Comparative anatomy of roots and mycorrhizae of common Ontario trees

MARK BRUNDRETT,[1] GRACIA MURASE, AND BRYCE KENDRICK[2]
Department of Biology, University of Waterloo, Waterloo, Ont., Canada N2L 3G1

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The structure of roots and mycorrhizae of trees belonging to 20 important Ontario tree genera were examined. During this study efficient methods for examining root anatomy were developed, and tree root information was compiled. The ultimate lateral roots of most species examined were consistently mycorrhizal, and many species had heterorhizic root systems with separate long and short lateral roots. Tree roots displayed enough structural diversity in features such as thickened, lignified, or suberized walls, and secondary metabolite-containing cells to allow identification of genera. The roots of trees belonged to four major anatomical groups as a result of the major differences between angiosperm and gymnosperm roots, and between those with ectomycorrhizal (ECM) or vesicular–arbuscular mycorrhizal (VAM) associations. (*i*) Members of the Pinaceae had structurally similar heterorhizic roots that had ECM with a cortical Hartig net. (*ii*) *Thuja* (Cupressaceae) had distinctive nonheterorhizic roots with phi thickenings and VAM. (*iii*) Angiosperms with ECM belonged to diverse families, but all had similar heterorhizic root systems with ECM short roots that had an epidermal Hartig net and a narrow cortex of thick-walled cells. (*iv*) Most angiosperms with VAM had nonheterorhizic roots, and this group had the greatest diversity of root structural features. Possible structural and defensive roles of root features and potential influences of these features on mycorrhizal formation are considered.

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Les auteurs ont examiné les structures des racines et des mycorhizes d'arbres appartenant à 20 genres d'arbres importants en Ontario. À l'occasion de cette étude, des méthodes efficaces pour étudier l'anatomie racinaire ainsi que des informations sur les racines des arbres ont été définies. Les racines latérales ultimes de la plupart des espèces examinées étaient normalement mycorhizées et plusieurs espèces montrent des systèmes racinaires hétérorhiziques où on retrouve des racines longues et des racines latérales courtes. Les racines montrent suffisamment de diversité structurale dans leurs caractéristiques telles que les parois cellulaires épaissies lignifiées ou subérifiées et dans le contenu cellulaire en métabolites secondaires pour permettre l'identification, du moins au niveau des genres. Les racines des arbres appartiennent à quatre groupes anatomiques majeurs; ainsi, les différences principales se retrouvent entre les racines des angiospermes et des gymnospermes aussi bien qu'entre les racines ectomycorhizées (ECM) et endomycorhizienne à vésicule et arbuscule (VAM). (*i*) Les membres des Pinaceae ont des racines hétérorhiziques de structure similaire, avec ECM et réseau de Hartig cortical. (*ii*) Le genre *Thuja* (Cupressaceae) montre des racines typiquement non-hétérorhiziques avec épaississement phi et mycorhizes de type VAM. (*iii*) Les angiospermes avec ECM appartiennent à diverses familles, mais toutes montrent des systèmes racinaires hétérorhiziques similaires, avec racines courtes ECM possédant un réseau de Hartig épidermique et un cortex mince à cellules avec parois cellulaires épaisses. (*iv*) La plupart des angiospermes avec VAM montrent des racines non-hétérorhiziques et ce groupe possède la plus grande diversité de caractéristiques structurales racinaires. Les rôles structuraux et défensifs des caractéristiques structurales et les influences que pourraient exercer ces caractéristiques sur la formation des mycorhizes font l'objet d'une discussion.

[Traduit par la revue]

Introduction

The fine roots of plants are often considered to express little structural diversity (Fitter 1987), but there is not much evidence to support this assumption. In fact, fine root anatomy has been successfully employed in a number of taxonomic surveys, including those by Noelle (1910), Wilder (1986), French (1987), and Stützel (1988). Structural features have also been used to identify fossil roots by comparing them with roots of extant species (Millay *et al.* 1987). It is now known that much of the competition between plants occurs in the soil and involves resources that are obtained by roots (Caldwell 1987). The identification of roots by chemical (Chilvers 1972) or structural properties would thus be of value in ecological studies, since it would allow belowground competition between species to be examined directly.

Earlier attempts to provide identification manuals for tree roots relied on superficial characteristics (McDougall 1921; Gilbertson *et al.* 1961). A recent root identification manual (Cutler *et al.* 1987) provided structural details, but only woody roots were examined. It is equally important that we be able to identify fine, nonwoody tree roots, since these are responsible for nutrient acquisition, mycorrhiza formation, and most exploration of the soil.

In forests throughout the world, tree roots normally contain fungal hyphae involved in mutualistic mycorrhizal associations (Meyer 1973; Kormanik 1981; Harley and Smith 1983; Le Tacon *et al.* 1987). In ectomycorrhizal (ECM) associations, the associated fungus encases the root in a mantle of interwoven hyphae and forms a Hartig net composed of labyrinthine hyphae surrounding root cells (Harley and Smith 1983; Kottke and Oberwinkler 1986; Massicotte *et al.* 1987). In vesicular–arbuscular mycorrhizal (VAM) associations, hyphae grow within the root cortex producing highly branched exchange structures (arbuscules) inside cells, and resting spores (vesicles) (Gallaud 1905; Harley and Smith 1983; Scannerini and Bonfante-Fasolo 1983).

Forests in Ontario form part of the deciduous, Great Lakes–St. Lawrence, and boreal forest zones (Hosie 1979). Trees in

[1]Present address: Soil Science and Plant Nutrition, University of Western Australia, Nedlands, W.A. 6009, Australia.

[2]Author to whom all correspondence should be addressed.

deciduous and boreal forests belong to genera such as *Quercus* and *Populus*, which have frequently been reported to have ECM associations (Meyer 1973; Harley and Harley 1987), and genera like *Acer* and *Fraxinus* with well-documented VAM associations (Kormanik 1981; Harley and Harley 1987). However, these assumptions are based on information gathered in Europe and elsewhere in North America, whereas the tree species found in Ontario have been the subject of only a few studies (Malloch and Malloch 1981, 1982; Brundrett and Kendrick 1988).

Roots in the primary state of growth can develop a number of characteristic structural features. The innermost cortex layer of roots, the endodermis, develops suberized Casparian bands and suberin lamellae (Esau 1965; Clarkson and Robards 1975). The hypodermis is the outermost cell layer of the cortex and in many cases differentiates as an exodermis with suberized Casparian bands and suberin lamellae (Perumalla 1986; Peterson 1988). The exodermis may be uniformly suberized or dimorphic with alternating suberized long and unsuberized short cells (Kroemer 1903; Shishkoff 1987). Cells in the cortex or hypodermis may form phi thickenings, localized deposits of lignified wall material, which form rings around cells (van Tieghem 1888; von Guttenberg 1968). Phi thickenings are considered to provide structural strength to roots (van Tieghem 1888), and do not function as a permeability barrier (Peterson *et al.* 1981). Cells in other cortex layers may also have modified cell walls, contain crystals, or accumulate secondary metabolites (von Guttenberg 1968; Esau 1965). Most of these anatomical features apparently provide structural strength or have defensive roles since they are often most highly developed in long-lived roots (Brundrett and Kendrick 1988). However, suberin in the endodermis and exodermis functions as a permeability barrier, blocking apoplastic (cell wall) transport of substances (Clarkson and Robards 1975; Peterson 1988).

Endophyte-associated mycorrhizal morphological features can be used to identify particular endophytes with one host (Abbott 1982; Agerer 1986), but root characteristics apparently cause more substantial morphology differences between hosts. Gallaud (1905) observed that VAM associations in different species formed two distinctive morphology types (the *Arum* and *Paris* series). In roots with *Arum* series VAM, hyphae proliferated in the cortex by growing between host cells, while in *Paris* series VAM associations, hyphae formed coils within cells. This distinction arises because hyphae grow through longitudinal intercellular air spaces when these are present (Brundrett *et al.* 1985; Brundrett and Kendrick 1988). Root features apparently also regulate root penetration by VAM fungus hyphae and the distribution of arbuscules within roots (Brundrett and Kendrick 1990). Angiosperms with ECM usually have a Hartig net that is confined to the epidermis (Alexander and Hogberg 1986; Massicotte *et al.* 1987), while in gymnosperms, Hartig net hyphae extend throughout the cortex (Harley and Smith 1983; Kottke and Oberwinkler 1986). Structural characteristics of host roots may result in these alternative types of ECM associations, but root structure – mycorrhizal morphology interactions require further investigation.

Fine tree roots are often differentiated into long, distributive roots and short lateral roots with determinate growth (Noelle 1910; Kubíková 1967; Sen 1980). This differentiation into long and short roots is called heterorhizy, and is usually highly pronounced in trees forming ECM associations that are restricted to the short roots (Kubíková 1967; Sen 1980). Trees with VAM associations usually have little or no heterorhizy and establish mycorrhizae in roots that often have pronounced epidermal and exodermal cell wall defences (Kubíková 1967).

The objectives of the current investigation were (*i*) to examine and illustrate the structural diversity of the roots of Ontario trees; (*ii*) to evaluate and develop efficient and effective staining procedures for examining root and mycorrhizal anatomy; (*iii*) to compile anatomical information that could be used to identify tree root samples and that could serve as background information for investigations of root or mycorrhizal morphology; and (*iv*) to gain an understanding of possible physiological, ecological, and phylogenetic significances of the root anatomy features observed.

Materials and methods

Sampling procedure

Because of logistical constraints, only 20 tree genera could be represented in this study. These genera were selected because they contain species that have widespread distributions and are major components of Ontario forests. It is hoped that less common species restricted to the Carolinian forest zone of southwestern Ontario, trees that grow in open sites, and shrubs can be examined in a future study. The nomenclature of trees used in this study is consistent with (Hosie 1979), and angiosperms are listed in taxonomic sequence after Cronquist (1981).

Most root samples were collected from natural stands. Many samples were obtained by excavating under mature trees and were identified by superficial root characteristics. In other cases, root identification was simplified by collecting in fairly pure stands, in plantations, or by excavating entire saplings. The consistency of root anatomy features was determined by examining many root sections with each staining procedure, and by using several root samples from different locations. This allowed representative images to be selected for each species. Larger numbers of separate samples were used to investigate mycorrhizal relationships using clearing and staining procedures (see Table 1).

Processing of whole roots

Roots were cleared using the chlorazol black E (CBE) staining procedure (Brundrett *et al.* 1984; Brundrett and Kendrick 1988). Root samples were preserved in 50% ethanol – 5% lactic acid and cleared in 10% KOH in an autoclave (121°C) for 15 min. After rinsing, samples were stained in 0.01% CBE, then transferred to glycerol in Petri dishes for observation and storage. Representative subsamples were mounted on slides using a polyvinyl alcohol – lactic acid – glycerine mountant (Koske and Testier 1983). Samples of root containing large amounts of tannins were bleached with 3% alkaline H_2O_2 (Kormanik *et al.* 1980; Nylund *et al.* 1982) before staining in CBE. These clearing and staining procedures were used to examine whole roots for the presence of mycorrhizal fungus hyphae and for root features stained by CBE (lignified or suberized walls).

Processing of root sections

Cross sections of roots were prepared using Frohlich's parafilm hand sectioning procedure (Frohlich 1984). Thin sections were then selected under a dissecting microscope. Some of these sections were examined unstained to observe natural coloration or UV-light induced autofluorescence. Other sections were placed in mesh-bottomed, multichambered section holders (Brundrett *et al.* 1988) for use in one of the following four staining procedures.

(*i*) Sections were stained with the fluorescent alkaloid berberine sulphate (0.1% in water) for 1 h, counterstained in 1% aqueous aniline blue for 30 min, and then mounted in 1% $FeCl_3$ – 50% glycerol (Brundrett *et al.* 1988). These berberine – aniline blue (BAB) stained sections were examined with UV-fluorescence to show cell walls containing Casparian band or lamellar suberin and lignin.

(*ii*) The fluorescent dye Fluorol (Solvent Yellow No. 43, obtained from BASF) was used at 0.01% in a staining solution consisting of 1:1 polyethylene glycol (mw 400) and 90% glycerol. Sections in holders were stained for 1 h, rinsed briefly, then mounted in glycerol. This stain provides high-contrast fluorescent images of nonpolar substances such as lamellar suberin and lipids (M. C. Brundrett, B. Kendrick, and C. A. Peterson, unpublished data).

(*iii*) Sections were also cleared in 10% KOH for 6–12 h at 90°C, then stained using the CBE staining procedure described above. This procedure reveals fungal structures and lignified or suberized plant cell walls (Brundrett and Kendrick 1989).

(*iv*) Phloroglucinol in HCl (Jensen 1962) was used to confirm the presence of lignin in cell walls.

Microscopy

All micrographs were taken with a Zeiss Photomicroscope III or Zeiss DRC dissecting microscope with a MC63 camera attachment. Fluorescence micrographs were taken with epifluorescence illumination on colour slide film (100 ASA) and then copied as black and white internegatives before printing. Illumination was provided by excitation filter G 365 (365 nm peak λ), chromatic beam splitter FT 395 (395 nm), and barrier filter LP 420 ($\lambda \geq 420$ nm). Results of other staining procedures were recorded on black and white negative film (32 ASA) using Nomarski interference-contrast optics.

Terminology

Terms and abbreviations used in this paper to describe root structures are provided here, others are explained in the introduction. The endodermis (innermost cortex layer) has three possible developmental stages: state I, with Casparian bands; state II, with Casparian bands and suberin lamellae; and state III, with additional wall depositions (Esau 1965; Clarkson and Robards 1975). "Casparian bands" are localized bands of suberin deposited within radial cell walls; "suberin lamellae" consist of concentric layers of suberin deposited on all inner wall surfaces. "Exodermis" refers to a hypodermis (subepidermal layer) with suberized Casparian bands and suberin lamellae (Perumalla 1986; Peterson 1988). "Phi thickenings" are localized deposits of lignified wall material that form longitudinal bands in the root cortex (van Tieghem 1888; von Guttenberg 1968). Cells containing these wall thickenings may occur in a cell layer external to the endodermis (supraendodermal), in the hypodermis (hypodermal), or may be present throughout the cortex (van Tieghem 1888). "Metacutinization" is the protection of dormant root tips by suberization of one or more root-cap cell layers (Wilcox 1954; Romberger 1963). Throughout this paper, the term ECM is used for ectomycorrhizae, and VAM for vesicular–arbuscular mycorrhizae. Gallaud's (1905) term "*Arum* series" is used to describe predominantly intercellular VAM associations, and "*Paris* series" refers to intracellular associations. Root systems with separate "long" roots with extended growth and "short" roots with limited apical growth are referred to as "heterorhizic" (Kúbiková 1967). "Secondary" roots have a periderm and additional xylem and phloem resulting from secondary growth. The names of other structures are consistent with Esau (1965, 1977).

Results and Discussion

Methodology

Methods used to examine whole roots for the presence of mycorrhizae have involved (*i*) clearing in KOH alone, and (*ii*) clearing in KOH followed by bleaching with H_2O_2 (Kormanik *et al.* 1980; Nylund *et al.* 1982). Tree roots were prepared by both of these methods and subsequently stained with CBE (Brundrett *et al.* 1984). Roots containing VAM were best observed after autoclaving in KOH, since further decolourizing with H_2O_2 apparently damaged mycorrhizal hyphae or interfered with subsequent staining so that details of fungal morphology were difficult to observe. Roots containing large amounts of suberin, lignin, or tannin, were examined by flattening KOH-cleared roots under a cover slip to expose VAM hyphae with minimal interference from other root structures (Brundrett and Kendrick 1988). Details of VAM morphology in roots prepared this way can be seen in Figs. 34, 35, 109, 110, 118, and 119.

Roots bearing ECM usually contained large amounts of tannins that could not be efficiently removed by KOH clearing alone. The use of alkaline H_2O_2 (Kormanik *et al.* 1980; Nylund *et al.* 1982) after KOH clearing made it easier to visualize Hartig nets in these roots, even though CBE staining of hyphae was reduced by this treatment (Figs. 51, 63, 79). However, it was difficult to get clear, high-contrast images of Hartig net structure in whole roots because of this reduced staining intensity and interference from mantle hyphae and other root structures. Details of Hartig net structure could be clearly seen when ECM were sectioned before clearing in KOH and staining with CBE (Figs. 2, 3, 9, 10). This procedure, in combination with Nomarski interference-contrast microscopy, revealed the labyrinthine arrangement of Hartig net hyphae with exceptional clarity (see Figs. 10, 15, 21, 28).

A numer of anatomical features can be observed in roots cleared and stained to reveal mycorrhizal hyphae (Brundrett and Kendrick 1988). Details of the structure of exodermis, endodermis, phi thickenings, xylem, periderm, and other cells with modified walls can be observed in whole roots or root sections stained with CBE (Figs. 36, 43, 73, 97, 111, 117, 120, 130, 131). A surprising amount of information on root anatomy is revealed when the natural pigmentation or refringence of unstained root sections are observed (Figs. 48, 49, 65, 77) and even more detail is produced by UV-induced autofluorescence of these sections (Figs. 5, 38, 84). However, it can be difficult to identify cell types and wall components using these procedures, and the resulting images often have low contrast.

Root sections produced by an efficient hand-sectioning procedure (Frohlich 1984) could be used with several new fluorescent staining methods that provide high contrast images of wall structures. Suberin deposited in walls to form Casparian bands and suberin lamellae can be identified with BAB staining, which also reveals lignin and callose (Brundrett *et al.* 1988). This procedure is of particular value for identifying exodermal cells in roots (Figs. 45, 55, 74, 98, 135). The fluorescent dye Fluorol, when used in a polyethylene glycol – glycerine staining solution (more information about this procedure will be published elsewhere), provided high contrast images of lipids, including those in suberin lamellae and stored in mycorrhizal hyphae (Figs. 31, 99, 125). In combination with CBE staining, these procedures detected most important anatomical features of tree roots. The use of hand sections with these procedures was found to be much more efficient than methods involving paraffin or plastic embedding of material.

Root anatomy and mycorrhizae of Ontario forest trees

The following descriptions of Ontario forest tree roots and mycorrhizae use methodology abbreviations and terminology defined in the Materials and methods section. Many root samples were examined for each of the 20 genera considered, but only a representative selection of pictures could be reproduced here. Since species within a genus were generally found to have similar root and mycorrhizal characteristics, most features are considered for only one species, to avoid repetition. The structure of primary and mycorrhizal roots is emphasized, but information on their secondary roots is available elsewhere

(Noelle 1910; Cutler *et al.* 1987). The endodermis and other tissues are described at the developmental state in which they were normally encountered. Root anatomy and mycorrhizal references, information on related species examined, and observations of other root features are also included for each genus.

