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Comparative studies of ectomycorrhiza formation in *Alnus glutinosa* and *Pinus resinosa* with *Paxillus involutus*

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Abstract Mycorrhiza ontogeny and details of Hartig net and mantle structure were compared in ectomycorrhizas synthesized in growth pouches between the broad host range fungus *Paxillus involutus* and the tree species European black alder (*Alnus glutinosa*) and red pine (*Pinus resinosa*). In *Alnus glutinosa*, a paraepidermal Hartig net was restricted to the proximal (basal) portion of first-order laterals; the hypodermal layer appeared to be a barrier to fungal penetration. Phi-thickenings were present in some cortical cells but these were not related to lack of fungal ingress into the cortex. The mantle was often present close to the root apex but in many roots it was loosely organized and patchy. In several instances, the mantle formed around the root apex was only temporary; renewed root growth occurred without the formation of a mantle. In *Pinus resinosa*, the Hartig net developed between cortical cell layers of monopodial and dichotomously branched first–order laterals. Fungal hyphae in the Hartig net exhibited a complex labyrinthine mode of growth. The mantle had a pseudoparenchymatous structure and covered the root, including apices of dichotomously branched roots. The *Paxillus–Pinus resinosa* interaction had all the characteristics of a compatible ectomycorrhizal association. The *Paxillus–Alnus glutinosa* interaction, however, showed only aspects of superficial ectomycorrhizas, including the presence of a minimal (sometimes absent) and mostly proximal Hartig net and variable mantle development. Sclerotia were produced in the extraradical mycelium of *Paxillus involutus* when associated with either *Alnus glutinosa* or *Pinus resinosa*.

Key words Black alder · Red pine · Structure · Hartig net · Compatibility · Mycorrhiza

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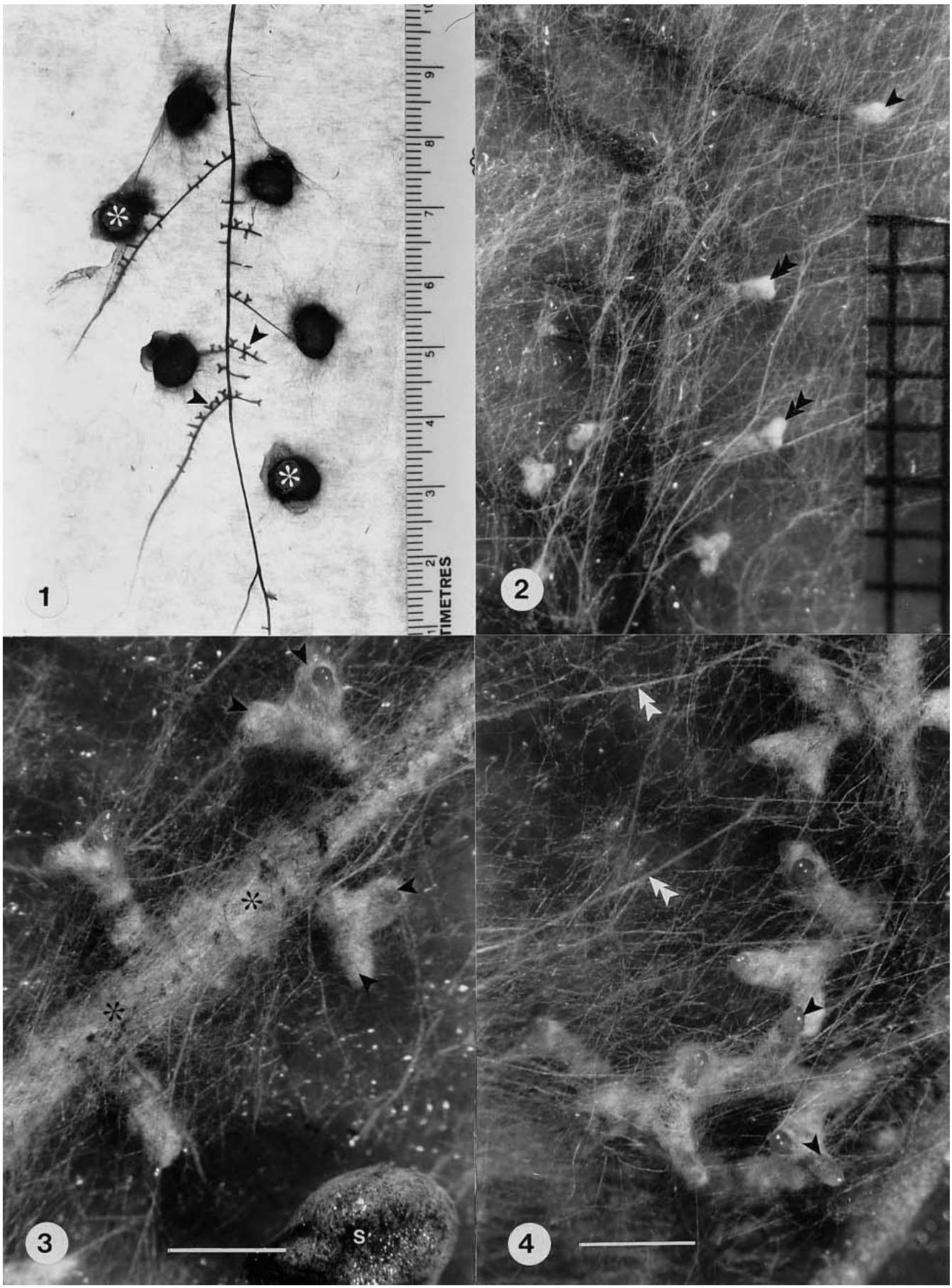
Introduction

Paxillus involutus, a fungus species most frequently found in temperate zones of the Northern Hemisphere (Laiho 1970), is regarded as a wide-host-range ectomycorrhizal symbiont associating with both angiosperm and conifer hosts (Trappe 1962; Laiho 1970). Some reports, however, have suggested that *Paxillus involutus* is a facultative symbiont, based on observations that sporocarps occur following trenching of host trees or in the absence of host trees, although this was questioned by Laiho (1970). *Paxillus involutus* is of interest as an ectomycorrhizal symbiont because of its potential use in biological control of various pathogenic fungus species (Duchesne et al. 1988, 1989) and its ability to degrade lignin (Haselwandter et al. 1990), cellulose, and proteins (Maijala et al. 1991). In addition, this species forms sclerotia (Grenville et al. 1985a; Moore et al. 1991) that can be induced in vitro by a cold temperature treatment (Moore and Peterson 1992). Sclerotia are of potential use as inocula and as a means of conserving fungus genotypes (Grenville et al. 1985b).

