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Mycorrhizal and Root Endophytic Fungi of Containerized *Picea glauca* Seedlings Assessed by rDNA Sequence Analysis

G. Kernaghan,[1] L. Sigler,[2] D. Khasa[3]

[1] Department of Renewable Resources, University of Alberta, Edmonton, Alberta, Canada T6G 2H1
[2] University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 2E1
[3] Centre de recherche en biologice forestière (CRBF), Pavillon C.-E.-Marchand, Université Laval, Québec, Canada G1K 7P4

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ABSTRACT

Fungi colonizing fine roots of containerized *Picea glauca* seedlings were assessed in four large conifer nurseries in northern Alberta. PCR amplification of fungal rDNA (internal transcribed spacer and a portion of the 5′ end of the large subunit gene) from random samples of fine feeder roots gave between 1 and 4 amplicons per seedling. Amplicons were either separated by electrophoresis and sequenced directly, or cloned and sequenced. The resulting sequences were compared to sequences obtained from cultures established from seedling roots and from GenBank by maximum parsimony analysis. ITS sequences formed 11 distinct clades, each including at least one reference sequence. The ectomycorrhizal basidiomycetes *Thelephora americana* and *Amphinema byssoides* were dominant, whereas ascomycetes were less common. Fungi with sequences similar to members of the *Heleotiales* which form ericoid mycorrhizas were also present. Correspondence analysis revealed strong positive and negative associations among fungal taxa as well as an influence of applied fertilizer level on fungal diversity and species composition.

Introduction

Canada produces close to 1 billion tree seedlings per year, which are used to reforest over 400,000 hectares. The province of Alberta contributes approximately 10% of this number, mostly as *Picea glauca* seedlings from large containerized forest nurseries [32]. The health and productivity of these seedlings is influenced by the community of fungi colonizing their root systems that form a continuum from pathogenic to mycorrhizal species [47]. Interspecific interactions within these fungal communities, including synergies among mycorrhizal fungi [27], synergies among pathogens [36], and negative interactions between mycorrhizal and pathogenic species [31], may all affect seedling growth.

Until recently, studies of fungi associated with roots of containerized seedlings have relied on cultural methods for the assessment of pathogenic fungi [25] and on a

Correspondence to: G. Kernaghan; E-mail: gavink@alcor.concordia.ca

combination of culturing and morphological characterization of mycorrhizas for ectomycorrhizal fungi [6, 7]. However, conifer roots are associated with a broad range of fungi [19, 37] and individual fine roots may be colonized by multiple species [20]. Also, culturing from root tips may be biased toward fast-growing saprophytes and against slow-growing symbionts, and the mycorrhizas of containerized seedlings may be too poorly developed for morphological differentiation [35].

More recently, molecular methods have been used to target particular pathogenic fungi colonizing nursery seedlings [10, 18] and to amplify the rDNA of all endomycorrhizal (VAM) fungi from root tissues [3, 17]. More complex fungal communities from environmental samples have also been assessed by analysis of total fungal, or "fungal community" DNA, from which sequences from all fungi [26, 34] can potentially be amplified.

The objectives of the present study were to compare the species composition and relative abundance of fungi colonizing roots of containerized *Picea glauca* seedlings from four conifer nurseries in northern Alberta. We chose to amplify fungal rDNA directly from roots (and from seedling root cultures for comparison), as this approach avoids cultural biases and the problem of multiple colonization of individual fine roots. This method can potentially detect all root-associated fungi, many of which are likely to be important to the health of conifer seedlings before and after outplanting.

Methods

Plant Material

White spruce seedlings were collected from four northern Alberta nurseries: (1) Pacific Regeneration Technologies Inc. (PRT), Beaverlodge AB (55° 13′ N 119° 26′ W); (2) Bonnyville Forest Nursery Inc., Bonnyville, AB (54° 16′ N 110° 44′ W); (3) Woodmere Nursery, Fairview, AB (56° 04′ N 118° 23′ W); and (4) Smoky Lake Forest Nursery, Smoky Lake, AB (54° 07′ N 112° 28′ W). Seedlings were grown in separate wells of styrofoam blocks containing a peat moss–vermiculite mixture for between 8 and 10 months with initial nitrogen fertilization levels of 55 ppm at Smoky Lake, 60 ppm at Woodmere, 80 ppm at Bonnyville, and 150 ppm at PRT. Twenty seedlings were selected at random from one seed lot per nursery, packed in plastic bags, and stored on ice.