GYMNOSPERMAE: Pinaceae

Larix laricina (Du Roi) K. Koch (tamarack, larch)

ROOT SYSTEM FORM: Heterorhizic. Long roots bear short roots that are thicker than those of most trees examined (0.25–0.50 mm wide). *Larix* short roots can grow to considerable lengths and often produce a second order of mycorrhizal laterals (Fig. 1). Inactive root tips become metacutinized by suberization of inner root cap cells.

ULTIMATE LATERALS: These short roots form ECM with a Hartig net that occupies most of the 4–6 cortex cell layers outside the state II endodermis (Figs. 2, 3). However, the innermost 1 or 2 rows of cortical cells have walls that are considerably thickened, highly refringent, and autofluorescent (Fig. 5) but that do not stain with BAB (Fig. 4) or phloroglucinol. The vascular cylinder consists of short tracheids in a diarch arrangement and 2 phloem poles (Fig. 4).

MYCORRHIZAE: *Larix* ECM structure is typical of members of the Pinaceae, consisting of a thick mantle and a Hartig net extending through all but the innermost cortex layer. Wall thickenings of innermost cortex cells apparently prevent further ingress by the fungus (Fig. 5). Hartig net hyphae develop complex labyrinthine branching patterns (Figs. 2, 3).

LONG ROOTS AND SECONDARY GROWTH: Long roots are usually much thicker than the ECM laterals. These roots have a large stele with 2 opposing xylem poles with adjacent resin ducts, and 2 alternating phloem poles (Fig. 6). Secondary growth results in the addition of much new xylem on the flanks of the primary xylem as well as secondary phloem tissue. The root periphery is then covered by a periderm consisting of tannin-filled phellem cells with large amounts of wall-deposited suberin (Fig. 6). In surface view the phellem cells are rectangular with somewhat convoluted radial walls and irregularly spaced pits. Many cells in the secondary phloem contain resin or tannins.

Larix long root anatomy has been described by Noelle (1910) and Jeffrey (1917). McDougall (1914) and Malloch and Malloch (1981) observed ECM in *L. laricina*, and the European species *L. decidua* has been repeatedly demonstrated to have ECM (Harley and Harley 1987).

Pinus strobus L. (eastern white pine), *P. resinosa* Ait. (red pine), and *P. banksiana* Lamb. (jack pine)

ROOT SYSTEM FORM: *Pinus* roots are clearly heterorhizic and characterized by the presence of several orders of dichotomously branched ECM short roots (this type of equal branching is very rare in higher plants). In *Pinus strobus* the dichotomous branches making up one ECM cluster are 0.30–0.60 mm wide and typically uniform in length (Fig. 7), but may be condensed into a tuberous structure. In *P. banksiana* these dichotomous laterals are narrower (0.17–0.40 mm wide), more elongated, and less regular in length than those of *P. strobus* (Fig. 8). Roots of *P. resinosa* are 0.25–0.50 mm wide and fairly compact, but usually have less regular dichotomous branching than in *P. strobus*. *Pinus* roots, like those of other Pinaceae, contain extremely large amounts of tannins. Metacutinization of old mycorrhizal root tips can be seen in whole roots cleared with KOH and H_2O_2.

ULTIMATE LATERALS: All dichotomously branched lateral roots observed were ectomycorrhizal. The epidermis consists of irregularly arranged, small, tannin-filled cells that become enveloped in the fungal mantle. The root cortex consists of relatively few cell layers (2–4), the innermost of which has no Hartig net hyphae (Figs. 9, 10). These cells have walls that are relatively thin, but are otherwise similar to those preventing further hyphal ingress in *Larix* roots. The stele occupies a large part of the root and consists of 2 xylem and phloem poles surrounded by a state II endodermis with thick suberin lamellae (Fig. 11).

MYCORRHIZAE: The characteristic dichotomous branching of *Pinus* ECM lateral roots allows the association to be recognised with a high degree of certainty without processing roots. Hyphal arrangement in the mantle and Hartig net was revealed by clearing and staining root sections with CBE (Figs. 9, 10). The branching pattern and course of individual hyphae could be followed throughout the cortex by changing the plane of focus (Fig. 10 shows only one focal plane).

LONG ROOTS AND SECONDARY GROWTH: Long roots are initially similar in structure to but much thicker than ECM laterals, with cortex and tannin-containing epidermal cells that are shed in strands from young roots. The anatomy of roots with secondary growth is similar to that of *Larix* (see Fig. 6), but primary xylem forms an arch around each resin duct. The periderm consists of rectangular cells with pits and thick, convoluted radial walls (Fig. 12).

Noelle (1910) compared the root anatomy of eight *Pinus* species. Wilcox (1964) found that *Pinus resinosa* long and short roots had two horseshoe-shaped xylem poles embracing

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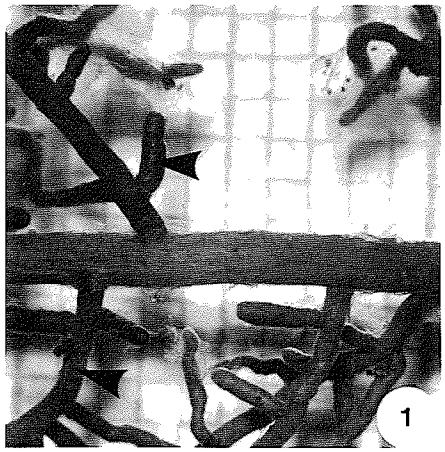
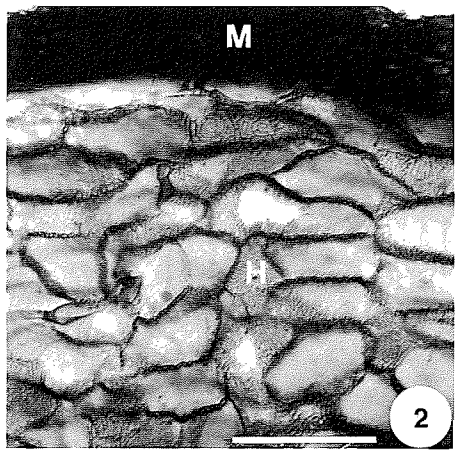
ABBREVIATIONS: Ep, epidermis; Hy, hypodermis; Ex, exodermis; C, cortex, En, endodermis; X, xylem; P, phloem; ⇐, Casparian bands; *s*, suberin lamellae; Sp, secondary phloem; B, periderm (bark); H, ECM Hartig net hyphae; M, mantle; A, arbuscule. In Figures, white scale bars = 100 μm and black bars = 10 μm.

FIGS. 1–6. *Larix laricina* (larch, tamarack) roots and ectomycorrhizae (ECM). Fig. 1. Root system with ECM short roots (arrows) (grid = 1 mm). Fig. 2. Tangential section of ECM Hartig net. This section was cleared in KOH, stained with chlorazol black E, and viewed with Nomarski interference-contrast optics (CBE–Nomarski). Fig. 3. Higher magnification view of labyrinthine Hartig net hyphae surrounding cortex cells (CBE–Nomarski). Fig. 4. Cross section of ECM root centre showing BAB-stained endodermis with Casparian bands, suberin lamellae, and passage cells (arrows), and xylem (UV fluorescence). Fig. 5. Unstained cross section with UV-induced autofluorescence of Hartig net hyphae, inner cortex (arrow), and endodermal walls. Fig. 6. Long-root cross section with Fluorol fluorescent staining of suberin in periderm cells and lipids in resin duct cells (arrows). Some staining of resin cells in the secondary phloem and autofluorescence of xylem are also visible. FIGS. 7–12. *Pinus strobus (white pine), P. resinosa* (red pine), and *P. banksiana* (jack pine) short roots (arrows) and ECM. Figs. 7 and 8. Root systems of *P. strobus* and *P. banksiana*, respectively, showing dichotomously branched ECM short roots (grid = 1 mm). Fig. 9. *Pinus strobus* ECM (CBE–Nomarski) cross section showing mantle and cortical Hartig net. Fig. 10. Details of hyphal branching in Hartig net. Fig. 11. Cross section of an ECM long root of *P. strobus* with Fluorol staining of endodermal suberin lamellae and xylem autofluorescence. Fig. 12. Periderm cells of a *P. strobus* root with thick, wavy radial walls (arrows) and pits (CBE–Nomarski).

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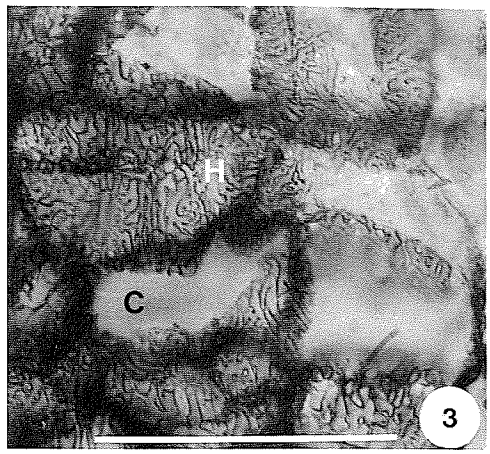
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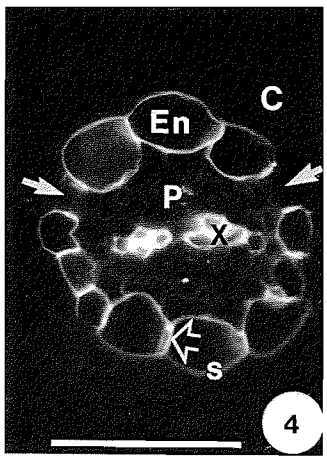
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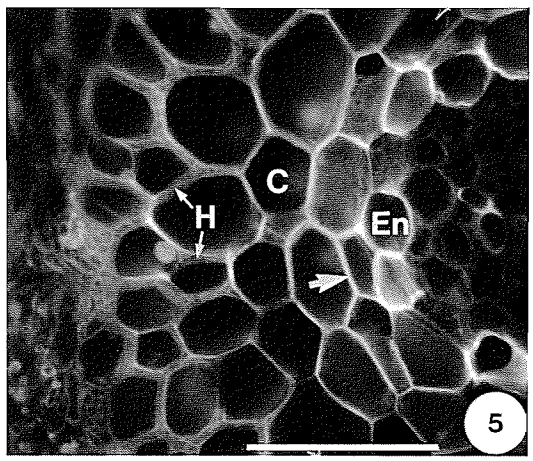
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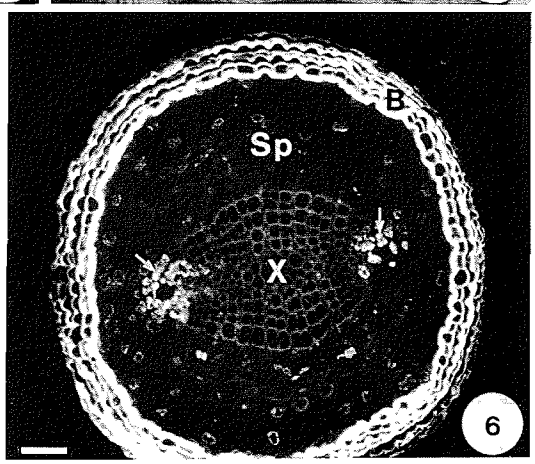
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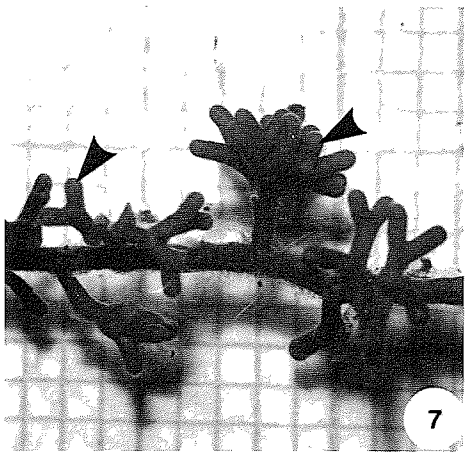

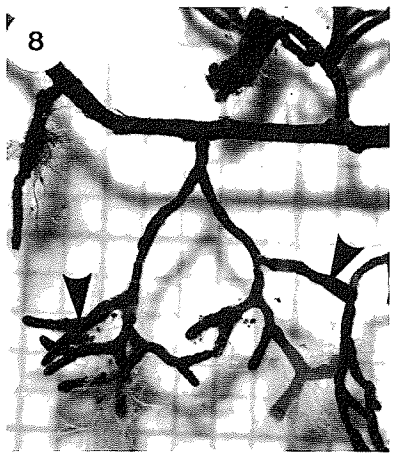
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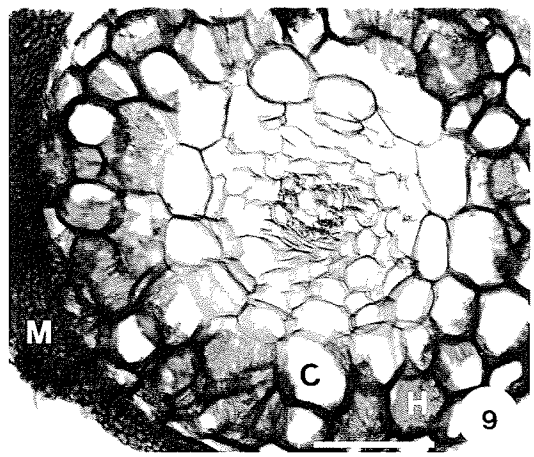
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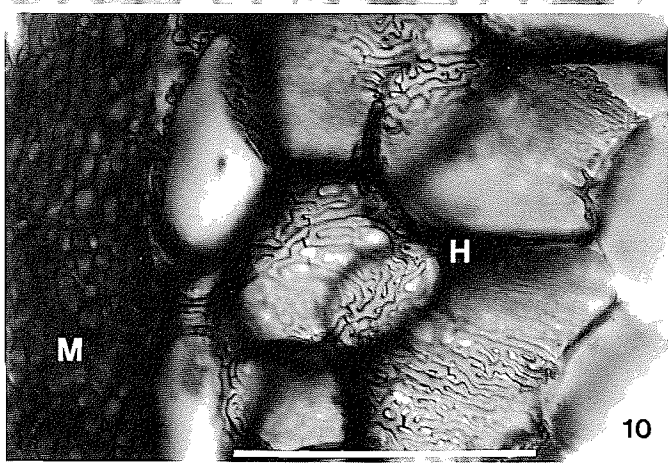
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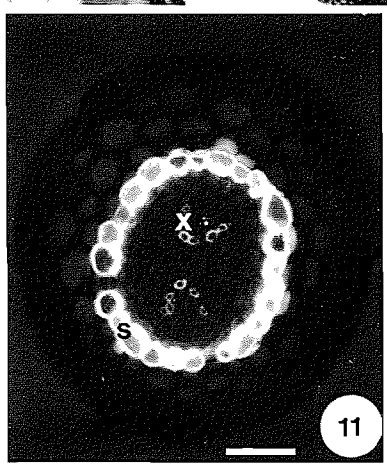
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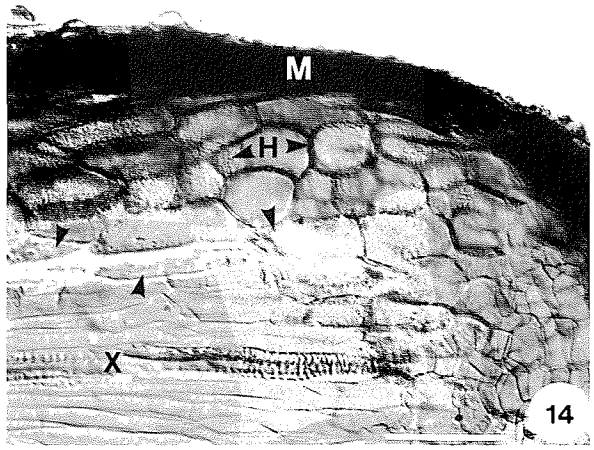
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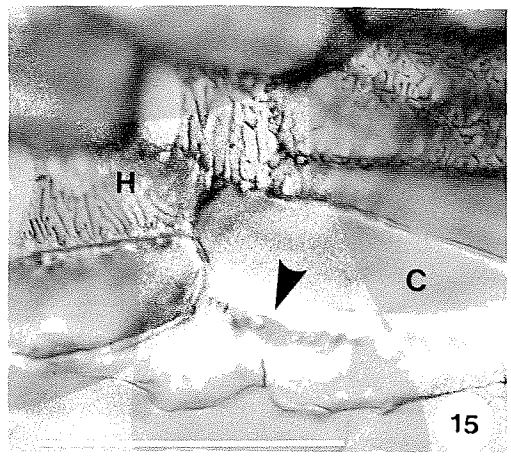
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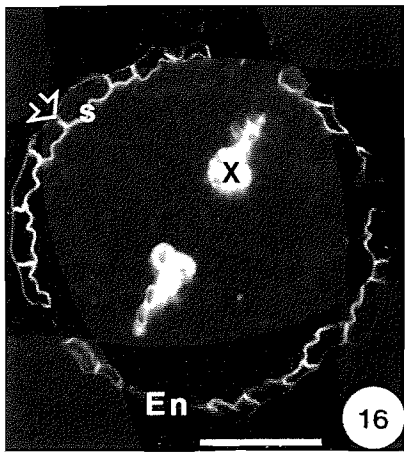
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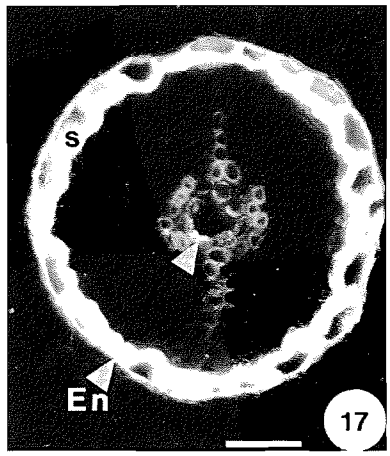
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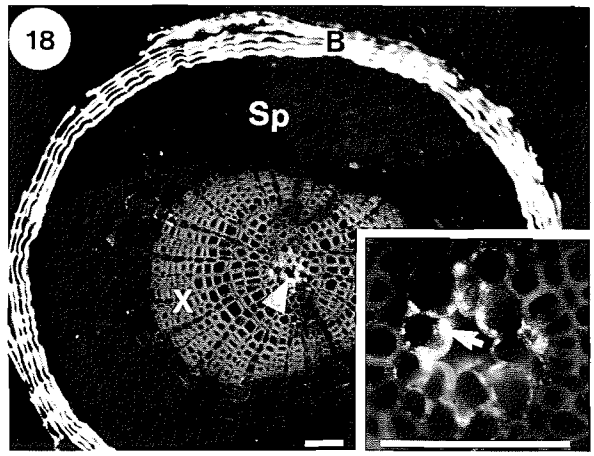
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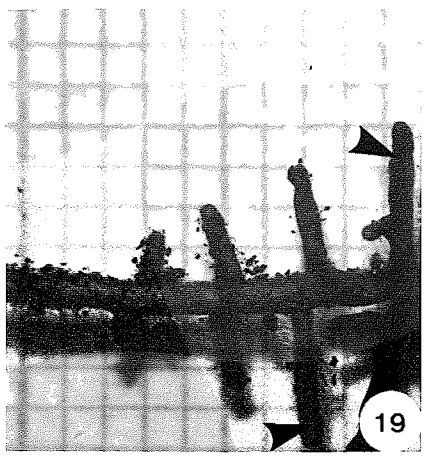
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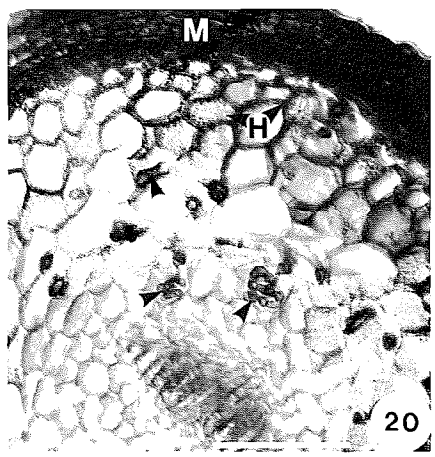
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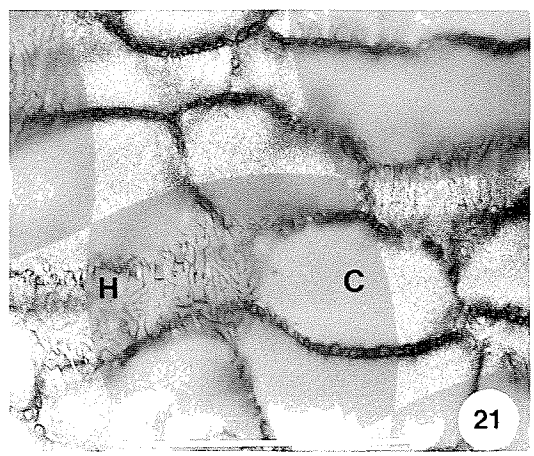
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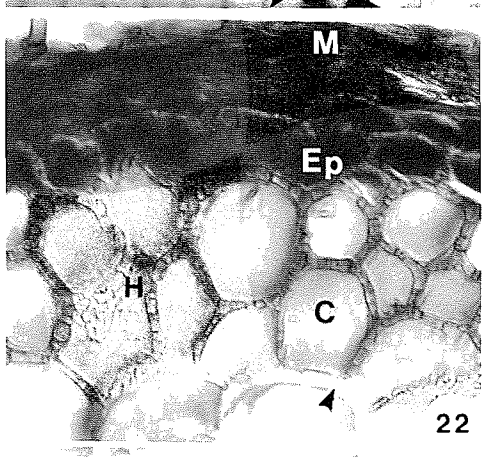
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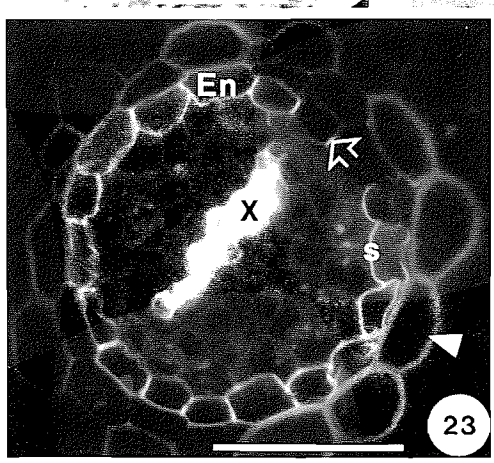
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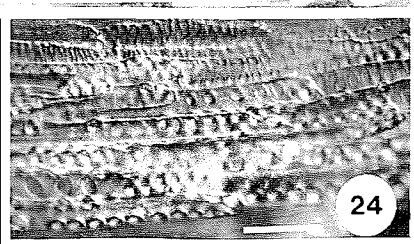
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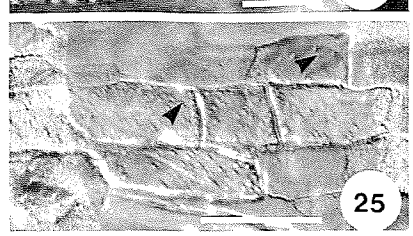
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resin canals, but these were often condensed together in short roots. Mycorrhizal morphology of this species is described by Wilcox (1968) and Wilcox and Wang (1987). Piché *et al.* (1983) investigated dichotomous branching in *P. strobus* short roots, and McKevlin *et al.* (1987) compared *P. taeda* roots growing in waterlogged and well-drained soil. ECM has been reported in *P. strobus* (Brundrett and Kendrick 1988), *P. banksiana* (Malloch and Malloch 1981), and *P. resinosa* (Wilcox 1968). Ectomycorrhizae of other *Pinus* species have been the subject of numerous reports as well as physiological and anatomical studies (see Harley and Smith 1983; Harley and Harley 1987).

