cankered branch segments were first soaked in a 5% solution of liquid bleach (0.26% NaOCl) for 10 min, rinsed with distilled water for 15 min, and placed into a moist chamber. Cirrhi of conidia could soon be seen on the surface of the branch, often within 30 min. Isolates were started from conidial cirrhi and/or from a single conidium. Mass conidial isolates were obtained by transferring an entire cirrhus to a petri plate containing PDA. Single conidium isolates were obtained by first placing a cirrhus into sterile distilled water, the resulting conidial suspension was diluted in series, and samples from each dilution were streaked onto PDA.

After initial isolation and identification, isolates were maintained and stored in petri plates containing spruce decoction agar (SDA) at 3 C. SDA was prepared by soaking 100 g of blue spruce bark shavings and twigs in 1,000 ml of distilled water at 95 C for 45 min. The solution was filtered through four layers of cheesecloth and 20 g of agar (Difco) was added per liter of decoction fluid. The medium was then autoclaved for 15 min.

Determination of v-c groups. Isolate pairings to establish vegetative compatibility groupings were performed using a modification of the system used with *C. parasitica* (1). Mycelial plugs (5 mm diameter) of *L. kunzei* were placed onto petri plates (100 × 15 mm) containing 40–45 ml of PDA. More consistent results were obtained when isolates of *L. kunzei* were grown on 2% water agar (WA) before the compatibility pairings. Isolates of *L. kunzei* grew very slowly on WA, but remained viable on this medium for several months. The isolates were grown on WA for a minimum of 2 wk at 26 C. Mycelial plugs were cut from the colony margins. The plugs were placed 1 cm from each other on the PDA plates, such that 21 plugs were placed on each plate. Each isolate

was paired with itself and with each of the other isolates. There were two replicates of each pairing plate. The pairing plates were sealed with Parafilm M, kept in low light at 26 C, and scored after 10 and 20 days.

Vegetative compatibility was determined as described by Anagnostakis (1). Briefly, two paired isolates were considered vegetatively compatible if their expanding colonies merged together uniformly upon contact. Isolates were considered incompatible if a dark 'barrage-like' reaction line formed along the line of contact between the paired colonies.

Vegetative compatibility and conidiogenesis. Conidial isolates of *L. kunzei* were used to determine the stability of v-c groups through conidiogenesis. In eight cases, vegetative compatibility between conidial isolates and tissue isolates from the same cankers was examined. In an additional experiment, 80 single-spore isolates from the same pycnidium were paired to determine the pattern of vegetative compatibility among the isolates.

RESULTS

Isolate collection. A total of 487 isolates of *L. kunzei* were obtained from 196 cankers taken from 121 trees. Most of the isolates were recovered from blue spruce, but *L. kunzei* was also recovered from other coniferous species (Table 1).

Vegetative compatibility groups. The mycelial colonies of *L. kunzei* grew uniformly from the WA plugs and generally made contact with adjacent colonies at a point midway between the plugs. The black reaction line characteristic of the incompatible response could be seen as early as 5 days after the pairings were made. After 10 days, the two distinct response types, compatible or incompatible, were scored (Fig. 1). Microscopically, the hyphae making up the reaction line were melanized, distorted, and some were lysed. Pycnidia did not form in a barrage along the reaction line.

Among the collection of 487 isolates, 44 v-c groups were identified. Of these 44 v-c groups, 36 single discrete groups (Table 2) and eight multi-merge groups were found (Table 3). Multi-merge groups showed compatible reactions to isolates contained in more than one v-c group (Fig. 2). An incompatible response that developed after 14 days was noted among some isolates found in the multi-merge groups.

Vegetative compatibility and conidiogenesis. In all eight examined cases, isolates of *L. kunzei* derived from conidia were always in the same v-c group as the tissue isolates from the same cankers. Conidial isolates, single or mass, from a given pycnidium were always in the same v-c group. The isolates from MSU-5B (v-c group 13) and BF-7D (v-c group 21) demonstrate the stability of v-c groups through conidiogenesis (Table 2). In the supporting experiment, all 80 single conidium isolates obtained from the same cirrhus produced by EL-16B were contained in v-c group 17 (Table 2).