Isolation of Fungal Cultures

Pure cultures of root-associated fungi were established using surface sterilization and specific media [4]. A total of 60 root tips were removed from five randomly chosen seedlings from each nursery. Roots were washed in tap water, surface sterilized with 30% hydrogen peroxide for between 0 and 30 s, and then rinsed in sterile distilled water. One-half of the roots were plated onto modified Melin Norkrans medium (MMN) [21] supplemented with 50 ppm oxytetracycline and 100 ppm streptomycin and the remainder were plated onto MMN also supplemented with 5 ppm benomyl, in order to select for growth of basidiomycetes [4]. Cultures were incubated for 3 weeks at 20°C, and slow-growing fungi were transferred onto fresh media lacking benomyl and antibiotics. Because our goal was to obtain cultures of root endophytes and mycorrhizal fungi from which reference sequences could be produced, fast-growing, ubiquitous saprophytes such as *Trichoderma, Penicillium*, and zygomycetes were not further analyzed. Reference isolates were deposited in the University of Alberta Microfungus Collection and Herbarium (UAMH) as *Amphinema byssoides* UAMH 9577 (Bonnyville), *Thelephora americana* UAMH 9578 (Smoky Lake), *Phialocephala fortinii* UAMH 9740 (Woodmere), and a sterile unidentified isolate (UAMH 9546) from PRT Nursery.

DNA Extraction and Amplification

Total genomic DNA was isolated from a random subsample of fine roots from each of 20 seedlings from each nursery using a modification of the protocol recommended for GlassMax (Gibco BRL Life Technologies, Burlington, ON). Eight to 12 mg of dried tissue was frozen in liquid nitrogen, pulverized in a ceramic mortar, and homogenized with 600 μL of 2× CTAB lysis buffer (2% CTAB, 100 mM Tris pH 8.0, 20 mM EDTA, 1.25 M NaCl, 0.2% β-mercaptoethanol). Samples were incubated for 1 h at 65°C, vortexed with 1 volume of chloroform, and centrifuged at 10,000 *g* for 15 min. DNA was then recovered from the aqueous phase using the GlassMax DNA isolation spin cartridge system.

For fungal cultures, portions of colonies were excised and the excess agar removed by heating in water and filtration under vacuum. Samples were dried by vacuum centrifugation for 30 min and stored at room temperature. DNA was extracted from 5–20 mg of dried hyphal tissue using the Qiagen DNeasy Plant Mini Kit following the manufacturer's recommendations (Qiagen Inc., Mississauga, ON).

DNA extracts were diluted 1:12.5 and 1:125 in order to reduce the effects of PCR inhibitors and a 25-μL aliquot was added to each PCR reaction tube containing 25 μL of reaction mixture. The PCR cocktail comprised 400 μM of each of dNTP (Pharmacia Biotech Inc., Baie d'Urfe, PQ), 100 mM KCl, 5 mM $MgCl_2$, 20 mM Tris (pH 9.0), 2.5% Triton X-100 (Caledon Laboratories Ltd., Georgetown, ON), 1 unit of *Taq* DNA polymerase (Promega Corp., Madison, WI), and 2 μM of each oligonucleotide primer: the fungal-specific ITS1-F [14] and a primer we refer to as "NL6C" (5′ CAAGTGTTTCCCTTTCAACA 3′), a modification of the fungal-specific primers NL6Amun and NL6Bmun [11].

Reactions were performed in a PerkinElmer DNA Thermal Cycler 9700 (PerkinElmer Corp., Norwalk, CT) with the following cycling parameters: initial denaturation at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for

1 min, extension at 72°C for 2 min, with a final extension at 72°C for 10 min.

PCR products from each seedling (1–4 amplicons) were simultaneously digested with the restriction enzymes *Hha*I and *Hinf*I (Pharmacia Biotech Inc., Baie d'Urfe, PQ) and the resulting fragments separated on 2% agarose in order to determine the number of RFLP types. Exemplars of each RFLP type were then separated on 2% MetaPhor agarose (FMC BioProducts, Rockland, MD) in 1× TBE buffer at 2.5 V/cm, run overnight. DNA from bands visualized with ethidium bromide were cored with a pipette tip and the agarose dissolved in 450 μL of warm water. The dissolved gel core then served as the template for a second, semi-nested, PCR reaction, using the universal primer ITS1 [46] (internal to ITS1-F) in combination with NL6C at an annealing temperature of 55°C. Levels of *Taq* polymerase were reduced (0.2, 0.1, or 0.05 units per reaction) in order to minimize non-specific amplification. This second amplification was necessary to produce enough PCR product for both RFLP analysis and sequencing. The resulting products were visualized on 2% agarose gels in 1× TBE run at 4 V/cm.