Abies balsamea (L.) Mill. (balsam fir)

ROOT SYSTEM FORM: Roots are heterorhizic, with short ECM roots that are 0.15–0.35 mm wide and arise from straight, long roots that are usually much thicker (Fig. 13). Ectomycorrhizal laterals often become considerably elongated and produce a second order of perpendicular ECM laterals (Fig. 13).

ULTIMATE LATERALS: Short roots have a cortex consisting of 5–6 cell layers, of which all but the innermost are enveloped by Hartig net hyphae (Fig. 14). The innermost cortex cell layer has thick, highly refringent cell walls that apparently prevent further hyphal ingress (Figs. 14, 15). The stele of these roots has a large central resin canal between 2 xylem poles, and is surrounded by a stage II endodermis with thick suberin lamellae (Figs. 16, 17).

MYCORRHIZAE: The mantle is usually thick. The Hartig net surrounding most cortical cells consists of hyphae with a convoluted or digitate branching pattern (Fig. 15).

LONG ROOTS AND SECONDARY GROWTH: Long roots are similar in structure to those of *Pinus*, but have a large central resin canal that becomes surrounded by secondary xylem (Fig. 18). The secondary phloem of these roots is well developed and the periderm consists of cells with large amounts of suberin (Fig. 18). In cleared whole roots, the rectangular periderm cells are without prominent wall ornamentations and have remnants of the abundant tannins they contained.

The long root anatomy of *Abies* species was investigated by Noelle (1910), who used the large, thin-walled central resin canal and numerous large mucilage cells in the secondary phloem to separate them from other members of the Pinaceae, and by Jeffrey (1905, 1917). Wilcox (1954) studied the anatomy of *A. procera* roots, describing long-root morphology and metacutinization of dormant roots. Ectomycorrhizal associations of *A. balsamea* roots have also been reported by McDougall (1928), and Malloch and Malloch (1981). There have been many reports of ECM in other *Abies* species.

Tsuga canadensis (L.) Carr. (eastern hemlock)

ROOT SYSTEM FORM: Roots are weakly heterorhizic, with ECM short roots that are similar to those of *Larix* and *Abies*, but usually coarser (0.35–0.60 mm wide). *Tsuga* short roots can grow to considerable lengths and produce perpendicular second-order ECM laterals, which in turn may produce third-order laterals. These higher order laterals become progressively longer closer to the base of the parent ECM (Fig. 19).

ULTIMATE LATERALS: Short roots have several layers of tannin-filled epidermal cells (Fig. 22), and 3–5 cortex cell layers, most of which are surrounded by Hartig net hyphae (Figs. 20, 21, 22). As with *Abies* roots, the innermost cortex layer consists of large cells with thick, modified walls that are highly refringent (Fig. 22) and stain with BAB as if partially lignified (Fig. 23). *Tsuga* short roots have a diarch stele without resin canals, inside an endodermis with suberin lamellae that has passage cell gaps (Fig. 23). Tracheids are shorter than those in long roots and consist of elements with spiral wall thickenings or bordered pits (Fig. 24), as is typical of conifers short roots. Old ECM laterals can undergo secondary growth.

MYCORRHIZAE: Labyrinthine Hartig net hyphae penetrate between cells in all but the innermost cortex layer, where cortex walls are thickened. These hyphae surround cortex cells that are rectangular in tangential view in the outer cortex (Fig. 21), but more rounded in the inner cortex.

LONG ROOTS AND SECONDARY GROWTH: These are similar in structure to ECM short roots but can be considerably thicker. Initially they have an epidermis and many layers of cortex cells with brown, tannin-incrusted walls. The stele is diarch, with a central resin canal that is less prominent than that in *Abies* roots. Tracheids are much longer than those in short roots. Developing secondary xylem, phloem, and periderm are similar to those illustrated for other conifers. Periderm cells are rectangular, suberized, and tannin-filled, with oval pits arranged in a regular array (Fig. 25).

An unidentified fungal parasite, which grew through the Hartig net to produce haustoria in inner cortex and stele cells, was also observed (Fig. 20). *Tsuga heterophylla* from western North America is also ectomycorrhizal (Molina and Trappe 1982). *Tsuga* long-root anatomy was described by Jeffrey (1905) and by Noelle (1910), who used thick, lignified resin duct epithelium to distinguish this genus from others with a central resin canal.

Picea mariana (Mill.) BSP (black spruce) and *P. glauca* (Moench) Voss (white spruce)

ROOT SYSTEM FORM: *Picea glauca* roots are heterorhizic with ECM short laterals that are typically elongate, narrow

FIGS. 13–18. *Abies balsamea* (balsam fir) roots and mycorrhizae. Fig. 13. Root sytem with branched ECM short roots (arrows) (grid = 1 mm). Fig. 14. Longitudinal section of a short root (CBE–Nomarski) with ECM mantle and Hartig net hyphae, xylem, and refringent inner-cortex cells (arrows). Fig. 15. Cortex cells with refringent walls (arrow) and Hartig net hyphae (longitudinal section, CBE–Nomarski). Fig. 16. Cross section of central zone of an *Abies* ECM root with BAB-induced fluorescence of xylem, and endodermal Casparian bands and suberin lamellae. Fig. 17. Similar section with Fluorol fluorescence of endodermal suberin lamellae and resin duct cells (arrow), and xylem autofluorescence. Fig. 18. *Abies* long root in cross section, with Fluorol-induced fluorescence of periderm suberin and resin duct cells (arrow). Inset shows detail of Fluorol staining of lipids in resin duct cells. FIGS. 19–25. *Tsuga canadensis* (hemlock) roots and mycorrhizae. Fig. 19. Perpendicular branches (arrows) on *Tsuga* ECM short root (grid = 1 mm). Fig. 20. Cross section of ECM short root (CBE–Nomarski) mantle and Hartig net hyphae, and the hyphae and haustoria of a parasitic fungus (arrows). Fig. 21. Tangential longitudinal section through Hartig net hyphae in cortex (CBE–Nomarski). Fig. 22. Cross section of ECM with tannin-filled epidermal cells, thick-walled inner-cortex cell (arrow), and Hartig net hyphae (CBE–Nomarski). Fig. 23. Cross section of ECM with BAB fluorescence of diarch xylem, endodermal Casparian bands and suberin lamellae, and inner-cortex cells (arrow). Fig. 24. Xylem tracheids with spiral thickenings and pitted walls (CBE–Nomarski). Fig. 25. Periderm cells with many pits; two are indicated by arrows (CBE–Nomarski).

(0.20–0.40 mm), and sparsely branched (Fig. 26). *Picea mariana* short roots are considerably narrower (0.10–0.20 mm), usually unbranched, and often have a narrower base and swollen ECM tips (Fig. 27).

ULTIMATE LATERALS: *Picea glauca* and *P. mariana* short roots have a narrow cortex with 2–4 cell layers enveloped by Hartig net hyphae that extend right up to the endodermis (Figs. 28, 29). The stele has 2 xylem poles, but often only 1 develops in *P. mariana* (Fig. 30). Some roots of this species have air spaces outside the primary phloem similar to those described in *Pinus* from waterlogged soil by McKevlin *et al.* (1987). The endodermis of both species has narrow Casparian bands and thick suberin lamellae (Figs. 30, 31).

MYCORRHIZAE: The mantle is usually thick in *P. mariana* and is often composed of dense black hyphae of a *Cenococcum* type association. Spherical cortex cells are surrounded by Hartig net hyphae with labyrinthine branching (Figs. 28, 29) that may accumulate lipids (Fig. 31).

LONG ROOTS AND SECONDARY GROWTH: In cross section these roots superficially resemble those of *Larix* and *Pinus* (Fig. 32). Most long roots of *Picea glauca* and *P. mariana* are encased in a periderm consisting of rectangular (suberized and tannin-filled) cells that are relatively thin walled and unpitted or have small pits in a regular array. Noelle (1910) examined the anatomy of long roots of nine *Picea* species that he could distinguish from *Larix* roots by the presence of tannin cells in the pericycle of the former and crystal cells and fibres in the phloem of the latter.

There are reports of ECM in *P. mariana* (Malloch and Malloch 1981) and *P. glauca* (Malloch and Malloch 1982) roots. Norway spruce (*P. abies*) ECM has been the subject of detailed structural investigations (Nylund and Unestam 1982; Kottke and Oberwinkler 1986; Nylund 1987) and many reports (Harley and Harley 1987). Johnson-Flanagan and Owens (1985) described metacutinization of dormant *P. glauca* roots.

GYMNOSPERMAE: Cupressaceae

Thuja occidentalis L. (eastern white cedar)

ROOT SYSTEM FORM: Nonheterorhizic. White cedar has fine lateral roots that are much coarser (0.40–0.80 mm wide) and have considerably fewer branches than those of the other conifers examined (Fig. 33). These roots are fairly straight and branch at a 45–90° angle to the parent root axis (Fig. 33). The epidermal layers consist of tannin-filled cells that give the roots their characteristic red-brown colouration and are shed in strips.

ULTIMATE LATERALS: Unlike other conifers, *Thuja* roots can form an exodermis with suberin lamellae deposition in walls of cells in the two outermost cortex layers, but this exodermis is without distinct Casparian bands (Figs. 37, 38). These exodermal wall modifications are present in older roots but usually contain gaps. Young and old roots contain a massive supraendodermal phi sheath and smaller phi thickenings throughout all but the 2 outermost of the 7–10 cortex layers (Fig. 39). These phi thickenings have lignified cell walls, as evidenced by autofluorescence (Fig. 38), BAB (Fig. 39), and phloroglucinol staining, and they are apparent in cleared whole roots (Figs. 34, 36). Phi thickenings of adjacent cells in the same cell layer are aligned to form a longitudinally continuous supporting structure (Fig. 36). Roots develop a state II endodermis with passage cells and have diarch xylem with long tracheids (Figs. 37–39). These tracheids were generally longer than those in the short ultimate laterals of the other conifers examined.

MYCORRHIZAE: Cedar roots harbour VAM with typical *Paris* series (essentially intracellular) morphology, but details of this association are not easily observed because of the high tannin content of roots and the refringence of cortex phi thickenings (Figs. 34, 35).

LONG ROOTS AND SECONDARY GROWTH: Secondary growth develops in higher order roots and some ultimate laterals. Xylem is usually polyarch and is surrounded by thick secondary phloem with orderly rows of phloem fibres that are square in cross section (Fig. 40).

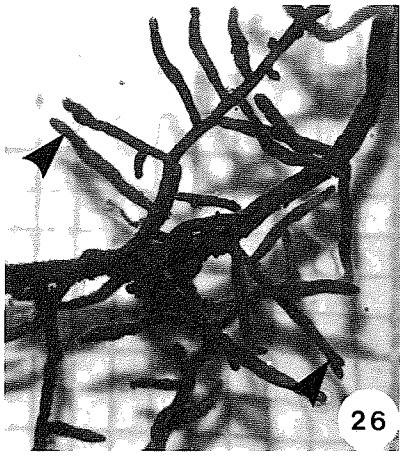
Juniperus virginiana L. (red cedar) and the shurbs *J. horizontalis* and *J. compressus* also have roots with similar phi thickenings and VAM associations. Malloch and Malloch (1982) found VAM in most of the *T. occidentalis* roots that they examined. Noelle (1910) reported that *Thuja* and other conifers outside of the Pinaceae had similar roots with an exodermis, prominent phi thickenings, and no primary xylem resin canals. Gallaud (1905) described *Sequoia* root anatomy and VAM, which closely resemble those of *Thuja*. Wilcox (1962) described similar root morphology in *Libocedrus decurrens*.

ANGIOSPERMAE: Salicaceae (Dilleniidae: Salicales)

Salix nigra Marsh. (black willow)

ROOT SYSTEM FORM: Heterorhizic with several orders of straight long roots bearing crooked, narrow short roots (0.15–0.25 mm wide) that gradually taper to an even narrower apex (0.08–0.15 mm wide) (Fig. 41). Many short roots do not establish ECM associations, but those that do are usually shorter and substantially thicker than nonmycorrhizal laterals (Fig. 42). Long, persistent root hairs are scattered throughout the root system. Scars are left on the surface of long roots after loss of short roots (Fig. 43).

