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Spatial distribution of discrete RAPD phenotypes of a root endophytic fungus, *Phialocephala fortinii*, at a primary successional site on a glacier forefront

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SUMMARY

Phialocephala fortinii is among the few identified hyphomycetes belonging to the *Mycelium radicis atrovirens* complex. This 'dark-septate endophyte' has a global distribution and colonizes a wide variety of host plants. In this study, the spatial distribution of discrete genets of *P. fortinii* on the forefront of a receding glacier was analysed using the randomly amplified polymorphic DNA (RAPD) technique to determine plants colonized and patterns of colonization. In two consecutive years of sampling, a total of 74 isolates of *P. fortinii* were obtained from nine plant species, typically ectomycorrhizal, ericoid mycorrhizal or non-mycorrhizal. The isolates showed substantial variation, sharing on average approx. half the RAPD markers. In the first year, three isolates belonging to a single genet were obtained from two plants separated by a distance of nearly 1.5 m. The continuity of this genet was assessed by a sampling the following year. No isolates similar to that, or any of the genets collected the year before were observed. Consequently, the identical isolates from the previous year were concluded to represent discontinuous ramets. Two additional large genets were observed during the second year of sampling, which inhabited roots of several plants representing three different species. These data suggest that the sharing of *P. fortinii* genets among plant species might play a fundamental role in adaptation and interaction within the whole plant community in a system undergoing primary succession.

Key words: *Phialocephala fortinii*, deuteromycetes, glacier forefront, RAPD-phenotypes, spatial distribution.

INTRODUCTION

Phialocephala fortinii is possibly the most abundant of the few known anamorphic taxa of root-colonizing hyphomycetes belonging to the *Mycelium radicis atrovirens* (MRA; Melin, 1922) complex (Ahlich & Sieber, 1996; Jumpponen & Trappe, 1998). Also referred to as 'dark-septate endophytes' (Haselwandter & Read, 1982; Jumpponen *et al.*, 1998a), the root-colonizing endophytes appear ubiquitous, with a circumboreal (Richard & Fortin, 1974) or global distribution (Jumpponen & Trappe, 1998) that includes maritime Antarctic (Christie & Nicolson, 1983; Laursen *et al.*, 1997) and extreme arctic habitats (Väre *et al.*, 1992). Although fungi belonging to the MRA complex frequently inhabit roots of many plant species (Haselwandter & Read, 1980, 1982; Currah *et al.*, 1988; Stoyke & Currah, 1991; Cázares, 1992; Jumpponen & Trappe, 1998), their ecological role remains poorly understood.

Cázares (1992) discovered that the dark-septate endophytes were common to most plants in primary and early successional communities on the forefront of the rapidly receding Lyman Glacier of the North Cascade Range in Washington State, USA. The plants harbouring the dark-septate endophytes included species that are usually considered ectomycorrhizal, vesicular-arbuscular (VA)-mycorrhizal, ericoid mycorrhizal or non-mycorrhizal. Cázares (1992) suggested that *Salix* spp. characteristically become established as individual shrubs in the proximity of which a variety of other plant species subsequently become established. Furthermore, Cázares (1992) concluded that dark-septate endophytes were common on roots of all species in these willow patch communities. *Phialocephala fortinii* appears to be among the major root-colonizing fungal components during the early stages of the primary successional alpine ecosystem at Lyman Glacier.

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The tools and principles of population ecology have only recently been applied to studying fungal communities, owing mainly to the inability to recognize fungal individualism and, furthermore, the difficulty of identifying individual units of fungal mycelium (Dahlberg, 1995). The terms genet and ramet, commonly used for vegetatively extending higher plants (Harper, 1977), have been adopted in describing genetically different mycelia and identical units resulting from asexual or vegetative propagation (Brasier & Rayner, 1987). Here, a genet is defined as a collection of isolates with identical phenotypes inferred from randomly amplified polymorphic DNAs (RAPDs), whereas a ramet refers to a collection of such identical phenotypes likely to be physically continuous.

Several approaches to the identification of fungal genets have been used. Somatic incompatibility, which refers to the fungal system of recognizing 'self' and 'nonself' mycelium, resulting in either acceptance or rejection, has been applied to fungi with both pathogenic (see Rayner *et al.*, 1984; Rayner, 1991) and mutualistic (see Dahlberg, 1995; Dahlberg & Stenlid, 1995) associations with their host. Similarly, allozyme patterns have in some cases enabled identification of genets in fungi (Sen, 1990; Rodrigues *et al.*, 1995; El Karkouri *et al.*, 1996). The recent, rapid evolution of molecular techniques has facilitated the development of tools that can be applied to fungal populations: RFLPs (Egger *et al.*, 1991; Egger, 1992; Smith *et al.*, 1992; Bae *et al.*, 1994; Matsumoto & Fukumasa-Nakai, 1995), randomly amplified polymorphic DNA (RAPDs) (Jacobson *et al.*, 1993; de la Bastide *et al.*, 1994; Liew & Irwin, 1994; Peever & Milgroom, 1994; Gosselin, Jobidon & Bernier, 1995; Raffle *et al.*, 1995; Perotto *et al.*, 1996) and amplified fragment length polymorphisms (AFLPs) (Mueller *et al.*, 1996) have been reported to be useful and efficient in analysis of both spatial and temporal distribution and variation of genets in populations of fungi.

The main objective of this study was to analyse the spatial distribution of discrete RAPD-phenotypes (genets) of *P. fortinii*. Studies described here were conducted to detect whether large, single genets of *P. fortinii* simultaneously colonize root systems of several established plant individuals in a developing primary successional plant community, or if *P. fortinii* comprises a fungal population of several small, genetically discrete individuals.