RFLP Analysis

Restriction fragment patterns of secondary, seminested, amplification products were obtained using the enzymes *Alu*I, *Hha*I, and *Hinf*I (Pharmacia Biotech Inc.). Digests were performed using 10 μL of PCR product, 7 μL deionized water, 2 μL of 10× reaction buffer, and 1 μL of enzyme. Restriction fragment patterns revealed by electrophoresis were compared to those generated from seedling root isolates (noted in bold type in Fig. 1) and from *Wilcoxina mikolae* (UAMH 6693).

Cloning and Sequencing

PCR products obtained from roots, and from cultures established from roots, were purified using the QIAquick PCR purification kit (Qiagen Inc., Mississauga, ON) and quantified with a TKO 100 fluorometer (Hoeffer, Scientific Instruments, San Francisco CA). In cases where amplicons were too similar in size for separation by electrophoresis (evidenced by the sum of the restriction fragment sizes exceeding that of the undigested product), separation was accomplished by cloning with pGEM-T easy vector kit (Promega Inc., Madison WI). PCR was then performed on 10 bacterial colonies from each ligation using the primers ITS1 and NL6C and the products compared on 2% agarose. One amplicon of each size class from each ligation was subsequently sequenced.

Sequencing reactions were performed with the Thermo-Sequenase II Dye Terminator Sequencing kit (Amersham Life Science Inc., Cleveland, OH), following the manufacturer's specifications and using ITS 4 [46] and NL6C as primers. Sequencing products were analyzed on an ABI 373 automated sequencer (PerkinElmer Corp., Foster City, CA).

ITS sequences were aligned with sequences obtained from root isolates, from Blast searches (http://www.ncbi.nlm.nih.gov/BLAST), and from GenBank accessions from known conifer root associates. Sequence alignments were first done automatically using Clustal X [39] and then manually adjusted using Bioedit (Ver. 5.0.6) [16]. Maximum parsimony analysis was then performed using PAUP* 4a [38]. If a given clade included sequences which did not closely align with any reference ITS sequences (clades B, C, and D), blast searches were conducted for representative taxa using the 360 bp region of the 5′ end of the large subunit (26S) rDNA (LSU rDNA), which was amplified along with the ITS region.

Ordination Analysis

Aligned ITS sequences were used in combination with the RFLP data from the secondary PCR products to construct a presence/absence matrix for the fungi detected on the 80 seedlings analyzed. Fungi and seedlings were ordinated by reciprocal averaging in PCord [28], then 95% confidence ellipses around the site scores from each nursery were calculated with Systat 10 (SPSS Inc., Chicago, IL.).

Results

RFLP Analysis

Electrophoresis of the initial PCR products obtained from the fine roots revealed that each PCR reaction produced 1 to 4 distinct amplicons ranging in size from 0.95 to 1.35 kb. Simultaneous restriction digestion of all amplicons from each sample revealed 5 distinct RFLP patterns from the Bonnyville samples, 6 from PRT, 8 from Smoky Lake, and 7 from Woodmere. Gel isolation of DNA from individual fragments from representative PCR reactions, followed by a second, seminested, PCR and restriction analysis, revealed 32 distinct RFLP patterns. DNA from 26 representative fragments was sequenced directly. Six others appeared to consist of more than one amplicon (based on RFLP analysis) and were cloned prior to sequencing. PCR-RFLP analysis of the cloned PCR products resulted in 2 to 3 distinct sequences from each ligation.

