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REVIEW

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Ectendomycorrhizal associations – characteristics and functions

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Abstract Mycorrhizal symbioses are widespread mutualistic associations of many plant hosts found in many habitats. One type of putative mycorrhizal association, ectendomycorrhiza, is confined to *Pinus* and *Larix* spp. and is common in conifer nurseries and in disturbed habitats. This association is characterized by the unique combination of a fungal mantle, Hartig net, and intracellular hyphae, the latter forming soon after Hartig net development. Many reports of the occurrence of ectendomycorrhizas from field-collected specimens are likely erroneous and instead may represent senescent ectomycorrhizas. The fungus species involved in the formation of ectendomycorrhizas were initially called E-strain fungi and their identification was based on characteristics of hyphae and chlamydospores. With the discovery of teleomorphs for some of these fungi, they were found to be ascomycetes. More recently, molecular methods have been used to clarify their systematics and phylogeny and it is apparent that most of the isolates belong to two species, *Wilcoxina mikolae* and *Wilcoxina rehmii*. Two species of dematiaceous fungi and a member of the Pezizales, *Sphaerosporella brunnea*, also have been reported to form ectendomycorrhizas. These fungi can form ectendomycorrhizas with their hosts over a broad pH range and may utilize many substrates as a carbon source. Ectendomycorrhizas may be important in the revegetation of disturbed sites and in the establishment of conifer seedlings in post-fire situations.

Keywords Chlamydospore · E-strain · Pinaceae · *Sphaerosporella* · *Wilcoxina*

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Introduction

Fungi are a major biotic component of the soil and thus plant roots interact with various fungus species throughout their development. Some interactions are deleterious and others beneficial. Many fungus species form mutualistic symbioses, mycorrhizas, with the majority of plant species (Smith and Read 1997) and develop various categories of mycorrhizas based on the identity of the fungal partner and the structural modifications of the symbionts during the formation of the association (Peterson and Farquhar 1994). Most research has focused on two categories of mycorrhizas, arbuscular mycorrhizas (AM) and ectomycorrhizas (ECM), because these are very widespread and are associated with many commercially important plant species (Smith and Read 1997). One category of mycorrhizas, first called "ectendotrophic mycorrhizas" by Melin (1923) and now usually referred to as ectendomycorrhizas (Egger and Fortin 1988), is of restricted occurrence and has received little attention. The definition of an ectendomycorrhiza includes the fungal taxa and host species involved in the symbiosis and the resulting structural features of the association. The fungal taxa usually involved are two species of *Wilcoxina* (*W. mikolae*, *W. rehmii*), *Sphaerosporella brunnea* and, to a lesser extent, *Phialophora finlandia* and *Chloridium paucisporum*. The hosts are primarily *Pinus* and *Larix* species. Structurally, ectendomycorrhizas are characterized by a thin mantle (sometimes absent), Hartig net, and various degrees of intracellular hyphal penetration into epidermal and cortical cells. These anatomical features led Egger and Fortin (1988) to suggest that ectendomycorrhizas should be considered a variant of ECM.

The early literature on ectendomycorrhizas was reviewed by Mikola (1965) and Laiho (1965). More recently, Mikola (1988) reviewed some aspects of the fungi involved, the distribution and ecology, as well as the confusion in terminology in early papers on mycorrhizas. He suggested that the term "pseudomycorrhiza" be dropped because it is not meaningful from a functional

point of view and that the term ectendomycorrhiza be restricted to those associations in which intracellular hyphal penetration occurs almost simultaneously with Hartig net formation. Although this restriction on the use of the term ectendomycorrhiza is important in distinguishing these associations from senescent ECMs, it remains to be determined whether there is nutrient exchange between intracellular hyphae and root cells, typical of a biotrophic association. If not, intracellular hyphae may represent a "latent pathogen" stage becoming active in nutrient absorption only after the onset of root senescence. Although arbutoid and monotropoid mycorrhizal associations have some features in common with ectendomycorrhizas, and have been referred to as ectendomycorrhizas by Zak (1974, 1976), they are not considered here because the fungus species involved are those typical of ectomycorrhizas (Zak 1974, 1976). Intracellular hyphae are confined to the epidermis and, in the case of monotropoid mycorrhizas, very specialized fungal structures ("pegs") are formed within epidermal cells; these become surrounded by an elaborate host-derived cell wall (Smith and Read 1997).

The objectives of this review are to bring together and critique information related to the occurrence, structure, and functions of ectendomycorrhizas, to discuss the current knowledge of the taxonomy of the fungi involved in the association, and to propose areas of research required to elucidate the role(s) of these mycorrhizas in natural and other ecosystems.

Occurrence of ectendomycorrhizas

Laiho (1965) summarized the gymnosperm and angiosperm tree species reported to form ectendomycorrhizas. Many of the early reports, however, are questionable because field-collected roots with any type of intracellular hypha development were often categorized as ectendotrophic. It was not until the pioneering work of Mikola (1965) and Laiho (1965) that the unique characteristics of the fungi involved in this association were recognized and the term E-strain applied to the isolates obtained. Mikola (1965) grouped all isolates showing similar gross morphological characteristics of mycelium in culture into a single species of E-strain fungus. The dimorphic mycelium consisted of pigmented, coarse aerial hyphae of variable thickness (straight and septate), and hyaline submerged hyphae, septate (winding and branching). Chlamydospore-like swellings often occurred.

Ectendomycorrhizas described by Mikola (1965) and Laiho (1965) from the field were confined to roots of pine seedlings from undisturbed natural sites, regenerating seedlings from burned sites, and nursery-grown *Pinus* and *Larix* seedlings in Finland and the United States (Table 1). In addition, both authors used E-strain isolates obtained from nursery seedlings in greenhouse and aseptic synthesis experiments (Table 1). They confirmed that the anatomical features of the ectendomycorrhizas in both *Pinus* and *Larix* species were typical of those described from field material.

Since these studies, additional reports of ectendomycorrhizas in *Pinus* roots collected from the field, or from seedlings grown in soil collected from forest sites, have been published (Table 1). Several of these (Danielson 1982; Ursic and Peterson 1997; Ursic et al. 1997; Massicotte et al. 1999) identified the fungus involved as E-strain, while others (v. Hofsten 1969; Wilcox 1971; Scannerini 1972; Pachlewski et al. 1991–1992) did not mention the fungus involved. McGee et al. (1999) reported structural features similar to those of ectendomycorrhizas in roots of one adult specimen of the recently discovered Wollemi pine (*Wollemia nobilis*, Araucariaceae) in Australia. Because the fungus involved was not determined, the occurrence was very restricted and most members of the Araucariaceae have AM associations, this finding is difficult to evaluate. The reports of ectendomycorrhizas in field collections of the angiosperm species *Uapaca kirkiana* (Högberg 1982) and the dipterocarp species *Shorea parvifolia* (Louis 1988) are based solely on the presence of intracellular hyphae; these may be hyphae of senescing ectomycorrhizas.