Paxillus involutus forms typical ectomycorrhizas with various host tree genera including *Pinus* (Laiho 1970; Grenville et al. 1985b; Pargney and Gourp 1991; Turnau et al. 1994; Shaw et al. 1995), *Betula* (Gaie 1977a; Brun et al. 1995), *Salix* (Gaie 1977b), *Picea* (Kifer 1974; Marschner and Godbold 1995) and *Quercus* (Branzanti and Zambonelli 1989). Reports on the association between *Paxillus involutus* and the genus *Alnus*, however, have been variable. Laiho (1970) did not find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Figs. 1–4 *Pinus resinosa* roots colonized with *Paxillus involutus*
Fig. 1 Portion of a growth pouch, 17 days after inoculation, showing numerous second-order mycorrhizal roots (*arrowheads*). Inoculum plugs (*) are present; *scale* mm
Fig. 2 Portion of a root system, 34 days after inoculation, showing monopodial (*arrowhead*) ectomycorrhizas and young dichotomous second-order mycorrhizal roots (*double arrowheads*). Roots are mostly colonized in the apical portions; *scale* mm
Fig. 3 Portion of a root system, 34 days after inoculation, showing well-developed dichotomous second-order mycorrhizal roots (*arrowheads*) as well as extensive colonization of the first-order root (*). Note the patchy texture of the mantle on this first-order root. A sclerotium (*S*) has developed; *bar* 1 mm
Fig. 4 Portion of a root system, 34 days after inoculation, showing older second-order dichotomous mycorrhizal roots that have dichotomized once again. Many apices have grown out of their mantle (*arrowheads*). Rhizomorphs (*double arrowheads*) are evident; *bar* 1 mm

Paxillus involutus – *Alnus* ectomycorrhizas in nature, and synthesis studies with *Alnus glutinosa* (L.) Gaertn. and *Alnus incana* (L.) Moench failed to result in mycorrhiza formation. Molina (1979, 1981), however, was able to synthesize ectomycorrhizas between *Paxillus involutus* and *Alnus rubra* Bong, *Alnus glutinosa* (L.) Gaertn., *Alnus incana* (L.) Moench, *Alnus sinuata* (Regel) Rydb., and *Alnus rhombifolia* Nutt. but all resulted in very poor Hartig net formation. Godbout and Fortin (1983), using the growth pouch method, obtained ectomycorrhizas with good paraepidermal Hartig net development when *A. rugosa* var. *americana* (Regel) Fern. and *A. crispa* (Ait.) Pursh. seedlings, inoculated with *Frankia* to induce nodule formation, were subsequently inoculated with *Paxillus involutus*. *Alnus serrulata* (Ait.) Willd. inoculated with *Paxillus involutus* formed ectomycorrhizas with sporadic Hartig net development (Murphy and Miller 1994). Several of the 16 field-collected morphotypes of *Alnus glutinosa* recently characterized in Germany exhibited a paraepidermal Hartig net (Pritsch et al. 1997) but none appeared to belong to *Paxillus involutus*.

Ectomycorrhiza formation between *Paxillus involutus* and *Alnus* species appears to be variable, but no detailed anatomical studies have been done. Differing results with *Alnus glutinosa* (Laiho 1970; Molina 1981), and the dependency of ectomycorrhiza formation on numerous abiotic and biotic factors (Smith and Read 1997), make it a good choice for a comparative anatomical study. *Pinus resinosa,* known to form ectomycorrhizas with *Paxillus involutus* (Grenville et al. 1985b) was included for comparison.

Materials and methods

Plant material and ectomycorrhiza synthesis

Red pine (*Pinus resinosa* Ait.) seeds, obtained from Petawawa, Ontario (45°24′ N, 75°33′ W, 70 m) and European black alder [*Alnus glutinosa* (L.) Gaertn.] seeds, obtained from Turkey (positional data unrecorded), were germinated as described for *Alnus crispa* by Godbout and Fortin (1983), with the exception that H_2O_2 surface-sterilization was performed for 40 and 20 min, respectively.

Seedlings of *Pinus resinosa* were transferred, 10 days after germination, into growth pouches containing 10 ml of modified Melin-Norkrans (MMN) solution without glucose (Marx and Bryan 1975). The mycobiont was grown and introduced as plugs into the pouches as described previously (Massicotte et al. 1986). Forty-two days after germination, seedlings were inoculated with *Paxillus involutus* (Batsch.) Fr. using the strain CG-9 (CRBF, University Laval, Québec, Canada) isolated in 1980 in the vicinity of *Populus tremuloides* Michx. (Godbout and Fortin 1983). Seedlings of *Alnus glutinosa* were transferred 8 days after germination into growth pouches containing 10 ml of modified Crone's mineral solution without glucose (Lalonde and Fortin 1972). Seedlings were then inoculated with *Frankia* 14 days after germination and with *Paxillus involutus* 32 days after germination. *Alnus glutinosa* and *Pinus resinosa* were also separately inoculated with a second strain of *Paxillus involutus* (CRBF-0262), isolated in the vicinity of *Larix laricina* in 1980. Differences in timing for inoculation between the two hosts depended on the degree of root development in the pouches.

Approximately 100 pouches of *Alnus glutinosa* out of 150 and 30 pouches of *Pinus resinosa* out of 50 were successfully colonized in four different experiments over 3 years.

Growth conditions

Seedlings were grown under light (130 $\mu E/m^{-2}sec^{-1}$) on a 16-h light–8-h dark cycle at 24 °C day–18 °C night temperatures. High levels of humidity (60–80% RH) were maintained with a humidifier. Additional nutrient solution was added to pouches as needed.