Identification of Fungi by Sequence Analysis

Alignment and maximum parsimony analysis of the ITS sequences from reference cultures and from GenBank with sequences obtained directly from root tips resulted in a phylogram with 11 clades, nine of which (B–F and I–K) contain at least one reference sequence from endophytic or mycorrhizal fungi (Fig. 1). The five sequences from root tips included in Clade B (59% bootstrap) are at least 99% similar to each other and 88–89% similar to the *Phialophora finlandia* sequence. This clade is sister to the ericoid

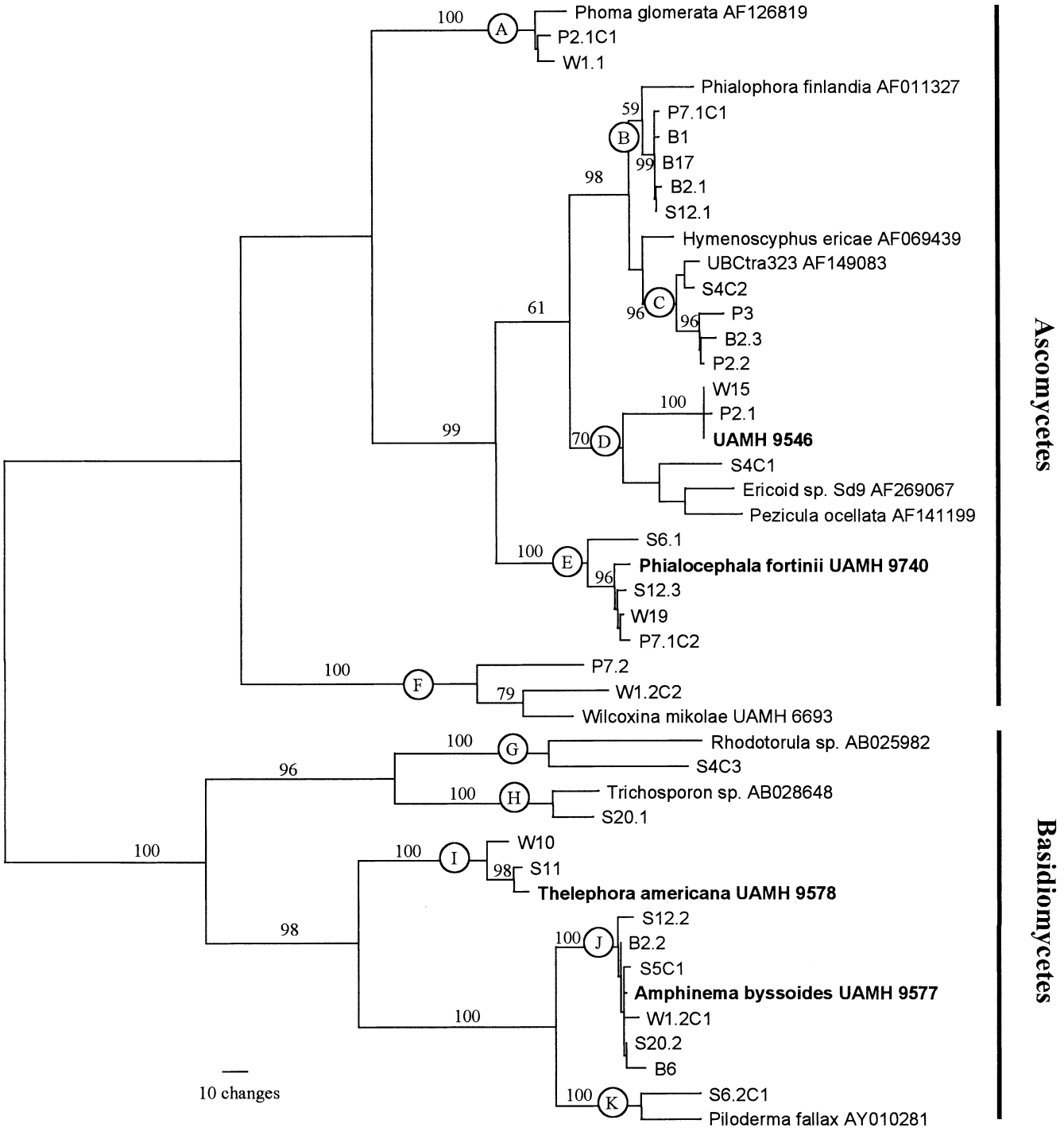

Fig. 1. One of the most parsimonious trees comparing ITS sequences from the fine roots of nursery grown *Picea glauca* seedlings to reference sequences. Sequences labeled with UAMH numbers and in bold type originate from root cultures obtained during this study (GenBank accession numbers Ay219837–Ay219840). UAMH 6693 *Wilcoxina mikolae* (Ay219841) was included for comparison. Sequences originating from the four nurseries are labeled as B for Bonnyville; P for PRT, S for Smoky Lake, and W for Woodmere. Other sequences are on deposit in GenBank. Cladesare marked by circled letters. Numbers above the lines refer to bootstrap values >50% (1000 replications). The tree was generated using the heuristic search and midpoint rooting options in PAUP [38].