MATERIALS AND METHODS

Site description

Lyman Glacier (lat. 48° 10′ 14″ N, long. 120° 53′ 44″ W) is located in the Glacier Peak wilderness area in the North Cascades Mountain range, WA, USA. The glacier and its forefront occupy a cirque and a north–south oriented, U-shaped valley bounded by cliffs that rise up to 600 m above the valley floor and culminate at the 2430-m-high Chiwawa Mountain at the head of the cirque. The glacier has not exceeded its terminal moraine of the late 1890s for the past *c.* 6900 yr, since beyond that moraine is an undisturbed deposit of volcanic ash from the Mount Mazama eruption in Oregon. The elevation of the present terminus is approx. 1800 m. The forefront parent material is a heterogeneous glacial till ranging from clay-sized particles to boulders, intermingled with deposits of glacial-fluvial sediments (for a description of the chemical characteristics see Cázares, 1992 and Jumpponen *et al.*, 1998b).

The basin contains distinctly different, primary and secondary successional habitats. The primary successional habitat is on the glacier forefront which has rapidly deglaciated during the past century. The glacier has been receding steadily since the 1890s, vacating an 1100 m forefront for invasion by pioneering colonizers. The steep cliffs surrounding the glacier and its forefront have facilitated the maintenance of the indigenous flora: no introduced, exotic weeds have been detected thus far (Jumpponen *et al.*, 1998b). Therefore, the Lyman Glacier forefront site supports a naturally evolved primary successional community. Vegetation is developing from the early colonization of barren parent material to a plant community resembling the secondary successional communities beyond the terminal moraine. The course of the vegetation change on the Lyman Glacier forefront is described in more detail in Cázares (1992) and Jumpponen *et al.* (1998b).

Sampling

Three representative willows were selected in 1993 in each of two areas deglaciated approx. 30 and 65 yr ago (one *Salix commutata* Bebb and two *Salix phylicifolia* var. *planifolia* (L.) Sm. individuals in the former, and three *S. phylicifolia* var. *planifolia* individuals in the latter). Nine randomly selected plant individuals were dug up in the immediate surroundings of each of the selected willows, and a single root sample was taken from each willow (Figs 1, 2). The soil and attached root systems were kept intact in order to minimize damage to the root systems or endophytes within the roots. The samples, kept cold with glacial ice, were sealed in plastic bags for transport to the laboratory for storage in a refrigerator at 4°C. All the root samples were processed within 2 wk of sampling.

Isolates of *Phialocephala fortinii* were analysed using RAPDs (see below) to detect identical phenotypes. Based on these findings, the site was revisited during summer 1994 and the same three willow shrubs in the area deglaciated 30 yr ago were resampled (Fig. 1). The area where a single genet was isolated in 1993 from the roots of two separated