Several cases of ectendomycorrhizas synthesized under laboratory conditions have been reported (Table 1) since the reviews of Mikola (1965) and Laiho (1965). The strength of these reports in most cases lies in the use of known fungus species and the determination of anatomical features of mycorrhizal roots. In the paper by Wilcox et al. (1974), fungus isolates (identified as BDG-58 and BDD-22) obtained from *Pinus resinosa* roots were used. BDD-22 was identified as *Chloridium paucisporum* Wang & Wilcox and the anamorph of BDG-58 was later identified as *Complexipes* sp. (LoBuglio and Wilcox 1988), of which the teleomorph is *Wilcoxina* (Yang and Korf 1985). Ectendomycorrhizas have been synthesized with *Pinus resinosa* (red pine) using various identified fungus species, including *Wilcoxina mikolae* var. *mikolae* (Yang and Wilcox 1984, as *Tricharina mikolae*; Piché et al. 1986; see our Fig. 1), *Phialophora finlandia* (Wang and Wilcox 1985; Wilcox and Wang 1987a, b), and *Chloridium paucisporum* (Wang and Wilcox 1985; Wilcox and Wang 1987b). Ectendomycorrhizas of *Pinus contorta* inoculated with *Sphaerosporella brunnea* (Egger and Paden 1986; Iwanyzki 1992) and *Pinus banksiana* inoculated with two varieties of *Wilcoxina mikolae* and *Wilcoxina rehmii* (Scales and Peterson 1991a) have been reported (Table 1). *S. brunnea* colonized short roots of *Pinus contorta* and formed a thin mantle (Figs. 2, 3).

A group of dematiaceous soil fungi also has been described in association with roots of many plant species from a variety of habitats (Jumponnen and Trappe 1998). Melin (1922) first described these fungi from the roots of *Pinus sylvestris* L. and *Picea abies* Karst. and placed them in the *Mycelium radicis atrovirens* (*Mra*) complex. Some of the members of the *Mra* form typical ectendomycorrhizas on members of the Pinaceae (Wilcox et al. 1974), whilst others, termed pseudomycorrhizas, appeared to be weak pathogens (Wang and Wilcox 1985; Wilcox and Wang 1987a, b). *Mra* fungi will not be discussed in detail in this review except when they have been shown to form ectendomycorrhizas.

Table 1 Reports of ectendomycorrhizas (1965–2000)

Host species	Fungus	Field/lab	Reference
Gymnosperms			
Larix occidentalis Nutt	E-strain	Semi-aseptic pot culture	Laiho (1965)
Larix leptolepis	E-strain	Nursery	Laiho (1965)
Pinus attenuata	E-strain	Nursery	Laiho (1965)
Pinus banksiana Lamb (*Wilcoxina mikolae*)	E-strain (R-947)	Field (mine spoils)	Danielson (1982)
Pinus banksiana	E-strain (R-947)	Oil sands tailings	Danielson and Visser (1989)
Pinus banksiana	*Wilcoxina mikolae* var. *mikolae*; var. *tetraspora Wilcoxina rehmii*	Semi-aseptic (growth pouches)	Scales and Peterson (1991a)
Pinus contorta Dougl.	E-strain	Forest/nursery	Laiho (1965)
Pinus contorta	*Sphaerosporella brunnea* (Alb. + Schwii Fr.) Svrcek & Kubicka	Aseptic culture	Egger and Paden (1986)
Pinus contorta	*Wilcoxina mikolae*	Semi-aseptic pot culture	Chanway and Holl (1991)
Pinus edulis Engelm.	E-strain	Semi-aseptic pot culture	Laiho (1965)
Pinus lambertiana	E-strain	Nursery	Laiho (1965)
Pinus monticola Dougl.	E-strain	Nursery	Laiho (1965)
Pinus nigra	E-strain	Nursery	Laiho (1965)
Pinus ponderosa Dougl. ex Laws.	E-strain	Forest/nursery/semi-aseptic pot culture	Laiho (1965)
Pinus ponderosa	E-strain (possibly *Wilcoxina mikolae*)	Greenhouse (forest soil)	Massicotte et al. (1999)
Pinus radiata D. Don	E-strain	Semi-aseptic pot culture	Laiho (1965)
Pinus resinosa Ait.	E-strain	Nursery	Laiho (1965)
Pinus resinosa	?	Field	Wilcox (1971)
Pinus resinosa	Isolates BDG-58 (*Wilcoxina*) and BDD-22 (*Chloridium paucisporium*)	Aseptic culture	Wilcox et al. (1974)
Pinus resinosa	*Tricharina mikolae*	Semi-aseptic pot culture; aseptic flask culture	Yang and Wilcox (1984)
Pinus resinosa	*Wilcoxina mikolae* var. *mikolae*	Semi-aseptic (growth pouches)	Piché et al. (1986)
Pinus resinosa	*Phialophora finlandia* Wang & Wilcox *Chloridium paucisporum* Wang & Wilcox	Field	Wang and Wilcox (1985)
Pinus resinosa	*Phialophora finlandia* Wang & Wilcox	Aseptic flask culture	Wilcox and Wang (1987a)
Pinus resinosa	*Phialophora finlandia* Wang & Wilcox *Chloridium paucisporum* Wang & Wilcox	Aseptic flask culture	Wilcox and Wang (1987b)
Pinus strobus L.	E-strain	Nursery/semi-aseptic pot culture	Laiho (1965)
Pinus strobus	?	Field	Scannerini (1972)
Pinus strobus	E-strain?	Nursery	Beckjord and Hacskaylo (1984)
Pinus strobus	E-strain	Nursery	Ursic et al. (1997)
Pinus strobus	E-strain	Semi-aseptic pot culture	Ursic and Peterson (1997)
Pinus strobus	*Phialophora finlandia*	Semi-aseptic pot culture	Ursic and Peterson (1997)
Pinus sylvestris L.	E-strain	Nurseries; aseptic flask culture; Hagem agar cultures	Mikola (1965)
Pinus sylvestris	E-strain	Nursery; semi-aseptic pot culture	Laiho (1965)
Pinus sylvestris	?	Field (one old tree)	v. Hofsten (1969)
Pinus sylvestris	?	Nursery	Pachlewski et al. (1991–1992)
Wollemia nobilis Jones, Hill & Allen	?	Field (one adult tree)	McGee et al.(1999)
Angiosperms			
Uapaca kirkiana Müll. Arg	?	Field	Högberg (1982)
Shorea parvifolia Dyer	?	Field	Louis (1988)
Helianthemum guttatum	*Terfezia arenaria, Terfezia claveryi, Tirmania pinoyi*	Lab (P-deficiency)	Fortas and Chevalier (1992)