mycorrhizal fungus *Hymenoscyphus ericae*. Clade C (96% bootstrap) contains four sequences from root tips which are 94% and 98% similar to each other and between 88 and 92% similar to another ericoid endophyte from the roots

of *Gaultheria shallon* (UBCtra323) described as "salal mycorrhizal fungus." An unidentified sterile fungus cultured from root tips during this study (UAMH 9546) is placed in Clade D and shows over 99% similarity with sequences W15 and P2.1. S4C1 is 84% similar to UAMH 9546 and 86% similar to *Pezicula ocellata* and "ericoid mycorrhizal species Sd9," from the ectomycorrhizal roots of *Quercus ilex* and the ericoid mycorrhizas of *Erica arborea* [2]. A Blast search performed using the LSU rDNA sequences matched sequence B2.1 from clade B with *Hymenoscyphus ericae* (AF284122, sequence derived from ex-type strain UAMH 6735) at 99% similarity (identities = 355/357). The closest matches to LSU sequences for S4C2 (clade C), S4C1, and UAMH 9546 (clade D) were *Chalara sessilis* (AF222476), with 96% similarity (identities = 345/358), *Ch. microchona* (AF222468), with 98% similarity (identities = 350/357), and *Ch. angustata* AF222448 with 93% similarity (identities = 319/341), respectively. Clade E contains the common root associated fungus *Phialocephala fortinii* and 4 root sequences and is supported by a 100% bootstrap value. Three sequences (S12.3, W19, and P7.1C2) are 99% similar to each other and 96% similar to *P. fortinii*. Sequence S6.1 is 92% similar to *P. fortinii* and 92–93% similar to the other root sequences. Clade F (100% bootstrap) includes the mycorrhizal ascomycete *Wilcoxina mikolae* and two sequences from root tips that are 79% similar to each other and between 82 and 88% similar to *W. mikolae*. Clades I and J (100% bootstrap) include reference ectomycorrhizal basidiomycete cultures *Thelephora americana* and *Amphinema byssoides*. The root tip sequences in Clade I are 94% similar to each other and 93–94% similar to *T. americana*. Six root tip sequences in Clade J are between 96 and 98% similar to each other and 97–100% similar to *A. byssoides*. Clade K contains *Piloderma fallax* and one root tip sequence showing 84% similarity. Clades A, G, and H are strongly supported with 100% bootstrap values. Two sequences from root tips group with the coelomycete *Phoma glomerata* and are 96–97% similar to *P. glomerata* and 97% similar to each other. Clades G and H contain the yeasts *Rhodotorula* sp. and *Trichosporon* sp. together with root sequences S4C3 (74% similar) and S20.1 (75% similar), respectively.

Fungal Community Composition

Estimation of the relative abundance of fungal species colonizing seedlings (done by relating representative sequence data back to RFLP data from each seedling) showed that sequences similar to those of the ectomycorrhizal basidiomycetes *Thelephora americana* and *Amphinema byssoides* (clades I and J) are most common (Fig. 2). Sequences in clades B and C, similar to some ericoid mycorrhizal fungi, are next in abundance, followed by sequences close to *Phialocephala fortinii* (clade E), UAMH 9546 (clade D), and *Piloderma fallax* (clade K). Sequences close to the ectomycorrhizal ascomycete W*ilcoxina mikolae* (clade F), *Phoma glomerata* (clade A), and *Trichosporon* sp. (clade H) were the least common.

Important differences could also be observed in relative proportions of root-colonizing fungi among nurseries (Fig. 2), with *Thelephora americana*–like sequences being found on a large proportion of seedlings from PRT and Bonnyville nurseries and *Amphinema*-like sequences dominating Woodmere and codominating Smoky Lake. Sequences in clades B and C were also common in PRT and Bonnyville, while *Phialocephala*-like sequences were most abundant in Smoky Lake. Sequences similar to *Phoma glomerata* (clade A), *Wilcoxina mikolae* (clade F), and UAMH 9546 were less abundant and were found in only some of the nurseries. Sequences similar to those of *Rhodotorula* sp. and *Piloderma fallax* were unique to Smoky Lake.