Distribution of vegetative compatibility groups. More than one isolate of *L. kunzei* was obtained from 154 of the 196 cankers. In 152 of the 154 cases, the isolates from a given canker were in the same v-c group. In two cases, isolates recovered from a single canker were in different v-c groups. Four isolates from EL-17B were in v-c group 11 (Table 2), and one isolate was in v-c group MM7 (Table 3). One isolate from H-11C was in v-c group 30, while the other was in v-c group 31 (Table 2).

Isolates obtained from different cankers on the same tree characteristically were found to be in only one or two v-c groups. For example, isolates from nine cankers on tree BF-7 were all in v-c groups 20 or 21 (Table 2). On 45 trees, isolates of *L. kunzei* were collected from more than one canker. Among the isolates obtained from a given tree, only one v-c group was represented in 30 cases, two v-c groups in 14 cases, and three v-c groups in one case.

Adjacent trees generally yielded isolates in the same v-c group or groups. For example, trees EL-26 through EL-30 were in a single yard in East Lansing, MI. The isolates obtained from these trees were all in v-c groups 17 and 21. More variety in v-c groups occurred as trees were located farther apart, as in different neighborhoods or cities. A given v-c group, however, could contain isolates that were obtained from widely separated sites (Fig. 3) For example, v-c group 7 contained isolates from Frankenmuth, Holt, and Franklin. Two v-c groups, 23 and 28, contained isolates from Michigan and Colorado (Table 2).

Fifteen of the v-c groups contained isolates that were collected from more than one host species. Isolates from blue spruce and Norway spruce (*P. abies* (L.) Karsten) were found in v-c groups 5, 7, 9, MM1, and MM2. Isolates from blue spruce and white spruce (*P. glauca* (Moench) Voss) were found in v-c groups 8, 11, 17, 26, and 30. Vegetative compatibility groups 12 and 13 contained isolates from blue, white, and Norway spruce. Isolates of *L. kunzei* from Engelmann spruce (*P. engelmannii* (Parry) Englem.) were found in v-c group 23 with isolates from blue and white spruce, and in v-c group 28 with isolates from blue and Norway spruce. The only non-spruce isolate in the same v-c group as spruce isolates was from eastern white pine (*Pinus strobus* L.). That isolate, BF-WP, was in v-c group 21 along with isolates from blue and Norway spruce. The isolates of *L. kunzei* from eastern hemlock (*Tsuga canadensis* (L.) Carr.) and Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco), in v-c groups 27 and 32, respectively, were not in the same v-c groups as any of the spruce isolates.

TABLE 1. Origin of isolates of *Leucostoma kunzei* used for vegetative compatibility tests

Host species	Number of Sites	Trees	Cankers	Isolates[a]
Picea pungens				
Colorado blue spruce	14	81	139	325t,8m,3s
P. abies				
Norway spruce	7	11	13	32t,2m,9s
P. engelmannii				
Engelmann spruce	1	5	5	23t
P. glauca				
White spruce	5	21	35	75t
Pinus strobus				
Eastern white pine	1	1	1	1t
Pseudotsuga menziesii				
Douglas-fir	1	1	1	2t
Tsuga canadensis				
Eastern hemlock	1	1	2	7t

[a] t = Tissue isolation, m = mass conidia isolate, s = single conidium isolate.

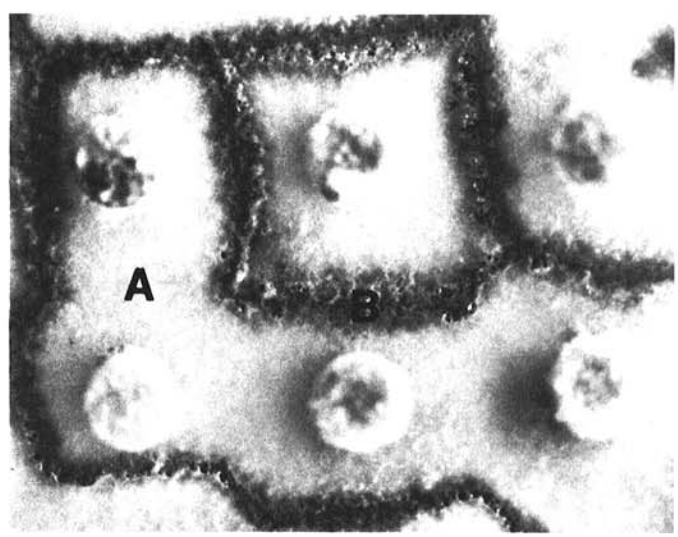

Fig. 1. Vegetative compatibility testing in *Leucocytospora kunzei*. After the pairings were made on potato-dextrose agar, the colonies expanded and made contact. Compatible colonies merge along the line of contact (A). In the incompatible reaction, a black 'barrage-like' reaction line develops along the line of contact of the two incompatible colonies (B).