Formation of ectomycorrhizas by ectendomycorrhizal fungi

One feature of E-strain fungi is their ability to form ectendomycorrhizas with some hosts and ectomycorrhizas with others. Danielson (1984) showed that *Sphaerosporella brunnea* forms typical ECMs with *Larix laricina*, *L. occidentalis*, *Picea glauca*, *Picea mariana*, four *Pinus* species and *Populus tremuloides* (Table 2). Later, Iwanyzki (1992) confirmed that this same fungus species formed ECMs with *Pinus banksiana. Wilcoxina mikolae* var. *mikolae* formed ECMs with both *Picea mariana* and the angiosperm species *Betula alleghaniensis* in growth pouches (Scales and Peterson 1991b). The dematiaceous

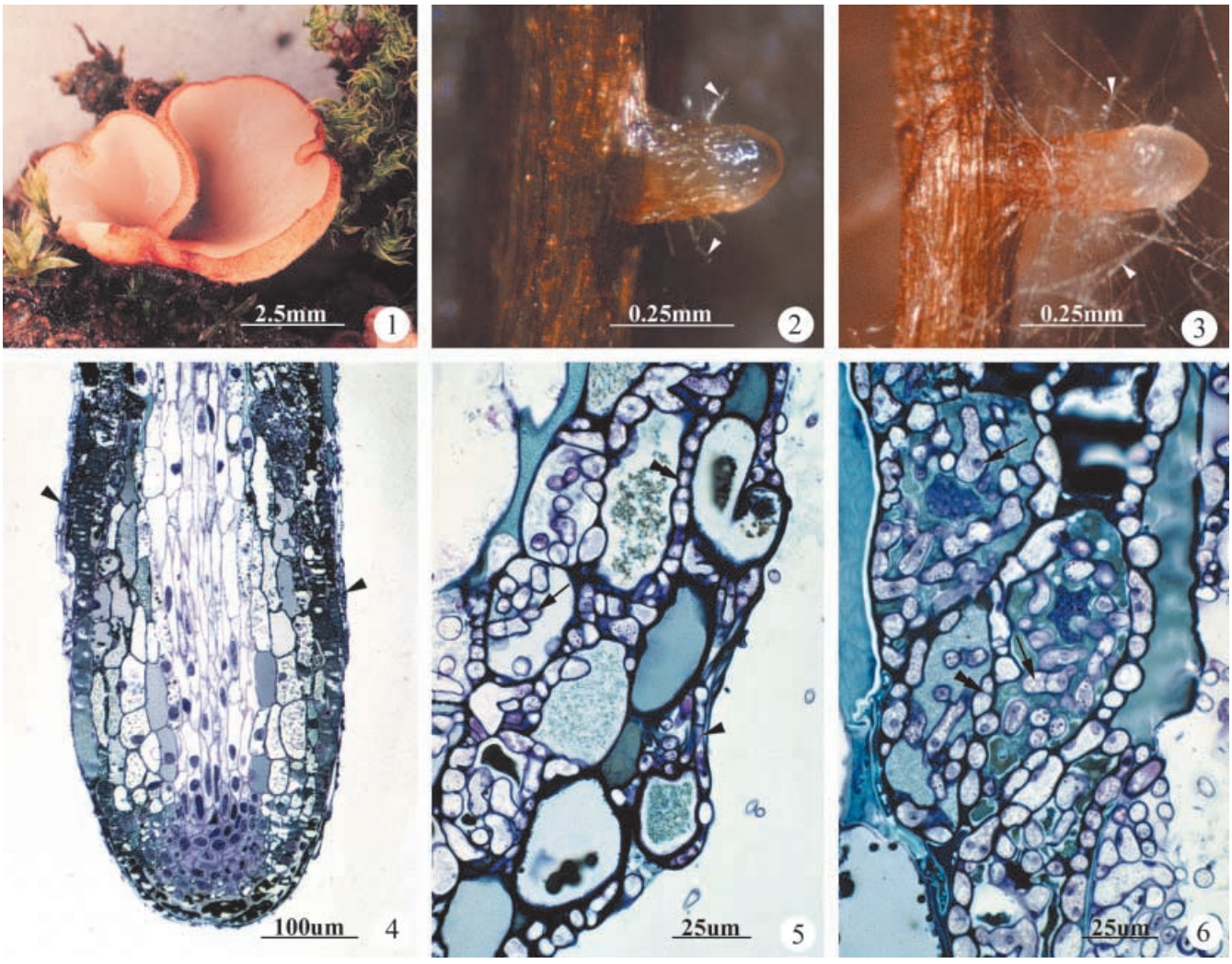

Fig. 1. Apothecium of *Wilcoxina mikolae* var. *mikolae*. Photo courtesy of Dan Luoma and Hugues Massicotte

Fig. 2 Short root of *Pinus contorta* showing root hairs (*arrowheads*)

Fig. 3 Short root of *Pinus contorta* colonized by *Sphaerosporella brunnea*. A loose weft of hyphae (*arrowheads*) is present

Fig. 4 Longitudinal section of *Pinus resinosa* short roots colonized by hyphae of *Wilcoxina mikolae* var. *mikolae*. A thin mantle (*arrowheads*) is present

Fig. 5 Longitudinal section of *Pinus resinosa* short roots colonized by hyphae of *Wilcoxina mikolae* var. *mikolae*. A thin mantle (*arrowheads*), Hartig net hyphae (*double arrowheads*) and intracellular hyphae (*arrow*) are present

Fig. 6 Longitudinal section of *Pinus resinosa* short roots colonized by hyphae of *Wilcoxina mikolae* var. *mikolae*. Hartig net hyphae (*double arrowheads*) and intracellular hyphae (*arrows*) are present

fungal species *Phialophora finlandia* formed ECMs with *Picea rubens* and *Betula alleghaniensis* (Wilcox and Wang 1987a, b), whereas *Chloridium paucisporum* only formed ECMs with *Picea rubens* (Wilcox and Wang 1987b).