FIGS. 26–32. *Picea glauca* (white spruce) and *P. mariana* (black spruce) roots and mycorrhizae. Figs. 26 and 27. White spruce and black spruce root systems (grid = 1 mm). *Picea mariana* ECM short roots (arrows) are finer (Fig. 27), while those of *P. glauca* are coarser and branched (Fig. 26). Figs. 28 and 29. Cross section of *P. mariana* ECM showing labyrinthine Hartig net hyphae extending to the endodermis (CBE–Nomarski). Fig. 30. Cross section of *P. mariana* short root with BAB-induced fluorescence of xylem, endodermal Casparian bands (arrow), and suberin lamellae. Fig. 31. Similar section with Fluorol fluorescence of lipids in Hartig net hyphae (arrow), endodermal suberin lamellae, and the contents of stele cells. Fig. 32. Long root of *P. glauca* stained with Fluorol, showing fluorescence of endodermal suberin and resin duct cells (arrows). FIGS. 33–40. *Thuja occidentalis* (white cedar) root anatomy and mycorrhizae. Fig. 33. Nonheterorhizic root system (grid = 1 mm). Fig. 34. Arbuscules of a VAM fungus and phi thickenings (arrow) in root cortex (CBE–Nomarski). Fig. 35. Vesicles (V) of a VAM fungus in *Thuja* (CBE–Nomarski). Fig. 36. Massive inner-cortex phi thickenings (arrows) in a squashed root preparation (CBE–Nomarski). Fig. 37. *Thuja* root cross section with Fluorol staining of suberin lamellae in the exodermis and endodermis (UV fluorescence). Staining of lipids in VAM hyphae (arrows), and xylem and phi thickening autofluorescence are also visible. Fig. 38. Similar root section with xylem, phi thickenings (arrows), endodermal and exodermal walls seen by UV autofluorescence. Fig. 39. Root cross section with BAB-induced fluorescence of phi thickenings (arrows) and other features. Fig. 40. *Thuja* secondary-root cross section with phloroglucinol staining periderm, xylem, and phloem fibres (arrows).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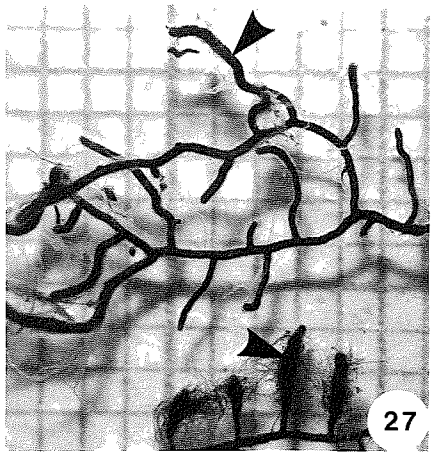
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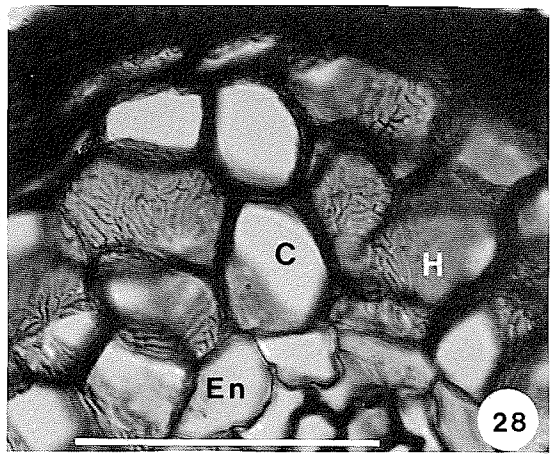
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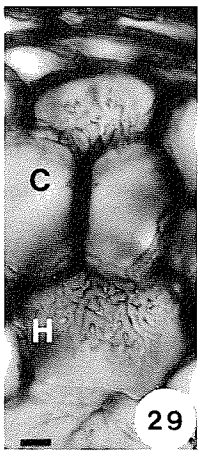
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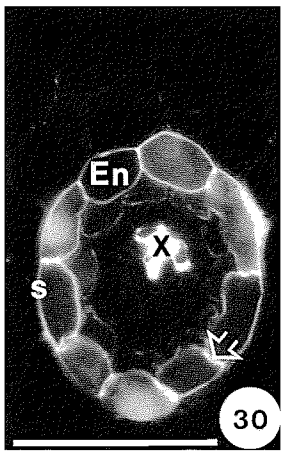
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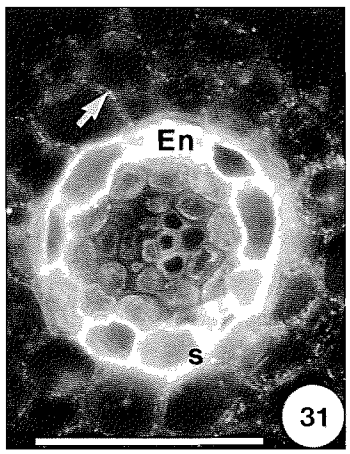
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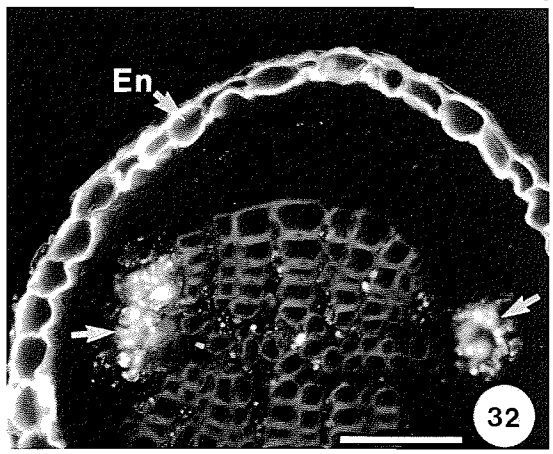
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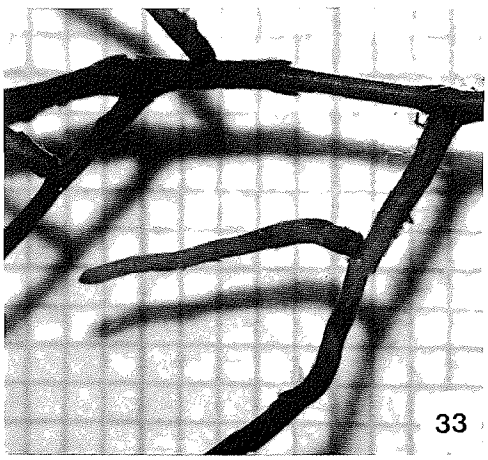
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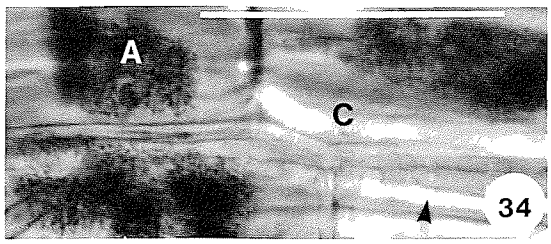
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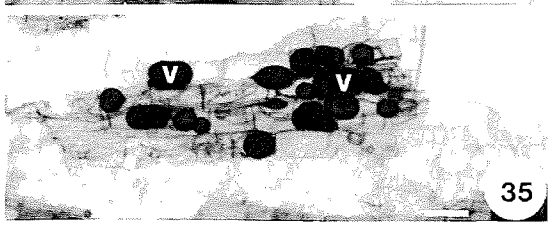
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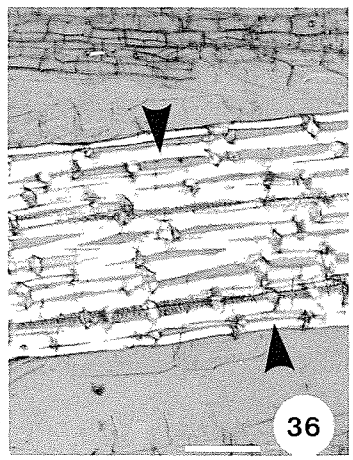
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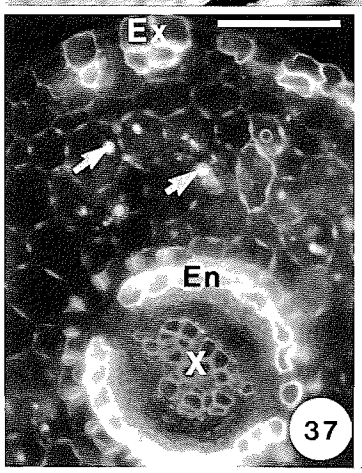
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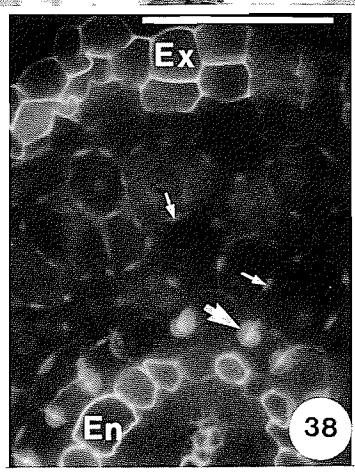
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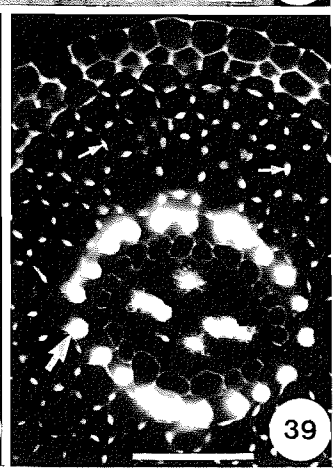
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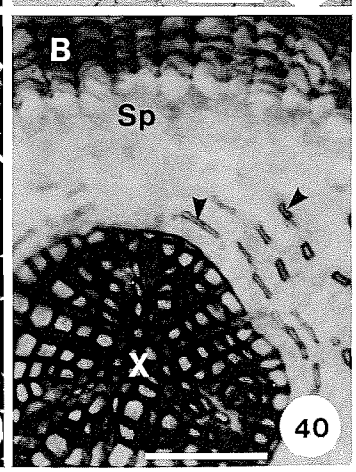
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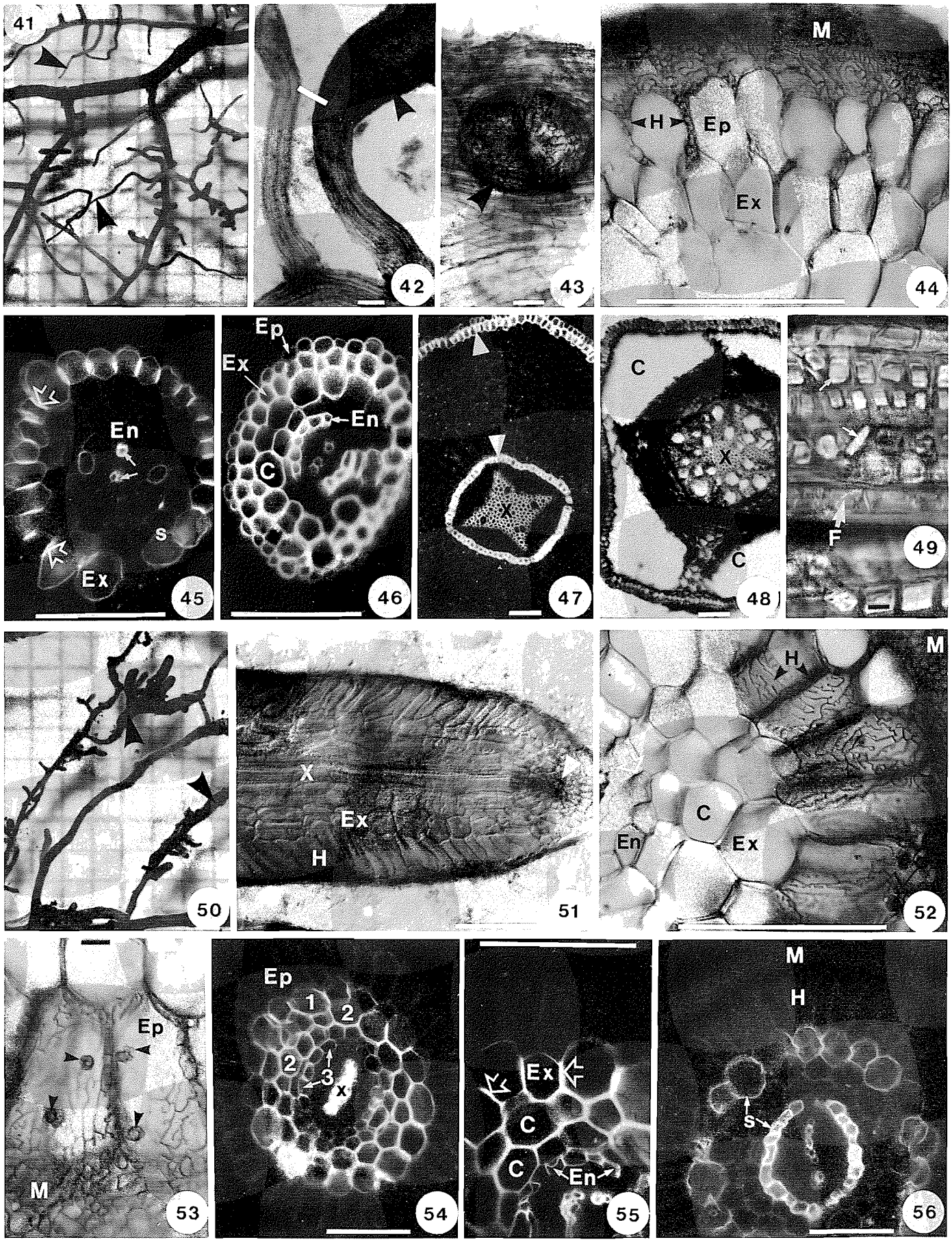
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ULTIMATE LATERALS: Lateral roots that form ECM have a somewhat enlarged epidermis with a Hartig net (Fig. 44) and an exodermis with Casparian bands and suberin lamellae, which often develops before the state II endodermis (Fig. 45). Nonmycorrhizal roots are similar but have small epidermal cells (Fig. 46). Mycorrhizal and nonmycorrhizal short roots also have 1 or 2 rows of other cortex cells, which may be suberized in older roots, and diarch xylem (Fig. 46).

MYCORRHIZAE: Only about 10% of the short roots examined were ectomycorrhizal, and VAM associations were not found. Roots with ECM often had a thin mantle and a Hartig net that failed to penetrate between some epidermal cells (Fig. 44). Epidermal cells in ECM roots were larger than those of nonmycorrhizal roots (Fig. 46) but small compared with those forming ECM in other Angiosperms. Some saprobic or parasitic fungi were seen in the cortex of short roots.

LONG ROOTS AND SECONDARY GROWTH: Long roots have 4 xylem poles and a heavily suberized exodermis and endodermis (Fig. 47). The cortex of these roots soon degenerates to form 3–5 large, longitudinal air chambers (Fig. 48). These features are eventually lost as result of secondary xylem, phloem, and periderm formation. Phloem fibres with walls that have oblique striations visible with polarized light are associated with parallel rows of cells containing cubical prismatic crystals (Fig. 49).

There are many reports of *Salix* species and other members of the Salicaceae having ECM, but many apparently can also have VAM (Harley and Smith 1987). The chambers in *S. nigra* long roots probably help to provide aeration.

Populus balsamifera L. (balsam poplar) and *P. tremuloides* Michx. (trembling aspen)

ROOT SYSTEM FORM: Heterorhizic. Long roots bear elongated, relatively unbranched short roots that are initially very narrow (0.10–0.15 mm wide), but become broader (0.15–0.30 mm wide) as a result of ECM formation (Fig. 50). Quiescent roots develop metacutinized root tips (Fig. 51).

ULTIMATE LATERALS: Most short roots develop ECM associations with a Hartig net surrounding obliquely elongated epidermal cells (Figs. 51, 52). These roots have a suberized exodermis and state II endodermis with passage cells (Figs. 54–56). The two intervening cortex layers have thick, highly refringent walls that may also become suberized (Figs. 54, 55). The stele has 2 xylem poles (Fig. 56).

MYCORRHIZAE: In *Populus* roots the Hartig net forms around sloping and much elongated epidermal cells, as is typical of Angiosperms with ECM (Figs. 51, 52). The fungal mantle may be thin or thick. In the root sample illustrated in Figs. 52 and 53, ECM hyphae produced projections that grew into the epidermal cells to form a type of ectendomycorrhizae. *Populus tremuloides* seedlings from a disturbed site were found to have VAM with normal hyphae and arbuscules in addition to ECM. *Populus* roots are similar in structure to those of *Salix nigra*, a member of the same family, but *Populus* short roots form ECM with a more extensive Hartig net and are rarely nonmycorrhizal.

LONG ROOTS AND SECONDARY GROWTH: *Populus* long roots soon develop a periderm consisting of tannin-filled cells that are rectangular in face view. In cross section, they are similar to secondary roots of other angiosperms.

In the present study several other species of *Populus*, *P. deltoides* Marsh. (eastern cottonwood) and *P. grandidentata* (Michx.) (largetooth aspen), were found to have similar roots and ECM associations. In North America, ECM has previously been reported in *P. deltoides* (Lohman 1927), *P. balsamifera* (Malloch and Malloch 1982), and *P. tremuloides* (Malloch and Malloch 1981), but Malloch and Malloch (1981, 1982) also found hyphae and vesicles of VAM fungi in some samples. Godbout and Fortin (1985) conducted a structural investigation of *P. tremuloides* ECM synthesized with a wide range of fungi. European *Populus* species have most often been reported to form ECM, but many apparently also form VAM (Harley and Harley 1987). Vozzo and Hacskaylo (1974) were able to experimentally synthesize ECM with *P. grandidentata, P. fremontii, P. tremuloides,* and *P. deltoides*, but they could form VAM only with *P. deltoides*.

ANGIOSPERMAE: Juglandaceae (Hamamelidae: Juglandales)

Juglans nigra L. (black walnut)

ROOT SYSTEM FORM: Nonheterorhizic. Fine roots are comparatively thick (0.3–0.5 mm) and branch infrequently (Fig. 57). Young roots are yellow-brown but soon become dark brown. Copious amounts of an orange-brown substance are released from roots in ethanolic fixatives. This substance is likely to be Juglone, a well-known allelopathic agent (Rice 1984).

