Studies in Mycology 53: 121–145. 2005.

Root endophyte and mycorrhizosphere fungi of black spruce, *Picea mariana*, in a boreal forest habitat: influence of site factors on fungal distributions

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Abstract: In a study of fungi growing in various root-associated habitats in and around *Picea mariana*, black spruce, in northern Ontario, Canada, an examination was made of the degree to which differences in growth sites within an area of a few square kilometers might influence the structure of root-associated filamentous microfungal populations. *Picea mariana* roots were collected at four strongly differing boreal forest sites: an undisturbed forest site with deep litter and humus layers; a recently regenerated forest; a clearcut, former portable sawmill site with a few small, naturally regenerated trees; and an open peat bog penetrated by roots from trees growing along the margin. Comparisons were done on isolate assemblages primarily from serially washed mycorrhizae, supplemented with comparison samples from washed root bark and adherent rhizosphere soil. The Bray & Curtis similarity index and nodal components analysis were utilised to identify trends within the data. Root endophyte fungi, mainly *Phialocephala fortinii* and *Meliniomyces variabilis*, were among the most common isolates from serially washed mycorrhizae and showed strong trends among the site types, with the former most common from sites low in humus and also low in known humus-associated microfungi, and the latter most common from the peat bog site. The overall composition of the isolate assemblages from washed mycorrhizae mainly reflected site factors, with assemblages from the undisturbed and regenerated forest sites similar to one another and those from the clearcut and peat bog sites strongly distinct. A major difference was also seen between two seasonal samples at the exposed clearcut site, but few seasonal differences were seen at the other sites. The regenerated and undisturbed forest sites were high in *Umbelopsis isabellina*, *Mortierella verticillata* and *Penicillium spinulosum*, fungi typical of humic horizons in boreal podzols; the clearcut yielded the greatest numbers of *Fusarium proliferatum*, *Umbelopsis nana* and *Penicillium montanense* isolates, an assemblage tending to indicate exposed mineral soil; while the peat bog was typified by the presence of characteristic northern peat inhabitants *Mortierella pulchella* and *P. spinulosum*, as well as temperate peat inhabitant *Penicillium lividum*. A synthesis of these results with other data suggests that as a microhabitat, the mycorrhizosphere, as originally defined by Foster & Marks, is of little significance in determining the structure of filamentous fungal populations in soil influenced by the presence of ectomycorrhizal forest tree roots. Edaphic and overall microbial community conditions are much more significant, but the influence of a "symbiorhizosphere effect" exerted by certain ectomycorrhizal symbionts within the whole soil volume they occupy is also known in some cases and worthy of further investigation.

Key words: DSE, ecology, forestry, rhizosphere, symbiosis.

INTRODUCTION

Roots of living plants give rise to a number of microhabitats, or, to put the matter more accurately, they introduce a number of ecological gradients that can be conceptually partitioned into microhabitats such as the rhizosphere and the rhizoplane. The range of microhabitats to be considered expands when discrete portions of root systems are involved in a biochemically and spatially complex symbiotic association with a mycorrhizal fungus. The present study is a continuation of a series of studies (Summerbell 1988, 1989) on a particular microhabitat, the mycorrhizosphere, and its significance in regard to the microfungal populations occurring in and around ectomycorrhizal roots.

Some clarification of the terminology involved appears necessary as a prelude to further discussion. For microhabitats around ectomycorrhizal roots, Hiltner's (1904) originally all-encompassing concept of the rhizosphere was adapted as the basis of several subsidiary concepts, most notably Foster & Marks' (1967) concept of the "mycorrhizosphere", the soil zone immediately influenced by the presence of the ectomycorrhizal mantle. This concept can be confused with Rawlings' (1958) quite different but homonymous concept of the "mycorrhizasphere", which, though introduced in a grammatically ambiguous sentence, appears to have meant the whole volume of the "organic soil horizons" influenced by the mycorrhizal association, including the nearby soil influenced by extending extramatrical hyphae. "Mycorrhizosphere" *sensu* Foster & Marks has become a well established term, whereas the term proposed by Rawlings has received little use. Somewhat confusingly, however, Filion *et al*. (1999) have recently revived the use of "mycorrhizosphere" *sensu* Rawlings to apply to the

total volume of soil influenced by an endomycorrhizal association. In fact, "mycorrhizosphere" is now the second term to be extracted from an established usage, differing in meaning, in an attempt to denote the total volume of mycorrhizally influenced soil. Tarafdar & Marschner (1994) had earlier used the term "hyphosphere" to indicate this concept, apparently unaware that this term was originally coined by Staněk (1984) to refer to something quite different, namely, the zone of influence of individual fungal hyphae on adherent and proximal bacterial populations. Its continued use in that original sense seems sensible, since the hyphosphere *sensu* Staněk is a distinct type of bacterial habitat that, intuitively, fits perfectly with the name given to it. To avoid further confusion, it is here proposed to follow Summerbell (1985) and restrict the use of "mycorrhizosphere" to the immediate area around the mycorrhizal feeder root zone (mantled area in ectomycorrhizae) as per Foster & Marks (1967) and to use the term symbiorhizosphere "for the totality of soil volume influenced by the presence of a mycorrhizal root system." Soil in this case must be broadly understood as also including organic debris in the process of comminution, and "the totality of soil volume influenced by a mycorrhizal root system" can either be taken holistically to include all mycobionts partnering with a particular plant (as in "the symbiorhizosphere of endomycorrhizal test plant #3") , or, where usefully applicable, can be partitioned into the zone of influence of particular mycobionts [as in "the symbiorhizosphere of *Laccaria bicolor* (Maire) P. D. Orton genet IIB partnered with *Picea mariana* in the northeast quadrant of plot 15"].

Two early researchers of the rhizosphere of ectomycorrhizae clearly stated what would be predicted about the mycorrhizosphere in terms of its significance to microbial populations. Harley (1948), a mycologist, said "we are dealing in ectotrophic mycorrhiza with a special case where, on a given root, one species of fungus in particular (the ectomycorrhizal partner) is so affected by local conditions as to become dominant in the root surface zone. We can only conclude, from what we know of the mutual competition and antagonism between species of microorganisms, that the whole population of the root region is, in consequence, profoundly modified." Similarly, bacteriologists Manteifel *et al*. (1950) stated that near ectomycorrhizal roots "distinct nutrient-exchanging (microbial) communities are undoubtedly found. The entry of plant-derived substances into the soil, a process having great consequence for rhizosphere bacteria, is entirely altered, since the substances must cross through the compact weft of fungal hyphae, and the fungus undoubtedly receives a portion of this exchange." In the time since these scenarios were outlined, many studies of mycorrhizosphere fungi and bacteria have been made (Rambelli 1973, Luppi Mosca & Filipello Marchisio 1985–86, Summerbell 1989, Garbaye 1994). The presence of distinctive bacterial populations on mycorrhizae has been unequivocally shown several times (Foster & Marks 1967, Rambelli *et al*. 1972) and is now generally accepted; indeed, very useful "helper bacteria" of ectomycorrhizal symbionts have been identified from mycorrhizosphere sources (Garbaye 1994). With fungi, however, as Summerbell (1989) pointed out, the number of control examinations included in most studies of ectomycorrhizosphere fungi was inadequate to truly show if any of the species seen was specific to, or quantitatively favoured by, the presence of the ectomycorrhizal symbiosis. Our knowledge of whether the mycorrhizal association "profoundly modifies" the root zone for microfungi, either in the mycorrhizosphere or in the symbiorhizosphere, is very limited [except in the special case of the highly allelopathic *Tricholoma matsutake* (S. Ito & S. Imai) Singer (Ohara & Hamada 1967)].

In a comparison of various microhabitats in the rhizosphere of the ectomycorrhizal tree black spruce [*Picea mariana* (Mill.) BSP] and the endomycorrhizal understory plant flowering bunchberry (*Cornus canadensis*) (Summerbell 1989), it was found that the only nonmycorrhizal fungi (defined non-globally as fungi occurring in association with one or both of the two plant species studied, but known or believed not to form mycorrhizae with either plant species) strongly statistically linked to isolation from mycorrhizal root tips, whether ecto- or endo-, were root endophytes that could be isolated after stringent root surface disinfection. These endophytes mainly consisted of two groups that in subsequent years have been identified as *Phialocephala fortinii* C.J.K. Wang & Wilcox [making up all or most isolates called *Mycelium radicis atrovirens alpha* by Summerbell (1989)] and *Meliniomyces variabilis* Hambleton & Sigler [called Sterile white 1 and Sterile dark 1 by Summerbell (1989)]. The former is an extensively studied root endophyte with various roles in different plant systems (Jumpponen & Trappe 1998), including weak pathogen of forest trees (Melin 1921, 1923, Levisohn 1960, Richard & Fortin 1974), protective endophyte (Narisawa *et al*. 2002, 2004), ectomycorrhizal symbiont (Jumpponen *et al*. 1988) and atypical ericoid commensal or symbiont (Currah *et al*. 1993, Stoyke & Currah 1993). The latter, newly named (Hambleton & Sigler 2005–this volume), is reviewed in the context of its formal taxonomic description in present volume.

In the present study, the biology of nonmycorrhizal fungi in the black spruce mycorrhizosphere was further studied by means of a comparison among very different types of habitats within an area of a few square kilometres. These habitats included two

forested sites, a hard-packed and sun-exposed former portable sawmill site in approximately the tenth year of natural regeneration, and a *Sphagnum* peat bog supporting spruce trees along its margin, with extensive root growth into the bog. The overall aim was to gain further insight into factors controlling the distribution of fungi in the rhizosphere of ectomycorrhizae by examining the effect of site-specific influences. For the most part, this involved determination of the extent to which distinct mycorrhizosphere mycota were found in the different forest habitats studied; in particular, the normal forest sites were contrasted to the rather extreme and perhaps nutritionally limited sites like the peat bog or sawmill sites. A second objective was to determine if there was a site effect on the prevalence of the root endophytes seen.