DISCUSSION

Vegetative compatibility in fungi, "refers to the characteristic enabling two fungi to make contact, fuse, and exchange cytoplasm or nuclear material" (9). Vegetative compatibility, therefore, is an important biological feature of fungi in that it governs both heteroplasmon and heterokaryon formation (5). Isolates that are vegetatively compatible make up a v-c group. Based on this survey of 487 isolates there were at least 44 v-c groups in *L. kunzei*. Large numbers of v-c groups have also been demonstrated in other members of the Ascomycotina including *C. parasitica* (1,5,10).

In order for two isolates to be vegetatively compatible they must have common alleles at one or more loci (1,2). Based on the number of v-c groups obtained in this study, there appear to be at least six loci involved in regulating vegetative compatibility in *L. kunzei*. This leaves the potential for at least 64 v-c groups to occur. In *C. parasitica*, studies indicated that homologous alleles at all compatibility loci were not always required for a compatible reaction to occur (2). The multi-merge groups found in *L. kunzei*, which were compatible to isolates in more than one v-c group, also demonstrate that complete allelic homogeneity is not required for vegetative compatibility. Multi-merge responses do indicate, however, a close genetic relationship between the interacting v-c groups, with homologous alleles at many but not all alleles (2). For example, v-c groups 20, 33, and MM-1 appeared to be closely related to each other (Table 3).

Unfortunately, vegetative incompatibility works against using viruses or viruslike agents for the biological control of fungi. Vegetative incompatibility between isolates of *C. parasitica* reduced the frequency of transmission of the dsRNA associated with hypovirulence from infected hypovirulent isolates to noninfected virulent isolates (2,5,8,9). Information from this study with *L. kunzei*, with regards to vegetative compatibility, will be useful in approaching future efforts in the biological control of this pathogen. Fortunately, multi-merge groups that occur in *L. kunzei* may serve as future 'delivery agents' for viruses or viruslike biological control agents. Encouragingly, it has been noted in *C. parasitica* that sometimes there is conversion between some hypovirulent and virulent isolates that are not vegetatively compatible (2,8).

Examining the distribution of v-c groups not only provided basic biological information about *L. kunzei*, it also provided information of interest to plant pathology. In this study, there was a tendency for a single or few v-c groups to predominate in localized areas. A similar situation was observed among vegetative compatibility groups of *Verticillium dahliae* Kleb. (11). With only two exceptions, all isolates of *L. kunzei* from a single canker were in the same v-c group. Cankered branches within a single tree or in adjacent trees often yielded isolates within the same v-c group or groups. Isolates from trees at different sites or from trees not adjacent to each other showed more variety with regard to v-c

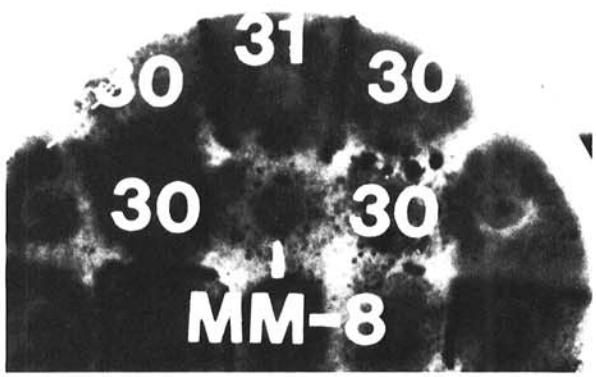

Fig. 2. The multi-merge response. The isolate in vegetative compatibility (v-c) group MM-8 was compatible with isolates in v-c groups 30 and 31 (no reaction lines), while groups 30 and 31 are incompatible with each other (black reaction lines).