ULTIMATE LATERALS: Roots usually contain VAM (Fig. 60). The exodermis is normally absent but may develop sporadically (Fig. 59). Most roots have a state II endodermis with pas-

FIGS. 41–49. *Salix nigra* (black willow) roots and mycorrhizae. Fig. 41. Narrow, tapering short roots (arrows) of willow (grid = 1 mm). Fig. 42. Whole CBE-stained roots showing narrow nonmycorrhizal lateral and wider ECM lateral (arrow). The root with ECM was squashed in slide preparation. Fig. 43. Short-root attachment scar (arrow) on a CBE-stained *Salix* long root. Fig. 44. Cross section of ECM (CBE–Nomarski) with mantle, incomplete epidermal Hartig net, and exodermis. Fig. 45. Cross section of BAB-stained ECM with exodermal as well as xylem and endodermis fluorescence (arrows). Fig. 46. Nonmycorrhizal short root in cross section with Fluorol fluorescence of suberin in the epidermis, exodermis, endodermis, and cortex. Fig. 47. Young long root in cross section with Fluorol fluorescence of the endodermis (inner arrow) and exodermis (outer arrow), and xylem autofluorescence. Fig. 48. Older long-root cross section with air chambers (C) and some secondary xylem formation (unstained). Fig. 49. Patterned phloem fibres (F) and associated cubical crystals from the secondary cortex. FIGS. 50–56. *Populus balsamifera* (balsam poplar) and *P. tremuloides* (trembling aspen) roots and mycorrhizae. Fig. 50. *Populus balsamifera* root system with swollen ECM short roots (arrows) (grid = 1 mm). Fig. 51. Whole ECM root of *P. balsamifera* with epidermal Hartig net, exodermis, xylem, and metacutinization (arrow) visible with CBE–Nomarski. Fig. 52. Cross section of *P. tremuloides* ECM (CBE–Nomarski) showing labyrinthine Hartig net hyphae, thick exodermal and cortex walls, and endodermal Casparian bands. Fig. 53. As Fig. 52, showing epidermal Hartig net and mantle hyphae and pegs (arrows) projecting into epidermal cells. Fig. 54. BAB fluorescence of *P. tremuloides* ECM cross section revealing xylem and suberization of (1) exodermal, (2) cortex, and (3) endodermal cell walls. Fig. 55. BAB fluorescence of exodermal Casparian bands and other suberized and lignified features of a *P. balsamifera* ECM cross section. Fig. 56. Section as in Fig. 54, but with Fluorol-induced fluorescence of exodermal (outer) and endodermal (inner) suberin lamellae, and autofluorescence of other structures.

sage cells, and 3 or 4 xylem poles (Figs. 58, 59). The root cortex has 8–10 cell layers and scattered idioblast cells containing multifaceted druse crystals like those of *Carya* (Fig. 58).

MYCORRHIZAE: Young roots contain VAM hyphae and arbuscules of an *Arum*-type association where the fungus spreads by coils in the outer cortex and by linear hyphae following air-channels in the inner cortex (Fig. 60). Similar associations were found in *Prunus* and *Fraxinus* roots. VAM has previously been observed in *J. nigra* roots (McDougall 1914; Brundrett and Kendrick 1988). Kormanik *et al.* (1982), and Kormanik (1985) investigated the physiology of this association.

LONG ROOTS AND SECONDARY GROWTH: roots with secondary growth have a thin periderm and a secondary phloem containing fibres and druse crystals. Unstained sections contain a dark brown pigment in their walls.

Carya ovata (Mill.) K. Koch (shagbark hickory)

ROOT SYSTEM FORM: Heterorhizic. Long roots bear narrow ECM short roots (0.15–0.25 mm wide) that usually have two orders of branching (Fig. 61).

ULTIMATE LATERALS: These roots form ECM with an epidermal Hartig net (Fig. 62), an unsuberized hypodermis, state II endodermis, and stele with 3 (or sometimes 2) xylem poles (Fig. 64). The root cortex consists of 3 or 4 layers of cells with moderately thick cell walls that show some autofluorescence and BAB staining (Fig. 64). Many cortex cells are occupied by multifaceted calcium oxalate druse crystals (Fig. 65).

MYCORRHIZAE: All short roots examined were ectomycorrhizal, but some also contained hyphae and vesicles of a VAM fungus that did not form a mycorrhizal association. Ectomycorrhizal fungi formed an external mantle and a labyrinthine Hartig net on moderately elongated epidermal cells (Figs. 62, 63).

LONG ROOTS AND SECONDARY GROWTH: Most long roots have a thick periderm and well-developed secondary phloem with fibres. In face view, phellem cells appear small and irregular, and many are arranged in pairs. Red tannins in pericycle cells give long roots a reddish colouration.

Carya cordiformis (Wang.) K. Koch (bitternut hickory) had similar short roots and ECM. This association was reported in *C. glabra* and *C. ovata* roots by McDougall (1914, 1928).

ANGIOSPERMAE: Tiliaceae (Dilleniidae: Malvales)

Tilia americana L. (basswood)

ROOT SYSTEM FORM: Heterorhizic with ECM short roots that are fairly long and narrow (0.20–0.30 mm). Short roots form loose clusters as a result of second- and third-order branching (Fig. 66).

ULTIMATE LATERALS: Short roots have an epidermal Hartig nets (Fig. 67) and no exodermis. Walls in the hypodermis and three other cortex layers are thick, highly refringent, and somewhat autofluorescent (Fig. 69). In older roots these walls are weakly lignified, as indicated by BAB (Fig. 68) and phloroglucinol staining. The endodermis slowly develops suberin lamellae (Fig. 69), pericycle cells may eventually also become suberized, and xylem is diarch (Fig. 68).

MYCORRHIZAE: *Tilia* short roots normally form ECM, often with a thin mantle, and a Hartig net surrounding epidermal cells that do not elongate much (Fig. 67). The Hartig net is not uniform in thickness because epidermal and cortex cells are arranged in irregular series (Fig. 67). MacKenzie (1983) conducted a structural study of mycorrhizal *T. cordata* roots. He considered the thick walls of cortex cells to be cellulosic.

LONG ROOTS AND SECONDARY GROWTH: *Tilia* long roots, when viewed in cross section, have a thick periderm of narrow, suberized phellem cells, secondary phloem with few fibres, and much secondary xylem (Fig. 70).

Ectomycorrhizae were found in *Tilia americana* by McDougall (1914), and Brundrett and Kendrick (1988), in *T. heterophylla* by Jackson and Driver (1969), and in the European species, *T. cordata*, in many investigations (Harley and Harley 1987).

ANGIOSPERMAE: Betulaceae (Hammamelidae: Fagales)

Betula alleghaniensis Britt. (yellow birch) and *B. papyrifera* Marsh. (white birch)

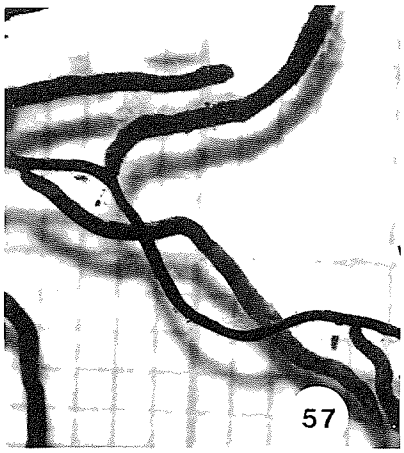
ROOT SYSTEM FORM: Heterorhizic with fine, elongate short roots (0.10–0.20 mm wide) that are somewhat wider (0.15–0.25) where ECM forms (Fig. 71), and usually have an additional order of sparse branches.

ULTIMATE LATERALS: These roots form ECM associations with Hartig nets surrounding elongated, sloping epidermal cells (Figs. 72, 73). The exodermis has well-defined Casparian bands and has suberin lamellae in older roots, and the xylem is diarch (Figs. 74–76). Below the hypodermis–exodermis is a single layer of cortex cells that form large phi thickenings, above a layer of smaller cells with unmodified walls and the state II endodermis (Fig. 75). The ECM laterals of both *Betula* species either had phi thickenings, a suberized exodermis, or both of these features, suggesting that these structures develop slowly and irregularly.

MYCORRHIZAE: *Betula* mycorrhizae were similar to those of other ECM angiosperms (Figs. 72, 73). Phi thickenings are the most characteristic feature of this otherwise typical association.

LONG ROOTS AND SECONDARY GROWTH: Long roots are initially protected by an outer primary cortex layer with phi thick-

FIGS. 57–60. *Juglans nigra* (black walnut) roots and mycorrhizae. Fig. 57. The root system of *J. nigra* (grid = 1 mm). Fig. 58. Unstained root cross section with Nomarski-induced refringence of crystals (arrows) and xylem. Fig. 59. Root cross section with Fluorol staining of suberin lamellae in the endodermis, except where passage cells occur (arrows) and one hypodermal cell. Fig. 60. Arbuscules and hyphae (arrows) of a VAM fungus in root (CBE–Nomarski). FIGS. 61–65. *Carya ovata* (shagbark hickory) roots and mycorrhizae. Fig. 61. Short roots (arrows) in heterorhizic root system (grid = 1 mm). Fig. 62. Longitudinal section of a hickory ECM showing mantle, Hartig net, and xylem (CBE–Nomarski). Fig. 63. Whole-root squashed preparation with CBE staining of Hartig net hyphae (Nomarski). Fig. 64. Cross section of ECM root with BAB fluorescence of xylem, endodermal Casparian bands and suberin lamellae, and some staining of cortex walls. Fig. 65. Unstained ECM cross section (Nomarski) showing Hartig net and large druse crystals in cortex (arrows). FIGS. 66–70. *Tilia americana* (basswood) root and mycorrhizal anatomy. Fig. 66. Branched ECM short roots (arrows) (grid = 1 mm). Fig. 67. Longitudinal section of ECM (CBE–Nomarski) showing mantle, epidermal Hartig net, and xylem. Fig. 68. Cross section of ECM root with BAB-induced fluorescence of xylem, suberin in the endodermis and pericycle (arrows), and modified cortex walls. Fig. 69. Similar section with Fluorol staining of lipid droplets (arrows) in immature endodermal cells. Autofluorescence of xylem and cortex cells is also visible. Fig. 70. Cross section of a *Tilia* long root with Fluorol staining of periderm suberin, lipid droplets in secondary phloem cells (arrows), and xylem autofluorescence.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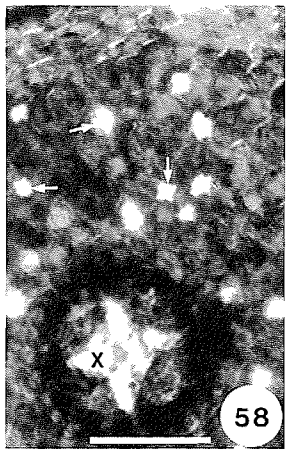
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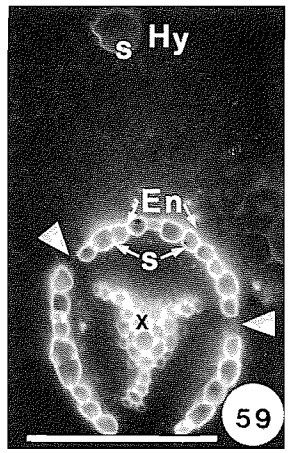
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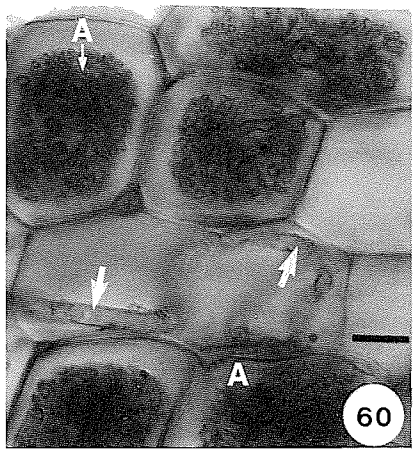
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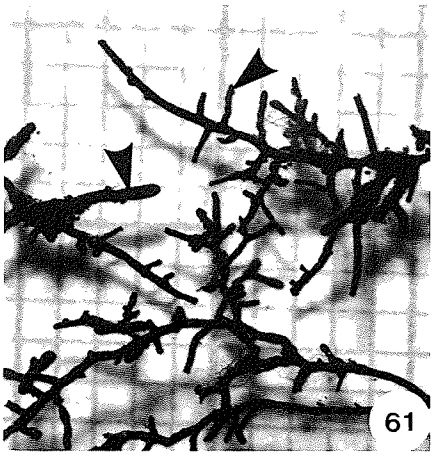
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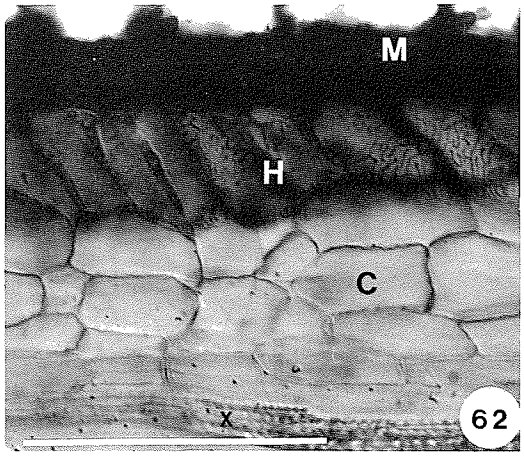
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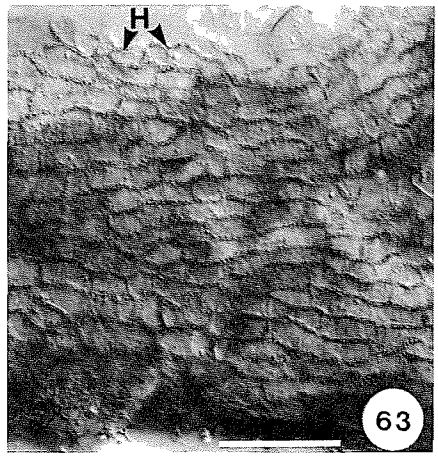
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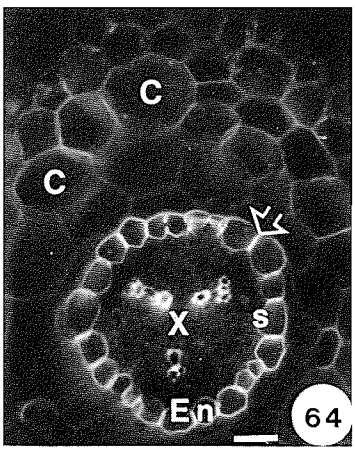
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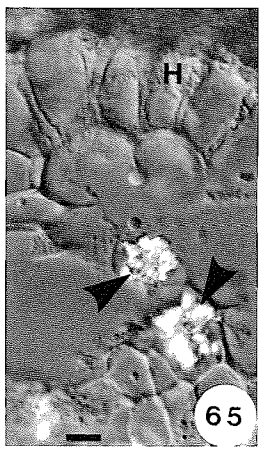
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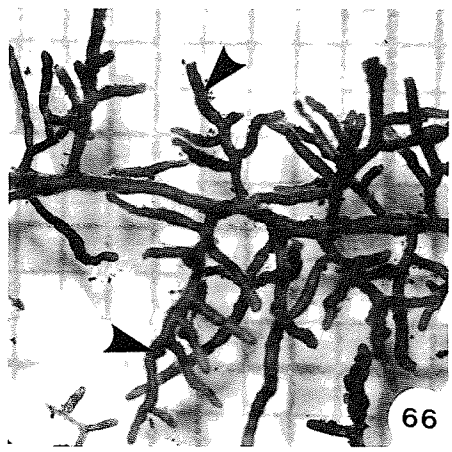
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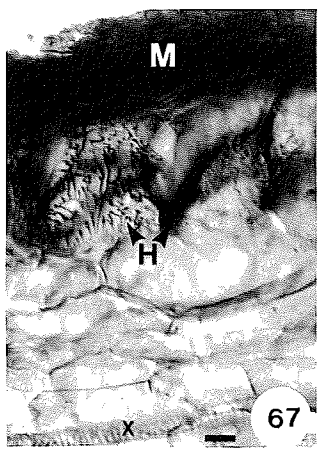
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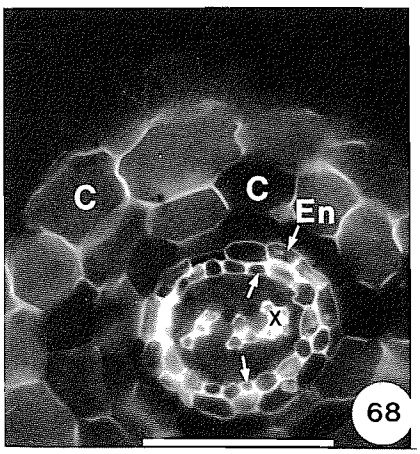
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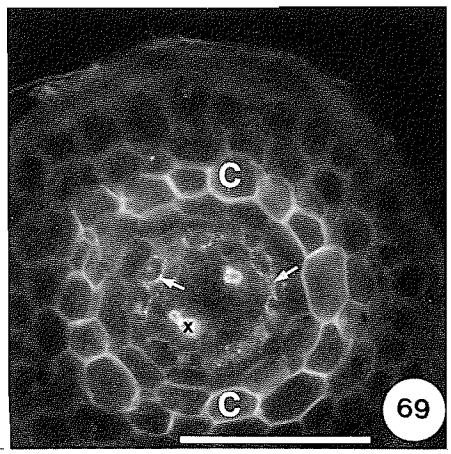
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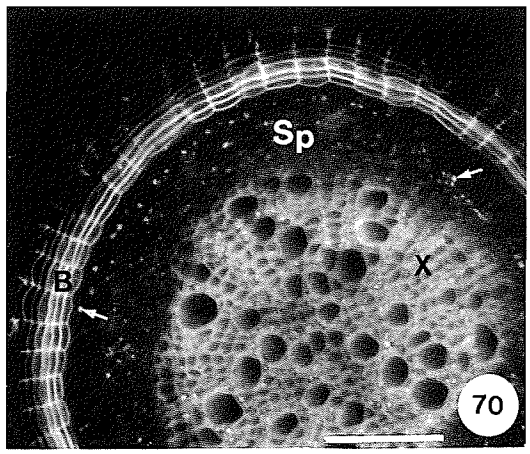
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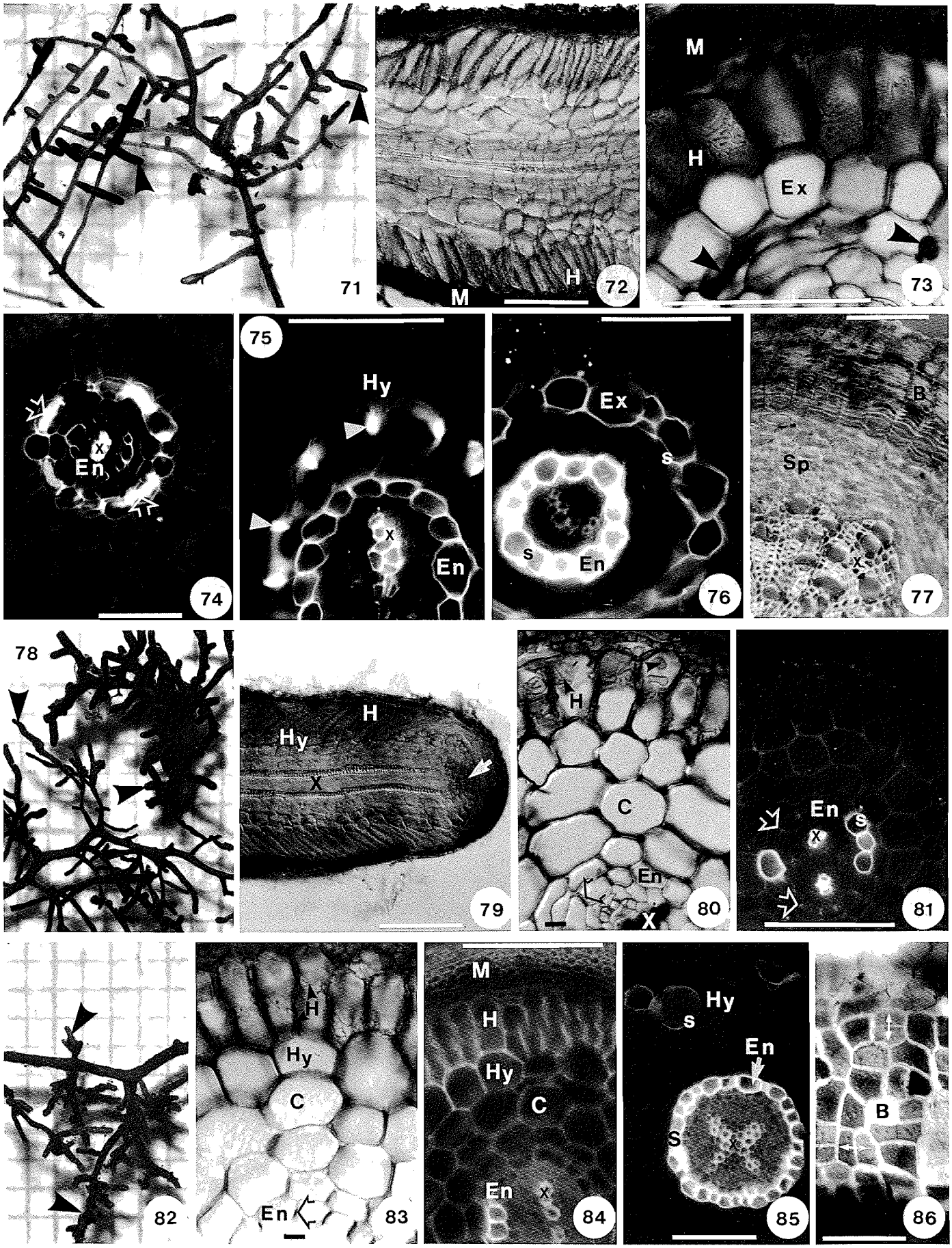
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enings. These roots later develop a thick periderm and other features resulting from secondary growth (Fig. 77).