MATERIALS AND METHODS

Study sites

The study sites investigated were located at Burt Lake, near Swastika, Ontario, Canada (Lat. 48.10, Long. -80.10 in decimal degrees). They were described in some detail by Summerbell (1988) and Summerbell (1989). In brief, site 1 was a moist but well-drained forest site consisting mostly of a dense stand of *Betula papyrifera*, *Picea glauca*, and *P. mariana*. Trees had been cut on the site in the past, and the current vegetation was 10–15 yr old at most. The *P. mariana* trees selected for sampling were approximately 15 years old. As at the other sites, except site 4, one tree was sampled in the spring season and an adjacent tree of similar age with a spatially overlapping root system was sampled in the fall season.

The second site was on a steep slope consisting of loose, stony soil. The forest stand consisted mainly of *P. glauca*, *Pinus banksiana*, *Abies balsamea*, *P. mariana*, and *B. papyrifera*. The stand was undisturbed, and trees were estimated to be 6080 years old. The sampled trees were approximately 60 years old and were moderately suppressed by taller, older trees. Their superficial roots grew within well established and clearly visible fermentation (A01) and humus (A02) soil horizons.

Site 3 was a former clearcut forming part of a disused portable sawmill site. The soil was slightly compacted and well drained. Sparse regeneration occurred; this consisted mainly of 5–10yr-old *P. glauca* and *P. mariana*. The sampled trees were approximately 10 yr old. The site was the only one included in this study in which the soil around the trees was fully exposed to the sun; it was also the only site that lacked significant litter and humus layers. In many areas, the ground cover consisted in large part of *Cladonia* lichens.

Site 4 was located at a small upland lake. Forest cover in this undisturbed area consisted mainly of 60–70-yr-old trees. Primarily, these included *P. banksiana*, *B. papyrifera*, and *P. mariana*. The single tree selected for sampling was at the margin of an open *Sphagnum* bog. Although a proportion of the roots from the sample tree were growing into mineral soil, only roots that extended directly into *Sphagnum* peat were sampled.

Sampling methods and study design

Picea mariana roots were collected for analysis in early June and late September. In order to be sure that each sample of these black spruce roots derived from the correct species, sampling was done by carefully exhuming, at each sampling occasion per site, one or two entire roots out of surrounding soil, debris and tangled heterogeneous root material. Sample roots were attached to the trees near ground level, and were 1.5–3 cm in diameter at the point of attachment. No attempt was made to sample roots only in a particular soil horizon. In general, however, the roots ramified within the lower litter layers and in the soil layers immediately beneath, which consisted of humus at some sites and mineral soil or peat at others, as noted above. This sort of root growth habit is typical of the characteristically shallow-rooted black spruce (Köstler *et al*. 1968, Hosie 1969). Some root branches extended into the upper litter layers, particularly in site 1. As mentioned above, at each site two trees with spatially overlapping root systems were used as sample sources, one in the spring and the other in the fall, except at site 4 where the same relatively isolated tree was used. The bulk of root washing needing to be done and the high number of isolates needed per sample for statistical purposes (80+ isolates per tree × 4 sites) prevented comparative sampling of additional trees at each site within seasonal samples; in any case, individual roots of the trees sampled compassed an area of up to 1 m^2 and interwove with roots of many other trees and understory plants, reducing the chance of any pernicious localized small-scale factors affecting the data. The need to assure plant species identity by laboriously excavating roots made it impractical to pool root tips from multiple trees. The impracticality of sampling multiple trees per seasonal site prevented directly controlling for the very unlikely possibility that the individual trees selected might have had unique, individually determined constellations of root fungal associates, not generally representative of conspecifics at the same site. However, this possibility and other selection artifacts (e.g., profound effects due to ages of individual trees, as distinct from effects largely ascribable to the overall ages of stands, such as shade levels/moisture content, soil composition, and mycorrhizal symbiont population structure) were indirectly controlled by showing high similarities and minimal statistical differences between fungal assemblages from *P. mariana* roots and assemblages

from *C. canadensis* roots sampled at distances of some metres from the sample trees at three of the four sites (Summerbell 1989). A comparison done with root-associated fungi of a *P. glauca* tree at one site (Summerbell 1985) also showed no significant differences with the fungal assemblage from *P. mariana* at the same site. Finally, in regard to the question of whether individual or growth-stage-specific mycorrhizal partners (late-stage on older trees, early-stage on younger) might have a profound effect on the mycorrhizosphere *sensu* Foster & Marks, the effect ascribable to the presence of the mycorrhizal mantle was studied by contrasting assemblages from washed mycorrhizal root tips with those from root bark and from stringently surface-sterilised roots (Summerbell 1989). Minimal effects ascribable to the presence of mycorrhizal mantles were seen.

Isolation and identification of root fungi

Soil and litter were gently shaken from the sample roots and large pieces of adherent litter were detached by hand. The roots were then placed into a sterile plastic bag and returned to the field laboratory.

At the field laboratory, roots were washed free of loosely-adhering matter by vigorous manual agitation in near-sterile water (due to the isolated location of the field station and the large volume of water required in these procedures, water had to be sterilised at the site. Prolonged boiling was used in lieu of standard sterilization techniques. Plating tests showed that the resulting water was entirely free of culturable fungi, but did contain a very small number of bacteria.) From these crudely washed whole roots, numerous small feeder-root branches 3–7 cm long were selected. These branches were placed into vials, one branch per vial, and subjected to a rigorous serial wash of the type suggested by Harley & Waid (1955). Twenty cycles of washing were conducted, each of four minutes' duration, under moderate agitation in a laboratory shaker. The water was changed between each wash.

From the serially washed roots, individual mycorrhizal root tips of healthy appearance were aseptically detached. An attempt was made to avoid including adjacent suberized root portions along with the mycorrhizal tips. "Beaded"-looking "pseudomycorrhizal" roots of the type described by Richard (1969) were also avoided. The isolated tips were then plated out onto Hagem agar (Modess 1941) modified by the addition of 1.0 g of yeast extract and 100 mg/L tetracycline hydrochloride. For each sampling, 44–52 washed root tips per site were plated out on Hagem agar. Plates were incubated at 18 ºC for 4 wk.

Summerbell (1989) showed that, at least in the present study area, there was a high degree of continuity between filamentous fungal assemblages from black spruce mycorrhizal root tips, and those of the bark of proximal fine roots, as well as those of the adherent rhizosphere soil (soil adhering to fine root surfaces after brisk preliminary washing). In order to obtain an overall representation of how these intergrading microhabitats of the "adherent rhizosphere" (*rizosfera adherente*, Rambelli *et al*. 1962) might differ from site to site, a composite sample was made at each site based on the root tip isolations described above, as well as isolations from root bark and from adherent rhizosphere soil. For the bark subsample, serially-washed spruce roots were used as sources of small (approx. 1 mm square) pieces of root bark that were removed from areas adjacent to the divergence points of the short lateral root branches that terminated in ectomycorrhizal tips. These pieces were plated on modified Hagem agar, at approximately 25 pieces per site per seasonal sample. Also for bark surface sampling, serially washed fine-root segments approximately 3–4 cm. long (aseptically dissected from the generally longer root segments washed) were plated out on sorbose-yeast extract-tetracycline (SYET) medium (Bandoni 1981), a medium restricting colonial overgrowth, and, following the techniques of Bandoni & Barr (1976) for isolating seldom-seen elements of the fungal community from leaf litter, these were moved to a new position on the agar surface at the beginning of each of four successive days while plates were held at 5 ºC. [This temperature, used to minimize overgrowth of the outgrowing fungal inocula, was conducive to (slow) growth of most or all species isolated in this study. At the beginning of the study, this was only partially known *a priori* based on information from, for example, Domsch *et al*. (1980), but was suspected based on generally low soil temperatures (data not shown); it was later confirmed by observing continued colony extension in subcultures stored at 5º C. Germination of conidia at this temperature was not studied.] The root pieces were inverted each day so that one surface was exposed to the agar on the first and third days after implantation, and the other was exposed on the second and fourth days. Subsequently, the root segments were removed and plates were incubated at 18 ºC. Such plating was done for 10 root pieces per sample and outgrowing colonies were subcultured for identification. Finally, from the water used in the twentyfold serial washing procedure, 1 mL subsamples of the first, fifth, and twentieth serial washes from each site were plated out on SYET medium and on another weak, low C/N ratio medium used for environmental isolation, Bandoni's Cjm ("conjugation medium")-tetracycline agar (Summerbell 1989). This wash material was considered conceptually to be a dilution of the adherent rhizosphere soil even though actual soil particles were not visible to the naked eye. A trend line showing fungal inoculum levels per wash in such twentyfold serial washing procedures used for

Table 1. Fungi isolated from washed *Picea mariana* mycorrhizae, proximal feeder root bark, and adherent rhizosphere soil at four different sites in a northern Ontario boreal forest.