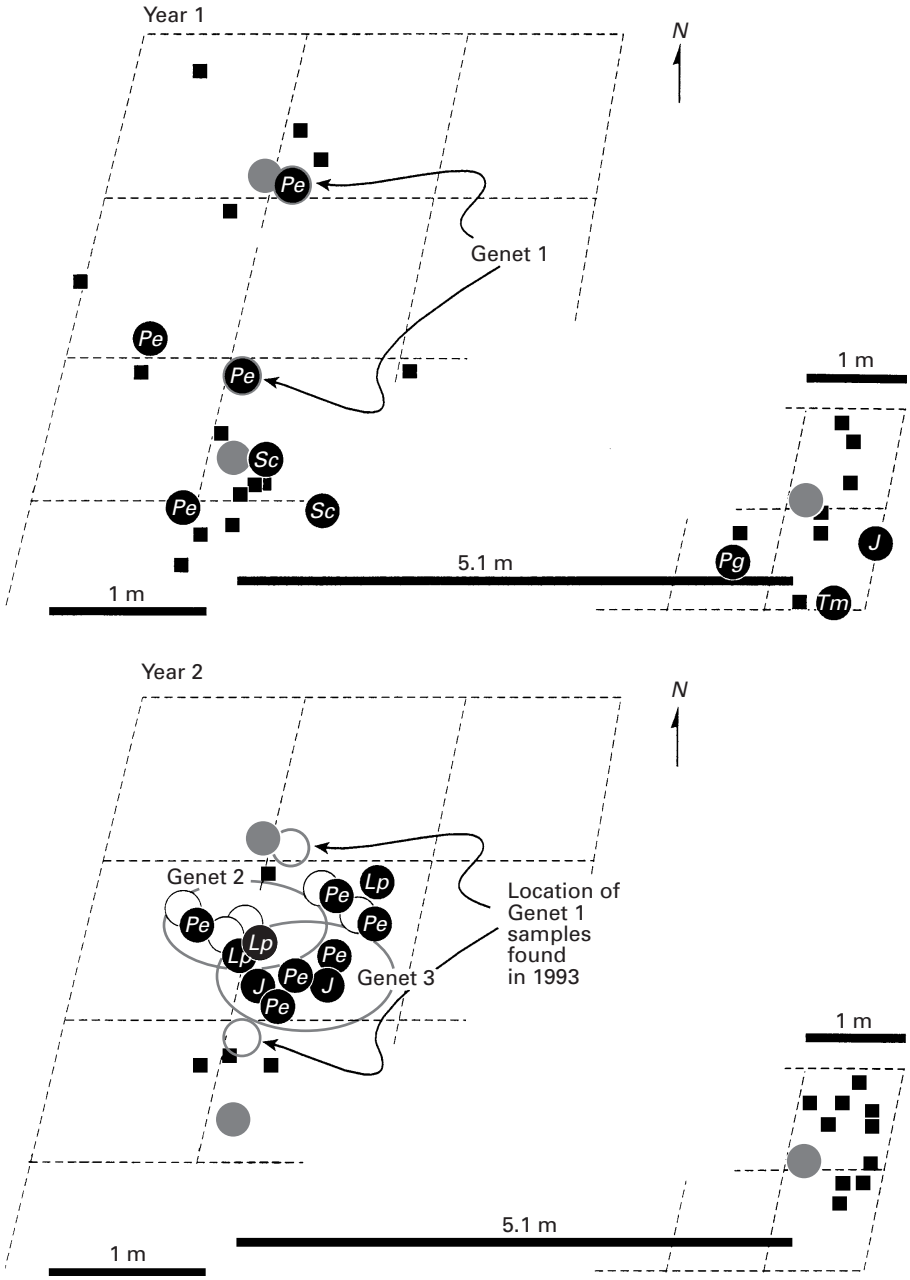

Figure 1. Distribution of plant individuals sampled in year 1 (1993) and year 2 (1994) at the forefront of the Lyman Glacier, Washington State, USA, in the area deglaciated for approx. 30 yr. Arrows indicate the location of two identical RAPD phenotypes (Genet 1) of *Phialocephala fortinii*. Samples colonized by *P. fortinii* Genets 2 and 3 are enclosed within large circles. Willow (●), plant sampled but no *P. fortinii* isolated (■), *Salix phylicifolia* root (year 2) (○); J, *Juncus* sp.; Pe, *Phyllodoce empetriformis*; Pg, *P. glandulifora*; Sc, *Salix commutata*; Tm, *Tsuga mertensiana*.

Phyllodoce empetriformis (Sw.) D. Don individuals was intensively sampled to test the physical continuity of this genet. In the 1994 sampling, the willow roots were excavated and followed within this area (see Fig. 1). Ten plants whose roots were intermingled with the willow roots were sampled together with the willow roots, resulting in 20 root samples from each of the three selected willow shrubs, 10 from the willow and 10 from adjacent indigenous species. Once collected, the samples were treated as during the 1993 sampling.

Isolation of the root endophytes

Each root sample was washed free of attached soil. Ten pieces of root, approx. 1 cm long, with dark-septate mycelium, were collected from each sample using a stereo microscope. Root pieces were stored in water until surface-sterilized in 30% H_2O_2 for 30–60 s and were transferred without rinsing to Petri plates containing 25 ml of Modified Melin Norkrans (MMN) media (Marx, 1969). The root pieces were incubated at room temperature until fungal colonies

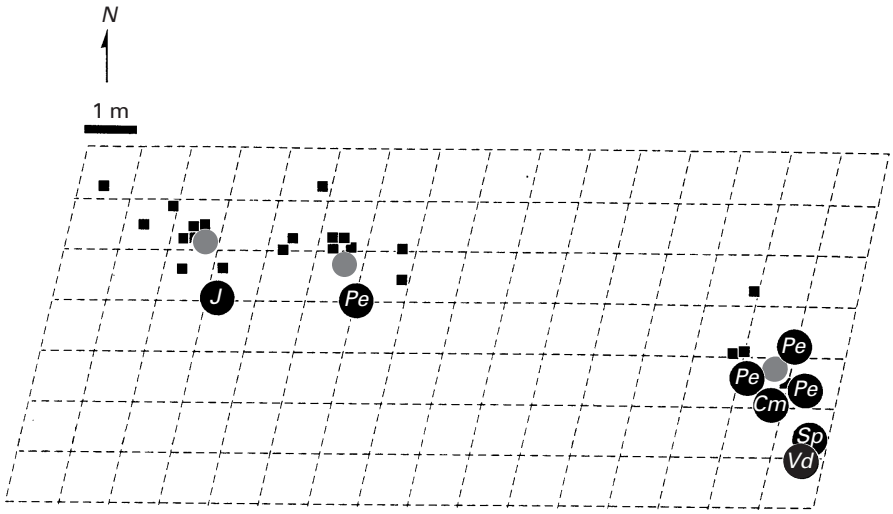

Figure 2. Distribution of plant individuals sampled in 1993 (year 1) at the Lyman Glacier forefront (Washington State, USA) in the area deglaciated for approx. 65 yr. Plant sampled; no *Phialocephala fortinii* isolated (■), plant sampled but *P. fortinii* isolated (●), willow (●). Cm, *Cassiope mertansiana*; J, *Juncus* sp.; Pe, *Phyllodoce empetriformis*; Sp, *Salix phylicifolia*; Vd, *Vaccinium deliciosum*.

were detected, usually within 2 wk. Fungal colonies possessing the macroscopic characteristics of *P. fortinii* (as described in Wang & Wilcox, 1985 and Currah & Tsuneda, 1993) were selected and transferred onto new plates. The macroscopic and microscopic characteristics were compared with those of the type culture (Wang & Wilcox, 1985) as isolates developed: the colour and texture of the colony, the presence of papillate hyphae, toruloid cells, and hyphal coils and strands (Currah & Tsuneda, 1993). Isolates differing from the type culture were omitted from further analyses: a uniform collection of similar isolates was obtained. Isolates were stored at 4°C for 3–6 months to enhance conidiophore production (Currah & Tsuneda, 1993); no conidiophores were detected.