External morphology and light microscopy

The external morphology of roots and ectomycorrhizas was examined with a Zeiss DR photodissecting microscope at intervals of 2–3 days after inoculation. Samples were collected from a period up to 2 weeks after the appearance of a woolly mantle on *Alnus glutinosa* and up to 8 weeks on *Pinus resinosa*. Tissue was fixed according to a procedure described previously (Massicotte et al. 1986), then dehydrated in a graded ethanol series and embedded in LR White Resin (London Resin Co.). Sections (1–1.5 μm) were cut with glass knives and stained for light microscopy with 0.05% toluidine blue O in 1% sodium borate. More than 20 samples of each ectomycorrhizal association were examined. The samples were collected from two separate sets of experimental syntheses for *Pinus* and from four separate experiments in the case of *Alnus.*

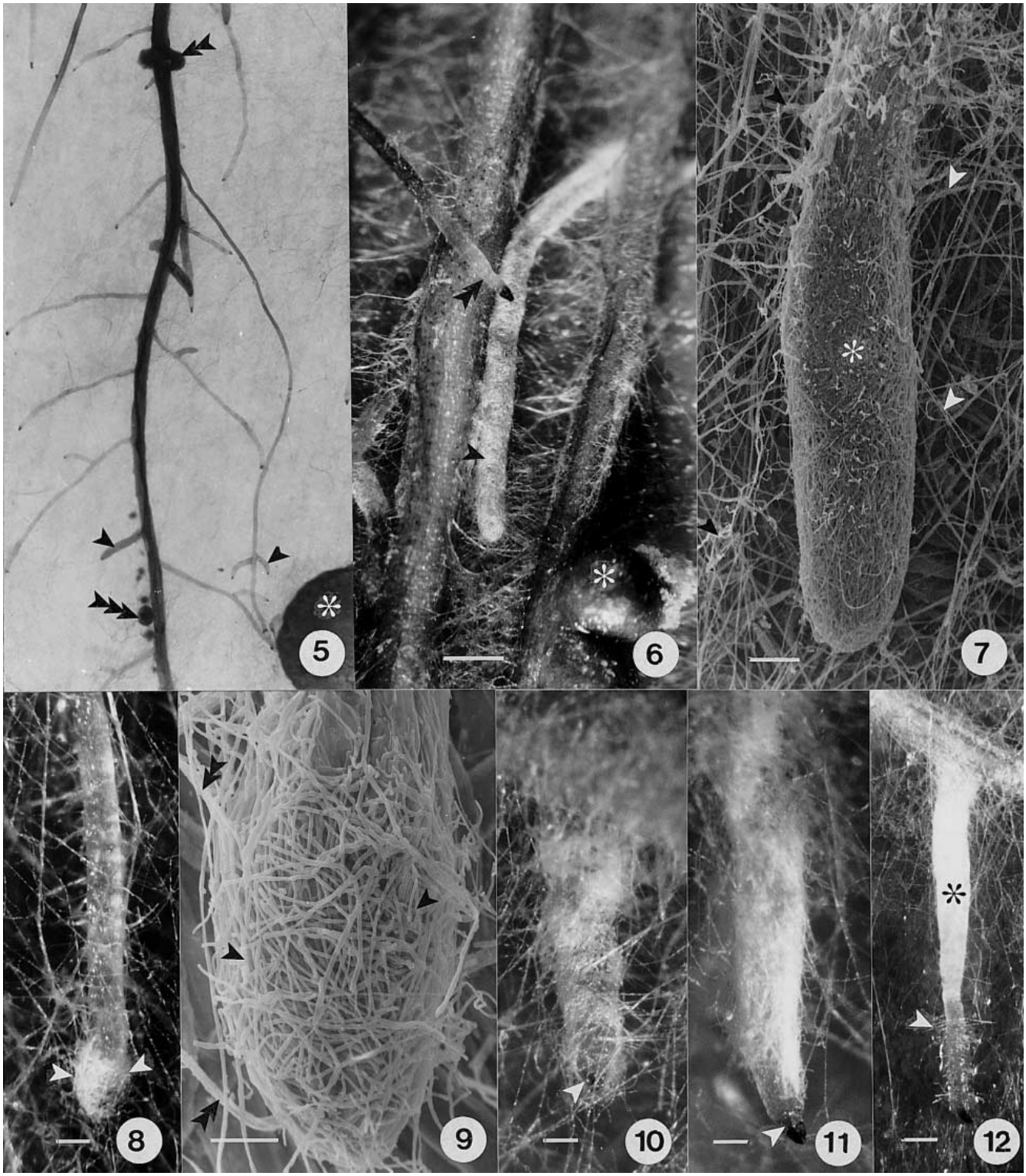
Scanning electron microscopy

Samples were fixed and dehydrated as above, critical point-dried, sputter coated with gold-palladium and viewed at 25 kV with a JEOL 35C scanning electron microscope.

Results

Strain variation

Only strain CG-9 was successful in establishing some level of root colonization on *Alnus glutinosa*. None of the pouches inoculated with strain CRFB-262 were successful. Both strains established mycorrhizas with *Pinus*



resinosa. We, therefore, focus the following descriptions using the CG-9 strain only.

External morphology

Seedlings of *Pinus resinosa* grew moderately well in plastic pouches and produced many first- and second-order lateral roots, the majority of which became mycorrhizal (Fig. 1). Typically, thin mantles were detected as early as 4 days after inoculation and well-developed mantles within 6–10 days after inoculation. Monopodial and dichotomous second-order laterals were often mycorrhizal within 1–2 weeks (Fig. 2). As the root system developed, once-dichotomized (Fig. 3) and twice-dicho-

Figs. 5–12 *Alnus glutinosa* roots inoculated with *Frankia* and colonized with *Paxillus involutus*
Fig. 5 Portion of a growth pouch showing *Frankia*-induced root nodules (*double arrowhead*), first-order and second-order mycorrhizal roots (*arrowheads*), 35 days after inoculation. An inoculum plug (*), approx. 6 mm in diameter, and sclerotium (*triple arrowhead*) are present
Fig. 6 Portion of a root system, 35 days after inoculation, showing a well-colonized first-order mycorrhizal root (*arrowhead*) adjacent to a non-mycorrhizal apex (*double arrowhead*) and a *Frankia*-induced nodule (*); *bar* 0.5 mm
Fig. 7 Scanning electron micrograph (SEM) of root similar to that indicated by the arrowhead in Figure 6. The mantle (*) adjacent to the root surface is compact and numerous loosely arranged hyphae (*arrowheads*) are attached to this; *bar* 50 μm
Fig. 8 A young mycorrhizal root, 13 days after inoculation, that has barely formed a mantle at the root apex (*arrowheads*); *bar* 100 μm
Fig. 9 SEM of root similar to that indicated in Figure 8 showing the interwoven mantle hyphae (*arrowheads*) confined to the root apex. Portions of rhizomorphs (*double arrowheads*) are evident; *bar* 25 μm
Fig. 10 A young mycorrhizal root, 13 days after inoculation, with a thin, patchy mantle covering most of the root. The pigmented apex (*arrowhead*) is still visible; *bar* 100 μm
Fig. 11 A young mycorrhizal root, 13 days after inoculation, with a pigmented apex (*arrowhead*) that had just started to grow out of a well-formed mantle; *bar* 100 μm
Fig. 12 An older mycorrhizal root with an apex that has grown out of its mantle (*). Root hairs (*arrowhead*) have formed on the exposed root tip; *bar* 250 μm

tomized (Fig. 4) mycorrhizal roots soon appeared. On older non-mycorrhizal portions of the roots adjacent to mycorrhizal roots (Fig. 3), hyphal growth on the root often appeared patchy. Mycorrhizal roots often appeared to have a fluffy, well-developed basal mantle and an apex free of hyphae (Figs. 3, 4).