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Acremonium cf. *pteridii* W. Gams & Frankland (CBS 562.84)	– [S: 1 j] 1 (0.5) [b]	–	–	–	
Acremonium sp. 1	–	–	– [B: 1 j] 1 (0.6) [b]	–	
Aureobasidium pullulans (de Bary) G. Arnaud	1 (1.2) [R: 1 j; S: 1 j] 2 (1.1) [b]	–	2 (2.4) [R: 2 j; B: 1 s] 3 (1.9)	– [S: 1 s] 1 (0.9)	
Basidiomycetous sp. 1	1 (1.2) [R: 1 s] 1 (0.5)	–	–	–	
Basidiomycetous sp. 2	–	1 (0.6) [R: 1 s] 1 (0.5)	–	–	
Basidiomycetous sp. 3	–	4 (2.8) [R: 4 s] 4 (2.0)	1 (1.2) [R: 1 j] 1 (0.6)	–	
Basidiomycetous sp. 4	1 (1.2) [R: 1 s] 1 (0.5)	–	–	–	
Candida ciferrii Kreger-van Rij	1 (1.2) [R: 1 j] 1 (0.5)	–	–	–	
Catenulifera rhodogena (F. Mangenot) Hosoya	–	– [S: 1 j] 1 (0.5)	–	–	
Cenococcum geophilum Fries : Fries	–	–	–	1 (1.2) [R: 1 s] 1 (0.9)	
Chaetomium cochlioides Palliser (TRTC 50638)	–	–	1 (1.2) [R: 1 j] 1 (0.6)	–	
Chaetomium globosum Kunze : Fries	–	–	1 (1.2) [R: 1 s] 1 (0.6)	–	
Cladosporium cladosporioides (Fresenius) de Vries (TRTC 49132)	–	–	– [B: 1 s] 1 (0.6)	–	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Cladosporium sphaerospermum Penzig	– [S: 1 j] 1 (0.5)	–	–	–	
Cryptendoxyla hypophloia Malloch & Cain (CBS 796.84; TRTC 49498)	– [S: 1 j] 1 (0.5)	–	–	–	
Epicoccum nigrum Link	–	1 (0.6) [R: 1 s] 1 (0.5)	–	–	
Exophiala lecanii-corni (Benedek & Specht) Haase & de Hoog	–	– [S: 3 s] 3 (1.5)	–	–	
Fusarium cf. *coeruleum* (Libert) Saccardo (TRTC 50604)	–	–	– [B: 1 j] 1 (0.6)	–	
Fusarium proliferatum[c] (Matsushima) Nirenberg (TRTC 50602, 50621, 50639, 50640)	1 (1.2) [R: 1 j; S: 1 s] 2 (1.1)	–	**7 (8.2)** [c] [R: 7 s; B: 1 s; S: 5 s] 13 (8.2)	–	
Geomyces pannorum (Link) Sigler & J.W. Carmichael complex (TRTC 49468, 49470)	2 (2.4) [R: 1 j, 1 s; S: 6 s] 8 (4.6)	– [S: 1 j, 2 s] 3 (1.5)	–	– [S: 1 s] 1 (0.9)	
Geotrichum-like	–	–	1 (1.2) [R: 1 j] 1 (0.6)	–	
Gliocladium-like			– [B: 1 j] 1 (0.6)	–	
Hormonema dematioides Lagerberg & Melin (TRTC 50618)	– [S: 1 j] 1 (0.5)	–	–	–	
Hormonema sp. 1		–	–	– [S: 1 s] 1 (0.9)	
Lecanicillium aphanocladii Zare & W. Gams	–	1 (0.6) [R: 1 j] 1 (0.5)	–	–	
Meliniomyces variabilis Hambleton & Sigler (UAMH 5900, 5979; TRTC 49471, 50623, 50645)	**13 (15.7)** [R: 2 j, 11 s] 13 (7.5)	**14 (9.8)** [R: 3 j, 11 s] 14 (7.0)	2 (2.4) [R: 2 j] 2 (1.2)	**31 (38.3)** [R: 14 j, 17 s; B: 2 s] 33 (29.7)	for root tips alone 0.001; all isolates 0.001

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Mortierella alpina Peyronel	–	1 (0.6) [R: 1 j] 1 (0.5)	– [S: 1 s] 1 (0.6)	2 (2.5) [R: 2 s; B: 1 j, 1 s] 4 (3.6)	
Mortierella humilis Linnemann ex W. Gams (TRTC 50620)	– [S: 4 s] 4 (2.3)	–	1 (1.2) [R: 1 s; B: 1 j, 2 s] 4 (2.5)	–	
Mortierella jenkinii (A.L. Smith) Naumov	– [B: 1 s] 1 (0.5)	–	–	–	
Mortierella macrocystis W. Gams (TRTC 50606)	2 (2.4) [R: 2 s] 2 (1.1)	4 (2.8) [R: 2 j, 2 s] 4 (2.0)	2 (2.4) [R: 2 s] 2 (1.2)	– [S: 1 j, 1 s] 2 (1.8)	
Mortierella parvispora Linnemann	3 (3.6) [R: 2 j, 1 s; B: 1 j, 2 s] 6 (3.4)	6 (4.2) [R: 6 j] 6 (3.0)	1 (1.2) [R: 1 s] 1 (0.6)	2 (2.5) [R: 1 j, 1 s] 2 (1.8)	
Mortierella pulchella Linnemann	1 (1.2) [R: 1 j] 1 (0.5)	1 (0.6) [R: 1 j; S: 1 j] 2 (1.0)	–	**10 (12.3)** [R: 3 j, 7 s; B: 1 j, 2 s; S: 2 s] 15 (13.5)	
Mortierella verticillata Linnemann (TRTC 49484, 49485, 50607, 50642)	1 (1.2) [R: 1 j; B: 5 j, 2 s; S: 1 j, 1 s] 10 (5.7)	**9 (6.3)** [R: 4 j, 5 s] 9 (4.5)	1 (1.2) [R: 1 s; B: 1 j] 2 (1.2)	–	for all isolates 0.05
Mucor hiemalis Wehmer	–	1 (0.6) [R: 1 j] 1 (0.5)	– [B: 1 s] 1 (0.6)	–	
Mucor silvaticus Hagem (TRTC 50671, 50673)	2 (2.4) [R: 1 j, 1 s; B: 2 j] 4 (2.3)	**8 (5.6)** [R: 6 j, 2 s; B: 1 j; S: 1 j] 10 (5.0)	–	–	
Mucor sp. 1	1 (1.2) [R: 1 s] 1 (0.5)	1 (0.6) [R: 1 j; B: 4 s; S: 2 s] 7 (3.5)	– [B: 2 s] 2 (1.2)	– [B: 1 s] 1 (0.9)	
Mucor zonatus Milko	1 (1.2) [R: 1 s; B: 1 s] 2 (1.1)	–	–	–	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Oidiodendron chlamydosporicum Morrall	-	-	2 (2.4) [R: 2 j] 2 (1.2)	–	
Oidiodendron cf. *griseum* Robak	-	-	2 (2.4) [R: 2 j] 2 (1.2)	–	
Oidiodendron cf. *tenuissimum* (Peck) S. Hughes (TRTC 49493, 50672)	1 (1.2) [R: 1 j] 1 (0.5)	3 (2.1) [R: 1 j, 2 s] 3 (1.5)	1 (1.2) [R: 1 j] 1 (0.6)	– [S: 2 s]	
Oidiodendron truncatum Barron	–	– [S: 2 s] 2 (1.0)	–	–	
Paecilomyces farinosus (Holmskjold : Fries) A.H.S. Brown & G. Smith	–	1 (0.6) [R: 1 s] 1 (0.5)	1 (1.2) [R: 1 s] 1 (0.6)	–	
Penicillium citrinum Thom	–	1 (0.6) [R: 1 s] (0.5)	–	–	
Penicillium dierckxii Biourge	–	–	1 (1.2) [R: 1 s] 1 (0.6)	–	
Penicillium expansum Link (TRTC 50625)	–	–	– [B: 2 s] 2 (1.2)	–	
Penicillium islandicum Sopp	–	–	– [S: 1 j] 1 (0.6)	–	
Penicillium janthinellum Biourge (TRTC 49482, 49483, 50653)	–	–	1 (1.2) [R: 1 j; B: 6 j; S: 5 j] 12 (7.6)	–	
Penicillium lanosum Westling	–	–	– [B: 1 s] 1 (0.6)	–	
Penicillium lividum Westling (TRTC 49159, 49474, 50651, 50652)	1 (1.2) [R: 1 s; B: 2 j; S: 3 j] 6 (3.4)	2 (1.4) [R: 1 j, 1 s] 2 (1.0)	– [S: 1 j, 1 s] 2 (1.2)	**5 (6.2)** [R: 1 j, 4 s; B: 2 s; S: 2 s] 9 (8.1)	
Penicillium melinii Thom (TRTC 49486, 50619)	3 (3.6) [R: 3 j; B: 2 j; S: 1 j] 6 (3.4)	– [B: 4 j, 1 s] 5 (2.5)	–	–	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Penicillium miczynskii K.M. Zalessky (UAMH 5593)	2 (2.4) [R: 2 s ; S: 1 s] 3 (1.7)	– [S: 2 s] 2 (1.0)	– [B: 3 s] 3 (1.9)	–	
Penicillium montanense M. Christensen & Backus (TRTC 50648, 50649)	–	6 (4.2) [R: 6 j] 6 (3.0)	**6 (7.1)** [R: 6 s; B: 1 s] 7 (4.4)	–	
Penicillium simplicissimum (Oudemans) Thom	–	–	– [B: 1 j] 1 (0.6)	–	
Penicillium soppii K.M. Zalessky (TRTC 50647)	– [B: 1 j, 2 s] 3 (1.7)	2 (1.4) [R: 2 j] 2 (1.0)	–	–	
Penicillium spinulosum Thom (TRTC 49131, 49158, 49489)	**7 (8.4)** [R: 4 j, 3 s; B: 2 s; S: 1 s] 10 (5.7)	**8 (5.6)** [R: 4 j, 4 s; B: 4 s; S: 1 s] 13 (2.5)	1 (1.2) [R: 1 s] 1 (0.6)	**8 (9.9)** [R: 1 j, 7 s; B: 5 s] 13 (11.7)	for root tips alone 0.05, all isolates 0.01
Penicillium thomii Maire (TRTC 49129, 49155 etc. [9 other specimens])	1 (1.2) [R: 1 s; B: 2 j] 3 (1.7)	4 (2.8) [R: 4 j, 1 s] 5 (2.5)	2 (2.4) [R: 2 s] 2 (1.2)	3 (3.7) [R: 3 s] 3 (2.7)	
Penicillium variabile Sopp (UAMH 6013, 6014)	–	–	1 (1.2) [R: 1 j; S: 2 j] 3 (1.9)	–	
Penicillium waksmanii K.M. Zalessky	–	–	– [B: 1 s] 1 (0.6)	–	
Penicillium sp. 1	–	–	–	– [S: 1 s] 1 (0.9)	
Penicillium sp. 2	–	–	– [B: 1 j] 1 (0.6)	–	
Phialocephala fortinii C.J.K. Wang & Wilcox (UAMH 5901, 5960; TRTC 49481, 49497, 50600)	**20 (24.1)** [R: 6 j, 14 s; B: 2 j] 22 (12.7)	6 (4.2) [R: 3 j, 3 s] 6 (3.0)	**19 (22.4)** [R: 13 j, 6 s; B: 15 j; S: 1 j] 35 (22.2)	**13 (16.1)** [R: 1 j; 12 s] 13 (11.7)	for root tips alone 0.01, all isolates 0.001
Phoma glomerata (Corda) Wollenweber & Hochapfel	–	–	–	– [S: 1 j] 1 (0.9)	
Phoma sp. 1	–	– [S: 5 j] 5 (2.5)	–	–	
Pochonia bulbillosa (W. Gams & Malla) Zare & W. Gams	1 (1.2) [R: 1 s] 1 (0.5)	–	1 (1.2) [R: 1 s] 1 (0.6)	1 (1.2) [R: 1 j] 1 (0.9)	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Pseudogymnoascus roseus Raillo (TRTC 49467, 49473)	–	–	– [B: 6 j] 6 (3.8)	–	
Rhinocladiella atrovirens Nannfeldt	–	– [S: 1 s] 1 (0.5)	–	–	
Rhizoctonia sp. 2	–	–	1 (1.2) [R: 1 j] 1 (0.6)	–	
Sagenomella verticillata W. Gams & Söderström	–	–	1 (1.2) [R: 1 j] 1 (0.6)	–	
Septonema chaetospira (Grove) S. Hughes var. *pini* Bourchier	– [S: 1 j] 1 (0.5)	– [S: 1 s] 1 (0.5)	–	–	
Sesquicillium microsporum (Jaap) Veenbaas-Rijks & W. Gams	–	– [S: 2 j] 2 (1.0)	–	–	
Tolypocladium inflatum W. Gams (CBS 670.83; TRTC 50646, 50647)	–	3 (2.1) [R: 2 j, 1 s; S: 1 s] 4 (2.0)	**5 (5.9)** [R: 5 j] 5 (3.1)	–	
Trichoderma citrinoviride Bissett	– [B: 1 s] 1 (0.5)	–	–	–	
Trichoderma hamatum (Bonorden) Bainier (TRTC [accession # not known])	2 (2.4) [R: 1 j, 1 s] 2 (1.1)	– [S: 1 j] 1 (0.5)	–	–	
Trichoderma koningii Oudemans (TRTC 49476, 49490)	2 (2.4) [R: 2 j] 2 (1.1)	–	2 (2.4) [R: 2 s; B: 2 s] 4 (2.5)	–	
Trichoderma polysporum (Link) Rifai (TRTC 49453, 49478, 49494)	5 (6.0) [R: 4 j, 1 s] 5 (2.8)	5 (3.5) [R: 5 j; S: 1 j] 6 (3.0)	–	–	
Trichoderma viride Persoon : Fries (TRTC 49433, 49435, etc. [5 other specimens])	5 (6.0) [R: 1 j, 4 s; B: 3 s; S: 1 s] 9 (5.2)	3 (2.1) [R: 2 j, 1 s; B: 2 s; S: 1 j] 6 (3.0)	**3 (3.5)** [R: 1 j, 2 s; B: 1 s] 4 (2.5)	– [B: 1 s] 1 (0.9)	
Trichosporon sporotrichioides (van Oorschot) van Oorschot & de Hoog	1 (1.2) [R: 1 j] 1 (0.5)	–	–	–	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
Umbelopsis angularis W. Gams & M. Sugiyama (TRTC 49434, 49449, 49469, 49472, 49475)	**9 (10.8)** [R: 4 j, 5 s; B: 1 j, 1 s; S: 1 s] 12 (6.9)	4 (2.8) [R: 4 s; B: 1 j, 1 s; S: 1 s] 7 (3.5)	**3 (3.5)** [R: 3 s; S: 1 s] 4 (2.5)	–	for all isolates 0.05
Umbelopsis isabellina (Oudem.) W. Gams (CBS 493.83; TRTC 49421, 49426, 49428, 49429, 49447)	**9 (10.8)** [R: 1 j, 8 s; B: 2 j, 1 s] 12 (6.9)	**31 (21.7)** [R: 17 j, 14 s; B: 1 j; S: 2 s] 34 (17.1)	1 (1.2) [R: 1 s] 1 (0.6)	–	for root tips alone 0.001, all isolates 0.001
Umbelopsis nana (Linnemann) von Arx [CBS 669.83 (atypical isolate); TRTC 49491, 50624]	–	–	**3 (3.5)** [R: 3 j; B: 2 j; S: 1 j] 6 (3.8)	–	
Umbelopsis ramanniana (A. Möller) W. Gams (TRTC 49465, 50605, 50643)	1 (1.2) [R: 1 s] 1 (0.5)	6 (4.2) [R: 4 j, 2 s; S: 1 j, 1 s] 8 (4.0)	1 (1.2) [R: 1 s] 1 (0.6)	–	
Umbelopsis vinacea (Dixon-Stewart) von Arx	4 (4.8) [R: 4 j] 4 (2.3)	–	2 (2.4) [R: 1 j, 1 s] 2 (1.2)	–	
Unidentified sp. 1	–	–	–	1 (1.2) [R: 1 j] 1 (0.9)	
Verticillium fungicola (Preuss) Hassebrauk	–	– [S: 1 j] 1 (0.5)	–	– [B: 1 s] 1 (0.9)	
Verticillium leptobactrum W. Gams (UAMH 5951)	–	– [S: 1 s] 1 (0.5)	–	2 (2.5) [R: 2 j] 2 (1.8)	
sterile dark 2	–	1 (0.6) [R: 1 j] 1 (0.5)	–	–	
sterile dark 3	–	1 (0.6) [R: 1 j] 1 (0.5)	–	–	
sterile dark 6	–	–	–	1 (1.2) [R: 1 s] 1 (0.9)	
sterile dark 7	–	–	– [S: 1 s] 1 (0.6)	–	