The identity of the isolates was confirmed by comparison of the RFLPs of PCR-amplified (Saiki *et al.*, 1988) ITS (Internal Transcribed Spacer) region of nuclear ribosomal RNA gene (Gardes *et al.*, 1991; Erland *et al.*, 1994; Gardes & Bruns, 1996a,b) with the type culture of *P. fortinii* (Wang & Wilcox, 1985). The ITS-region was amplified with primers ITS1 (White *et al.*, 1990) and NL6Amun (Egger, 1995) with the cycle parameters described in Lee & Taylor (1990) and digested according to the manufacturer's instructions with 5 units of restriction enzymes, *Msp*I, *Rsa*I and *Taq*I (Promega Corporation, Madison, WI, USA). Only two of the macroscopically prescreened isolates possessed an RFLP pattern different from that of the type culture (data not shown); these were omitted from further analyses. Representative isolates are deposited and maintained in the culture collection at the Pacific Northwest Research Station, Forestry Sciences Laboratory, Corvallis, OR, USA.

DNA extraction and RAPD analysis of isolates

The fungal tissue for DNA extraction was grown on a permeable cellophane membrane covering MMN Petri plates. The whole colony, with the transfer plug excluded, was scraped from the membrane with a microscope slide within 2 wk after transfer and placed into a 1.5-ml Eppendorf tube. The tube and its contents were frozen in liquid nitrogen and stored at −20°C until the time of DNA extraction.

The protocol of Lee & Taylor (1990) with the following modifications was used to extract DNA: the tissue was ground with a micropestle attached to an electric drill in 50 µl of the lysis buffer (Lee & Taylor, 1990), then 350 µl of the buffer was added before incubation of 30 min at 65°C. The phenol/chloroform/IAA (25/24/1) extraction was repeated twice before precipitation with −20°C 90% ethanol and vacuum drying. DNA was resuspended in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) and 4 µg RNAse-A was added. The RNA was digested for 60 min, the phenol/chloroform/IAA extraction repeated twice then precipitation and drying took place as already described, followed by resuspension in 25 µl of TE. The DNA concentration was measured with a fluorometer and each extract diluted to a final concentration of 0.5 ng per µl.

Next, RAPDs were generated by PCR with four (UBC409, UBC431, UBC438 and UBC450; TAG GCG GCG G, CTG CGG GTC A, AGA CGG CCG G and CGG AGA GCC C, respectively) arbitrary decamers (Williams *et al.*, 1990) chosen from the 50 that were included in the preliminary screening (UBC401–UBC450; University of British Columbia, Vancouver, BC, Canada). Other primers (UBC413, UBC432, UBC444 and UBC446; GAG

Table 1. Phialocephala fortinii-*harbouring plant species sampled at Lyman Glacier forefront, Washington State, USA during 1993 and 1994*

Sample number	Time since deglaciation (yr)	Year sampled	Host plant and mycorrhizal habit	No. of isolates	No. of genets	Genet number*
1	30	1993	*Phyllodoce empetriformis* (ericoid)	4	2	1
2	30	1993	*P. empetriformis* (ericoid)	1	1	–
3	30	1993	*P. empetriformis* (ericoid)	2	1	1
4	30	1993	*Salix commutata* (ecto-)	2	1	–
5	30	1993	*S. commutata* (ecto-)	2	1	–
6	30	1993	*P. empetriformis* (ericoid)	2	1	–
7	30	1993	*Juncus* sp. (non-)	1	2	–
8	30	1993	*Tsuga mertensiana* (ecto-)	3	1	–
9	30	1993	*Phyllodoce glanduliflora* (ericoid)	2	1	–
10	30	1994	*S. phylicifolia* (ecto-)	7	4	2
11	30	1994	*Luzula piperi* (non-)	4	2	2, 3
12	30	1994	*P. empetriformis* (ericoid)	3	1	3
13	30	1994	*Juncus* sp. (non-)	4	1	3
14	30	1994	*P. empetriformis* (ericoid)	4	1	3
15	30	1994	*Juncus* sp. (non-)	5	1	3
16	30	1994	*P. empetriformis* (ericoid)	2	2	3
17	30	1994	*L. piperi* (non-)	3	2	2, 3
18	30	1994	*P. empetriformis* (ericoid)	3	2	2
19	30	1994	*L. piperi* (non-)	2	1	–
20	30	1994	*P. empetriformis* (ericoid)	1	1	–
21	30	1994	*P. empetriformis* (ericoid)	2	1	2
22	65	1993	*Juncus* sp. (non-)	3	2	–
23	65	1993	*P. empetriformis* (ericoid)	1	1	–
24	65	1993	*P. empetriformis* (ericoid)	2	1	–
25	65	1993	*Cassiope mertensiana* (ericoid)	2	2	–
26	65	1993	*S. phylicifolia* (ecto-)	1	1	–
27	65	1993	*P. empetriformis* (ericoid)	2	2	–
28	65	1993	*P. empetriformis* (ericoid)	3	2	–
29	65	1993	*Vaccinium deliciosum* (ericoid)	1	1	–

Number of isolates indicates total number of isolates obtained from the root system of a given plant individual, number of genets refers to discrete RAPD phenotypes identified among these isolates. A genet number was assigned only for genets colonizing more than one plant individual. Nomenclature follows Hitchcock & Cronquist (1973).
*Genet number indicates which of the three larger genets in Figure 1 were isolated from the plant individual.