In the correspondence analysis of species abundance data pooled across all nurseries (Fig. 3), the first three axes explained 22.4% ($\lambda_1 = 0.842$), 18.4% ($\lambda_2 = 0.693$) and 14.2% ($\lambda_3 = 0.536$) of the total variation in root colonization, respectively. The analysis indicates positive interspecific associations among clades A, B, C, D, and I. Positive associations are also apparent among members of clade H, G, and J, while members of clades E and K appear to be negatively associated with all other taxa. Calculation of 95% confidence limit ellipses around the site scores from each nursery indicate the level to which the species compositions of the four nurseries overlap, with Bonnyville being completely encompassed by PRT; PRT and Woodmere, as well as Woodmere and Smoky Lake, overlap to some degree. The standard deviations in site scores along the first ordination axis (44.15 for Bonnyville, 89.6 for PRT, 121.12 for Woodmere, and 288.53 for Smoky Lake) indicate a gradient in fungal community variability, from nurseries with high fertilizer levels (e.g., Bonnyville) to those with low fertilizer levels (e.g., Smoky Lake). The correspondence analysis also indicated an influence of the level of applied nitrogen on the fungal species distribution, with fungi toward the right of the diagram being more

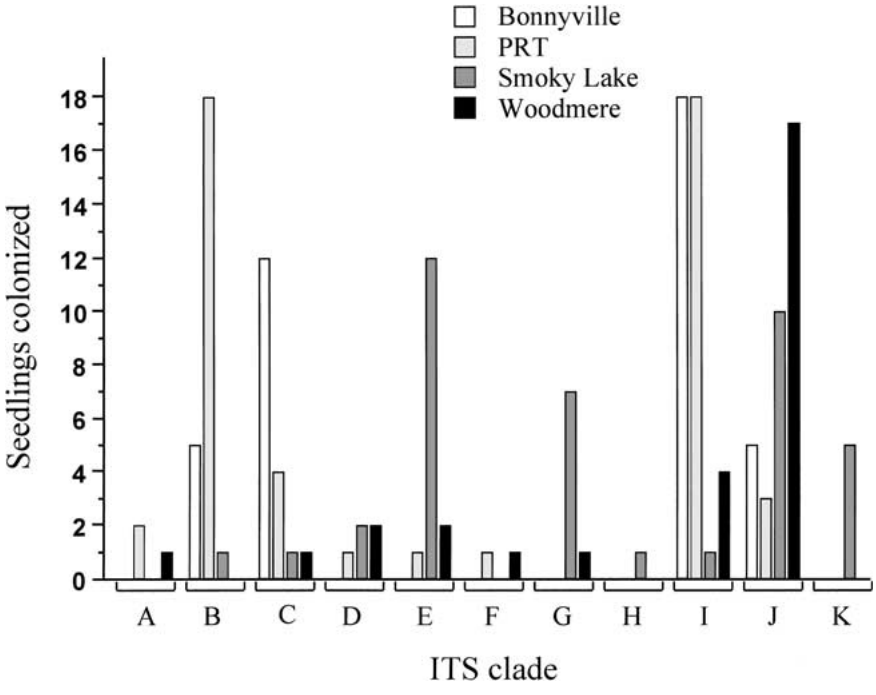

Fig. 2. Relative abundances of fungi corresponding to ITS clades A to K for each of the four nurseries. Abundances are based on the frequencies of RFLP patterns produced from PCR products.

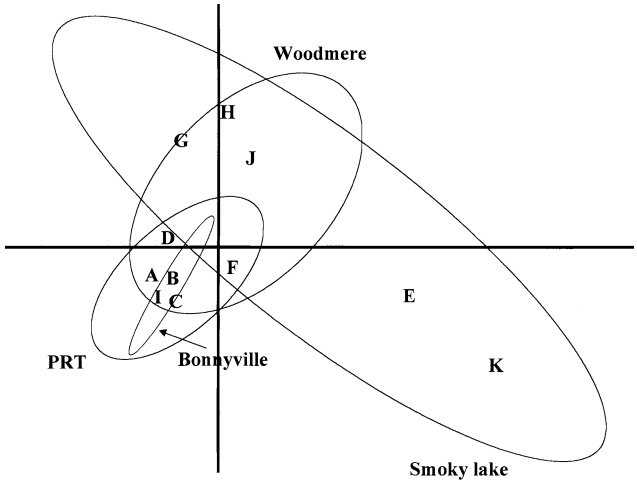

Fig. 3. Correspondence analysis of fungi belonging to ITS clades A to K and seedlings analyzed. Ellipses circumscribe the 95% confidence limit of site scores from each nursery. Abundances are basedon the frequencies of RFLP patterns produced from PCR products.