Seedlings of *Alnus glutinosa* grew more rapidly than seedlings of *Pinus resinosa* under similar conditions, and produced numerous first-, second-, and third-order laterals, some of which became mycorrhizal (Fig. 5). Nodules formed in more proximal regions of the primary root (Fig. 5), and thin mantles were detected as early as 5 days following inoculation. On mycorrhizal roots, well-developed fluffy mantles surrounded the apex within 7–14 days (Fig. 6). SEM images revealed compact hyphae on the root surface and loose mantle hyphae at the periphery (Fig. 7). Fungus colonization pattern varied on different root tips (Fig. 8–12). Some young mycorrhizal tips were colonized at the apex (Fig. 8) and had a mantle of interwoven hyphae (Fig. 9). Other roots were completely surrounded by a patchy mantle (Fig. 10), or displayed renewed growth of an apparently uncolonized root apex from a root with a basal mantle (Fig. 11). Renewed apical root growth was often followed by root hair initiation (Fig. 12). *Alnus* seedlings did not always form mycorrhizas when mycelium was present on the root system and a general darkening of the root system was often observed.

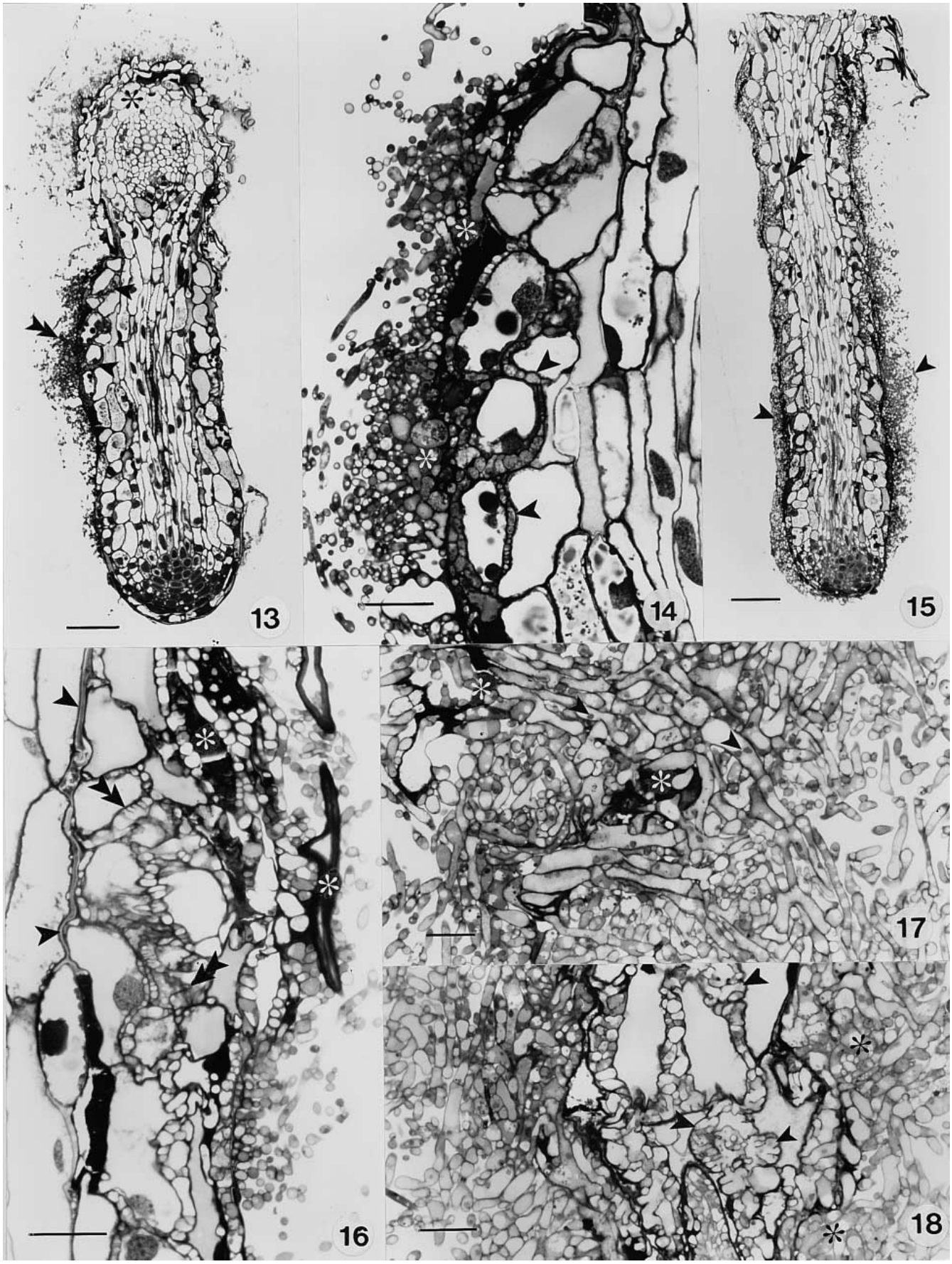
Light microscopy

In *Pinus resinosa*, colonized lateral roots formed ectomyccorhizas with varying degrees of mantle and Hartig net development (Figs. 13–15). Hartig net hyphae showing characteristic labyrinthic growth occurred up to the collapsed and "phenolized" endodermis (Fig. 16). The mantle was unequal in thickness and often incorporated dark, collapsed, root cap cells (Fig. 16). Paradermal sections revealed a pseudoparenchymatous layer consisting of wide-diameter hyphae in the inner mantle (Fig. 17), and the labyrinthic growth of hyphae in the cortex (Fig. 18). Many ectomycorrhizal roots dichotomized to produce two apical meristems (Fig. 19). Extension growth of the two axes occurred and each had a well-developed mantle and intercellular penetration of Hartig net hyphae behind the apex (Figs. 20, 21) in all cortical cell layers up to the collapsed endodermal layer (Fig. 22). Septa and nuclei were present in Hartig net hyphae (Fig. 22), and the inner mantle often incorporated densely staining material, presumably collapsed root cap cells (Fig. 22).