GCG GCG A, AGC GTC GAC T, GCA GCC CCA T and GCC AGC GTT C, respectively) also produced strong amplicons but these were either monomorphic or were not reproduced consistently. Amplification was performed in 25 µl reaction volumes (1 × reaction buffer supplied with Taq polymerase, 1.3 µg per µl BSA, 100 µM dNTPs, 1.8 µM $MgCl_2$, 0.2 µM primer, 1 unit of Taq polymerase (Promega, Madison, WI, USA) and 2 ng of template DNA). The cycle consisted of 3 min at 93°C (predenaturation), followed by 44 cycles of 1 min at 93°C (denaturation), 1 min at 37°C (annealing) and 2 min at 72°C (extension). The final extension step was set to 10 min at 72°C to assure the completion of the reaction. Electrophoresis of the reaction products was performed in 2% agarose gels in 1 × TBE buffer. PCR products were visualized in the gel by staining with ethidium bromide. Gels were photographed on a UV illuminator.

The samples from 1993 and 1994 were analysed separately. The loci from the two separate samplings were not considered comparable because of potential reproducibility problems (see Meunier & Grimont, 1993; Tommerup *et al.*, 1995). Consequently, continuity of the identical isolates detected in 1993 was tested by side-by-side comparison with the samples from 1994. All isolates were amplified as two replicates from two separate extractions within a reaction and all the reactions were performed twice, making a total of four repeated reactions per sample. Only the consistently reproduced amplicons were scored for presence (1) or absence (0). Approximately 80% of the amplicons were scored because of reproducibility and consistency; amplicons that were poorly reproduced in the four separate reactions were excluded. This resulted in a total of 45 fragments (6–18 fragments per primer) in the analysis of 34 isolates obtained in the 1993 sampling and 44 fragments (4–18 fragments per primer) in the analysis of the 40 isolates from the 1994 sampling.

A pairwise Jaccardian similarity matrix† was

†Jaccardian pairwise similarity for two isolates x and y: $F_{xy}(F_{xy}+F_x+F_y)^{-1}$

(F_{xy} = total number of amplicons common to both isolates x and y; F_x = total number of amplicons unique to isolate x; F_y = total number of amplicons unique to isolate y).

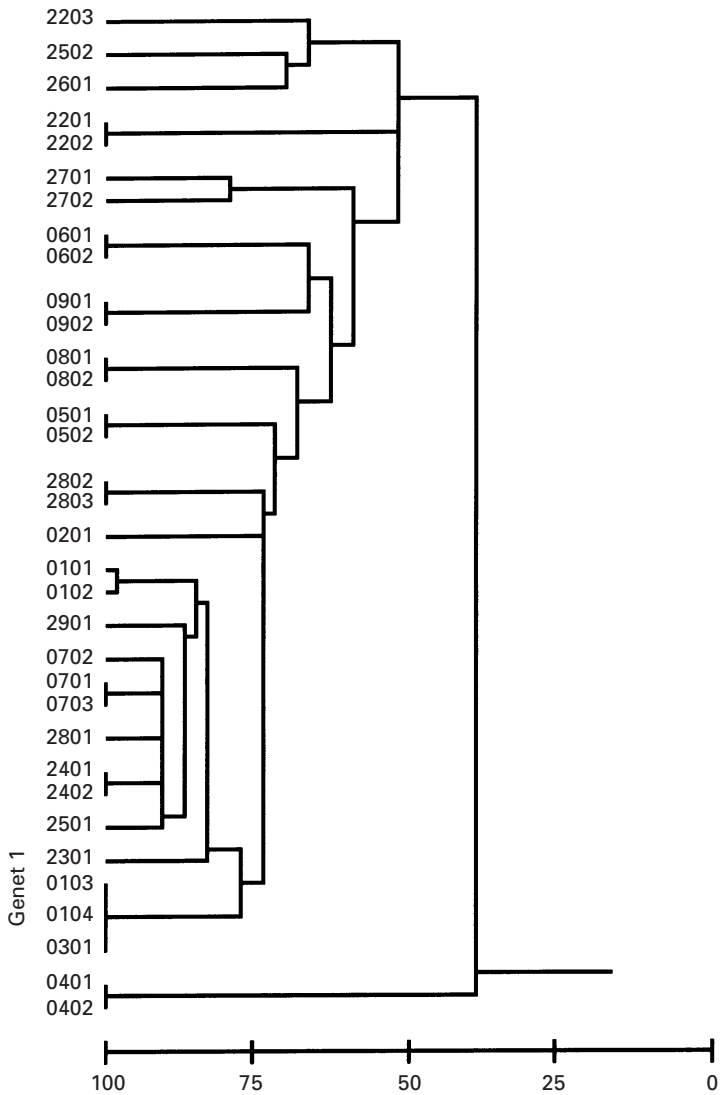

Figure 3. Clustering of 34 isolates of *Phialocephala fortinii* isolated from the roots of 17 individual plants at Lyman Glacier forefront, Washington State, USA in 1993. Branch lengths indicate Jaccardian similarities (%). Simple linkage method (nearest neighbour) was employed. The first two digits refer to the plant individual from which the isolates were obtained (Table 1), the following two to individual isolate numbers from that plant individual.

calculated from the binary (presence or absence) data using the CORR procedure in SYSTAT (1992).

This matrix was used to generate a phenetic dendrogram by the simple linkage method (nearest neighbour linkage) using the CLUSTER procedure (SYSTAT, 1992).

RESULTS

Nine different plant species on the glacier forefront were found to be colonized by *P. fortinii*. These species are known as ectomycorrhizal (*Salix commutata*, *S. phylicifolia* and *Tsuga mertensiana*), ericoid mycorrhizal (*Phyllodoce empetriformis*, *P. glanduliflora*, *Cassiope mertensiana* and *Vaccinium deliciosum*) or non-mycorrhizal (*Juncus* sp. and *Luzula piperi*).