Table 1. (Continued).

Taxa present (voucher specimen or culture[a])	Site 1 (regenerated forest)	Site 2 (undisturbed forest)	Site 3 (sparsely regenerated clearcut / portable sawmill site)	Site 4 (open *Sphagnum* bog, near margin)	Statistical significance level (p value ≤)
sterile dark 8	–	1 (0.6) [R: 1 j] 1 (0.5)	–	–	
sterile dark 10	1 (1.2) R: 1 j] 1 (0.5)	–	2 (2.4) [R: 2 s] 2 (1.2)	–	
sterile white 2	– [S: 1 s] 1 (0.5)	–	–	–	
sterile white 6	– [B: 1 j] 1 (0.5)	–	–	–	
sterile white 7	1 (1.2) [R: 1 j] 1 (0.5)	–	–	–	
sterile white 8		1 (0.6) [R: 1 j] 1 (0.5)	–	–	
sterile white 10	–	–	–	1 (1.2) [R: 1 j] 1 (0.9)	
sterile white 11		1 (0.6) [R: 1 s] 1 (0.5)	–	–	
Totals	R: 83 (all: 173)	R: 143 (all: 198)	R: 85 (all: 157)	R: 81 (all: 111)	Root tips alone 392; all isolates 639

[a]Voucher cultures or specimens: CBS = culture in Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; TRTC = dried herbarium specimen in Mycology Herbarium, Royal Ontario Museum, Toronto, Ont., Canada; UAMH = culture and dried herbarium specimen in University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, AB, Canada.

[b]Notations and symbols in the data: R = roots, i.e., serially washed ectomycorrhizal root tips, B = bark, i.e., serially washed fine feeder root bark, S = soil of adherent rhizosphere as obtained in 1st, 5th and 20th serial washing steps carried out with previously rinsed root segments. Numbers before square brackets refer to isolates from washed root tips (R) alone and indicate the number of isolates, followed in parentheses by the percentage of total isolates obtained from root tips within that site. Numbers within square brackets give a breakdown of isolates from R, B and S for each of two seasonal sampling times, June (j) and September (s), e.g., "R: 1 j, 2 s " indicates 1 isolate from root tips in the June sample and 2 from root tips in the September sample. Numbers after the square brackets give the overall total number of isolates from the site from R+B+S, with the percentage of the total isolates for the site following in parentheses.

[c]For isolates from R only, numbers and names for the five most common species at each site are highlighted in bold; in case of ties for a place in the rank order, all isolates at the tied value are bold-faced.

ectomycorrhizal root segments is given by Harley & Waid (1955).

Plates were incubated at 5 ºC for 14 d, then moved to 18 ºC and incubated an additional 28 d, with contamination-minimising subculture of colonies done throughout the period. Numbers of colonies obtained from the bark and wash samples varied, as did the number of mycorrhizal root tips yielding colonies, but ultimately, the composite samples from each site were composed of 50-75 % isolates from mycorrhizae, with the remainder consisting of bark and wash isolations in relatively equal proportions.

Most fungi were identified following Domsch *et al*. (1980) with updated information from recent literature. *Penicillium* spp. were preliminarily identified using the media and techniques described by Pitt (1979), though for final species identification all applicable literature was consulted. Names have been updated to the present date where possible, based not just on following nomenclatural changes but also on continued employment of notes made and species concepts developed while this study was in progress. So far, however, only *M. variabilis* isolates from this study appear to have been sequenced (see Hambleton & Sigler 2005–this volume). Voucher cultures and specimens for the species obtained are listed in Table 1.

Analysis of data

The data analysed in the present study includes a portion of the data treated by Summerbell (1989) as well as previously unpublished data. The assemblages of fungal isolates obtained from the various sites were compared by means of a similarity index (C) (Bray & Curtis 1957). This procedure was done with the composite samples; samples from washed mycorrhizae alone gave highly similar results (data not shown). In order to provide a rough internal control as to what level of similarity in this index would indicate a similar site, the June and September samples were kept separate in this analysis. The contrast between within- and among-site similarities was also designed to control for anomalies deriving from the individual trees and microsites chosen for sampling; it was expected that within-site similarities would be highest, especially in the deeply forested sites in which microclimate was moderated by canopy cover, followed by between-site similarities for the similar sites 1 and 2. Similarities between the extreme sites 3 and 4 and between these sites and the moderate sites 1 and 2 were expected to be relatively low. For taxa present in sufficient numbers to be statistically tested, differences within the washed-mycorrhiza sample and the composite sample were assessed by the chi-square test following Söderström (1975); to attain sufficient numbers, this analysis was done combining the seasonal results from each site.