Alnus glutinosa first-order mycorrhizal roots showed varying degrees of mantle development, ranging from patchy (Fig. 23) to complete (Fig. 24). In sub-apical regions, the mantle was apposed against epidermal cells, some of which had intensely staining walls (Fig. 24, 25). In more basal portions, intercellular penetration of Hartig net hyphae was sporadic and confined to the epidermis (Fig. 26). In the sub-apical regions (Fig. 27), the hyphae did not penetrate between epidermal cells, the outer tangential wall of epidermal cells stained intensely, the hypodermal layer showed shrinkage, and thickenings (Phi-thickenings) occurred in the radial walls in the second row of cortical cells (Fig. 30). More proximally, the mantle was still apposed to epidermal cells, some of which differentiated into root hairs, and the Phi-thickenings were thinner and fewer in number (Fig. 28). A still more proximal section revealed a compact mantle and a well-developed intercellular epidermal Hartig net that showed both paraepidermal and periepidermal characteristics (Fig. 29). Higher magnification also indicated a darkening of tangential walls between the second and third layer of cells (Fig. 31) and a mucilaginous matrix embedding the hyphae in the mantle (Fig. 31).

Discussion

Isolate CG-9 of *Paxillus involutus* used in this study formed ectomycorrhizas with a full mantle and an Hartig net around epidermal and cortical cells in roots of *Pinus resinosa* seedlings. This is consistent with results obtained with a different *Paxillus involutus* isolate on this tree species (Grenville et al. 1985b). Cortical cell walls did not show thickening and vacuolar deposits were not synthesized in response to colonization by the fungus. These results, along with work on other *Pinus*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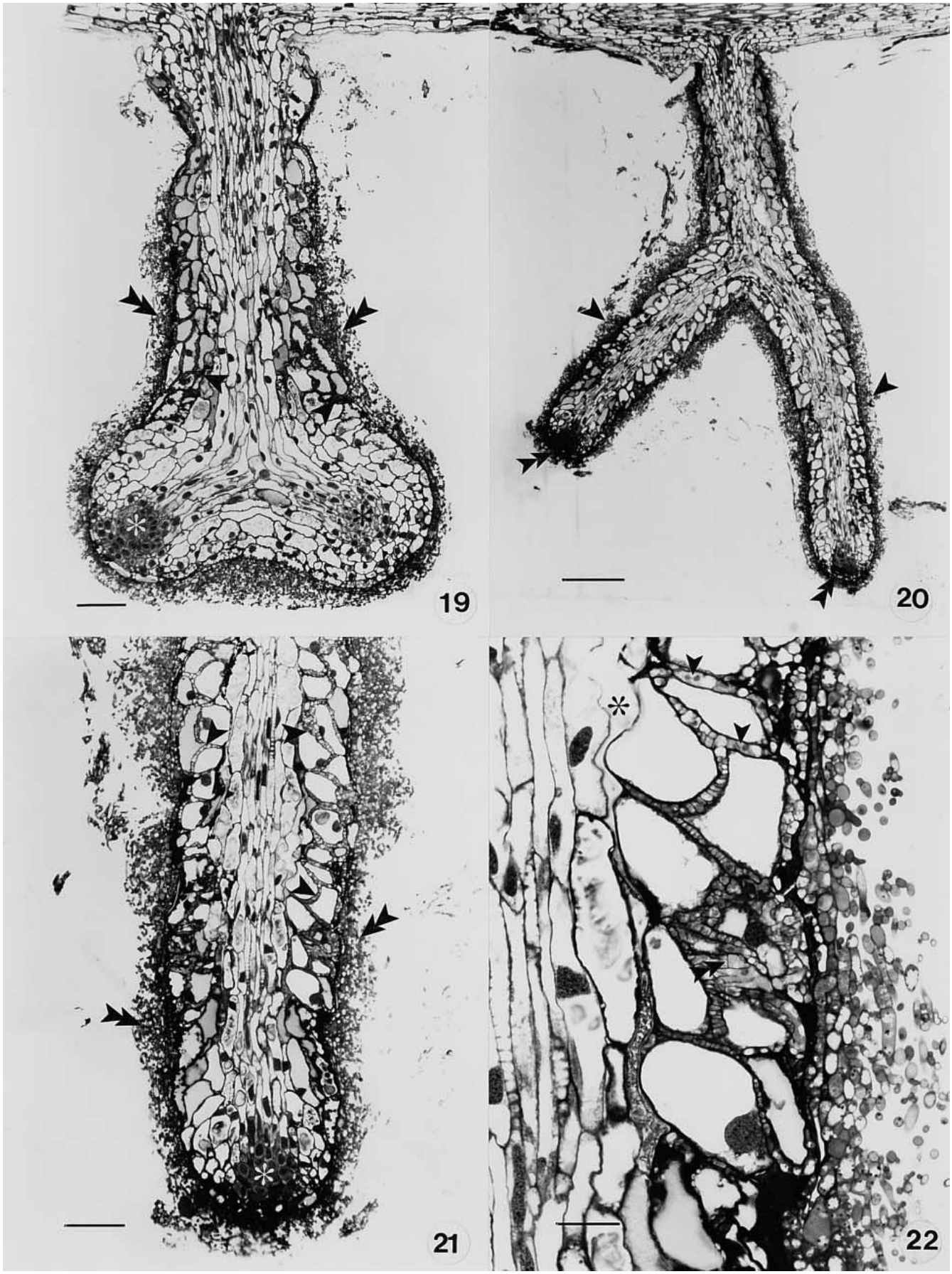
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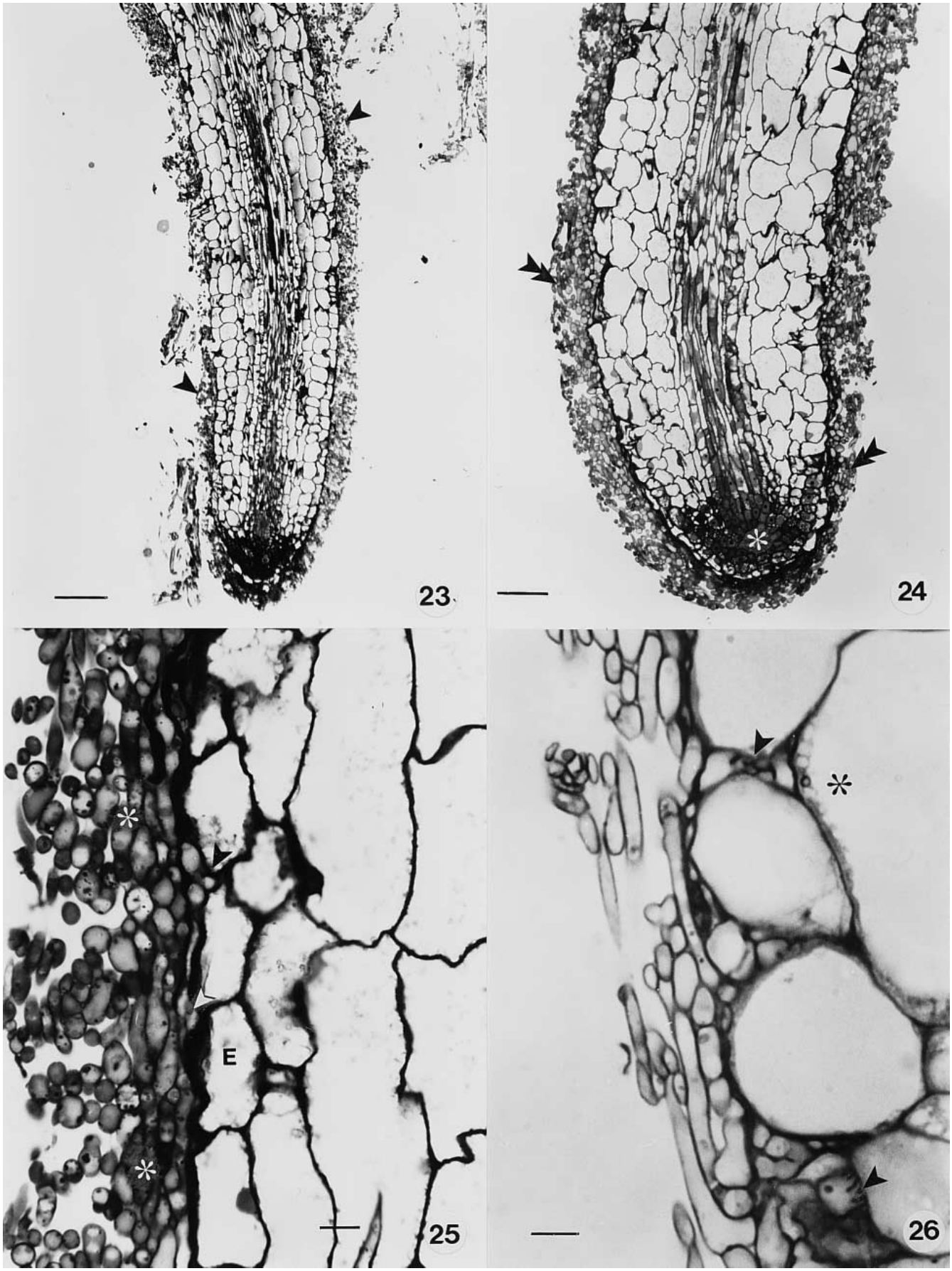
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Figs. 13–18 Light microscopy of toluidine blue O (TBO)-stained sections of *Pinus resinosa* monopodial second-order mycorrhizal roots colonized with *Paxillus involutus*
Fig. 13 Longitudinal section of partially colonized root. A portion of the mantle (*double arrowhead*) and intercellular penetration (*arrowheads*) can be seen on one side of the root. A few hyphae are also present on the first-order root (*); *bar* 100 μm
Fig. 14 Higher magnification of an adjacent section of the same root as in Figure 13 showing a localized, partially-formed mantle (*) and intercellular penetration (*arrowheads*); *bar* 25 μm
Fig. 15 A well-colonized longer root with a mantle (*arrowheads*) enveloping most of the root and a well-developed Hartig net mainly in the proximal region (*double arrowhead*); *bar* 100 μm
Fig. 16 A higher magnification of a proximal portion of Figure 15 showing intercellular penetration of the Hartig net up to the collapsed endodermis (*arrowhead*). Note the labyrinthic mode of growth (*double arrowheads*). Sloughed cells with dense material (*) are present among mantle hyphae; *bar* 25 μm
Fig. 17 A paradermal section (at the inner mantle level) of a root similar to the one shown in Figure 15. Hyphae have started to swell and have formed a compact pseudoparenchymatous layer. Nuclei (*arrowheads*) are obvious in some hyphae and dark material, likely sloughed root cap cells, are included within the mantle (*); *bar* 25 μm
Fig. 18 A paradermal section of a root similar to the one shown in Figure 15, taken at the epidermal level. The labyrinthic growth (*arrowheads*) of the Hartig net is obvious between the epidermal cells. Note the compact portion of the mantle (*); *bar* 25 μm