Attempts at isolation from 17 of a total of 60 plant individuals sampled in 1993 resulted in one or more *P. fortinii* isolates per plant, 34 isolates in total (Table 1). *Phialocephala fortinii* was isolated at a similar frequency from areas deglaciated 30 and 65 yr ago: 9 vs. 8 plant individuals were colonized by *P. fortinii*, resulting in 19 vs. 15 isolates, respectively (Table 1). One to four isolates of *P. fortinii* were obtained from a single root system in the 1993 sampling (Table 1). The isolates from the root systems of individual plants represented one or two genets. In the subsequent sampling in 1994, up to seven isolates per root system were obtained from 12 of the 20 plants sampled within the area where the recovery rate was greatest in 1993 (Table 1, Fig. 1). These isolates represented up to four genets per root system.

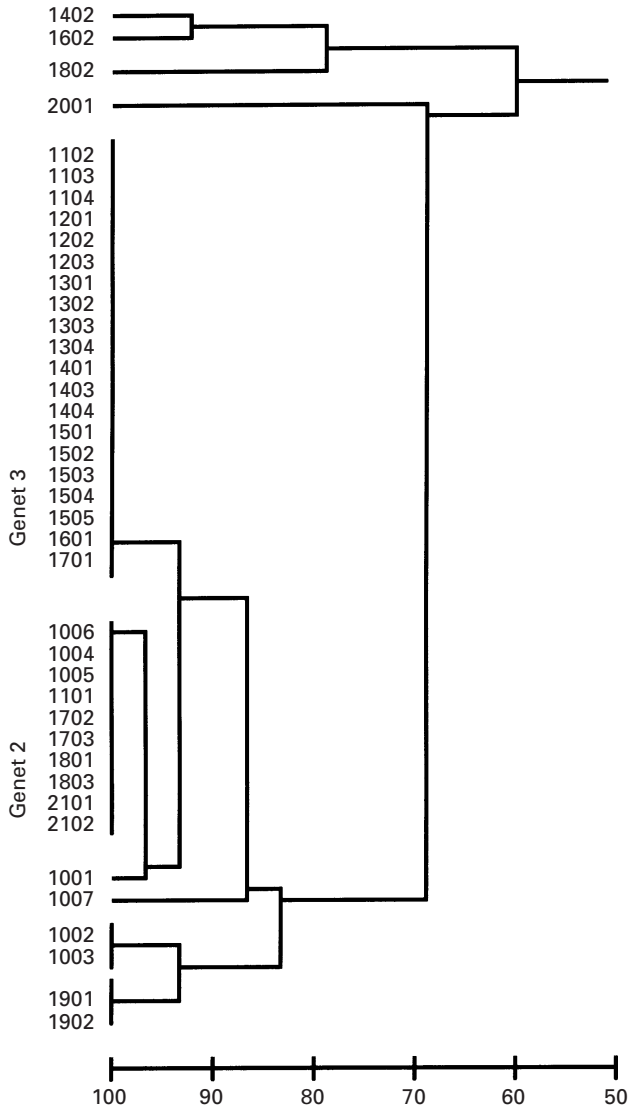

Figure 4. Clustering of 40 isolates of *Phialocephala fortinii* isolated from the roots of 12 individual plants at Lyman Glacier forefront, Washington State, USA in 1994. Branch lengths indicate Jaccardian similarities (%). Simple linkage method (nearest neighbour) was employed. The first two digits refer to plant individual from which the isolates were obtained (Table 1), the following two to individual isolate numbers from that plant individual.

In the 1993 sampling, a single genet was detected from root systems of two *Phyllodoce empetriformis* individuals separated by almost 1.5 m (Genet 1 in Fig. 1). The following year, this area was intensively sampled to test the continuity of this genet. Forty isolates of *Phialocephala fortinii* were obtained from 12 colonized plant individuals. A *Salix phylicifolia* shrub, whose roots were followed in the 1994 sampling, shared a genet (Genet 2 in Fig. 1) with two *Phyllodoce empetriformis* and two *Luzula piperi* individuals, in addition to three genets unique to the *S. phylicifolia* roots alone. The shared fungal genet was thus isolated from the roots of five different plants representing three different taxa. The two *L. piperi* plants that shared Genet 2 harboured another common genet (Genet 3 in Fig. 1), which was found to be shared among three individual *P. empetriformis*, two *L. piperi* and two *Juncus* sp. plants, but was not detected in the *S. phylicifolia* roots. This genet was thus isolated from the roots of a total of seven individual plants also representing three different taxa. Neither of these larger genets was identical to the isolates obtained during the earlier sampling. No *P. fortinii* isolates were obtained from the roots of the other two adjacent willow shrubs included in the 1994 sampling (Fig. 1).

Despite the monomorphism in ITS-RFLPs, the genets inferred from RAPD phenotypes were highly variable, sharing an average of 52.8% (±15.2% SD) and 85.0% (±16.1% SD) of the fragments scored in the analysis of 1993 and 1994 data, respectively (Figs 3, 4). The genet-corrected (i.e. with monomoprhic RAPD phenotypes removed) averages were 51.7% (±15.5%) and 78.5% (±15.2%) for the data from 1993 and 1994, respectively. Even the genets obtained from a single root system occasionally shared

less than 40% of the fragments (e.g. isolates 2201, 2202 and 2203 from *Juncus* sp. in Fig. 3). The data contained no visible hierarchical pattern attributable to physical distance between isolates.

DISCUSSION

The molecular tools used in this study proved powerful in identifying those isolates similar to *Phialocephala fortinii*. Macromorphological and micromorphological characteristics were usually reliable for identification of the obtained isolates despite the absence of asexual reproductive structures (conidiophores); polymorphisms between isolates morphologically similar to the type culture of *P. fortinii* were observed only infrequently in the ITS-RFLP assay (data not shown). The RAPD assay reproducibly identified a range of discrete phenotypes.