Structure of ectendomycorrhizas

Mikola (1965) microscopically examined the structure of ectendomycorrhizas in controlled synthesis experiments with E-strain fungus isolates and described the presence of Hartig net hyphae, intracellular hyphae in epidermal and cortical cells, and either a thin mantle or lack thereof in roots of *Pinus sylvestris.* Laiho (1965) confirmed these observations with *Pinus sylvestris* and extended them to *Larix occidentalis* roots. Wilcox (1969) showed essentially the same features in nursery seedlings of *Pinus resinosa*, although the fungus involved was not identified. Later, Wilcox and Ganmore-Neumann (1974) and Wilcox et al. (1974) synthesized ectendomycorrhizas on *Pinus resinosa* with two fungus isolates obtained from this host. A feature mentioned by Wilcox et al. (1974), but not in previous studies, was the presence of chlamydospore-like intracellular structures of fungal origin in cortical cells. Ectendomycorrhizas of *Pinus resinosa* seedlings inoculated with E-strain *Wilcoxina mikolae* (Yang and Wilcox 1984 as *Tricharina mikolae*), and the two *Mra* species *Phialophora finlandia* and *Chloridium paucisporum* (Wilcox and Ganmore-Neumann 1974;

Table 2 Formation of ectomycorrhizas by verified E-strain fungal species

Host	Fungus	Reference
Gymnosperms		
Larix laricina (DuRoi) K. Koch	*Sphaerosporella brunnea* (Alb. & Schw.:Fr.) Svrcek & Kubicka	Danielson (1984)
Larix occidentalis Nutt.	*Sphaerosporella brunnea*	Danielson (1984)
Picea abies (L) Karst	E-strain	Laiho (1965)
Picea engelmanii Parry	E-strain	Laiho (1965)
Picea glauca (Moench) Voss	*Sphaerosporella brunnea*	Danielson (1984)
Picea mariana (Mill.) B.S.P.	*Sphaerosporella brunnea*	Danielson (1984)
Picea mariana	*Wilcoxina mikolae* var. *mikolae*	Scales and Peterson (1991a)
Picea pungens Engelm.	E-strain	Laiho (1965)
Picea rubens Sarg.	*Phialophora finlandia* Wang and Wilcox	Wilcox and Wang (1987a)
Picea rubens	*Chloridium paucisproum*	Wilcox and Wang (1987b)
Pinus banksiana Lamb.	*Sphaerosporella brunnea*	Danielson (1984)
Pinus contorta Loudon var. *latifolia* Engelm.	*Sphaerosporella brunnea*	Danielson (1984)
Pinus flexilis James	*Sphaerosporella brunnea*	Danielson (1984)
Pinus ponderosa Dougl.	*Sphaerosporella brunnea*	Danielson (1984)
Pseudotsuga menziessii (Mirb.)	E-strain	Laiho (1965)
Angiosperms		
Betula alleghaniensis Britton	*Phialophora finlandia* Wang and Wilcox	Wilcox and Wang (1987a)
Betula alleghaniensis	*Wilcoxina mikolae* var. *mikolae*	Scales and Peterson (1991b)
Populus tremuloides	*Sphaerosporella brunnea*	Danielson (1984)

Wang and Wilcox 1985; Wilcox and Wang 1987a, b) were described as having a mantle, Hartig net hyphae, and intracellular hyphae. Roots of *Pinus contorta* seedlings inoculated with the post-fire ascomycete *Sphaerosporella brunnea* showed the basic anatomical features of ectendomycorrhizas (Egger and Paden 1986; Iwanyzki 1992). Recently, Ursic and Peterson (1997) showed structural features of *Pinus strobus* ectendomycorrhizas collected either from a nursery or synthesized in the greenhouse using an unidentified E-strain isolate from nursery seedlings. Again, a thin mantle, Hartig net hyphae and intracellular hyphae were present.

The development and ultrastructure of ectendomycorrhizas examined to date is described in a few publications. v. Hofsten (1969) examined the ultrastructure of ectendomycorrhizas of *Pinus sylvestris* collected from a mature tree, but the publication makes no mention of the fungus mantle. Most of the preparations in v. Hofsten (1969) are poorly preserved and observations of the ultrastructural features suffer as a result; the structural features shown are typical of senescent ECM. Likewise, the micrographs included in the ultrastructural study of ectendomycorrhizas of *Pinus strobus* roots (Scannerini 1972) suggest that the cells examined were from old root segments and it is difficult to determine the nature of the interaction of intracellular hyphae with root cells. In two later studies (Piché et al. 1986; Scales and Peterson 1991a), the synthesis of ectendomycorrhzas in growth pouches between known fungus species and two pine species, *Pinus resinosa* (Figs. 4, 5, 6) and *Pinus banksiana*, allowed for detailed analysis of the development of the association and structural changes in both symbionts.

Short roots were colonized (Figs. 7, 8, 9, 10, 11, 12) and, similar to ECM, meristems were induced to dichotomize (Fig. 8). In both pine species, typically a thin fungal mantle developed (Figs. 4, 10) that consisted of branched hyphae. Although the function of the mantle has not been examined, its loose organization probably precludes any roe in blocking apoplastic transfer of nutrients from the soil solution, or physical protection from pathogenic organisms. Scales and Peterson (1991a) suggested that the thick mucilage on the root surface plays a role in preventing transfer of nutrients from the soil solution to the root. Tannin-filled cells that form at the periphery of colonized roots (Figs. 4, 5, 6, 12) also may impede nutrient transfer into root tissues. Histochemical staining for polysaccharides revealed dense bodies, probably glycogen, in the mantle and adjacent Hartig net hyphae, providing circumstantial evidence for movement of carbon compounds from host cells to fungus hyphae (Scales and Peterson 1991a). The relatively wide intercellular hyphae of the Hartig net penetrated into the epidermal and cortical cell layers in both monopodial roots (Figs. 4, 5, 6) and dichotomously branched roots (Fig. 12). This occurred very close to the root apical meristem (Piché et al. 1986). The entire cortex up to the endodermis was frequently colonized (Scales and Peterson 1991a). In *Pinus banksiana* roots colonized by *W. mikolae*, Hartig net hyphae close to the root meristem contained numerous mitochondria and endoplasmic reticulum cisternae; adjacent cortical cells also had organelles indicating their viability. Further from the root apex, Hartig net hyphae became more vacuolated but still retained numerous mitochondria.