Associational structure within the data of the (numerically maximal) composite sample was further explored by means of nodal components analysis [NCA; Ecosurvey, T. J. Carleton, Dept. of Botany, University of Toronto (Carleton 1985)]. The analysis was conducted using a noncentred principal components analysis followed by varimax rotation of the axes (Noy-Meir, 1971; Carleton, 1980, 1985; Carleton & Maycock, 1980). Data from NCA are presented in the form of principal component loadings postnormalized to fall on a scale between 1 and –1.

RESULTS

Table 1 shows the isolates obtained in all procedures. In the Table, each entry representing a particular species at a particular site first gives the number of isolates of the species obtained from washed mycorrhizae alone, and then follows this, in non-zero cases, with the percentage of total washed-mycorrhizal isolations at the site that are accounted for by this species. Next, in square brackets, a breakdown is given of the number of isolates of the species obtained from roots (R), bark (B) and wash soil (S) in the June and September samples (represented by small letters j and s after isolation numbers). Finally, after the material in square brackets, the total number of isolates of the species from the site is given, followed by its corresponding percentage of all isolates from the site. The five most common species from washed mycorrhizae at each site are indicated by placing the relevant numerical values in bold-face type; in one case where three species are tied for the fifth-most-common rank, the species involved are all indicated with bold-face numbers. Names of species attaining top-five rank from mycorrhizae at any site are also bold-faced.

All four sites investigated gave rise to highly distinctive arrays of isolates from washed mycorrhizae. Site 2, the undisturbed forest site, was characterized by particularly high levels of *Umbelopsis isabellina* (Oudem.) W. Gams, as well as considerable *M. variabilis* and *Mortierella verticillata* Linnem. *Umbelopsis isabellina* fell in proportion by over 50 % in the regenerated forest site, site 1, but remained one of the most common isolates. This site also featured *M. variabilis*, *Umbelopsis angularis* W. Gams & M. Sugiyama and *Trichoderma viride* Pers. : Fr., but the most commonly seen isolate was *P. fortinii*, which had been uncommon at site 2. *Mortierella verticillata* remained common at site 1, but mainly from root bark rather than ectomycorrhizae. Site 3, the highly disturbed, clearcut site, yielded a great predominance of *P. fortinii*, but also other distinctive fungi seldom or not at all isolated at other sites, such as *Fusarium proliferatum* (Mats.) Nirenb. and

Table 2. Matrix of Bray and Curtis similarity index values for fungal assemblages isolated from washed *Picea mariana* ectomycorrhizae in two seasonal samplings at boreal forest sites 1 (disturbed forest), 2 (undisturbed forest), 3 (highly disturbed portable sawmill site) and 4 (*Sphagnum* bog). Axes are labelled with site # followed by sampling month. Within-site comparisons are bold-faced.

Dates	1 (Sept)	2 (Jun)	2 (Sept)	3 (Jun)	3 (Sept)	4 (Jun)	4 (Sept)
1 (Jun)	**0.49**	0.30	0.29	0.23	0.22	0.14	0.27
1 (Sept)		0.34	0.45	0.22	0.18	0.23	0.36
2 (Jun)			**0.53**	0.05	0.29	0.08	0.19
2 (Sept)				0.07	0.19	0.26	0.36
3 (Jun)					**0.06**	0.04	0.10
3 (Sept)						0.06	0.09
4 (Jun)							**0.55**

Umbelopsis nana. *Penicillium montanense* M. Chr. & Backus and *Tolypocladium inflatum* W. Gams were also unusually common. Most common species other than *P. fortinii* were distinctly seasonal in occurrence at this site. Site 4, the peat bog site, was outstanding in the predominance of *M. variabilis*, which made up over one-third of isolates. Also prominently present were *Mortierella pulchella* Linnem., a species only uncommonly found at other sites, and the somewhat more evenly distributed *P. fortinii*, *Penicillium lividum* Westl. and *Penicillium spinulosum* Thom.

In the composite samples, with fungi from root bark and root washing included along with isolates from ectomycorrhizae, some additional distinctive trends emerged. Particularly in the clearcut site 3, distinctive species such as *Penicillium janthinellum* Biourge and *Pseudogymnoascus roseus* Raillo were seasonally common from bark. Members of the *Geomyces pannorum* (Link) Sigler & J.W. Carmichael complex, particularly the greyish *Geomyces vulgaris* Traaen, were common in the spring soil washings at site 1. More than half of *P. lividum* isolates (11/19), a species present at all sites, were from bark or soil rather than mycorrhizae, even though the overall ratio of bark+soil: washed mycorrhiza isolates in the combined sample was approximately 5 : 8. In apparent contrast, 24/37 *P. spinulosum* isolates (65 %) were from mycorrhizae, as were 10/13 *Penicillium thomii* Maire isolates (76.9 %); however, values for these *Penicillium* species from mycorrhizae vs. combined bark and soil samples are not significantly different in chi-square testing.

As detailed above, several analytical techniques were used to analyse the distribution of isolates across the four sites, including chi-squared analysis, similarity comparisons, and a variant of principal components analysis.

Of the 97 filamentous fungal taxa recognised among the 639 isolates obtained from composite samples at the four test sites (including a few species highly filamentous in appearance but arguably classifiable as yeasts, e.g., *Candida ciferrii* Kreger-van Rij, seen as its *Sporothrix* synanamorph), only six were isolated in high enough numbers to be individually testable by chi-squared analysis for significant differences among all sites. All six did show significant differences (Table 1, right column). The single most common species overall, *P. fortinii*, was most abundantly isolated from the most disturbed site, clearcut site 3; it was by far the most common species from this site, making up 22.2 % of isolates. It also made up approximately 12 % of isolates at the peat bog site, site 4, and the regenerated forest site 3, but was relatively uncommon in the undisturbed forest site 2, making up only 3 % of isolates. The second most common fungus overall was *M. variabilis*; it was particularly predominant in the peat bog site, site 4, making up 29.7 % of isolates, but also made up approximately 7 % of isolates from the two fully forested sites, sites 1 and 2, and a minor 1.2 % of isolates from the clearcut site. The third most common fungus, *U. isabellina*, was mainly associated with the two fully forested sites 1 and 2, while the fourth, *P. spinulosum*, was most strongly associated with the clearcut site (11.7 % of isolates) and also relatively strongly with the regenerated forest (5.7 % of isolates). Of the other two testable fungi, *M. verticillata* was mainly associated with the fully forested sites, while *U. angularis* was moderately common at all sites except the peat bog site, which yielded no *Umbelopsis* isolates whatsoever. If the chi-square analysis of site differences was restricted to isolates from mycorrhizae alone, significant differences were seen as for the composite samples, sometimes with an incremental change to a higher or lower significance level. *Mortierella verticillata* and *U. angularis*, however, were not isolated from enough root tips to be validly tested in the restricted sample.

When sites 1 and 3 were combined in chi-squared analysis as sites visibly showing signs of previous logging disturbance, in apposition to 2 and

Table 3. Nodal components analysis of spruce rhizosphere fungal populations in seasonal at various boreal forest sites. Values are derived from non-centred principal components analysis with subsequent Varimax rotation of axes (see text). Part 1. Ranked, normalised loadings for individual seasonal samplings at sites 1 (disturbed forest), 2 (undisturbed forest), 3 (highly disturbed portable sawmill site) and 4 (*Sphagnum* bog). To make trends in the data easier to discern, loading values with an arbitrarily chosen, approximately median (absolute) value of 0.15 or above are in bold-face.

Principal component number							
1		2		3		4	
sample	loading	sample	loading	sample	loading	sample	loading
4 (Jun)	**–.847**	3 (Jun)	**.801**	2 (Sept)	**–.533**	3 (Sept)	**.910**
4 (Sept)	**–.476**	1 (Sept)	**.238**	2 (Jun)	**–.526**	1 (Sept)	**.278**
2 (Sept)	**–.155**	4 (Sept)	**.200**	1 (Sept)	**–.306**	3 (Jun)	**.188**
1 (Sept)	**–.150**	1 (Jun)	.118	1 (Jun)	**–.221**	1 (Jun)	**.167**
3 (Jun)	–.072	3 (Sept)	.081	4 (Jun)	**–.154**	2 (Jun)	.090
1 (Jun)	–.040	2 (Sept)	.037	4 (Sept)	.133	2 (Sept)	.078
3 (Sept)	–.012	2 (Jun)	.026	3 (Sept)	.087	4 (Sept)	.066
2 (Jun)	.006	4 (Jun)	.021	3 (Jun)	.024	4 (Jun)	.025

4 as apparently undisturbed sites, it was seen that *F. proliferatum* was significantly associated with the former type of site ($p < 0.001$), as was *P. fortinii* ($p < 0.001$). *Meliniomyces variabilis* was equally significantly associated with undisturbed sites in this calculation, but this result may to some extent be an artifact of its high affinity for the peat bog site, since it was almost equally prevalent at the disturbed and undisturbed forest sites 1 and 2.

In similarity analyses (Table 2), as mentioned above, data were analysed seasonally in respect to June and September isolation times. As Table 2 shows, sites 1, 2 and 4 showed approximately 50 % self-similarity between seasons. Such a result is expected at sites resampled and compared with the Bray & Curtis index, which deducts all fortuitous differences in isolate numbers, for a given species obtained in parallel samples, from the overall value given for similarity. All species common in June samples at sites 1, 2 and 4 were also common in September samples, and chi-squared tests for seasonal differences at these sites yielded almost entirely insignificant results (data not shown), except that *M. variabilis* was significantly more commonly obtained in September at site 2 and in June at site 4. Site 3, the highly sun-exposed clearcut site, deviated strongly from this pattern of seasonal stability by showing only 6 % within-site similarity in seasonal samples. *Fusarium proliferatum* was only isolated in September, and this result was significant ($p < 0.01$), while *P. fortinii* was significantly more commonly isolated in June ($p < 0.001$). Several fungi not numerically predominant enough for individual chi-squared analysis, such as *P. roseus* and *P. janthinellum*, were only isolated in June, while other such fungi such as *Penicillium montanense*, *U. angularis* and *Trichoderma koningii* Oudem., were only isolated in September. These apparent differences are mentioned because they may collectively have had a strong effect on similarity results.