common in nurseries with higher levels of applied N and those toward the left of the diagram being more common in nurseries with less applied N (a linear regression of seedling scores along the first ordination axis against nitrogen gave $r^2 = 0.946$, $p = 0.027$). The diagram also indicates a possible effect of the source of sphagnum used for growing media, as Bonnyville and PRT obtain their sphagnum from a local wetland different than Woodmere and Smoky Lake.

Discussion

The direct amplification of fungal DNA combined with isolation from root tips allowed us to make a detailed assessment of the community of root-associated fungi in the conifer nurseries studied. The use of either of these methods on its own, however, would have resulted in very different pictures of fungal species composition and abundance. Fungi detected by PCR either were not isolated by pure culture techniques or were isolated at very low frequencies. PCR did not detect fast-growing ubiquitous saprophytes that were frequently isolated in culture. From this, it would seem that PCR is more sensitive and specific since it is not dependent on the ability of the fungi to grow in culture and detects only those fungi actually colonizing healthy roots. At present, however, neither approach is definitive in providing full identification of the fungi. Even though a high degree of genetic diversity can be recognized with PCR, sequences cannot always be matched unambiguously to known fungi. As well, cultural studies are required to explore the symbiotic potential of identified taxa.

Previous studies of containerized seedlings have focused on either mycorrhizal or pathogenic fungi. Morphology of ectomycorrhizas has been used to identify many of the same species that we detected using our combined approach. The ectomycorrhizal basidiomycetes *Thelephora americana* (as *T. terrestris*) and *Amphinema*

hyssoides have been identified on containerized *Picea engelmanii* seedlings in British Columbia [20]. These two species, and the ascomycete *Wilcoxina* (as E-strain), were also identified in a prior morphology based study of containerized *Picea glauca* seedlings from Alberta nurseries [6], A notable difference between our survey and the prior survey was the detection of fungi of ascomycetous affinity usually associated with ericoid plants [29, 30] (Clades B, C, D, Fig. 1). However, it is possible that some of these fungi were present at other nurseries and identified as "Mycelium radicis atrovirens," a form name encompassing dark septate root endophytes (DSE) [23]. Recent studies indicate that DSE include a large number of taxa having different ecological functions; neither the taxa nor their functions are fully resolved [23, 30, 43, 44].

Our finding of sequences of *Phialophora finlandia* as well as other fungi close to the well-known ericoid mycorrhizal fungus *Hymenoscyphus ericae* (*Leotiomycetes, Helotiales*) (Clades B and C) [12] correlates with results of Vrålstrad et al. [43]. They used ITS sequences to examine genetic diversity among the mycobionts of a common ectomycorrhizal morphotype ("*Piceirhiza bicolorata*") of both conifers (including *Picea*) and hardwoods [42, 43]. Over 75% of the mycobionts occurred within the *H. ericae* aggregate which included *Phialophora finlandia*. Tested isolates formed either ecto- or ericoid mycorrhizas depending upon their original host plant [44]. *Phialophora finlandia* has previously be shown to form ectomycorrhizas on *Pinus strobus* seedlings [40] and to improve root growth of inoculated *Picea rubens* seedlings [48]. A Blast search using the LSU rDNA sequence confirmed affinity of a Clade B representative to the *H. ericae* aggregate. Sequences in our Clade C were most similar to the unpublished sequence AF149083 obtained from the ericoid mycorrhiza of *Gaultheria shallon* "salal mycorrhizal fungus" (UBCtra323). The placement of the salal fungus sister to *H. ericae* was also found by Vrålstrad et al. [43]. Results of Blast searches using LSU rDNA sequences from selected taxa of Clades C and D suggested affinities with some anamorphic *Chalara* species (*C. sessilis, C. microchona, C. angustata*), recently shown to be allied to teleomorphs in the Leotiomycetes rather than to those in *Ceratocystis* as found for other *Chalara* species [33].