Ectomycorrhizae of these two *Betula* species was initially reported by McDougall (1914, 1928) and more recently by Malloch and Malloch (1981). Wilcox and Wang (1987) examined the structure of ECM produced by *Phialophora finlandia* in *B. alleghaniensis* and observed phi thickenings in the cortex. There are numerous reports of ECM in European *Betula* species (Harley and Harley 1987).

Carpinus caroliniana Walt. (blue-beech, ironwood)

ROOT SYSTEM FORM: Heterorhizic, with short ECM roots that are 0.07–0.20 mm wide and have several orders of clustered branches (Fig. 78).

ULTIMATE LATERALS: Most short roots have ECM with an epidermal Hartig net (Figs. 79, 80). The root cortex has 3 or 4 layers of cells with walls that show little or no autofluorescence (Fig. 81), BAB, or phloroglucinol staining. Hypodermis cells have thickened walls that can be distinguished in cleared roots (Fig. 79), but do not become suberized (Fig. 81). Roots also have an endodermis, which develops thick suberin lamellae, and diarch xylem (Fig. 81).

MYCORRHIZAE: These are similar to the ECM of other angiosperms examined, but the mantle and epidermal Hartig net are often relatively thin. Ectendomycorrhizal associations, where mycorrhizal hyphae penetrate epidermal cells, are observed in some *Carpinus* roots (Fig. 81). *Carpinus caroliniana* was reported to have ECM by McDougall (1914).

LONG ROOTS AND SECONDARY GROWTH: Long roots are initially narrow with a thin periderm and secondary phloem but much secondary xylem. These long roots superficially resemble those of other ECM angiosperms.

Ostrya virginiana (Mill.) K. Koch (hop hornbeam, ironwood)

ROOT SYSTEM FORM: Heterorhizic, with long, narrow ECM short roots (0.15–0.30 mm wide), without much second-order branching (Fig. 82).

ULTIMATE LATERALS: Most short roots have ECM with enlarged epidermal cells enveloped in a Hartig net (Fig. 83). The cortex has 3 or 4 cell layers with thickened walls that show some autofluoresence (Fig. 84) and become partially lignified in older roots. The hypodermis is unsuberized in many cases, but some cells eventually form suberin lamellae (Fig. 85). The endodermis has state II suberin lamellae and the xylem has 1–3(–4) poles (Fig. 85).

MYCORRHIZAE: Short roots have an ensheathing mantle and a Hartig net surrounding somewhat elongated, sloping epidermal cells (Fig. 83). In cleared whole roots the Hartig net appears uneven because of the irregular arrangement of epidermis and cortex cells. McDougall (1914) and Lohman (1927) have also reported ECM in *O. virginiana*.

LONG ROOTS AND SECONDARY GROWTH: *Ostrya* long roots have secondary xylem, phloem, and a periderm similar to those of *Populus* and other angiosperms examined. The periderm cells are thin and irregular in face view, with some cells arranged in pairs (Fig. 86).

ANGIOSPERMAE: Fagaceae (Hamamelidae: Fagales)

Fagus grandifolia Ehrh. (beech)

ROOT SYSTEM FORM: Heterorhizic, with elongate short roots, 0.10–0.35 mm wide, that may be infrequently branched or form ECM clusters (Fig. 87).

ULTIMATE LATERALS: Short roots have ECM with an epidermal Hartig net (Figs. 88, 89). The hypodermis has wall modifications but usually no suberin lamellae (Fig. 91). There is one subhypodermal cortex layer that has thick walls that are highly refringent (Fig. 89) and lignified, as evidenced by phloroglucinol and BAB staining (Figs. 91, 92). These walls are thickest on the side facing the endodermis and have pit field gaps (Figs. 89, 92). The endodermis initially has small Casparian bands (Fig. 91), then develops thick suberin lamellae (Fig. 93), while the xylem and phloem have 2 or 3 poles.

MYCORRHIZAE: Epidermal cells beneath the mantle are surrounded by labyrinthine Hartig net hyphae. These host cells are irregularly arranged (Fig. 89) and often become considerably elongated. The thick walls of cortex cells are also clearly visible in cleared whole roots.

LONG ROOTS AND SECONDARY GROWTH: Long roots develop a periderm with tannin-filled and suberized cells that are irregularly shaped in face view. The secondary phloem is fairly narrow (Fig. 94).

Ectomycorrhizal lateral roots may contain swellings caused by the root-knot nematode, *Meloidogyne haela* (Fig. 90). *Fagus grandifolia* ECM was previously reported by McDougall (1914), Vozzo and Hacskaylo (1964), and Brundrett and Kendrick (1988). The European beech (*Fagus sylvatica*) has been the subject of many physiological and structural ECM investigations; Harley and Harley (1987) cite 75 *Fagus* ECM references. Clowes (1951) compared the structure of *F. sylvatica* mycorrhizal and nonmycorrhizal roots.

FIGS. 71–77. *Betula alleghaniensis* (yellow birch) and *B. papyrifera* (white birch) roots and mycorrhizae. Fig. 71. Yellow birch root system with swollen mycorrhizal short roots indicated by arrows (grid = 1 mm). Fig. 72. White birch ECM Hartig net and mantle (longitudinal section, CBE–Nomarski). Fig. 73. Yellow birch ECM cross section showing phi thickenings (arrows), and labyrinthine Hartig net hyphae (CBE–Nomarski). Fig. 74. White birch ECM cross section with BAB fluorescence of exodermal Casparian bands, as well as the endodermis and xylem. Fig. 75. Yellow birch ECM cross section with BAB fluorescence of phi thickenings (arrows), endodermal suberin, and xylem. Fig. 76. Section as in Fig. 74, but stained with Fluorol to show exodermal and thick endodermal suberin lamellae (UV fluorescence). Fig. 77. Unstained long-root cross section (Nomarski) showing refringence of xylem, secondary phloem, and periderm cell walls. FIGS. 78–81. *Carpinus caroliniana* (blue beech) short root and mycorrhizal anatomy. Fig. 78. Clusters of ECM short roots (arrows) (grid = 1 mm). Fig. 79. Whole ECM root (CBE–Nomarski) with hypodermis, xylem, metacutinization (arrow), and Hartig net. Fig. 80. Cross section of root showing xylem, endodermal Casparian bands, cortex, and Hartig net hyphae (arrows) that have penetrated into epidermal cells (CBE–Nomarski). Fig. 81. Cross section of ECM with Fluorol staining of incomplete suberin lamellae and weak Casparian band autofluorescence of the endodermis. FIGS. 82–86. *Ostrya virginiana* (hop hornbeam) roots and mycorrhizae. Fig. 82. Short roots (arrows) (grid = 1 mm). Figs. 83–85. Cross sections of ECM short roots. Hartig net hyphae, endodermal Casparian bands, and thick hypodermal and cortex cell walls (CBE–Nomarski). Fig. 84. Autofluorescence of hyphae in the mantle and Hartig net, and walls in the exodermis, cortex, and endodermis. Fig. 85. Fluorol-induced fluorescence of suberin lamellae in endodermal and some hypodermal cells. Fig. 86. Periderm cells of long roots contain tannins, are irregular in outline, and occur in pairs (↔) (CBE–Nomarski).

Quercus alba L. (white oak), *Q. rubra* L. (red oak), and *Q. velutina* Lam. (black oak)

ROOT SYSTEM FORM: Heterorhizic, with long, fairly narrow short roots (0.15–0.35 mm wide) that often branch to form large ECM clusters (Fig. 95).

ULTIMATE LATERALS: Short roots have ECM with an epidermal Hartig net (Figs. 96, 97). Below this layer is an exodermis with Casparian bands but no suberin lamellae (Figs. 98, 99). Between the hypodermis and state II endodermis are 1–3 layers of cortex cells with much thickened inner walls similar to those of the single layer in *Fagus* (Figs. 97, 98). These wall modifications have pit field gaps, are autofluorescent, and stain with phloroglucinol and BAB (Fig. 98). The stele usually has 3 or 4 xylem poles.

MYCORRHIZAE: *Quercus* roots are typical of angiosperms with ECM, having a Hartig net composed of a single layer of labyrinthine hyphae forming between the radial walls of considerably elongated epidermal cells (Figs. 96, 97).

LONG ROOTS AND SECONDARY GROWTH: Long roots develop a thick periderm consisting of tannin-filled cells with suberized walls (Fig. 100). The narrow secondary phloem eventually contains large clusters of fibres (Fig. 100).

Two additional species of *Quercus*, *Q. macrocarpa* Michx. (bur oak) and *Q. palustris* Muenchh. (pin oak), were also examined and found to have similar root structures and ECM associations. Wasp-containing galls were observed on *Q. alba* roots. Mycorrhizae have been documented in the roots of *Q. macrocarpa*, *Q. alba*, *Q. rubra*, and *Q. velutina* (McDougall 1914; Lohman 1927; Jackson and Driver 1969). Rothwell *et al.* (1983) found ECM and hyphae and vesicles of a VAM fungus in *Q. imbricaria* roots. *Quercus* and *Fagus*, both members of the Fagaceae, have short roots that can be distinguished from other ECM angiosperms by the sclerenchyma layer(s) in their cortex.

ANGIOSPERMAE: Ulmaceae (Hamamalidae: Urticales)

Ulmus americana L. (white elm)

ROOT SYSTEM FORM: Heterorhizic, consisting of numerous, small, short, obclavate lateral roots scattered on long roots (Fig. 101). Short roots are wider at the base (0.15–0.30 mm), tapering to a narrower (0.09–0.15 mm) tip (Fig. 102). Most short roots contain VAM associations and some are considerably swollen. *Ulmus* short roots are usually determinate but may resume growth, forming constrictions or beads like those of *Acer*.

ULTIMATE LATERALS: Most of the unusually shaped short roots of *Ulmus* contain VAM associations (Figs. 103, 104). In Fig. 103, small, narrow epidermal cells (which still contain some tannins after KOH clearing) and larger rectangular exodermal cells can be seen. The exodermis of *Ulmus* is similar in structure to that found in *Acer* roots, with a thick lignified and suberized external wall (Fig. 105). Many exodermal cells in older roots also form Casparian bands and suberin lamellae (Figs. 106, 107). The cortex consists of 3–5 (swollen roots may have up to 7) layers of large cells that are not separated by air-channels and have walls that are somewhat modified, as shown by BAB staining and autofluorescence (Figs. 105, 106). Mature *Ulmus* roots have a stage I or II endodermis and a diarch stele (Figs. 105–107).

MYCORRHIZAE: *Ulmus* has a *Paris*-type VAM association in which hyphae form coils as they spread throughout the cortex by growing within cells (Fig. 104). Some mycorrhizal lateral roots are considerably swollen, with extra cortex layers, perhaps to accomodate more VAM arbuscules. Most arbuscules form within inner cortex cells, which are larger than outer cortex cells containing hyphal coils.

LONG ROOTS AND SECONDARY GROWTH: Most long roots have a periderm and a thin secondary phloem that contains large fiber clusters.

Roots of *Ulmus thomasii* Sarg. (rock elm) were also examined and found to have similar anatomical features and mycorrhizae, as should roots of *U. rubra* Muhl. (slippery elm), which were not sampled. Population levels of all *Ulmus* species in Ontario have been drastically reduced by Dutch elm disease. *Ulmus americana* was previously observed to have VAM (Vozzo and Hacskaylo 1964: Brundrett and Kendrick 1988), as were European *Ulmus* species (Harley and Harley 1987). It is interesting to note that *Ulmus* roots have many anatomical similarities to those of *Acer*, even though the families Ulmaceae and Aceraceae are not closely related.

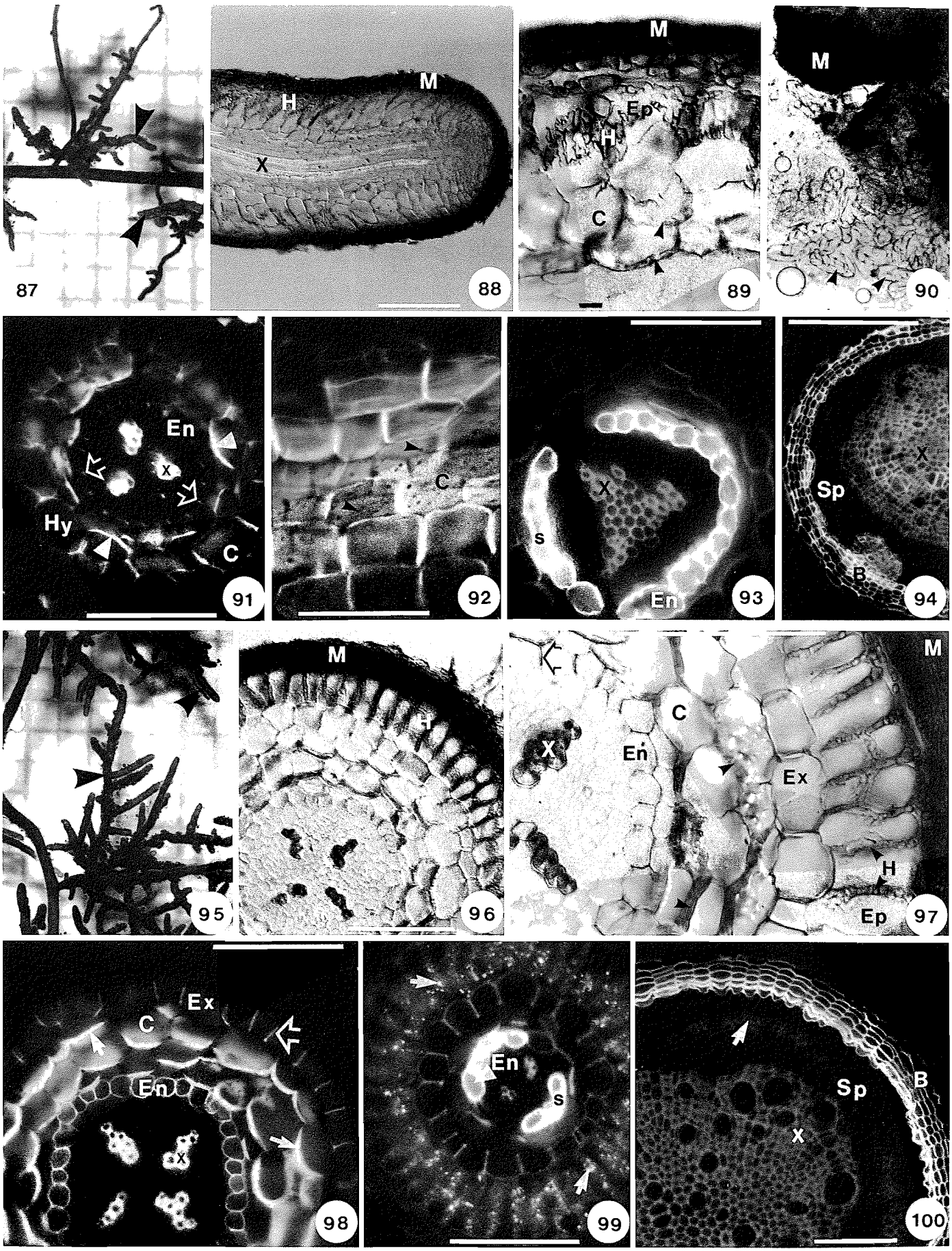
ANGIOSPERMAE: Rosaceae (Rosidae: Rosales)

Prunus serotina Ehrh. (black cherry)

ROOT SYSTEM FORM: Nonheterorhizic; the infrequently branched roots are red-brown in colour and 0.20–0.55 mm wide (Fig. 108).