Similarity values among sites showed that fully forested sites 1 and 2 overall were highly similar to one another, with values ranging from 29–45 % similarity (with the high end of that range approaching within-site similarities), even across seasons. The clearcut site 3 showed similarities in an intermediate 18–29 % range with the two fully forested sites except in its June sample, which was markedly distinct from the June sample taken at undisturbed forest site 2. Site 3 showed very little similarity with the peat bog site 4. Site 4 showed mainly intermediate similarities of 14–36 % with the fully forested sites, with one exception (site 2 June vs. site 4 Jun, a difference partially accounted for by heavy isolation of *M. variabilis* in the latter sample and sparse isolation in the former).

The differences seen among sites and seasons in isolations from washed mycorrhizae tended strongly to be reflected in isolations from bark and surrounding rhizosphere soil, except that the normally nonsporulating root fungi *P. fortinii* and *M. variabilis* were seldom obtained from soil and only the former was commonly isolated from bark in at least one study site.

NCA of the sites (Tables 3, 4) yielded a first principal component strongly correlated both with the peat bog site 4 itself (Table 3) and with fungi notably common at this site, such as *M. variabilis*, *P. fortinii*, *M. pulchella* and *P. spinulosum* (see Table 4, showing NCA values for the ten most common fungi in the study as a whole, plus all species having a high loading value on any of the principal components). A second principal component was mainly associated with samples, such as the June site 3 sample and the September site 1 sample, in which *P. fortinii* isolation was markedly high; the next most strongly associated fungus was *M. variabilis*.

Table 4. Nodal components analysis of spruce rhizosphere fungal populations in seasonal at various boreal forest sites. Part 2. Normalised loadings of fungal taxa on principal components. The four highest loading values (in absolute value) on each component are in boldface.

	Principal component number			
Taxon	**1**	**2**	**3**	**4**
Fusarium proliferatum	–.007	.034	–.042	**.437**
Meliniomyces variabilis	**–.857**	**.217**	**–.407**	.179
Mortierella pulchella	**–.288**	.059	–.099	.042
M. verticillata	–.028	.058	–.152	.119
Penicillium janthinellum	–.016	**.208**	–.006	.069
P. lividum	–.133	.077	–.091	.097
P. montanense	–.001	.017	–.075	**.242**
P. spinulosum	**–.205**	.125	**–.267**	.161
Phialocephala fortinii	**–.271**	**.857**	**–.447**	**.614**
Pseudogymnoascus roseus	–.008	.104	.003	.034
Tolypocladium inflatum	–.016	.128	–.080	.057
Trichoderma viride	–.043	.091	–.147	.191
Umbelopsis angularis	–.041	.079	–.176	**.232**
Umbelopsis isabellina	–.075	**.214**	**–.573**	.218

The third principal component was strongly linked to the two fully forested sites 1 and 2 and their typical association of *U. isabellina*, *P. fortinii*, *M. variabilis*, and *P. spinulosum*. Finally, principal component 4 was most strongly associated with September samples at the two disturbed sites, especially the clearcut site 3, with highest loading values for *P. fortinii*, *F. proliferatum*, *P. montanense* and *U. angularis*.

DISCUSSION

Molecular biosystematic studies have now succeeded in clarifying the identities of a number of biologically important fungi that are normally isolated as nonsporulating mycelia from roots of trees and other plants, and this has allowed researchers to confidently assign taxonomic names to these fungi. Included are the two most common species isolated in the present study, *P. fortinii* and *M. variabilis*. *Phialocephala fortinii* was in fact described purely on the basis of morphology by Wang & Wilcox (1985), following up on the discovery of Gams (1963) that such isolates occasionally sporulated as a *Phialocephala*, as well as on the later discovery of Richard & Fortin (1973) that a proportion of isolates would reliably do so after 6 mo at 5 ºC. The ability, however, to link the name *P. fortinii* phenotypically, with no more presumption than is used in many other morphological identifications, to the many completely nonsporulating but otherwise-identical isolates that are obtained from the same habitats, has been facilitated by extensive molecular studies such as those of Addy *et al*. (2000) and Grünig *et al*. (2001, 2002, 2004). As with many current names, *P. fortinii* has been revealed as having been applied to several entities potentially definable as cryptic species in a closely related species complex (Grünig *et al*. 2004). Even those cryptic species, however, may become morphologically recognisable after correlation of morphology with molecular data has been done (Grünig 2003). In the present study, just a small number of the *P. fortinii* isolates obtained could be induced to sporulate, but since it was not practical to do supplementary molecular identification, application of the name to other isolates was restricted to those that were strictly morphologically identical in all features except this sporulation. This rather conservative procedure would certainly have excluded biotypes such as Grünig's "type 1" (Grünig *et al*. 2001, 2002), a distinct genetic group closely related to *P. fortinii* but forming little aerial mycelium in culture. At the present study sites, however, such deviating sterile dark isolates were very uncommon. Criteria for recognising sterile isolates of *P. fortinii* morphologically have been given by Currah & Tsuneda (1993). This species in the past usually made up most or all the isolates referred to in various studies as *Mycelium radicis atrovirens*, and our ongoing investigations at CBS show that many historic collection isolates retained under this name, such as those of Levisohn (1954), can indeed be molecularly confirmed as *P. fortinii* (unpubl. data).

Meliniomyces variabilis, newly described by Hambleton & Sigler (2005) in the present volume, is an easily recognizable but, so far as is known, completely

nonsporulating taxon that has previously been referred to by several tentative names, including both "sterile white 1" and "sterile dark 1" (Summerbell, 1989), D. S. type 2 (Schild *et al*. 1988) and Variable White Taxon (Hambleton & Currah 1997). Typical isolates from the present study sites appear in the molecular analysis of Hambleton & Sigler (2005–this volume).

Both *P. fortinii* and *M. variabilis* show isolation patterns typical of potentially root-penetrating, endophytic or endorhizosphere fungi, particularly in their regular outgrowth from surface-sterilized roots (Summerbell 1989, Hambleton & Currah 1997, Jumpponen & Trappe 1998). The former species has been demonstrated to penetrate roots of many plant species, forming hyphae, microsclerotia and, in some cases, mycorrhiza-like structures within root tissues (Melin 1921, 1923, Richard & Fortin 1974, Currah *et al*. 1993, Stoyke & Currah 1993, Jumpponen *et al*. 1998, Jumpponen & Trappe 1998, Sieber 2002). Some isolates of the latter fungus have been shown to penetrate roots of *Picea* and of ericoid plants, apparently harmlessly, as is summarized by Hambleton & Sigler (2005–this volume), while others appear merely to partially ensheathe root surfaces (Summerbell 1987). Despite the access of these two species to the somewhat uniform and controlled conditions found within plant tissues, they nonetheless, as is underlined by the present study, appear to be very responsive to site conditions limiting their distribution. With *P. fortinii*, this may relate to extensive colonization of microhabitats outside the vital regions of roots, particularly colonization of root bark, as seen at one site in the present study and also in various studies done elsewhere [Mańka & Truszkowska 1958, Mańka & Rząsa 1961, Mańka & Gierczak 1961, Mańka *et al*. 1968a, b, Kowalski 1974a, b, 1980a, see also the fungi labelled "SDF" and "DRP" by Parkinson & Crouch (1969), and Whitney (1962), respectively]. *Phialocephala fortinii* is also reliably isolated from washed organic or mineral soil particles from various forest soil types (Gams 1963, Söderström & Bååth 1978). With regard to the occurrence of *M. variabilis* outside roots, the situation is not so clear, as discussed below.

The distribution of *P. fortinii* in our study was consistent with what has been seen in some other studies: this species, while broadly distributed, was distinctly favoured (or at least readily isolated) in disturbed, humus-poor soils as compared to the mature humus-rich podzols typical of boreal forests. Söderström & Bååth (1978) and Bååth (1981) found *P. fortinii*-like *Mycelium radicis atrovirens* (hereafter PFMRA) isolates in Swedish *Picea abies* (L.) H. Karst stands to be obtainable mainly from soil layers beneath the humus layer. Similarly, Richard & Fortin (1974) found PFMRA (partially confirmed morphologically as *P. fortinii*) to form so-called pseudomycorrhizae on spruce seedlings in the disturbed soils of tree nurseries, but not in undisturbed spruce forest soils, while Hambleton & Currah (1997) obtained *P. fortinii* more commonly from ericaceous roots in a forested sand dune area than in nearby alpine heath and bog areas. The generalization linking the *P. fortinii* complex to areas relatively free of organic buildup should not be thought to apply to all forest and edaphic zones: PFMRA, which grows optimally at pH 4 (Mańka 1960), is well known to colonize roots mainly in the more acidic upper soil layers in soil types where the lower horizons are near-neutral in pH, as in many areas of Poland and Germany (Mańka & Rząsa 1961, Holdenrieder & Sieber 1992, Sieber 2002). In near-neutral soils, fungal communities antagonistic to PFMRA and conducive to *Cylindrocarpon destructans* (Zins.) Scholt. may develop (Mańka *et al*. 1968a, 1968b, Kowalski 1980b). The distribution of PFMRA is thus of particular interest since this fungus, whether just through its tendency to indicate areas low in *C. destructans* or through other factors, seems to be an indicator of good sites for the regeneration of both ectomycorrhizal (Kowalski 1980b, 1982; compare also Levisohn 1960, Bloomberg & Sutherland 1971) and endomycorrhizal (Mańka *et al*. 1968a, 1968b) conifers. As ecological microarrays and similar techniques begin to make microbial soil health profiling accessible, such factors may become steadily more prominent in fields such as silviculture.

Some of the fungi co-occurring with *P. fortinii* also showed a pattern consistent with the idea that the presence and integrity of the humus layer was a principal factor influencing the patterns of fungal site distribution seen in this study. *Penicillium janthinellum*, for example, co-occurred strongly with *P. fortinii* in the clearcut site 3, forming a pattern reflected to some extent in principal component 2. This fungus is not typically found in undisturbed northern podzols, but "appears in large numbers when these soils are brought under cultivation" (Mirchink *et al*. 1981) or otherwise disturbed. A typical record is that of Carter (1978), who found *P. janthinellum* to be one of the most common species in sites damaged by metal pollution near Sudbury, Ontario, Canada. In undisturbed soils, *P. janthinellum* is typical of more southerly soil types such as chernozems (Mirchink *et al*. 1981), that is, black grassland prairie soils of the type predominant in Russia. In forest soils, it may be excluded from the fermentation layer (Widden & Parkinson 1973). When previously found in association with spruce, it was mainly isolated from suberized root surfaces (Mańka & Truszkowska 1958), as in the present study.