Clade D includes some other helotialean fungi isolated from both ecto- and ericoid mycorrhizal hosts. "Ericoid mycorrhizal species Sd9" was found on roots of the ectomycorrhizal *Quercus ilex* and the ericoid mycorrhizal *Erica arborea* [2]. *Pezicula ocellata* and *Cryptosporiopsis* species (anamorphic *Pezicula* species) [1, 41] have been isolated from the rhizospheres of *Cornus canadensis* growing in the understory of *Picea mariana* [31]. A *Cryptosporiopsis* sp. commonly isolated from nonmycorrhizal fine roots of *Picea mariana* in natural environments was subsequently found to form a *Pezicula* teleomorph in culture [19]. A subgroup in Clade D includes a fungus detected at three nurseries, but isolated only once in culture (UAMH 9546) from Smoky Lake. This nonsporulating fungus demonstrated hyphal features (loops, terminal swellings) similar to those of some ericoid root-associated fungi. The fact that it was obtained from surface-sterilized root tissue implies that it is endophytic in nature. The local *Sphagnum* used as growth medium for the *Picea* seedlings is a likely inoculum source for many of these fungi [8].

Phialocephala fortinii (Clade E) is a common helotialean root-associated fungus reported from many plant hosts [13, 23, 43]. Although it has been shown to improve conifer seedling growth in some cases [23], its ability to form mutualistic symbioses may be strain dependent and in some cases it may be pathogenic or commensal [13].

PCR detected a few saprophytic fungi (Clades A, G, H), but none regarded as pathogenic. The coelomycete, *Phoma glomerata*, is a ubiquitous soil saprophyte found on dead material of many host plants [9] and has been isolated from *Picea mariana* roots [37]. The basidiomycete yeasts *Trichosporon "beigelii"* (now recognized as a complex encompassing several species), *Rhodotorula* spp., and *Cryptococcus albidus* have been isolated from *Picea mariana* rhizospheres in natural environments [37].

Amplification of community DNA can produce artifacts such as chimeric sequences [45]. Chimera formation did not appear to be a problem in our study, as virtually all of our sequences aligned well with reference sequences. We did, however, see a much greater variation in RFLP patterns than in ITS sequences (i.e., RFLP patterns were an overestimate of actual community variation). This may have been due, in part, to intragenomic sequence variation within individuals [15], to carryover during electrophoretic separation of amplicons, or to stalling during the elongation of PCR products, leading to size variations in the final amplicons. Regardless, total fungal community DNA analysis appears to be an efficient method for analyzing fungal communities associated with conifer roots without previous knowledge of species composition and allows for the subsequent targeting of species of interest.

Hunt [20] studied the ectomycorrhizal fungi of conifer nurseries in British Columbia and found that *Thelephora americana* (as *T. terrestris*) dominated under conditions of poor aeration, water-logging, and high fertilizer rates. When these conditions were ameliorated, the mycorrhizal community became more diverse and included larger proportions of *Amphinema byssoides* and *Wilcoxina* sp. We also found similar trends. Seedlings in nurseries with higher fertilizer regimes (Bonnyville and PRT) showed much greater colonization by *T. americana* (clade I) and members of clades B and C, while those in nurseries with lower rates (Smoky Lake and Woodmere) supported a more diverse fungal community and were more likely to be colonized by *A. byssoides* (clade J) and *Phialocephala fortinii* (clade E). The latter situation is likely preferable in terms of seedling quality, as colonization by *Amphinema* results in greater seedling growth over colonization by *Thelephora* [7, 20]. *Amphinema byssoides* is also an extremely common associate of boreal *Picea* [24] and is therefore likely to persist on outplanted seedlings, whereas *Thelephora* has been shown to decrease in abundance with time after outplanting [5, 22]. Our results, therefore, support prior suggestions that both mycorrhizal colonization and seedling growth can be optimized by limiting levels of applied nitrogen [20].

The positive correlation between colonization by *A. byssoides* and *P. fortinii* in the low fertilization nurseries (Smoky Lake and Woodmere) and between the helotialean taxa (clades B, C, and D) and *Thelephora* in the higher fertilization nurseries (Figs. 2, 3) might be due to differences in seedling nutrient status or to a positive biological association between the fungal species. Alternatively, there may be negative associations between *Phialocephala* and *Thelephora* and between helotialean taxa and *Amphinema*, respectively. This question is best addressed by determining the ecological function of the helotialean fungi, which may be forming mutualistic, commensal, or antagonistic symbioses with their host roots.

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