ULTIMATE LATERALS: Most roots without secondary growth establish VAM associations (Figs. 109, 110). *Prunus* roots are characterized by prominent supraendodermal phi thickenings (Figs. 111–113). Many roots also have smaller phi thickenings in their hypodermal and subhypodermal layers, but these develop slowly and irregularly (Fig. 113). Exodermal wall modifications are absent. The phi thickenings are highly refringent (Fig. 111), can be observed by autofluorescence (Fig. 112), exhibit intense BAB-induced fluorescence

FIGS. 87–94. *Fagus grandifolia* (beech) roots and mycorrhizae. Fig. 87. Branched ECM short roots (arrows) and long roots (grid = 1 mm). Fig. 88. Longitudinal section of ECM short root showing mantle, epidermal Hartig net, and xylem (CBE–Nomarski). Fig. 89. Similar section viewed at higher magnification to show Hartig net hyphae and thick inner walls of cortex cells (arrows). Fig. 90. Root-knot nematodes (arrows) from squashed ECM short root (unstained–Nomarski). Fig. 91. Cross section of ECM with BAB-induced fluorescence of xylem, endodermal Casparian bands, cortex cell inner-wall thickenings (arrows), and hypodermal cells. Fig. 92. Glancing tangential section showing pits (arrows) in thick cortex cell walls (BAB fluorescence). Fig. 93. Cross section of ECM root centre with Fluorol staining of endodermal suberin lamellae and xylem autofluorescence. Fig. 94. Cross section of root with secondary growth showing Fluorol staining of the periderm and autofluorescence of the xylem. FIGS. 95–100. *Quercus rubra* (red oak), *Q. velutina* (black oak), and *Q. alba* (white oak) roots and mycorrhizae. Fig. 95. Typical view of root system (black oak) with ECM short root clusters (arrows) (grid = 1 mm). Fig. 96. Cross section of short root (CBE–Nomarski) showing ECM mantle, Hartig net, and other root structures. Fig. 97. Detail of ECM section in Fig. 96, showing xylem, endodermal Casparian bands, thick cortex walls (arows) with pits, exodermis, epidermal Hartig net, and mantle. Fig. 98. White oak ECM cross section with BAB-induced fluorescence of exodermal Casparian bands, cortex sclerenchyma (arrows), the endodermis, and xylem. Fig. 99. Red oak ECM in cross section with Fluorol staining of thick endodermal suberin lamellae and lipids in Hartig net hyphae (arrows) (UV-fluorescence). Fig. 100. Black oak secondary-root cross section with Fluorol staining of periderm suberin and autofluorescence of xylem and phloem fibres (arrow).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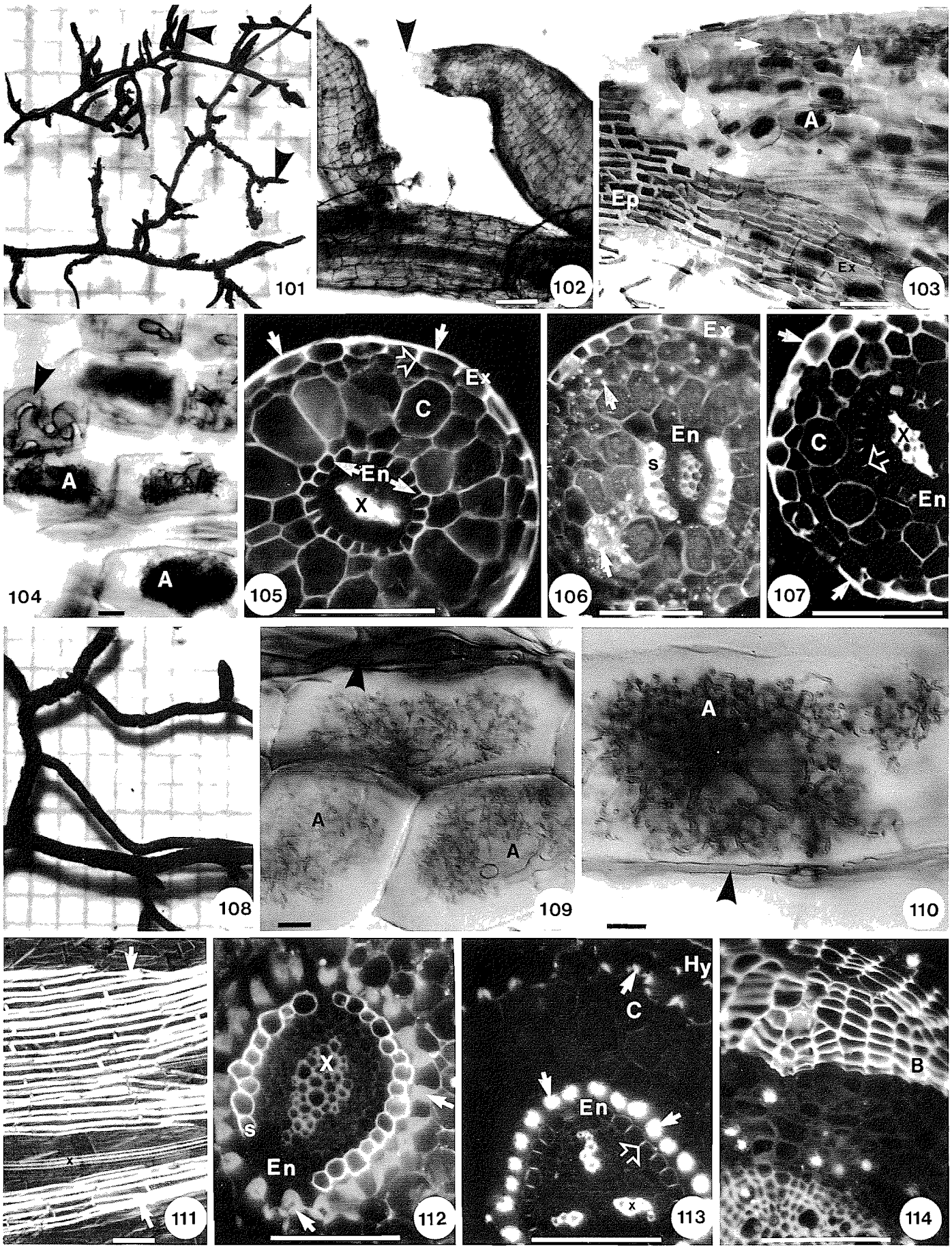
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(Fig. 113), and stain with phloroglucinol, indicating that they are lignified. Approximately 4 other VAM-containing cortex layers, arranged in irregular series, occur between the inner and outer phi layers. *Prunus* roots usually have a triangular stele with 3 xylem poles (but may have 2 or 4) and a stage II endodermis (Figs. 112, 113).

MYCORRHIZAE: *Prunus* mycorrhizae are very similar to those of *Fraxinus*, with wide cortical air spaces that are occupied by VAM hyphae. Arbuscules (Figs. 109, 110) are produced throughout the cortex. These features are typical of intercellular *Arum*-series VAM association. Mycorrhizal structures in *Prunus* roots can be difficult to observe because of the high tannin content of the roots.

LONG ROOTS AND SECONDARY GROWTH: Older roots that do not senesce develop a thick, highly suberized periderm (Fig. 114). The secondary phloem of *Prunus* roots contains scattered groups of fibres and druse crystals (Cutler *et al.* 1987).

Prunus pensylvanica L.f. (pin cherry) and *P. virginiana* L. (choke cherry) roots had similar structural features and mycorrhizal relations. *Prunus serotina* (Kormanik *et al.* 1982; Brundrett and Kendrick 1988) and *P. pensylvanica* (Malloch and Malloch 1981) were also previously reported to have VAM. Phi thickenings in *Prunus* roots are similar to those described in other members of the Rosaceae (MacKenzie 1979; Weerdenburg and Peterson 1983).

ANGIOSPERMAE: Aceraceae (Rosidae: Sapindales)

Acer rubrum L. (red maple), *A. saccharinum* L. (silver maple), and *A. saccharum* (Marsh. (sugar maple)

ROOT SYSTEM FORM: Relatively heterorhizic roots, with short or considerably elongated fine roots. All *Acer* species examined had similar root systems consisting of relatively coarse (0.10–0.45 mm wide) ultimate lateral roots that often have irregular constrictions or beads (Figs. 115, 116). *Acer* roots are initially yellow-brown but soon become dark brown or black as large amounts of phenolics are deposited. Maple roots have large, rectangular exodermal cells that are heavily stained by CBE, giving cleared roots a distinctive appearance.

ULTIMATE LATERALS: Most fine roots contain VAM associations (Fig. 118). Epidermal cells are small with relatively unmodified walls, becoming colonized by saprobic fungi in many older roots. Exodermal cells are 50–150 μm long and 30–50 μm wide, several times larger than those of the epidermis, with a very thick outer wall layer like that found in *Ulmus* roots (Figs. 117, 123). This exodermal wall is autofluorescent and stains with BAB (Figs. 123, 124) and also to some extent with phloroglucinol, which indicates that both suberin and lignin are probably present. Some cells in the exodermal layer also eventually develop radial wall modifications and suberin lamellae (Figs. 123–125). Some roots of *A. saccharinum* (and many *A. pensylvanicum* roots) also develop additional phi thickenings in exodermal cell walls (Fig. 120). The cortex of maple roots consists of 2 or 3 layers of large cells with walls that are autofluorescent and in older roots, stain as if lignified and (or) suberized (Figs. 121, 124). The stele of fine *Acer* roots usually has 2 xylem and phloem poles surrounded by a narrow state II endodermis with tannin-filled cells (Figs. 123, 124).

Quiescent maple roots have metacutinized root apices with deposits of suberin in root-cap cell walls (Fig. 121). These suberin deposits result in the beads or constrictions observed (Fig. 122), since they inhibit lateral expansion of root tips resuming growth (Kessler 1966). Kessler (1966), Beslow *et al.* (1970), and Brundrett and Kendrick (1988) consider that both environmental and physiological factors are responsible for the beading of maple roots.

MYCORRHIZAE: Maple roots contain hyphae of VAM fungi forming coils in the outermost layer(s) of the cortex and arbuscules in inner layers (see Kessler 1966). This VAM spread by coiling hyphae and localized arbuscule formation is typical of intracellular *Paris* VAM associations. Arbuscules and hyphae are very difficult to observe within cleared and stained maple roots as a result of CBE staining of exodermal and modified cortex cell walls (Brundrett and Kendrick 1988). These fungal structures can be seen in squashed roots (Figs. 118, 119). Mycorrhizal colonization of *Acer saccharum* fluctuates depending on the time of sampling, but these variations may be caused by the difficulty of detecting VAM structures in old roots (Brundrett and Kendrick 1988). In the present study, moderately high colonization levels were found in most *Acer* species, but *A. rubrum* and *A. spicatum* roots contained less VAM and had many root hairs.

LONG ROOTS AND SECONDARY GROWTH: Most fine roots remain in a primary growth state until death, but some roots undergo secondary growth, sloughing off their exodermis and forming a thick periderm (Fig. 126). The secondary phloem is narrow and has few fibres.

Acer nigrum Michx. f. (black maple), *A. pensylvanicum* L. (striped maple), and *A. spicatum* Lam. (mountain maple) also to have VAM and similar root anatomy features. Vesicular–arbuscular mycorrhizae have previously been reported in *A. saccharum* (Kessler 1966; Kormanik *et al.* 1982; Brundrett and Kendrick 1988), *A. rubrum* (McDougall 1914; Vozzo and Hacskaylo 1964; Kormanik *et al.* 1982), *A. saccharinum* (McDougall 1914), *A. nigrum* (McDougall 1914), *A. spicatum* (Malloch and Malloch 1981), and most European *Acer* species examined (Harley and Harley 1987). Endrigkeit (1933) conducted anatomical and permeability investigations on *A. pla-*

FIGS. 101–107. *Ulmus americana* (white elm) roots and vesicular–arbuscular mycorrhizae (VAM). Fig. 101. Oval short lateral roots (arrows) produced by elm (grid = 1 mm). Fig. 102. Elm short root (CBE-stained) with swollen base and narrow tip that is resuming growth (arrow). Fig. 103. CBE-stained elm root squashed to reveal VAM hyphal coils (arrows), arbuscules, and tannin-filled epidermal cells above the larger exodermal cells. Fig. 104. Arbuscules and coiled hyphae (arrow) in *Ulmus* (CBE–Nomarski). Fig. 105. Root cross section with BAB fluorescence of exodermis with Casparian bands and thick outer walls (arrows), the endodermis, and cortex walls. Fig. 106. Similar section with Fluorol staining of endodermis, lipids in VAM fungal hyphae (arrows), and suberin in some exodermal cells (UV fluorescence). Fig. 107. Old *Ulmus* root cross section with BAB fluorescence of thick outer exodermal walls (arrows), cortex walls, the xylem, and endodermal Casparian bands. FIGS. 108–114. *Prunus serotina* (black cherry) root anatomy and VAM morphology. Fig. 108. Root system (grid = 1 mm). Figs. 109 and 110. Intercellular hyphae (arrows) and arbuscules with numerous fine branches (CBE–Nomarski). Fig. 111. Supraendodermal phi thickenings (arrows) are apparent in whole roots (CBE–Nomarski). Fig. 112. Root cross section with endodermal suberin lamellae revealed by Fluorol fluorescence. Autofluorescence of phi thickenings (arrows) and xylem can also be seen. Fig. 113. Similar section with BAB fluorescence of xylem, endodermal Casparian bands, and phi thickenings (arrows). Fig. 114. Secondary-root cross section with Fluorol staining of periderm suberin (UV fluorescence).

tanoides roots and found the exodermis to be impermeable to the dyes he used; after histochemical tests, he considered its walls to contain both lignin and suberin.

ANGIOSPERMAE: Oleaceae (Asteridae: Scorphulariales)

Fraxinus americana L. (white ash), *F. nigra* Marsh. (black ash), and *F. pennsylvanica* Marsh var. *subintegerrima* (Vahl.) Fern. (green ash)

ROOT SYSTEM FORM: Nonheterorhizic. *Fraxinus americana* and *F. pennsylvanica* have very coarse roots (0.25–0.55 mm wide), while *F. nigra* has narrower (0.12–0.35 mm) roots (Figs. 127–129). Fine roots are long and relatively unbranched. Ash roots are yellow or light brown and have a low tannin content in contrast with the roots of other trees examined. Root tips have a conical root cap that becomes metacutinized (Figs. 130, 131). These root apices and the dimorphic exodermis distinguish ash from other CBE-stained tree roots (Fig. 13).

ULTIMATE LATERALS: These typically have VAM associations (Figs. 132–134) and are similar in structure in all *Fraxinus* species examined. *Fraxinus* roots have a dimorphic exodermis with Casparian bands and alternating long and short cells (Figs. 130, 135). The long cells have thick suberin lamellae while the short cells or passage cells are without suberin lamellae and have a thick outer wall cap (Figs. 135–137). The cortex proper consists of 5–10 series of regularly arranged cells and large intercellular air spaces (Fig. 135). The endodermis reaches state II development, and the xylem usually has 4 or 5 (2–3 in *F. nigra*) xylem poles bridged by xylem cells to form an polygon. Prominent metacutinization of several inner and outer root-cap cells occurs in quiescent roots (Figs. 130, 131). These suberized root caps are shed when roots resume growth, so there are no root constrictions like those in *Acer*.

MYCORRHIZAE: Hyphae of VAM fungi penetrate through unsuberized short cells, produce several intracellular coils in the outer cortex, then form intercellular air channel hyphae in the inner cortex (Fig. 134). Several hyphae follow each crooked air channel (Fig. 134), and arbuscules (Figs. 132, 133) form in cells throughout the cortex. *Fraxinus* VAM morphology is similar to that in *Prunus* roots and is typical of *Arum*-series associations, with rapid intercellular spread of the VAM fungus.

LONG ROOTS AND SECONDARY GROWTH: Young roots of higher branching orders are initially similar in structure to ultimate laterals, but their exodermis is eventually lost as a result of secondary growth and periderm formation. These secondary roots have a relatively thin periderm and a broad secondary phloem with many fibres (Fig. 138).

Fraxinus pennsylvanica Marsh var. *austinii* Fern. (northern red ash) roots were similar in structure and had VAM. Some *F. nigra* roots had swellings (Fig. 129) where hypertrophied cortex cells contained clusters of small spores presumed to belong to a chytrid. *Fraxinus americana* (Furlan *et al.* 1983; Brundrett and Kendrick 1988), *F. nigra* (Malloch and Malloch 1982), and *F. pennsylvanica* (Kormanik *et al.* 1982) were previously reported to have VAM. Kubíková (1968) carried out a detailed histochemical investigation of *F. excelsior* exodermis structure, reported that long cells contained suberin lamellae and that the thick caps of short cells mostly contained cellulose. Ash roots may be associated with the ash bolete fungus (*Boletinellus merulioides*) which forms hollow aphid-containing sclerotia on ash roots (Brundrett and Kendrick 1987).