Once the Hartig net is established, relatively wide intracellular hyphae develop in epidermal and cortical cells (Piché et al. 1986; Scales and Peterson 1991a; Fig. 6) and these show various degrees of branching within cortical cells. Intracellular hyphae are surrounded by host-derived membrane and an interfacial matrix material

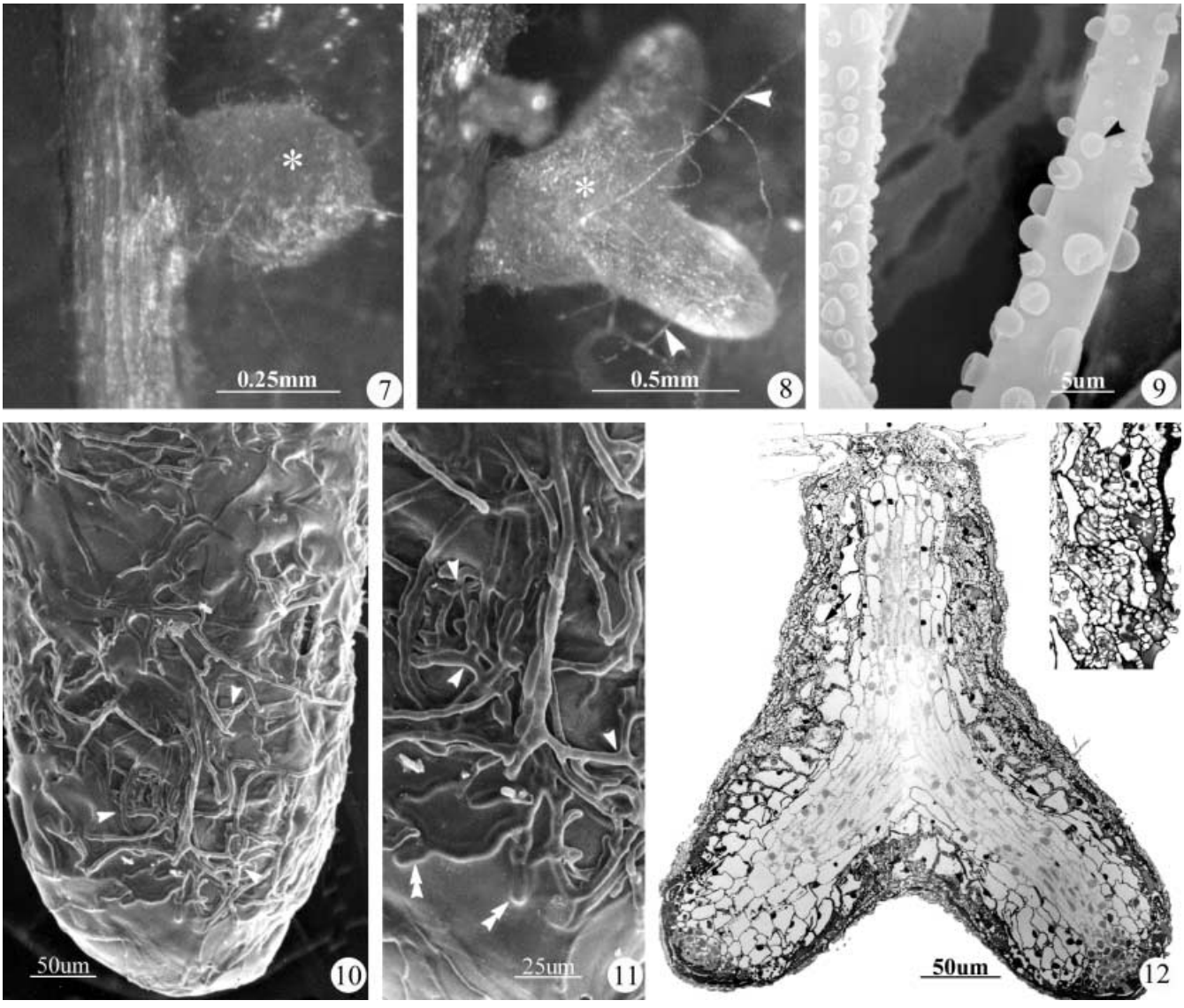

Fig. 7 Short roots of *Pinus banksiana* colonized by hyphae of *Wilcoxina mikolae* var. *mikolae*. The monopodial mycorrhizas have a mantle (*asterisk*). Reproduced from Scales and Peterson (1991a) with permission

Fig. 8 Short roots of *Pinus banksiana* colonized by hyphae of *Wilcoxina mikolae* var. *mikolae*. The dichotomously branched mycorrhizas have a mantle (*asterisk*). Extraradical hyphae (*arrowheads*) are evident

Fig. 9 Hyphae of *Sphaerosporella brunnea* showing ornamentations (*arrowhead*) typical of this genus and other E-strain fungal species

Fig. 10 Scanning electron micrographs of the mantle of *Pinus banksiana–Wilcoxina mikolae* var. *mikolae* mycorrhizas. Hyphae are branched (*arrowheads*). Reproduced from Scales and Peterson (1991a) with permission

Fig. 11 Scanning electron micrographs of the mantle of *Pinus banksiana–Wilcoxina mikolae* var. *mikolae* mycorrhizas. Hyphae are branched (*arrowheads*) and are embedded in mucilage (*double arrowheads*). Reproduced from Scales and Peterson (1991a) with permission

Fig. 12 Longitudinal section of dichotomously branched *Pinus banksiana–Wilcoxina mikolae* var. *mikolae* mycorrhiza. Hartig net hyphae are present in the epidermis and cortex (*arrows*) and intracellular hyphae (*inset*) are present. Tannin-filled cells (*) are evident. Reproduced from Scales and Peterson (1991a) with permission

(Scales and Peterson 1991a) and contain numerous organelles and electron-dense vacuolar deposits that are likely phenolic in nature (Piché et al. 1986; Scales and Peterson 1991a). The cytology of intracellular hyphae suggests that they play an active role in the transfer of substances between the symbionts, but there is no direct evidence for transfer of nutrients across this interface. The observations to date provide a firm basis for further cytological studies such as examining the nature of the interfacial matrix between the perifungal membrane and the hyphal cell wall for comparison with other biotrophic interfaces in parasitic and mutualistic symbioses (Bonfante and Perotto 1995; Harrison 1999), the interaction between the intracellular hyphae and root cell cytoskeleton (see Peterson et al. 2000), and determining in detail the nature of the electron-dense vacuolar granules of the Hartig net and intracellular hyphae.