TABLE 2. Vegetative compatibility groups in *Leucocytospora kunzei*, isolate composition and geographic distribution

V-c group	Isolates within the v-c group[a]
1	R-1A(1,2)
2	R-1B(1,2,3,4)
3	R-2A(1,2,3,4), R-2B(1,2,3), R-2C(1,2,3)
4	F-2C(1,2), HL-7B(1,2)
5	F-1A(1,2), F-4B(1,2), M-3A(1,2), H-9A(1,2), H-11A(1,2)
6	F-3A(3), F-4A(1,2)
7	F-5B(1,2), F-7A(1,3,4), FK-1A(1,2,3,4,5), FK-1B(1,2,3,4), HL-2C(1,2,3,4), HL-3A(1,2,3,4,6,7)
8	F-6A(1,2), F-8B(1,2,3), H-1B(1,2), H-2B(1), H-3A(3)
9	F-9A(1,2,3), F-12A(1,2,3,4), M-4B(1,2), M-5A(1), M-6B(2), M-7A(3,4), F-10A(1,2), F-11A(1)
10	DG-1A(1,2,), DG-1B(1,2), DG-2A(1,2,3)
11	DG-3A(1,2,3,4), V-4E(1,2), V-7A(1,2,3), V-8A(1,2,3), EL-17B(1,3,4,5)
12	DG-4A(1,2), DG-4B(2), V-11A(2,3), H-4A(2), H-12A(1), EL-25A(1,2,3)
13	DG-4D(1,2), M-7B(1,2,3), MSU-5B(1,2m,3m,4s,5s,6s,7s, 8s,9s,10s,11s,12s)
14	M-1A(1,2,3), V-15A(1,2), V-16B(1,2), V-17A(1)
15	M-2A(1,2)
16	MSU-2A(1,2,3)
17	MSU-2B(1,2,3), FK-2B(1,2), EL-15A(3,4), EL-16B(1,2,3,4, 5,10-90s), EL-27C(1,2), EL-28A(1,2), EL-30A(1,2,3), EL-30B(1,2,3,4), EL-30C(1,2)
18	BC-3A(1), BC-3B(2)
19	EL-3A(1,2,3,4), EL-6A(1,2,3)
20	BR-6A(1,2,3m), BF-6B(1,2,3,4), BF-6C(1), BF-6E(1), BF-7A(1,2m), BF-7B(1)
21	BF-7C(1,2,5,6,7m), BF-7D(1,2,3,4,5m,10s,11s), BF-7E(1,2m,10s), BF-7F(1,2,3m), BF-7G(1,5,6), BF-7H(1,2,3m,4m), BF-7I(1,2), EL-29A(1,2), EL-29B(1,2), EL-29C(1,2), EL-31A(1), EL-31B(1,2), EL-31C(1,2), EL-26B(1,2), EL-27A(1,2,3), EL-27B(1,2), EL-27D(1,2), EL-21A(3), EL-21B(1,2,3), EL-22A(2,3), OK-1A(1,2), OK-1B(1,2,3), OK-4A(1,2), OK-4B(1,2,3,4), OK-5A(2), OK-6C(1), OK-7A(1,2), OK-8C(1,2), OK-9A(1,2,3), OK-9B(1,2), OK-9C(1,2,3), OK-10A(1,2), OK-10B(1,2,3), OK-10C(1,2), OK-11A(1,2), OK-11B(1,2), OK-12A(1,2), OK-12B(1,2,3), BF-WP(white pine isolate)
23	V-5B(1,2,3), V-5C(1,2,3,4), V-6B(2), V-12A(1,2,3), V-2C(1,2), V-2E(1,2,3), HL-5C(1,2,3), HL-5D(1,2,3), HL-6A(1,2,3,4), HL-7A(1,2,3,4), HL-7C(1,2,3,4), COL-1A(1,2,3,4,5)
24	V-9A(1,2), V-9D(1,2), V-8C(1,2,3)
25	V-4D, V-10D(1,2)
26	V-3A(1), V-12A(1), V-14A(1,2,3)
27	HL-8A(1), HL-8B(1,2,3,4,5,6)
28	COL-4A(1,2,3,4), MAS-1B(3), MAS-2A(1), MAS-4B(1,2,3,4,5,6), MAS-4D(1,2,3,4,5)
29	MAS-3A(5,6,7,8)
30	H-5A(2), H-5B(2), H-5C(1,2,5), H-5D(1,2,3), H-6A(1,2), H-8C(1,2), H-10A(1,2), H-10B(1,2), H-10C(1), H-11C(2)
31	H-9B(1,2), H-11B(1), H-11C(1)
32	EL-13B(1,2)
33	EL-23A(1), EL-23B(1,2)
34	EL-24A(1,2)
35	COL-3A(1,2,3,4,5)
36	COL-5A(1,2,3), COL-6A(1,2,3,4,6,7)