The ITS-RFLPs (Gardes & Bruns, 1996a) employed in this study have been shown to vary little within a species, but typically vary even between closely related species (Gardes *et al*., 1991). In this study, isolates grouped by their macromorphological and micromorphological characteristics were further tested by restriction digestion of the PCR-amplified ITS-region. The preliminary screening by the visual macroscopic and microscopic characteristics seemed to exclude isolates deviating from the type culture of *P. fortinii*. Several unidentified macroscopically distinct colonies were observed emerging from the surface-sterilized roots. Colonies with macroscopical characteristics similar to those of the *Phialocephala fortinii* type culture (Wang & Wilcox, 1985), however, appeared to be the most frequent individual type. The areas where the isolates were obtained were deglaciated approx. 30 and 65 yr ago. During 8 yr of field collecting, Jumpponen (1998) observed only a single fruiting mycorrhizal species, *Laccaria montana* Singer, in that area. It is in the regions deglaciated approx. 45 yr ago, that other fruiting species (*Inocybe lacera* (Fr.:Fr.) Kumm. and *Cortinarius tenebricus* Favre) have been observed. Given the possible low diversity in the fungal flora, *P. fortinii* might be an essential root colonizer in the early successional community.

Phialocephala fortinii was isolated at a similar frequency from the areas deglaciated for 30 and 65 yr, suggesting that *P. fortinii* is present throughout the glacier forefront. It has also been frequently isolated from material collected from mature forest soils (see Currah *et al*., 1987, 1988; Ahlich & Sieber, 1996). Root colonization by dark-septate endophytes similar to *P. fortinii* have been observed in habits ranging from South African coastal plains and lowlands (Allsopp & Stock, 1993) to tropical (e.g. Thomazini, 1974; Sengupta *et al*., 1989), temperate (Ahlich & Sieber, 1996), subalpine (Cázares, 1992; Stoyke *et al*., 1992) alpine (Bisset & Parkinson, 1979a,b,c; Haselwandter & Read, 1980; Read & Haselwandter, 1981; Allen *et al*., 1987; Haselwandter, 1987; Blaschke, 1991a,b; Stoyke & Currah, 1991), maritime antarctic (Christie & Nicholson, 1983; Laursen *et al*., 1997) and arctic (Bisset & Parkinson, 1979c; Bäre *et al*., 1992; Ahlich & Sieber, 1996) zones. Jumpponen & Trappe (1998) tabulated almost 600 plant species, representing about 300 genera and 140 families, reported to be colonized by dark-septate endophytes. *Phialocephala fortinii* alone has been isolated from field-collected root samples or shown to be able to colonize a variety of species in controlled bioassays (Jumpponen & Trappe, 1998). Taken together, results presented here and observations discussed previously indicate that *P. fortinii* is likely to have no, or very little, host specificity. Furthermore, it does not seem to be restricted to any of the known host–fungus associations (i.e. it seems able to colonize plant species with different mycorrhizal habits).

The areas studied here have been deglaciated fairly recently and therefore have allowed establishment of fungal individuals only for a limited time. The population of *P. fortinii*, however, expressed a substantial variation, as inferred from the RAPD markers used in this study. Even the fungal individuals obtained from a single root system seemed distant, based on the proportion of shared RAPD fragments. Several possible reasons are suggested for the high diversity observed.

First, the most variable RAPD-fragments are likely to represent non-coding DNA and, as a result, are not affected by natural selection. These neutral loci might bear more variation within and among populations than functional gene products such as allozymes. Jacobson *et al*. (1993) showed that somatically compatible isolates, which are frequently interpreted as representatives of a single genet, of *Suillus granulatus* (L.:Fr.) Kuntze might have distinct RAPD phenotypes. Similarly, Rizzo *et al*. (1995) found that somatically compatible isolates of *Armillaria ostoyae* (Romagn.) Henrik from a mixed *Pinus* stand in Minnesota were distinguishable by nuclear DNA fingerprints. This indicates larger variation in RAPD or fingerprint phenotypes than in the expression of the genes which code for somatic compatibility systems, for example.

Second, the mitochondrial genome might contribute to the variation observed in RAPD phenotypes (e.g. Aagard *et al*., 1995; Aagard, 1997). A colony with a uniform nuclear genotype could be a mosaic of mitochondrial genomes owing to different patterns of nuclear and mitochondrial migration after anastomosis of hyphae of compatible mating types (Hintz *et al*., 1988; May & Taylor, 1988; Specht, Novotny & Ullrich, 1992; Anderson & Kohn, 1995). Although nuclearly uniform, mitochondrial mosaics might be identified as distinct individuals on the basis of RAPD phenotypes,

resulting in overestimation of both numbers of individuals and genetic variation in the studied population.

Third, the studied population at the glacier forefront might result from asexual propagation from a large and diverse population surrounding the forefront. The high degree of observed variation in the forefront suggests a large number of vegetatively dispersing individuals able to invade and become established at the study site. Evidence of mycelial expansion or asexual (clonal) dispersal in this study was provided by isolation of identical genets from root systems of several plant individuals. In at least one case, these genets were probably discontinuous and resulted from asexual propagation (isolates representing Genet 1 in Fig. 1). Alternatively, based on estimated growth of 0.3–1.6 m per yr for various species of *Armillaria* in natural conditions (Shaw & Roth, 1976; Kile, 1983; Risbeth, 1991; Smith *et al*., 1992), it is possible that a single genet might have vegetatively expanded over this distance. However, given the short period of plant colonization in the glacier forefront, short snow-free growing season, and relatively harsh site conditions, vegetative expansion seems an unlikely explanation.