Taxonomy and structural characteristics of fungus species involved in ectendomycorrhizas

Early descriptions of E-strain fungi implicated in ectendomycorrhiza formation were based on morphological characteristics of sterile hyphae of isolates plated on agar media (Mikola 1965; Laiho 1965). The presence of characteristic chlamydospores either sieved from soil, isolated from pot cultures, or from cultures of isolates on agar plates was used later (Wilcox et al. 1974; Danielson 1982). Hyphae were described as being septate, with septa approximately 66 µm apart, 3–9 µm in diameter, possessing Woronin bodies and with various ornamentations on aerial hyphae (Danielson 1982). Danielson (1982) hypothesized that the ornamentations (Fig. 9) provide a compartment for the deposition of diffusable waste products, which replace root tissue or culture media as sinks. The ornamentations were formed on aerial hyphae in culture or on extraradical hyphae from roots of container-grown spruce (Danielson 1982). Submerged-intercalary chains or aerial-terminal, thick-walled chlamydospores 45–100 µm in diameter from culture and 80–120 µm from soil are also formed by E-strain fungi (Wilcox et al. 1974; Danielson 1982). Based on the similarity of their chlamydospores to those of the Glomales, Walker (1979) assigned E-strain fungi the species name *Complexipes moniliformis* and erroneously placed them in the Endogonaceae. However, septation of hyphae in all E-strain fungi examined (v. Hofsten 1969; Mikola 1965, 1988; Wilcox 1971; Danielson 1982) and the presence of Woronin bodies (Danielson 1982) is evidence that these fungi do not belong in the Endogonaceae. Terminal chlamydospores of E-strain fungi have ornamentations similar in structure to those on aerial hyphae, which were suggested as a taxonomic character in the identification of this fungus group by Danielson (1982). Based on the structure of hyphae and, in particular, septal pores, Danielson (1982) hypothesized that E-strain fungi have ascomycetous teleomorphs. This proved to be the case and Yang and Wilcox (1984) described a new species, *Tricharina mikolae* Yang & Wilcox, based on ascocarps appearing in pot cultures of red pine inoculated with E-strain chlamydospores isolated from a Douglas-fir nursery. Synthesis experiments with red pine and these chlamydospores confirmed ectendomycorrhiza formation. Later, in a monographic treatment of the genus *Tricharina*, Yang and Korf (1985) erected a new genus in the Pezizales, *Wilcoxina*, with three species (one with two varieties): *W. mikolae* (Yang & Wilcox) Yang & Korf var. *mikolae* = *Tricharina mikolae* Yang & Wilcox, *W. mikolae* (Yang & Wilcox) Yang & Korf var. *tetraspora*, *W. rehmii* Yang & Korf and *W. alaskana* Kempton, Yang & Korf (=*W. sequoiae* according to Schumacher 1988). Of these three species, only the first two form anatomical features with roots that are typical of either ecto- or ectendomycorrhizas.

In a comparison of E-strain fungi isolated from mycorrhizal roots and ascocarps, Egger and Fortin (1990) confirmed that most of the isolates could be placed into the two mycorrhizal *Wilcoxina* taxa, *W. mikolae* and *W. rehmii* based both on general culture characteristics and restriction fragment polymorphisms within the nuclear ribosomal RNA (rRNA) genes. One isolate was intermediate between these taxa and the authors speculated that this could be a hybrid. Culture characteristics were found to be somewhat variable and less reliable than the molecular data for the identification of taxa. A further study (Egger et al. 1991), comparing polymorphisms in both nuclear and mitochondrial rRNA genes, supported the placement of the majority of E-strain isolates into these two *Wilcoxina* species. Interestingly, two isolates obtained from root cultures were again intermediate between these taxa in terms of their molecular characters. Because of this and differences in habitat, these authors suggested the possibility of a new *Wilcoxina* taxon. In a phylogenetic analysis using nuclear-encoded rRNA gene sequences, Egger (1996) confirmed that *Wilcoxina* and *Tricharina* should be maintained as separate genera and that the *Wilcoxina* taxa formed a distinct group with the non-mycorrhizal species *W. alaskana* being the most divergent taxon. The two anomalous isolates identified previously (Egger et al. 1991) remained outside *W. mikolae* and *W. rehmii* in this analysis and, based upon the degree of nucleotide divergence, were hypothesized to be cryptic species of *Wilcoxina* that were not known from teleomorph collections.

The formation of a mycorrhizal association by E-strain fungi typical of either an ectomycorrhiza or an ectendomycorrhiza may have a genetic component. Support for this comes from recent molecular studies. Based on comparison of mitochondrial ribosomal RNA gene RFLPs (Egger et al. 1991) from different *Wilcoxina* isolates of spruce ectomycorrhizas and pine ectendomycorrhizas (Egger 1996), evidence was found to suggest that *W. mikolae* and *W. rehmii* populations subdivided by host, but it was not clear whether population subdivision was due to differences in host compatibility or host geographical distribution.

Fungi that form ectendomycorrhizas appear to be phylogenetically related to ascomycetes in the orders Pezizales (operculate discomycetes) and Leotiales (inoperculate discomycetes). Stoyke et al. (1992) compared nuclear rRNA gene restriction fragments of many dematiaceous isolates from mycorrhizas of subalpine plants. Included in their analysis was *Phialophora finlandia*, which is known to form ectendomycorrhizas (Wang and Wilcox 1985). The majority of isolates examined by Stoyke et al. (1992) belonged to *Phialocephala fortinii*. The phylogenetic affinities of *Phialocephala fortinii* are not well resolved, this taxon being placed between Onygenales and Leotiales by Monreal et al. (1999), occupying an unresolved position between Leotiales and Pezizales in Jumpponen and Trappe (1998), and within the Leotiales (although with weak bootstrap support) by Hambleton (1998). Of these three studies, the most convincing is Hambleton (1998) because it was based upon the nearly complete small subunit rRNA sequence, rather than a partial sequence (Jumpponen and Trappe 1998) or ITS sequence (Monreal et al. 1999). Placement of *Phialocephala fortinii* in the Leotiales is also consistent with morphological observa-

tions of immature fruit bodies of the inoperculate type (Currah et al. 1993). Although *Phialocephala fortinii* does not form ectendomycorrhizas, based upon restriction fragment length polymorphisms of the rDNA internal transcribed spacer (ITS) region, it forms part of *Mra* fungi (Harney et al. 1997). These include *Phialophora finlandia* and *Chloridium paucisporum*, which do form ectendomycorrhizas. Interestingly, *Phialophora finlandia* clustered closely to *Hymenoscyphus ericae*, the predominant ericoid mycorrhizal fungus endophyte and a member of the Leotiales, in the study of Stoyke et al. (1992). The close relationship between *Phialophora finlandia* and *H. ericae* has been confirmed by DNA sequence analysis (Monreal et al. 1999). The other taxa forming ectendomycorrhizas, *Wilcoxina* and *Sphaerosporella*, are clearly members of the Pezizales (Landvik et al. 1997). Therefore, evidence of a close phylogenetic relationship between *Mra* fungi and E-strain fungi is lacking in the literature, despite some members of each group forming ectendomycorrhizas on similar plant hosts. However, recent research by Williams (1999), based upon PCR-RFLP matching of sporocarps and *Mra* root tips, suggests that some members of the genus *Helvella* (Pezizales) form *Mra* root associations. The exact nature of these associations remains to be determined and it is not known whether they resemble ectendomycorrhizas, but further research may indicate that some members of the highly diverse *Mra* group are related to Pezizales.