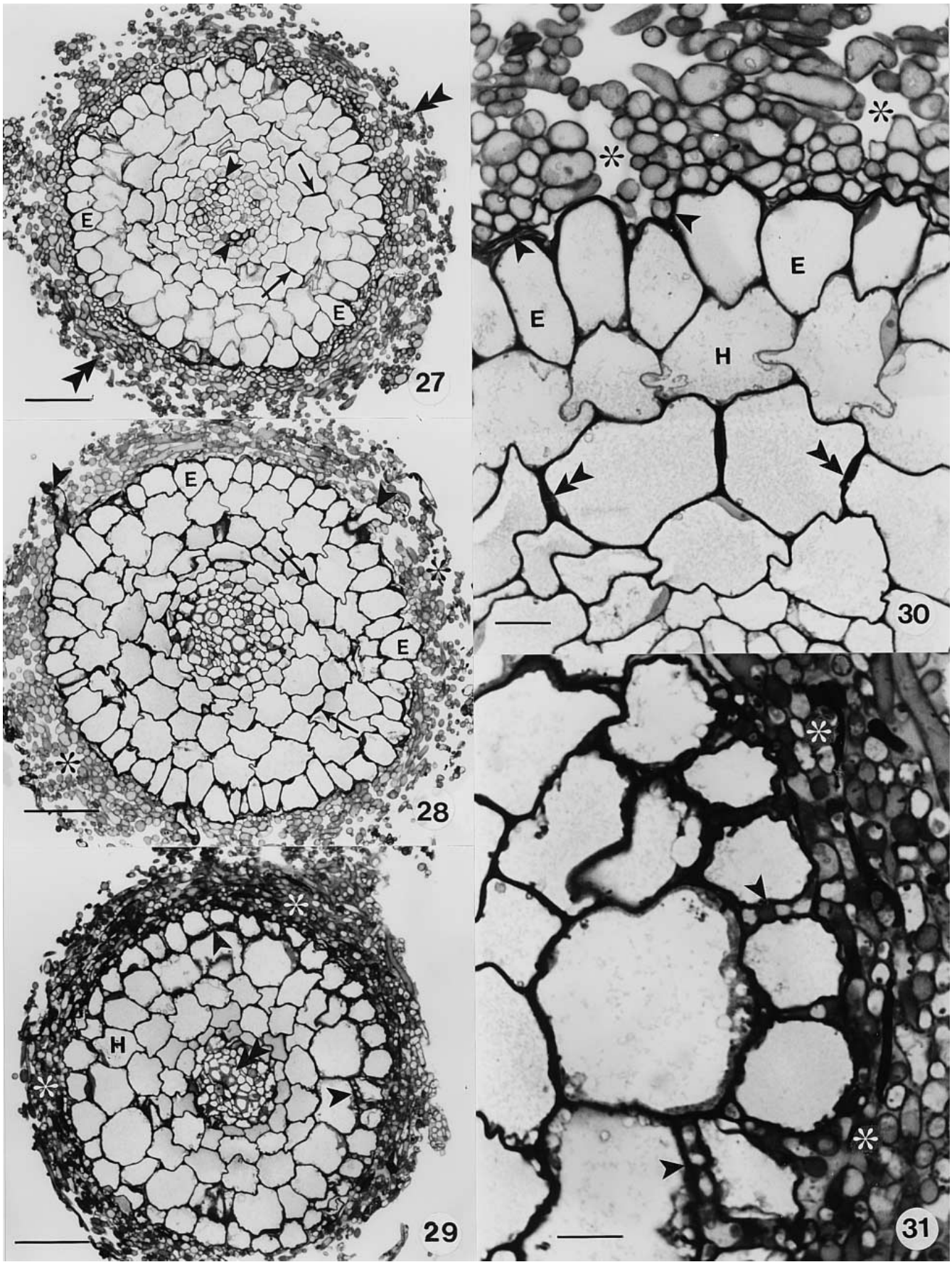
Figs. 19–22 Light microscopy of TBO-stained sections of *Pinus resinosa* second-order mycorrhizal roots colonized with *Paxillus involutus*
Fig. 19 A root that has dichotomized showing two apical meristems (*). The root is well covered with mantle (*double arrowheads*) and has intercellular hyphal penetration (*arrowheads*) present up to the level of root splitting; *bar* 100 μm
Fig. 20 Older dichotomous root with elongated branches. A mantle (*arrowheads*) surrounds both branches and the unbranched base. Two meristems (*double arrowheads*) are present; *bar* 250 μm
Fig. 21 Higher magnification of an elongated branch shown to the left in Figure 20 with a well-developed meristem (*). The mantle (*double arrowheads*) envelopes the branch and Hartig net hyphae (*arrowheads*) are present close to the meristem; *bar* 100 μm
Fig. 22 Higher magnification of a portion of root indicated in Figure 21 showing Hartig net up to the collapsed endodermis (*). Nuclei (*arrowheads*) and septa (*double arrowhead*) are present in branched Hartig net hyphae; *bar* 25 μm