Fourth, the great variation and high number of individuals obtained in this very limited sampling could result from frequent sexual recombination and propagation. Isolates obtained during the 1994 samping were more similar than the diverse isolates from 1993 (average of 78.5% vs. 51.7% of the RAPD fragments were shared, respectively; see also Figs 3, 4), suggesting a possible sibling relationship and colonization of adjacent vacant root systems. Spores might disperse over great distances, but most probably land near the sporocarp, potentially resulting in clusters of closely related individuals. Although only asexual reproduction is known for *P. fortinii*, sexual propagation cannot be ruled out. No connection between the anamorphic *P. fortinii* and any teleomorphic taxon is known. However, Currah *et al*. (1993) observed small, dark, inconspicuous apothecia in pots of *Rhododendron brachycarpum* inoculated with *P. fortinii*. The aphothecia did not mature, but Currah *et al*. (1993) suggested *P. fortinii* as an anamorph of an apothecial member of the Leotiales. Jumpponen *et al*. (1997) pointed out that some members of the Leotiales are very inconspicuous and dark, easily escaping detection. Therefore, sexual reproduction is possible, and could explain the observed diversity, despite the lack of a known teleomorph. Small variation among the 40 isolates obtained in the 1994 sampling from a limited area indicates a close relatedness between these isolates.

Several plant individuals of different species were observed to be colonized by the same fungal genets. For adjacent plant individuals with intermingled root systems, these genets are likely to be physically continuous, i.e. ramets, as defined earlier. It is unlikely that plant individuals separated by a considerable distance would share a continuous, single ramet. Rather, as shown by the one example in the presented data, identical genets can be expressions of two or more independent ramets of a genet. Fragmentation is a common feature in clonal plants (Harper, 1977). Several examples of filamentous fungi suggest that fragmentation might also be a frequent phenomenon in fungi in their natural environments (Thompson & Rayner, 1980; Holmer & Stenlid, 1991; Dahlberg & Stenlid, 1994; Rizzo *et al*., 1995). The likelihood of fragmentation increases with age of fungal genet. Division of a single, continuous genet into genetically identical, discontinuous ramets might result from physical division of the vegetative mycelium (Dickman & Cook, 1989) or from asexual propagation (Anderson & Kohn, 1995). Division by dispersal of asexual propagules (conidiospores, hyphal fragments or sclerotia) seems more likely, given the considerable distance between the observed ramets at the harsh alpine site with extended dry summer. Currah *et al*. (1993) hypothesized that the intracellular sclerotial bodies, frequently produced by *P. fortinii* while colonizing host root tissue, can be effective propagules when root cells filled with mycelium are loosened and sloughed off the root. The heavily melanized mycelium and sclerotial bodies of *P. fortinii* might play an essential role in increasing the longevity of such propagules by discouraging grazing and protecting against desiccation (Kuo & Alexander, 1967; Bell & Wheeler, 1986). However, studies on the feeding of some common soil fungivores show preference for darkly pigmented fungi (Mitchell & Parkinson, 1976; Visser & Whittaker, 1977; Parkinson, 1988).

It is likely that adjacent plant individuals are connected via a shared mycelial ramet. The significance and ecological role of dark-septate endophytes, including *P. fortinii*, is presently unclear. Various studies of the interaction between the endophytes and their hosts have yielded conflicting results. Fernando & Currah (1996) concluded that inoculation of a given host species with *P. fortinii* might result in increase or decrease in growth, depending on the strain of the fungus used in the study. Jumpponen *et al*. (1998a) observed a substantial increase in biomass and foliar concentrations of nitrogen and phosphorus as a result of inoculation of *Pinus contorta* with a strain of *P. fortinii*. The increased foliar nutrient concentrations suggest involvement of the fungal endophyte in nutrient acquisition. It is possible that under some circumstances colonization by a root endophyte could result in a positive (mutualistic), mycorrhiza-like host response. Extramatrical mycelium penetrating into soil matrices inaccessible to the host plant and transporting nutrients to the host seems possible. Similarly, a connection between plant individuals via

shared mycelium might allow flow of photosynthates, as suggested for ectomycorrhizal systems (Read *et al.*, 1985; Simard *et al.*, 1997). Such transport of carbohydrates might be significant for the development of early successional plant communities.

The role of root-associated micro-organisms in plant succession has been poorly studied. Allen & Allen (1984, 1988) have hypothesized that VA-mycorrhizae might partially regulate the successional change of plant communities. Similarly, root endophytes, *P. fortinii* included, might affect the successional change in plant communities, serving as a fungal network providing means of facilitation via the shared mycelium, or change the relative competitive fitness as a result of host response to colonization. Alternatively, they could be involved in nutrient acquisition from organic debris. *Phialocephala fortinii* and *Leptodontidium orchidicola* have been shown to possess enzymatic activities allowing utilization of detrital organic compounds (Currah & Tsuneda, 1993; Fernando & Currah, 1995; Caldwell *et al.*, 1996). Such abilities could be crucial in early successional communities where supply of nutrients, nitrogen in particular, might be sparse (Matthews, 1992; Jumpponen *et al.*, 1998b) and where nutrients bound to recently deposited litter can be elementary for successful establishment and survival of new plant individuals. Data are too few to allow any definite answers about the potential role of the dark-septate endophytes, especially in the successional context. However, whatever their precise function in natural environments, the sharing of genets among plant species suggests that *Phialocephala fortinii* might play a fundamental role in adaptation and interaction among the entire plant community during the primary succession.

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