[a] Isolates are identified using the following coding system: site code-tree number-canker letter-individual isolate number [for example DG-1A(1)= Dow Gardens:tree 1:canker A:isolate 1]. Where more than one isolate from a single canker was in the same v-c group they are listed within parentheses to shorten the table length. Site codes in Michigan are: BC—Bay City; BF—Botany Farm site, Michigan State University, East Lansing; DG—The Dow Gardens, Midland; EL—East Lansing, residential areas; F—Frankenmuth; FK—Franklin; H—Haslett; HL—Holt; M—Midland, residential areas; MAS—Mason; MSU—Michigan State University, main campus, East Lansing; OK—Okemos; R—Richville; V—Vassar. The site code in Colorado is COL—Kenosha Pass, Pike National Forest. Isolate numbers alone represent tissue isolates, m—mass conidia, s—single conidium.

groups. Short-range spread via conidia could explain the predominance within cankers, trees, and adjacent trees of isolates in a single or few v-c groups.

Isolates of *L. kunzei* started from conidia obtained from a pycnidium on a cankered branch were always in the same v-c group as a tissue isolate from that canker. From any given pycnidium, all conidial isolates were in the same v-c group. Vegetative compatibility groups do not appear to segregate during conidiogenesis. This has also been noted with *C. parasitica* (1). Perithecia of *L. kunzei* were not found during the 3 yr of this research and are rare on blue spruce (12). It has not been shown if v-c groups segregate during ascosporogenesis in *L. kunzei* as they do in *C. parasitica* (1). The variety of v-c groups of *L. kunzei* found in this study suggests that perithecia do form and that ascospores play a role in the dissemination of this fungus.

The tendency for a single or a few v-c groups of *L. kunzei* to occur in a given tree or adjacent trees is similar to the situation with *C. parasitica* in Europe (9). On chestnuts in Europe, a single or a few v-c groups of *C. parasitica* occur in a given tree or adjacent trees. In the United States, however, many v-c groups of *C. parasitica* commonly are recovered from a single tree or even a single canker (9,10). Perithecia of *C. parasitica* are common in the United States and ascospores are an important means of dissemination, but in Europe, perithecia are rare and conidia are the primary means of dissemination (9). If parallels can be drawn between *C. parasitica* and *L. kunzei*, conidia appear to play an important role in the short-range dissemination of *L. kunzei* in Michigan.

The host distribution of *L. kunzei* found in this survey agrees with earlier work (12). In Michigan, species of spruce were the primary hosts of this fungus. *L. kunzei* was also isolated from eastern hemlock, Douglas-fir, and eastern white pine, but in the residential areas studied, Cytospora canker of those hosts is infrequent. Based on v-c groups and inoculation studies (Proffer, *unpublished*) most if not all species of spruce may be serving as reservoirs of inoculum of *L. kunzei* capable of infecting other spruces. Other coniferous species do not appear to serve as such reservoirs based on this vegetative compatibility study or on inoculation studies (12; Proffer, *unpublished*). The isolate from white pine that was in the same v-c group (v-c group 21) as spruce isolates was probably atypical. It was recovered from a single dead branch of an eastern white pine adjacent to several infected spruces. Isolations from eastern white pines in other locations in the course of this study did not yield isolates of *L. kunzei*.

Another use for vegetative compatibility grouping is as a marker for inoculation studies. By surveying the v-c groups present on trees before inoculation trials, it is then possible to use isolates known to be in different v-c groups for inoculation studies. Using v-c group analysis, one could check the identity of any isolates