Figs. 23–26 Light microscopy of longitudinal TBO-stained sections of *Alnus glutinosa* first-order mycorrhizal roots colonized with *Paxillus involutus*
Fig. 23 Long root showing a sporadically-developed mantle (*arrowheads*), without detectable intercellular penetration; *bar* 100 μm
Fig. 24 Shorter root showing a well-developed mantle (*double arrowheads*) covering the root apex. The root apical meristem (*) is obvious. Sporadic intercellular penetration (*arrowheads*) is present in the basal portion of the root; *bar* 50 μm
Fig. 25 Higher magnification in the sub-apical portion of a root similar to the one shown in Figure 24 showing the mantle (*) apposed on epidermal (*E*) cells. Hyphal tips (*arrowheads*) have started to penetrate between epidermal cells; *bar* 10 μm
Fig. 26 Higher magnification in basal portion of a root similar to the one shown in Figure 24 showing intercellular penetration up to the hypodermis (*) and some labyrinthic growth (*arrowheads*) of Hartig net hyphae; *bar* 10 μm

and *Picea* species (Kiffer 1974; Marschner and Godbold 1995), indicate that this fungus forms typical ectomycorrhiza features, and presumably is an effective symbiont with these conifer genera. The situation is similar to that with several angiosperm genera including *Betula* (Gaie 1977a; Brun et al. 1995), *Salix* (Gaie 1977b) and *Quercus* (Branzanti and Zambonelli 1989), and confirms the broad host range typical of *Paxillus involutus* (Laiho 1970).

In specific conditions tested in the synthesis experiments (MMN without glucose), we observed two different outcomes with *Paxillus involutus* CG-9 and *Alnus glutinosa*. The first outcome was observed repeatedly in several pouches: the entire root system was colonized by a thin covering of hyphae, but root tips were not colonized to form an ectomycorrhiza structure. The second outcome was the formation of more typical ectomycorrhizal rootlets, with a patchy and variable mantle. In some cases, the appearance of stable ectomycorrhiza was very transitory, as the apex would often grow through the mantle. These mycorrhizas usually had a sporadic paraepidermal Hartig net in the basal (proximal) portions of the rootlet. This is in agreement with previous work (Godbout and Fortin 1983; Massicotte et al. 1986, 1989a,b; Pritsch et al. 1997), even though deeper Hartig net penetration has been reported on *Alnus rubra* (Miller et al. 1991) and *Alnus sinuata* (Helm et al. 1996). Pritsch et al. (1997) also documented an unusual instance of intracellular penetration in epidermal and cortical cells in a *Lactarius lilacinus* morphotype. More observations are required from field and lab synthesis material to clarify the pattern of root colonization on alder.