Physiology: in vitro growth of ectendomycorrhizal fungi

E-strain fungi are comparable to most ectomycorrhizal fungi in their ability to utilize various simple sugars, but not cellulose, as a carbon source (Mikola 1965). In contrast, the dematiaceous root endophyte *Phialophora finlandia*, shown to form ectendomycorrhizas with some hosts (Wilcox and Wang 1987a), is capable of using cellulose, laminarin, starch, and xylan (Caldwell et al. 2000). An ascomycete, *Sphaerosporella brunnea*, also shown to form ectendomycorrhizas with at least *Pinus contorta* (Egger and Paden 1986; Iwanyzki 1992), converts most of the glucose provided in the medium into mannitol, with minor amounts into trehalose, glycogen and free amino acids. This indicates that mannitol is the major soluble storage carbon compound in this fungus species (Martin et al. 1988). Egger (1986) had shown previously that this fungus species was capable of hydrolyzing gelatin, cellulose, and to a lesser extent oil, at acidic pH, and gelatin and pectin at neutral pH.

A comparison of various E-strain isolates, *Mra* isolates, and *Boletus variegatus* for their ability to grow on various nitrogen sources showed that E-strain isolates were able to use ammonium and nitrate, with some variation in growth response to nitrate among isolates (Mikola 1965). An NADPH-specific nitrate reductase has been extracted and characterized from the ectendomycorrhizal fungus *Wilcoxina mikolae* var. *mikolae* (Prabhu et al. 1995). This enzyme was shown to be induced specifically by nitrate and repressed by ammonium (Prabhu et al. 1996b).

The siderophore, ferricrocin, was produced by two isolates of *W. mikolae* and one of *W. rehmii* in culture medium lacking iron after 20 days of mycelial growth (Prabhu et al. 1996a). A comparison of the in vitro biomass accumulation of the three isolates with or without added iron showed no difference for the first 20 days, but biomass was significantly lower after 20 days in medium lacking iron. Thus, there was a good correlation between induction of the siderophore in the culture medium lacking iron and reduction in biomass accumulation. Prabhu et al. (1996a) suggested that the production of this siderophore by ectendomycorrhizas formed on conifer seedlings in mine spoils imparts some protection against heavy metals.

E-strain fungi, like many ascomycete fungi, are sensitive to benomyl (Danielson 1982). This was shown experimentally in a study of the effect of two fungicides, benomyl and oxine benzoate, on four isolates of E-strain fungi (*W. mikolae* var. *mikolae*, *W. mikolae* var. *tetraspora*, *W. rehmii* and an unnamed isolate (Chakravarty et al. 1990). When tested in vitro, all isolates showed a significant reduction in mycelial biomass at benomyl or oxine benzoate concentrations above 10 ppm. Benomyl alone had a stimulatory effect on biomass of *W. rehmii* at 0.1 ppm; the reason for this is unknown.

Temperature had a differential effect on growth of six E-strain fungus isolates, depending on the source of the isolate (Wilcox et al. 1983). The peak growth rate of isolates from more northern sites was at 20°C, whereas that of southern isolates was at 24°C. Only isolates from northern sites were able to grow at 4°C.

Physiology: ectendomycorrhiza formation and function

Mikola (1965) tested the pH tolerance of E-strain fungi and found that their optimal range for ectendomycorrhiza formation with *Pinus sylvestris* lies between pH 3.9 and 8.0 in axenic culture with Hagen agar medium as the substrate, and pH 2.2 and 12.6 in pot culture with peat as the substrate. However, the degree of short root dichotomization and fungus mantle thickness showed some dependency on pH. These features were most developed between pH 4.0 and 5.5, whereas at pH 8.0 the onset of mycorrhization was delayed and a thin mantle resulted. Interestingly, ECM-forming members of the *Mra* have a similar pH range (Wilcox and Wang 1987b). *Chloridium paucisporum* formed typical ectendomycorrhizas with *Pinus resinosa* and ECMs with *Picea rubens* at pH 5.7 but mycorrhization did not occur in *Pinus resinosa* at pH 3.0. These authors found also that *Chloridium paucisporum* was “pseudomycorrhizal” on *Betula allegha-niensis*. This conclusion was based on the lack of radial elongation of epidermal cells and hypha penetration into the root apex. There was no indication given on the effect of this type of colonization on seedling growth. As indicated by Mikola (1988), and discussed earlier in this review, the term pseudomycorrhiza probably has little value in terms of function. Perhaps the term “weak pathogen” should be

used when the invasion of root meristems and vascular cylinders described by Wilcox and Wang (1987b) occurs.

Pachelewski et al. (1991–1992) found that an increase in phosphorus (in the form of $AlPO_4$ or P_2O_5) or nitrogen (urea) fertilization caused an increase in the amount of intracellular colonization by ectendomycorrhizal hyphae in *Pinus sylvestris* roots. An interesting study of the formation of mycorrhizas between *Helianthemum guttatum* and three desert truffle species (*Terfezia aenaria*, *Terfezia claveryi*, *Tirmania pinoyi*) showed that the type of mycorrhiza formed depended on the nutrient level in the substrate (Fortas and Chevalier 1992). Specifically, with both axenic and gnotoxenic conditions, low phosphate levels resulted in Hartig net and intracellular hypha development, whereas high phosphate resulted in Hartig net development only. In neither case was a mantle formed. These results indicate that the type of root colonization is dependent, in part, on the nutrient level present during the interaction between fungus hyphae and roots. On the other hand, Mikola (1965) reported that nitrogen (in the form of ammonium) caused a decrease in intracellular colonization, but even heavy applications of the fertilizer did not completely inhibit mycorrhization. Clearly, nutrition experiments need to be repeated under controlled conditions with identified E-strain fungus species within appropriate experimental designs.

Bacteria commonly are found in the mycorrhizosphere. Many of these can influence the formation of ECMs and some are known to fix atmospheric nitrogen (Smith and Read 1997). Pachlewski et al. (1991–1992) isolated many strains of *Bacillus polymyxa* from ectendomycorrhizas formed between E-strain fungal isolates and roots of *Pinus sylvestris*, the majority of which had the ability to decompose cellulose. An isolate of *Bacillus*, probably *B. polymyxa*, was inoculated onto roots of *Pinus contorta* seedlings either alone or in combination with an isolate of the E-strain fungus *Wilcoxina mikolae* (Chanway and Holl 1991). As expected, *W. mikolae* formed ectendomycorrhizas and the number of mycorrhizal root tips was the same in treatments with and without inoculation with *Bacillus*. The authors did not comment on whether there were differences in structural features of the ectendomycorrhizas formed. Inoculation with *Bacillus* alone did not affect shoot or root biomass or total foliar nitrogen content, but inoculation with *W. mikolae* alone reduced shoot biomass and total foliar nitrogen content relative to controls. Coinoculation resulted in higher shoot and root biomass than with *W. mikolae* alone and lower total foliar nitrogen than controls. A continuation of this line of research might discover effective E-strain fungus-bacteria combinations that enhance seedling growth in the field.