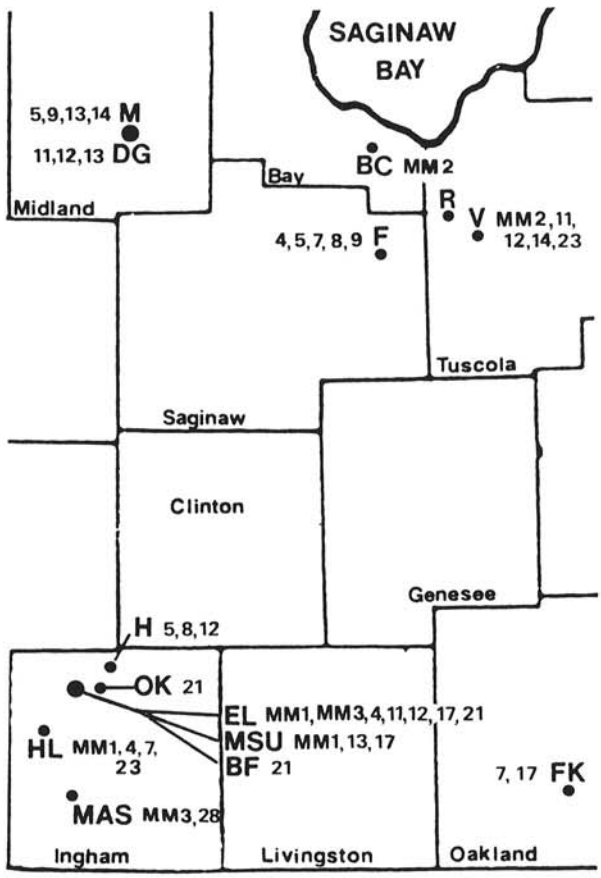

Fig. 3. Vegetative compatibility (v-c) groups of *Leucocytospora kunzei* recovered from more than one site are labeled on the map. For example, v-c group 12 was recovered from three sites: DG, V, EL. Isolates from Colorado were found in v-c groups 23 and 28. Site codes in Michigan are: BC—Bay City; BF—Botany Farm site, Michigan State University, East Lansing; DG—The Dow Gardens, Midland; EL—East Lansing, residential areas; F—Frankenmuth; FK—Franklin; H—Haslett; HL—Holt; M—Midland, residential areas; MAS—Mason; MSU—Michigan State University, main campus, East Lansing; OK—Okemos; R—Richville; V—Vassar. Multi-merge groups are identified by MM before the v-c group number.

TABLE 3. Vegetative compatibility groups in *Leucocytospora kunzei*, isolate composition and distribution in multi-merge groups

Multi-merge group[a]	Isolates in the group[b]	Compatible v-c groups	Late incompatiblity[c]
MM1	EL-2B(3,4), EL-9A(1,2), EL-9B(1,2,3), EL-9C(1), EL-12A(1), HL-1B(1,2,3,4), EL-12B(1,2), MSU-1A(1), HL-4A(2), HL-4D(1,2,3), HL-4C(1,3), MSU-3A(1), HL-4B(1,2,3,4), MSU-4A(1)	20,33	
MM2	BC-1A(1), BC-1B(1), V-1A(1,2,3), V-1D(1,2), V-2B(1,2,3)	1,19	
MM3	MAS-5A(1,2), MAS-5B(1,2,3,4,5,6), MAS-5C(1,2,3,4), EL-14A(1,2,3,4)	2,10	
MM4	OK-2A(1,2), OK-2C(1,2)	30,MM6, MM7,MM8	31,MM5
MM5	OK-3C(1,2,3)	30,MM6, MM7,MM8	31,MM4
MM6	EL-8A(1,2,3), EL-10A(1), EL-10B(1,2)	30,MM4, MM5, MM7,MM8	31
MM7	EL-17A(1,2,3), EL-17B(6)	30,31, MM5,MM6 MM8	MM4
MM8	H-8A(1), H-8B(1,2)	30,31, MM4, MM5, MM6, MM7	

[a] Multi-merge groups contain isolates that are vegetatively compatible with isolates in more than one v-c group.

[b] Isolates are identified using the following coding system: site code-tree number-canker letter-individual isolate number [DG-1A(1) = Dow Gardens:tree 1:canker A:isolate 1]. Where more than one isolate from a single canker was in the same v-c group they are listed within parentheses to shorten the table length. Site codes in Michigan are: BC—Bay City; EL—East Lansing, residential areas; H—Haslett; HL—Holt; MAS—Mason; MSU—Michigan State University, main campus, East Lansing; OK—Okemos; V—Vassar.

[c] Late incompatible reactions occurred between some of the multi-merge groups. In this case, the black reaction line appeared much later than normal. Regular reaction lines were visible at 7 days, in the late incompatibility response; however, the black reaction lines did not appear until 14–20 days after pairing.

recovered from the inoculations done on the tree. In this way vegetative compatibility can serve as a nonmutational marker for epidemiological studies. Use of v-c groups as markers would presumably avoid any unintentional selective pressures that might occur when using mutational markers.

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