The mantles we observed were often patchy and very loosely organized. Molina (1981) described the mantle formed between these symbionts as 'irregular'. Where the mantle interfaced with epidermal cells, the latter often developed intensely staining walls, a feature indicated as well in the micrograph of an ectomycorrhiza between *Paxillus involutus* and *Alnus serrulata* (Murphy and Miller 1994) and in written descriptions of ectomycorrhizas between *Paxillus involutus* and a number of *Alnus* species (Molina 1981). Duddridge (1986) reported similar results in interactions between *Suillus grevillei* (Klotzsch) Sing. and seedlings of both *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus sylvestris* L. Deposition of phenolic compounds in plant cell walls and vacuoles frequently indicates an incompatible interaction between ectomycorrhizal fungi and host roots (Nylund and Unestam 1982; Malajczuk et al. 1984; Duddridge 1986). It is noteworthy that several attempts in processing our mycorrhizal alder roots from this experiment, using either Spurr's or Epon as an embedding medium, failed. Only protocols using LR White resin were successful for this recalcitrant material, perhaps because of the presence of phenol-like compounds in the epidermis. In spite of the deposition of intensely staining material in epidermal and cortical cell walls, some hyphae were able to penetrate between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Figs. 27–31 Light microscopy of transverse TBO-stained sections of *Alnus glutinosa* first-order mycorrhizal roots colonized with *Paxillus involutus*

Fig. 27 Sub-apical portion of a root similar to the one shown in Figure 24 showing a well-developed mantle (*double arrowheads*) apposed against the epidermis (*E*). Note the maturing protoxylem elements (*arrowheads*) in the stele and Phi-thickenings (*arrows*) along cortical cell walls; *bar* 50 μm

Fig. 28 Section taken more proximally than the one in Figure 27 showing the loose mantle (*) apposed to epidermal (*E*) cells, some of which have developed into root hairs (*arrowheads*). No obvious intercellular penetration is present at this level. Phi-thickenings are sporadic (*arrows*); *bar* 50 μm

Fig. 29 Section taken more proximally than the one in Figure 28 showing a compact mantle (*) and obvious intercellular penetration of Hartig net hyphae (*arrowheads*) up to the hypodermis (*H*). Note the differentiated metaxylem elements (*double arrowheads*) in the stele. Root hairs and Phi-thickenings are not present at this level; *bar* 50 μm

Fig. 30 Higher magnification of Figure 27 showing the loose hyphal structure of the mantle (*), hyphae that had started to penetrate between epidermal cells (*E*), collapsed cells (likely due to suberin walls) of the hypodermis (*H*), a third layer of cells with Phi-thickenings (*double arrowheads*). Note the deposition of extracellular material (*arrowheads*) between the epidermis and penetrating hyphae; *bar* 10 μm

Fig. 31 Higher magnification of Figure 29 showing the intercellular penetration of Hartig net hyphae (*arrowheads*) up to the second layer of root cells, and a thick and compact mantle (*) surrounding the root. Tangential walls between the second and third layer of cells stain intensely; *bar* 10 μm

epidermal cells to form a limited Hartig net; wall appositions, a frequent incompatible response (Nylund et al. 1982), were not formed. The Hartig net found in some roots was never very extensive and rarely showed labyrinthic branching, in agreement with the observations of Molina (1981) for these same symbionts.

In the *Paxillus involutus–Alnus glutinosa* mycorrhizas synthesized in this study, epidermal cells did not show marked radial enlargement, a feature typical of some *Alnus* species colonized by other fungus symbionts e.g. *Alnus crispa* colonized by *Alpova diplophloeus* (Godbout and Fortin 1983; Massicotte et al. 1986), and of most angiosperm species forming ectomycorrhizas (Smith and Read 1997).

In a recent descriptive study of *Alnus glutinosa* field morphotypes (Pritsch et al. 1997), photographic evidence suggests that for almost all morphotypes, a paraepidermal Hartig net develops, and radial enlargement of epidermal cells is variable among morphotypes. A comparison of other fungus symbionts of *Alnus glutinosa* for this feature might determine if radial elongation of epidermal cells can be used as a good indicator for *Alnus*--fungus symbiont compatibility. Also, monitoring the formation of extracellular fibrillar material that bridges hyphae and the root surface during contact (Lei et al. 1991) could help determine the compatibility of symbioses with *Alnus* species. In the present study of root apices of *Alnus glutinosa*, the formation of what appeared to be transient ectomycorrhizas, i.e. the apex was able to grow out of the mantle and initiate root hairs from the protoderm, may indicate a certain degree of incompatibility. The observations made in the present study support the view that ectomycorrhiza formation in the genus *Alnus* is more fungus-specific than with many tree species (Molina 1981).

In previous synthesis experiments with *Alpova diplophloeus*, a reputedly genus-specific fungus for *Alnus* spp. (Molina et al. 1992), ontogenetic analysis revealed that the fungus colonized the root readily and initiated the paraepidermal Hartig net in close proximity to the apex, both on *Alnus crispa* (Massicotte et al. 1986) and *Alnus rubra* (Massicotte et al. 1989a,b). In the experiments reported here on *Alnus glutinosa*, the location of the minimally developed Hartig net was confined to proximal (basal) regions of the root. Godbout and Fortin (1983) reported similar observations with *Alnus crispa* and *Alnus rugosa* colonized by *Paxillus involutus*. The colonization pattern within the root between the broad-host-range *Paxillus involutus* and the genus-specific *Alpova diplophloeus* is different, and seems to be independent of the host tested.

Evidence that a functional relationship can be established between *Paxillus involutus* and *Alnus glutinosa* is provided by the work of Arnebrant et al. (1993) in which nitrogen fixed by the actinorrhizal species *Alnus glutinosa* was translocated to *Pinus contorta* Doug. Ex Loud seedlings via an interconnecting mycelium. However, it was demonstrated recently that the net transfer of nitrogen between *Alnus incana* and *Pinus sylvestris* is dependent upon the nutritional status of pine and that mycorrhiza-mediated nitrogen transfer is low and may not be significant to the fitness of the "receiver" plant (Ekblad and Huss-Danell 1995).

In the present study, the formation of sclerotia in the extensive extraradical mycelium network associated with both *Alnus glutinosa* and *Pinus resinosa* might be indirect evidence that *Paxillus involutus* acquires carbon from these hosts. However, labelling experiments would be required to confirm this because this fungus is known to be able to degrade lignin (Haselwandter et al. 1990) and cellulose (Maijala et al. 1991), which are components of the paper wick in the growth pouches.

Paxillus involutus, a broad host range ectomycorrhizal fungal species, exhibits properties such as sclerotium production (Grenville et al. 1985a), potential use in biocontrol of pathogenic fungal species (Duchesne et al. 1988, 1989), and the ability to sequester heavy metals (Turnau et al. 1994). These features, and the different patterns of colonization between gymnosperms and angiosperms such as *Alnus* spp. make it an important fungus species for further work in the context of biochemical and genetical responses between host and fungus.

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