In principal component 4, reflecting in part the pattern of isolations seen at the clearcut site 3, especially in early autumn, *P. fortinii* is prominently associated with *F. proliferatum* and *U. angularis*.

The former associate, a member of a *Fusarium* group long subject to taxonomic confusion, is little known from northern soils, but, in general, fusaria tend to be inhibited in coniferous forest soils wherever there is a buildup of litter and litter-inhabiting microbiota (Schisler & Linderman 1984). There were also some indications of mineral soil-associated mycota in the spring sample from site 3. *Umbelopsis nana*, in particular, was a frequent isolate from both roots and from a dilution of bulk soil plated out as an auxiliary study (data not shown). This species appears to be an indicator of mineral soil habitats in many northern forests (Wicklow & Whittingham 1974, Söderström 1975, Bååth 1981), supporting the general picture of site 3 as a site where the presence of mineral soil mostly stripped of humus was a strong influence on fungi of the mycorrhizosphere. A microfungal assemblage evocatively similar to that of site 3, especially the spring sample, was seen in the study of Bååth (1981) on soil fungi of a Swedish clearcut pine forest. In that study, the lower soil layers (A2 and B) yielded PFMRA, *U. nana* (listed as *Mortierella nana* Linnem.), and *Sagenomella verticillata* W. Gams & Söderström as the most common species. In our study, *S. verticillata* was isolated only once, but was from a sample also yielding *U. nana* and abundant *P. fortinii*. The seasonality seen with *U. nana* isolation in the present study has also been seen elsewhere: this species was found by Mańka & Gierczak (1961) to be abundant on healthy and decaying pine roots in spring, but to decline drastically in numbers over the course of the Polish summer. On the other hand, Bååth (1981) found high numbers of *U. nana* isolates in Swedish clearcut soils in September. *U. nana* may undergo a seasonal decline only in relatively warm, sun-exposed sites, or perhaps only in association with roots. In overview, the following fungi obtained in the spring clearcut site have been found to be significantly associated with mineral soil or the lower A horizons in acid soils: PFMRA (Söderström 1975), *Oidiodendron chlamydosporicum* Morrall [called *Oidiodendron* sp. 1 in Söderström 1975, later described under the synonymous name *O. scytaloides* W. Gams & Söderström – see Rice & Currah (2005–this volume)], *P. roseus* [see *Gymnoascus roseus* (Raillo) Apinis in Wicklow & Whittingham 1974], *S. verticillata* (Bååth 1981), *U. nana* (see *Mortierella nana* in Wicklow & Whittingham 1974, Söderström 1975, Bååth 1981) and *U. vinacea* (Dixon-Stewart) Arx (see *Mortierella vinacea* Dixon-Stewart in Wicklow & Whittingham 1974, Söderström 1975). Taking these fungi into consideration with *F. proliferatum* from the autumn sample, and an overall picture of a mycorrhizosphere and proximal rhizosphere effect due to low-humus, high mineral-content soils is inescapable. It is clear that though the presence of such soils may increase isolation of *P. fortinii*, especially from bark and adherent soil but also from root tips, they are unlikely to be a prerequisite for the occurrence of *P. fortinii* in root tips. At least some *P. fortinii* was obtained from ectomycorrhizae at all sites.

On the opposite side of the coin, high-humus sites in the present study were linked to *U. isabellina*, *M. variabilis*, *M. verticillata*, *Mucor silvaticus* Hagem, and, in general, principal component 3. *Phialocephala fortinii* was present but was relatively infrequently isolated. *Umbelopsis isabellina* and another fungus well represented in the high-humus, undisturbed forest site 2 in the present study, *P. spinulosum*, were also typical of humic layers in the spruce forest data of Söderström & Bååth (1978) as well as the pine forest study of Bååth (1981). Similar findings have also been obtained in other studies (Wicklow & Whittingham 1974), including Canadian studies (Singh 1976). *Umbelopsis ramanniana* (A. Möller) W. Gams also appeared to be most abundant in the undisturbed forest site, though its numbers were too low to allow statistical testing. This fungus, regarded as a specific indicator of podzolic soils by Mirchink *et al*. (1981), appears, according to that author, to be most abundant where continuous cover by conifers has profoundly affected the podzolization process. With regard to *P. fortinii*, this species at site 2 was isolated only from mycorrhizae, and though the isolates obtained could have derived from individual rootlets that had grown out of the humus layer, this layer was relatively deep at that site. The most plausible interpretation may be that humus does not prevent endophytic growth of *P. fortinii*, but does provide biotic or chemical conditions that severely limit the ability of this species to grow in or across feeder root bark.

Relatively few data are available on the distribution of *M. variabilis*, but a clear similarity is seen between the present results and those of Hambleton & Currah (1997): these authors, if one does calculations based on their data, can be seen to have found that this fungus grew from 40 % of ericaceous root collections in a bog site, as opposed to 8.8 % of collections in a sand dune area and 13.2 % of collections in an alpine heath. In the present study, the overwhelming predominance of this fungus from washed ectomycorrhizae at the peat bog site by no means indicates restriction to similar sites, as the fungus was also one of the most common taxa associated with ectomycorrhizal root tips in the regenerated and undisturbed forest sites, and was not excluded from the clearcut site. *Meliniomyces variabilis* has been observed growing on peat moss *in vitro* (Summerbell 1987, Piercey *et al*. 2002) and has pectinolytic ability, though no known cellulolytic ability (Summerbell & Malloch 1988), but despite these preliminary signs of saprobic competence, it was scarcely isolated in the present study from materials

other than root tips. Only in the peat bog site were two isolates obtained from bark, and no isolate was obtained from adherent rhizosphere soil excepting a single isolate from a *Picea glauca* site, similar to site 1, that was not included in the present study on *P. mariana* roots. Though as a nonsporulating organism, *M. variabilis* might have been largely excluded from wash water through its lack of disseminable propagules, there is no obvious explanation for why it would not regularly grow out from mixed mycelial and chlamydospore inoculum in bark if it was present in or on that material. Also, since small fragments of peat-derived organic material would be expected to be present in the wash water from site 4, and since mutual colonial overgrowth was not a major problem in root wash plates from this site, *M. variabilis* would have been expected to grow out if it were extensively colonizing the peat. This fungus, then, appears likely to be the most purely endophytic fungus encountered in the present study. Its ability to grow within root tissue was repeatedly demonstrated by isolation from roots surface-sterilized with $HgCl_2$ (Summerbell 1989). At the study sites, it was not restricted to *Picea* species, but also grew from serially washed roots of the endomycorrhizal species *Cornus canadensis* (Summerbell 1989), which only occurred at sites 1, 2 and 3. The study of Summerbell (1989) was thus the first of three (see also Hambleton & Currah 1997, Hambleton & Sigler 2005–this volume) so far consistently showing that *M. variabilis*, like *P. fortinii*, appears to be a root endophyte with a broad distribution among plant hosts from various different families.

The associates of *M. variabilis* in the peat bog site together constituted the most distinct assemblage of fungi seen at any of the study sites. They were grouped together with relatively strong loading values on the most strongly supported principal component, component 1. The composition of the washed root and rhizosphere assemblage, with *M. pulchella* and *P. spinulosum* both present in relatively high density, was notably similar to the fungal assemblages found by Nilsson *et al*. (1992) in bulk peat sampled from relatively dry parts of an ombrotrophic (i.e., purely precipitation-nourished) bog site in northern Sweden. Interestingly, the "dry" community studied by these authors was found in the upper parts of bog hummocks, whereas lower, wet ombrotrophic sites had distinctly different communities. The roots in the present study were from a relatively level and always palpably moist area of the bog. Our site, however, was in the open, near the bog margin, and it did not yield pools of water when trodden upon; thus it may have been relatively dry.

Little is known about fungi associated with ectomycorrhizal roots in bogs, and it is worthwhile to give our results a context within the modest amount of data that have been obtained for peat bog microfungi in general. As would be expected, it would appear that fungal assemblages of relatively southerly peat bog sites are less similar to the present study sites than were the northern Swedish sites. However, there are also some interesting similarities. Christensen & Whittingham (1965) investigated the soil fungi of mesic *P. mariana* stands in boggy sites in Wisconsin. These authors listed the characteristic species of these stands as *Mortierella* (= *Umbelopsis*) *vinacea*, *Periconia* sp., *Penicillium odoratum* M. Christensen & Backus (= *P. lividum*), *Oidiodendron flavum* von Szilvinyi [possibly = *O. griseum* (Sigler & Gibas 2005–this volume) though morphologically marginally separable from it (Rice & Currah 2005–this volume)], *Cephalosporium* (probably = *Acremonium*, *Pochonia* or *Lecanicillium*) sp., *Cryptococcus laurentii* (Kufferath) C.E. Skinner, and several other species known only by culture numbers. Although, overall, this list strongly diverges from the list of bog species obtained in the present study, one component, *P. lividum*, was common on roots in our peat bog site, especially in the September sample, but was not recorded from the Swedish bogs studied by Nilssen *et al*. (1992). It may represent a relatively 'southern' element in the mycota of our study area, with its occurrence enhanced by the warm summer months. It was also found commonly by Moore (1954) in a study of peats in the relatively temperate Dublin Mountains of Ireland. *Oidiodendron flavum* and the genus *Periconia*, frequent in Wisconsin as noted above, were not represented in the present study at all (unless our *O*. cf. *griseum* from the clearcut site was conspecific with it in a morphologically and ecologically variable species). Most historic identifications of *Oidiodendron* species, however, need to be treated with some caution (see Rice & Currah 2005–this volume). Christensen & Whittingham's sites also differed by lacking *M. pulchella* and by yielding few isolates of *P. spinulosum*, showing that the overall correspondence between mycotas of these sites and those of more northerly peats was low. These authors did not try to identify nonsporulating fungi, but it should be noted that a fungal taxon listed as "*Mycelia sterilia* 3157" was the most frequently occurring species in open bog sites. Unfortunately, no diagnostic characters were recorded for this taxon.