Occurrence and effects of ectendomycorrhizas

Although ectendomycorrhizal associations may be limited to a few fungus species that associate with *Pinus* and *Larix* to form characteristic structural features, the fungi involved are able to form ectomycorrhizas with a number of conifer and angiosperm genera and, therefore, have a broad host range (Molina et al. 1992). The wide global distribution of these fungi and the diverse habitats in which they are found suggest that they play a more significant role in the survival and growth of tree species than previously recognized. A recent study (Massicotte et al. 1999) confirmed the presence of E-strain fungi in a variety of habitats and their ability to colonize a range of hosts. These authors showed that an E-strain fungus, probably *Wilcoxina mikolae*, colonized roots of *Abies grandis* (Dougl.) Lindl., *Pseudotsuga menziesii* (Mirb.) Franco, *Pinus ponderosa* Dougl. ex Laws., *Lithocarpus densiflora* (Hook. & Arn.) Rehd. and *Arbutus menziesii* Pursh. when seedlings were planted in soil taken from three forest sites ranging from a clearcut site to a mature Douglas-fir-pine forest in southwestern Oregon, USA. Obviously, propagules of E-strain fungi were present in all of these soils and, under the experimental methods used for the synthesis of mycorrhizas, they colonized both conifer and angiosperm tree species.

Early work indicated that E-strain fungi and ectendomycorrhizas were mostly found in conifer nurseries and disturbed sites (Mikola 1965, 1988) and, consequently, most of the research on the potential role of ectendomycorrhizas has involved these situations. LoBuglio and Wilcox (1988) found that *Pinus resinosa* seedlings inoculated with either an E-strain isolate or *Phialophora finlandia* and planted on iron tailings tended to show better survival rates than controls.

Seedlings of *Pinus banksiana* inoculated with a variety of mycorrhizal fungi including an E-strain isolate and outplanted into peat-amended oil-sand tailings showed a progressive loss of most of the inoculated fungus species over 3 years (Danielson and Visser 1989). Interestingly, only E-strain ectendomycorrhizas were present in substantial numbers over the full 3 years, but it was not determined whether the mycorrhizas formed were by the inoculated isolate or by indigenous E-strain fungi. Inoculation with the E-strain isolate resulted in an increase in shoot growth relative to controls after 2 years, suggesting that at least this isolate could be useful in enhancing early seedling growth under these conditions. This contrasts with results of Levisohn (1954) with *Pinus sylvestris* seedlings in which inoculation with colonized root pieces from the same species resulted in a decrease in shoot growth.

Seedlings of *Picea glauca* planted in coal spoil were colonized solely by E-strain fungi for the first 2 years and only by year 4 were E-strain fungi gradually replaced by the basidiomycete *Amphinema byssoides* (Fr.) J. Erikss. (Danielson 1991). Even after 10 years, 25% of root tips were still colonized by E-strain fungi. In contrast, *Pinus banksiana* seedlings grown on oilsand tailings only developed mycorrhizal root tips with E-strain fungi if the tailings were amended with peat; these fungi then dominated root systems (Danielson 1991). The experiment was not set up to test the effects of E-strain fungi on seedling growth so nothing can be concluded about their physiological role.

In a conifer seedling nursery in Ontario, Canada, Ursic et al. (1997) found a high rate of root rot symptoms on *Pinus strobus* seedlings and a high incidence of root tips

colonized by E-strain and other ascomycetous fungi, including *Phialophora finlandia,* a member of the *Mra* known to form ectendomycorrhizas. Although there was no statistical correlation between the two, the authors suggested that the dominance of E-strain mycorrhizas in 2- and 3-year-old seedlings excluded beneficial basidiomycetous fungi from colonizing roots. However, in preliminary in vitro tests to determine the interaction between E-strain fungi and several species of ectomycorrhizal fungi, Mikola (1965) found no inhibitory effects when various combinations of E-strain fungi and ectomycorrhizal fungi were grown together on culture plates. These types of tests may not be representative of what occurs in natural settings, where other abiotic and biotic factors are involved. Indeed, the studies by Danielson and Visser (1989) and other authors suggest that there is competition between inoculated and indigenous fungi when seedlings are outplanted.

An important consideration when discussing any mycorrhizal association is the cost-benefit balance of the relationship (Johnson et al. 1997). This has not been studied in any detail for ectendomycorrhizas. In other mycorrhizal systems such as arbuscular mycorrhizas, early colonization of seedlings is costly to the plant because seedlings may depend primarily on resources in the seed for carbon compounds, and the fungus competes for the limited carbon available (Johnson et al. 1997). E-strain fungi often predominate on tree seedlings found in nurseries and degraded sites (Mikola 1988; Danielson 1991), but the cost to seedlings has not been measured. Ectendomycorrhizas can develop under conditions of very low irradiance and presumably low photosynthate production (Mikola 1965), which suggested to Mikola (1988) an explanation for the early colonization of nursery seedlings by E-strain fungi and not ectomycorrhizal fungus species. The latter require considerable carbon for their development. Because some fungus species involved in forming ectendomycorrhizas are capable of hydrolyzing complex polysaccharides (Egger 1986; Caldwell et al. 2000), it is possible that carbon compounds are absorbed by hyphae and translocated to seedlings before they become autotrophic. Alternatively, these fungi may be able to acquire their carbon from the breakdown of complex carbohydrates in the soil while establishing themselves on roots and thus not be a carbon drain on seedlings. To date, the extent of extraradical mycelium and its role in nutrient absorption and translocation in ectendomycorrhizas have not been determined.

Research is required to determine the role of E-strain fungi during early seedling growth in a variety of habitats. In very disturbed sites, such as heavily burned areas, perhaps propagules, i.e. chlamydospores, of E-strain fungi are among mycorrhizal perennation structures present when the first tree seedlings emerge. Research on the tolerance of hyphae and chlamydospores of *Wilcoxina* and other E-strain fungi to high temperatures, heavy metals, and other soil pollutants is required.

Although Mikola (1988) suggested that tree seedlings benefit from colonization by fungus species such as *Wilcoxina*, more research is required on the basic biology of the fungus symbionts and the ectendomycorrhizas they form, before their utilization in forestry becomes a reality.

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