Mortierella pulchella, although not recorded in Wisconsin (Christensen & Whittingham 1965), was found (as *M. sossauensis* Wolf) to be common in Irish peats (Dickinson & Dooley 1969), just as it was on roots and rhizospheres from peat in the present study. Apart from the examples already mentioned, previous studies on various peat and *Sphagnum* habitats have frequently shown the presence of a substantial portion of the fungal types seen in the present study, especially *P. spinulosum* (Moore 1954, Dickinson &

Dooley, 1969) *P. thomii* (Bisby *et al*. 1935, Moore 1954, Christensen & Whittingham 1965, Dickinson & Dooley 1969), *Oidiodendron* isolates resembling *O. tenuissimum* (Peck) S. Hughes, *O. griseum* Robak or *O. maius* Barron (Christensen & Whittingham 1965; see also comments on *O. griseum* in Dickinson & Dooley 1969) and, curiously, *Mortierella alpina* Peyr. (Dickinson & Dooley 1969, Holdenrieder & Sieber 1992, Thormann *et al*. 2001, 2003), even though this last species is normally considered an indicator of alkaline sites (Luppi Mosca 1972, Domsch *et al*. 1980).

It was striking that all members of the genus *Umbelopsis*, including *U. isabellina*, the most abundant sporulating fungus in the study, appeared to be excluded from the peat bog site. *Umbelopsis* species have been recorded, mostly under their previous *Mortierella* names, as common in some peat bog sites and as uncommon or rare in others. *Umbelopsis isabellinus*, as *Mortierella isabellina* Oudem., was listed as common by Bisby *et al*. (1935) in Manitoba acid peats and was also occasionally found by Dickinson & Dooley (1969) in Irish peat. Christensen & Whittingham (1965), on the other hand, found it to be absent from four of five open bog sites. It was more common in boggy black spruce-tamarack stands, and still more common in drier cedar-fir sites. Holding *et al*. (1965) found the fungus in humic and degraded iron podzols, but not in nearby peats. Thormann *et al*. (2003) found it to be associated with one of several microsite-specific substrates they examined in a Canadian riverine sedge fen, namely *Carex aquatilis* Wahl. rhizomes, but also to be one of the elements characteristically involved in early decay of *Sphagnum fuscum* (Schimp.) Klinggr. at their fen sites. In the Swedish study of Nilsson *et al*. (1992), *U. isabellina* was relatively common in wet bog sites but very uncommon in the dry ombrotrophic site type that was otherwise mycologically similar to the present study's site 4. This association with wet sites is surprising in that, for example, in Singh's (1976) study of Newfoundland forest plantations, both *U. isabellina* and *U. ramanniana* were present only in well-drained humus, and absent from moist humus. As for *U. ramanniana*, it was common in the hill peats studied by Moore (1954) and also in one of two Scottish lowland peat bogs examined by Holding *et al*. (1965). It was, however, rare in the studies of Christensen and Whittingham (1965) and Dickinson & Dooley (1969). Apparently, *U. isabellinus* and *U. ramanniana* can both respond to certain local conditions, possibly involving complex microbial competition patterns rather than simple physical factors, and can grow in some but not all peat and other *Sphagnum* sites of widely varying moisture content.

A similar complexity of site distribution is shown by *P. fortinii*/PFMRA in peat and other *Sphagnum* sites. In most studies of northern peats, in contrast to those of northern soils, this species complex is simply not mentioned, nor is any compatible sterile fungus described. Moore (1954) did record the presence of dark sterile fungi from Irish upland blanket peats; however, Dickinson & Dooley (1969) noted that the sterile fungi predominant on serially washed roots of *Juncus* sp. and other herbaceous bog plant roots in Irish raised bog peats were hyaline types. Nilsson *et al*. (1992) studying Swedish peat, found PFMRA to be most common in wet sites at lower (10–20 cm) depths and quite uncommon in dryer sites. On roots, Schild *et al*. (1988), studying *Picea sitchensis* (Bong.) Carr. plantations in Ireland, and Hambleton & Currah (1997), studying boreal *Ericaceae* in Alberta, Canada, found *P. fortinii*/PFMRA to be very uncommon from roots in peaty soils and bog sites, respectively, but more common from drier sites. Addy *et al*. (2000), using the same surface sterilization procedures as those used by Hambleton & Currah (1997), namely 3 min of 1 % sodium hypochlorite, found *P. fortinii* to be relatively common on both ericaceous and *Carex* roots in sites characterized as bog/poor-fen mosaics, noted as being somewhat less acidic than bogs. Likewise both *Carex aquatilis* and *Salix planifolia* Pursh root materials from bog-like riverine sedge fens yielded *P. fortinii* as a characteristic species (Thormann *et al*. 2003). Nonmycorrhizal *Picea abies* roots studied by Holdenrieder & Sieber (1992) yielded PFMRA as the greatly predominant colonizer of roots collected at a site with peat soils: this organism made up 73.4 % of all isolates collected. The peaty site in question, however, was able to support a stand of *P. abies*, making it less extreme than bog sites where growth of trees may be restricted. The present study's site 4 was an open bog more like the sites at which *P. fortinii*/PFMRA is often found to be uncommon, and yet *P. fortinii* was among the most common fungi from roots. It is possible that because the roots studied came from a large tree with half its root system in the soil at the bog margin, the contiguity of the root system between soil and bog areas allowed *P. fortinii* to colonize roots in the bog areas mainly or entirely via endophytic growth. If true, this would suggest that successful plant-to-plant dispersal of *P. fortinii* in the more extreme bog sites may be restricted to a greater extent than is successful endophytic growth within individual plants. This growth may be continuously maintained in or near meristematic areas producing new fine roots, as outgrowth of PFMRA from roots larger than fine feeder roots is relatively uncommon (Mańka 1960).

In overview, the present study's results further reinforce the conclusion of Summerbell (1989) that there is no evidence of a mycorrhizosphere effect on filamentous fungi. Apart from a significant but

relatively minor enhancement of the occurrence of the chitinolytic *U. isabellina* on ectomycorrhizal roots as opposed to bark, soil or nearby endomycorrhizal roots (Summerbell 1989), all aspects of the variation seen in fungal assemblages from washed *P. mariana* ectomycorrhizae clearly relate to considerations other than the presence of mycorrhizal symbionts. The two most common fungi, *P. fortinii* and *M. variabilis*, are regulated in distribution by their endophytic habit of growth, the degree of their affinity for root bark (high only in conducive, mineral soil sites for the former fungus, low in general for the latter fungus), and, very notably, by site-specific edaphic and/or soil microbial community factors, mostly poorly understood but demonstrably high in impact. The distribution of other fungi found on the washed mycorrhizae mainly reflects site factors, most notably effects due to mineral soil exposure, humus levels, and presence of acid peat, along with the occasional influence of root bark as a microhabitat – as seen, for example, with *P. janthinellum*. The mycorrhizosphere, as a microbial microhabitat, is mainly of bacteriological interest, though the level of that interest with respect to helper bacteria is quite high (e.g., de Oliveira & Garbaye 1989, Garbaye 1994). With hindsight, this inapplicability of the mycorrhizosphere concept to filamentous microfungi seems obvious, in that many fungal colonies within a few days in artificial culture can cover an area into which hundreds of mycorrhizal root tips could fit. Mycorrhizal root tips, dispersed as they are through the matrix of soil and litter, are too small in scale to be expected to regularly support haloes of specific fungal rhizosphere colonizers or strongly locally increased microfungal colony or propagule numbers, except perhaps in dry soils due to their moisture content, or in moribund root tips in the process of turnover. However, the opacity of soil and the inventiveness of biology make it somewhat dangerous to make presumptions about such matters *a priori*, and it is better practice to obtain evidence, as has now been done here and by various other authors.

Though the mycorrhizosphere effect on filamentous microfungi can arguably be set aside, the extent to which there may be a symbiorhizosphere effect on fungi of northern forest soils remains largely to be explored. Indeed, it is not generally known whether ectomycorrhizal extramatrical mycelia commingle harmoniously in soil and humus or whether they generally tend to delimit and occupy zones of specific influence. There is one well-documented extreme case concerning *Tricholoma matsutake*, where a very strongly delimited colonization zone (aptly described as a *shiro* or "castle" based on its ring-like structure) surrounds an area of profoundly reduced and altered bacterial and microfungal colonization (Ohara & Hamada 1967, Ogawa 1976a, b). The related species *Tricholoma fulvocastaneum* Hongo (Ogawa 1977, 1978), *T. bakamatsutake* Hongo (Ogawa & Ohara 1978), and *T. caligatum* (Viv.) Rick. (Ohara & Ogawa 1982) exert a similar, but somewhat less pronounced, effect on the biology of the surrounding soil. Studies of ectomycorrhizal fruiting body and specific root tip mycobiont distributions show that some degree of zonation or of areas of overlapping growth may commonly occur in forest sites (Ogawa 1985, Bruns 1995, Fiore-Donno & Martin 2001, Redecker *et al*. 2001, Bergemann & Miller 2002); however, to our knowledge, the effect of these distribution patterns on nearby soil matrices has not been studied. In the present study, it is possible that some of the complex patterns of site distribution seen in fungi like *U. isabellina* may relate to the tolerance of these fungi for predominant ectomycorrhizal fungal species or communities as these symbionts influence the whole symbiorhizosphere, rather than just the mycorrhizosphere immediately adjacent to root tips. It would be of great interest if molecular techniques, greatly facilitating the study of fungi in soil, could now shed some light on co-distribution of ectomycorrhizal and rhizosphere fungi, as part of a general approach to studying the contribution of symbiotic and non-symbiotic fungi to soil quality and soil health.

ACKNOWLEDGEMENTS

Discussion with David Malloch, Terry Carleton, Randy Currah and Sarah Hambleton was instrumental in improving this study. Mia samseksama vivkunulo Ross Fraser is thanked for generous assistance with data analysis.

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