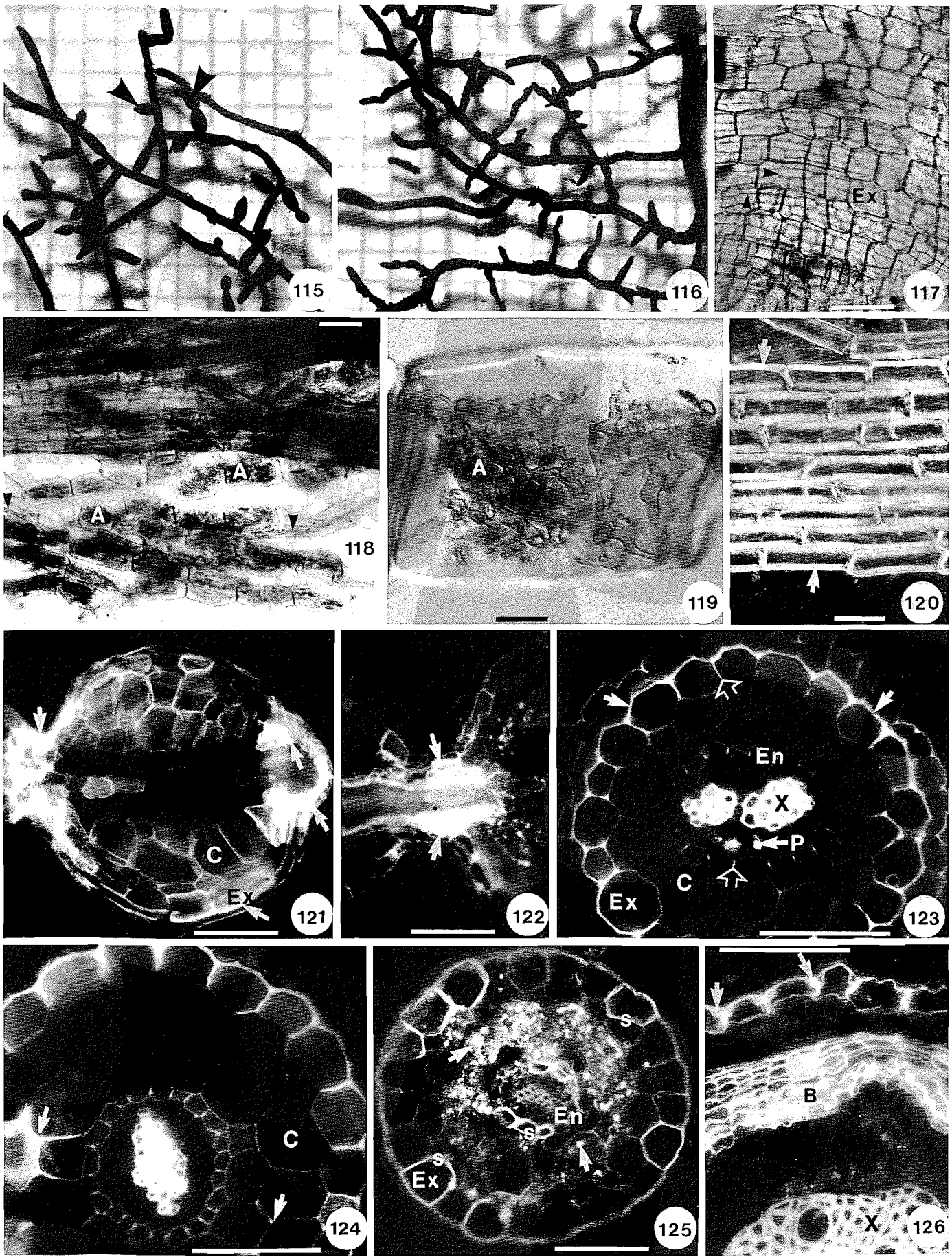
General discussion

Until recently, most data gathered for studies of plant communities have been primarily concerned with the aboveground parts of plants. This probably happened because the leaves and stems of plants are readily accessible, and much work has been spent on providing systems for their identification. In recent years, rapidly increasing interest in mycorrhizal phenomena and belowground aspects of competition has focused more attention on root ecology. The anatomy of belowground plant structures is much neglected in comparison with our knowledge of flowers, leaves, and aerial stems, but some information has been gathered in taxonomic and mycorrhizal studies (see Introduction). There is much need for further investigations of root structure for purposes of identification and to examine physiological roles of root features.

Roots are typically elongate, radially symmetrical organs that come in a relatively narrow range of sizes, colours, and textures, so that roots of different species may be superficially similar. These superficial characteristics have been used to separate the roots of some trees (McDougall 1921; Gilbertson *et al.* 1961). The identity of herbaceous plant roots can often be confirmed by tracing them back to the base of a plant. Tree root systems in forests can present more of a challenge because they grow to considerable distances from tree bases, and those from different trees intermingle (Lyford and Wilson 1964). In a recent study of a hardwood forest community, Brundrett and Kendrick (1988) found that the roots of herbaceous species and trees exhibited enough anatomical diversity to allow their identification during examinations of mycorrhizal phenomena.

In the present study it was found that the primary roots of most of the trees examined had characteristic features that could be used in their identification, at least to genus. This information could be obtained by using the methodology

FIGS. 115–126. *Acer rubrum* (red maple), *A. saccharinum* (silver maple), and *A. saccharum* (sugar maple) root and VAM morphology. Figs. 115 and 116. Root systems of silver maple and sugar maple, respectively (grid = 1 mm). Note prominent beads in silver maple roots (arrows). Fig. 117. Large rectangular exodermal cells in sugar maple root (CBE–Nomarski). Walls of smaller epidermal cells are visible in background (arrows). Fig. 118. VAM hyphae (arrows) and arbuscules from a squashed sugar maple root. Note intense CBE staining of root. Fig. 119. Arbuscule from sugar maple (CBE–Nomarski). Fig. 120. Exodermis with phi thickenings (arrows) from silver maple root (CBE–Nomarski). Figs. 121 and 122. Longitudinal section of a sugar maple beaded root stained with Fluorol to show suberin deposition (arrows) around the apical meristem (metacutinization), in the exodermis, and in the cortex (UV fluorescence). Figure 122 shows a constriction resulting from suberin deposition (arrows) where a metacutinized root resumed growth. Fig. 123. Cross section of a young sugar maple root with BAB fluorescence of exodermis with thick outer walls (arrows), xylem, and endodermal Casparian bands, and phloem sieve tube callose. Fig. 124. Older red maple root in cross section with BAB fluorescence as Fig. 123, but with additional modification of some cortex walls (arrow). Fig. 125. Silver maple root cross section with Fluorol staining of suberin lamellae in some exodermal cells, lipids in VAM hyphae (arrows), and in endodermal suberin lamellae (UV fluorescence). Fig. 126. Cross section of a silver maple root with secondary growth showing Fluorol fluorescence of periderm and sloughing exodermis cells (arrows). Autofluorescence of xylem cells and exodermal phi thickenings (arrows) are also visible.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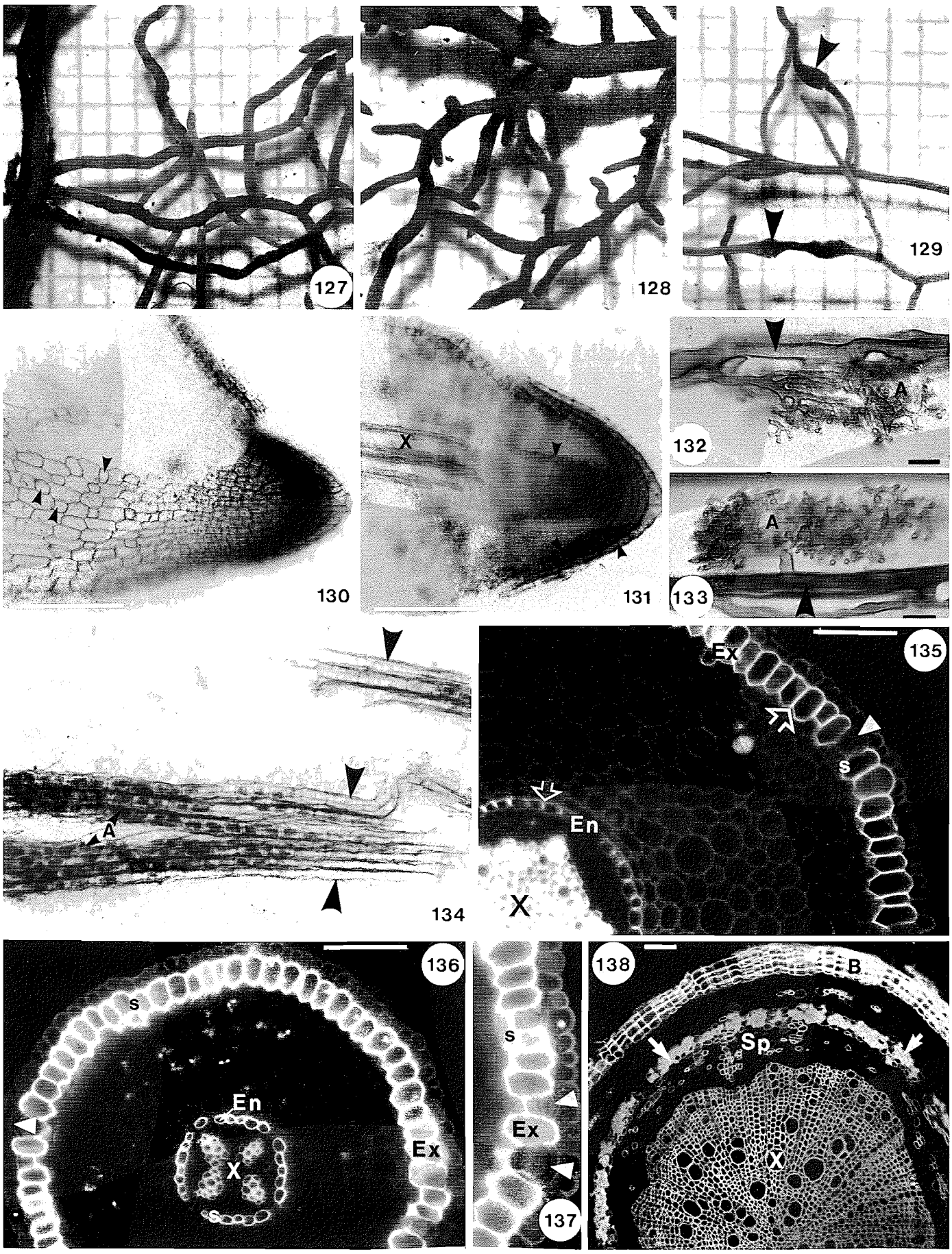
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described above, often during the course of mycorrhizal assessment. The root features summarized in Table 1 can be used to identify tree root samples directly and have been incorporated into a synoptic key (available from the authors on request). Root identification will often be much less complex than Table 1 would suggest, since it is only necessary to distinguish between the trees present at a given site, and with some practice, many of these will be found to be easily separable by visual appearance alone.

The tree roots examined in this study belong to four distinct anatomical groups: (*i*) gymnosperms with ECM; (*ii*) gymnosperms with VAM; (*iii*) angiosperms with ECM; and (*iv*) angiosperms with VAM. The most fundamental differences occur between angiosperms and gymnosperms with either type of mycorrhizae. Within the two groups of trees with ECM, the angiosperms and the gymnosperms, roots were often similar. Ectomycorrhizal gymnosperms with superfically similar roots could be differentiated by the arrangement of resin ducts and xylem in long and short roots (see Table 1). Angiosperm trees with ECM were structurally the most homogeneous group. These ECM angiosperms had superficially similar short roots, which could be identified by details of cortex structure (Table 1). Angiosperm trees with VAM exhibited the greatest structural diversity and were readily identified by their superficial appearance, as well as by anatomical features visible in sections or after clearing (Table 1).

Gymnosperm roots had a fundamentally different structurae than those of angiosperms. In gymnosperm roots, epidermis and cortex cell layers are arranged in stepwise series beginnning in the root cap and are shed from the roots in strips (Noelle 1910; Wilcox 1962). Thus the epidermis of conifer roots consists of a variable number of cell layers that have a gradual transition into cortex cells, and root hairs are often produced by a subsurface layer (Noelle 1910). Other characteristics of gymnosperm roots include a high cell wall tannin content, very thick endodermal suberin lamellae, and the presence of bordered pits in tracheid walls.

Gymnosperm trees can be separated into two distinct groups on the basis of their root anatomy features. One group consists of members of the family Pinaceae that have heterorhizic roots, relatively unmodified cortex cells, and normally form ECM associations. Mycorrhizae of Pinaceae have received considerable attention and the near-universal occurrence of ECM associations in this group is well established (Harley and Smith 1983). Noelle (1910) was able to construct a key that distinguished genera and many species of gymnosperms based on primary and secondary long-root anatomy. This key provided clear distinctions between genera but at the species level used details of the distribution of secretory cells, crystals, etc., that may be too variable to be reliable.

The second group of gymnosperms consists of families other than the Pinaceae, including the Cupressaceae and Taxodiaceae, that typically have nonheterorhizic roots with cortex phi thickenings, an exodermis, and VAM associations (Noelle 1910). The hypodermis of trees in this group was considered to be an exodermis by Noelle (1910), despite its irregular structure and formation. However, there are problems with the use of this term because the hypodermis is defined as a subepidermal layer, but these layers are indistinct in conifer roots (Noelle 1910). The exodermis in angiosperms is defined by the presence of Casparian bands that function as an apoplastic permeability barrier (Peterson 1988). The *Thuja* roots examined in the present study had hypodermal cells that slowly developed suberin lamellae, but Casparian bands were not detected by the staining procedures used. The presence of this permeability barrier needs to be confirmed in *Thuja* and other conifers with similar roots.

Angiosperms with ECM were structurally the most homogeneous group, even though they belonged to families that were often distantly related. Mutualistic relationships with ECM fungi have apparently resulted in covergent evolution of root features. In general, ECM angiosperms have heterorhizic root systems, with narow short roots with limited apical growth and an epidermal Hartig net. Epidermal cells usually elongate to increase the exchange area in contact with labyrinthine Hartig net hyphae (Massicotte *et al.* 1987). The stele of these roots is normal, but the cortex is usually reduced in thickness and composed of cells with variously modified walls.

There was considerable diversity among angiosperm trees with VAM associations. In general, roots of these trees were nonheterorhizic, with an exodermis and many cortex layers containing VAM arbuscules or hyphae. However, *Ulmus* and *Acer* had heterorhizic or somewhat heterorhizic roots, and *Prunus* and *Juglans* roots were usually without an exodermis.

The anatomical diversity of tree roots results because some features occur only within certain families or genera. Phi thickenings primarily occur in the Rosaceae, Cruciferae, Caprifoliaceae, and conifers outside the Pinaceae, but their location in the cortex varies between families (van Tieghem 1888). Other primary root features that appeared to be taxonomically restricted in distribution include druse crystals in the cortex of the Juglandaceae, thick inner-cortex wall sclerenchyma in the Fagaceae, and resin ducts in the Pinaceae (see Table 1). The exodermis was present in some angiosperm genera with ECM and most trees with VAM but had thick, somewhat lignified outer walls in roots of the unrelated genera *Acer* and *Ulmus* and was dimorphic in *Fraxinus* roots. It is interesting to note that many of the anatomical features that apparently were subject to the greatest selective pressures probably have structural or defensive roles, but the endodermis and exodermis function as permeability barriers (see Introduction).

Tree roots in natural ecosystems generally live for several years (Lyr and Hoffmann 1967), as do roots of most herbaceous plants in a hardwood forest community (Brundrett and

FIGS. 127–138. *Fraxinus* roots and VAM. Figs. 127–129. Root systems of *Fraxinus americana* (white ash), *F. pennsylvanica* (red ash), and *F. nigra* (black ash), respectively (grid = 1 mm). Note swellings caused by chytrid infections on black ash roots (arrows). Figs. 130–138. Details of white ash root anatomy. Figs. 130 and 131. Metacutinization of root tips stained with CBE. Figure 130 is focused on the root surface, showing the dimorphic exodermis with long and short (arrows) cells. Figure 131 focuses at a lower plane to reveal xylem and suberization of inner and outer root cap layers (arrows). Figs. 132 and 133. Fine arbuscule branch hyphae and coarse intercellular hyphae (arrows) of a VAM association (CBE–Nomarski). Fig. 134. Low magnification view of VAM showing longitudinal intercellular hyphae (arrows) and arbuscules (CBE). Fig. 135. Cross section of root with BAB-induced fluorescence of xylem, endodermal, and exodermal walls. Figs. 136 and 137. Similar sections with Fluorol staining of endodermal and exodermal suberin lamellae, and lipids in VAM hyphae (UV fluorescence). Note passage cells that have thick caps (arrows) and are without suberin lamellae in Figs. 135–137. Fig. 138. Secondary-root cross section with Fluorol staining of periderm suberin and autofluorescence of xylem and phloem fibres (arrows).

TABLE 1. Mycorrhizae, heterorhizy, and diagnostic anatomical features of lateral roots of Ontario forest trees

Tree	n^a	Mycorrhizae[b]	Heterorhizy[c]	Root width (mm)[d]	Exodermis[c,e]	Cortex rows[d,f]	Cortex features[g]	Endodermis state[d,h]	Xylem poles[d]	Diagnostic features of roots[i]
Larix laricina	6	ECM, C	+	0.25–0.50	−	4–6	In–Th	II	2	Two resin ducts in Lr stele
Pinus strobus	10	ECM, C	+	0.27–0.60	−	2–4	± In–Th	II	2	Arched xylem, two resin ducts in Lr dichotomous branching
Pinus resinosa	7	ECM, C	+	0.25–0.50	−	2–4	± In–Th	II	2	
Pinus banksiana	4	ECM, C	+	0.17–0.40	−	2–4	± In–Th	II	2	
Abies balsamea	6	ECM, C	+	0.15–0.35	−	5–6	In–Th	II	2	One central resin duct in Lr stele
Tsuga canadensis	12	ECM, C	+	0.35–0.60	−	3–5	In–Scl	II	2	Inner-cortex sclerenchyma in Sr
Picea mariana	5	ECM, C	+	0.10–0.20	−	2–3		II	1–2	Narrow, elongate, sparsely branched Sr
Picea glauca	9	ECM, C	+	0.20–0.40	−	3–4		II	2	
Thuja occidentalis	10	VAM, P	−	0.40–0.80	±	7–10	Phi	II	2	Phi thickenings throughout cortex
Salix nigra	6	ECM, Ep	+	0.08–0.25	+	2–3	± Sb	II	2	Very narrow, elongate Sr
Populus balsamifera	4	ECM, Ep	+	0.10–0.30	+	3	Th, ± Scl	II	2	Elongate, more or less unbranched Sr
Populus tremuloides	5	ECM, Ep	+	0.10–0.30	+	3	Th, ± Scl	II	2	
Juglans nigra	7	VAM, A	−	0.30–0.50	−(±)	8–10	Drs	II	3–4	Orange-brown substance in cortex
Carya ovata	4	ECM, Ep	+	0.15–0.25	−	3–4	Drs	II	3	Druse crystals in Sr cortex
Tilia americana	12	ECM, Ep	+	0.20–0.30	−	4	Th, ± Scl	I–II	2	Narrow, irregular Hartig net
Betula alleghaniensis	3	ECM, Ep	+	0.10–0.25	+	3	Phi	II	2	Phi thickenings and (or) exodermis in Sr cortex
Betula papyrifera	5	ECM, Ep	+	0.10–0.25	+	3	Phi	II	2	
Carpinus caroliniana	7	ECM, Ep	+	0.07–0.20	−	3–4	± Th	II	2	Relatively unmodified Sr cortex
Ostrya virginiana	4	ECM, Ep	+	0.15–0.30	−(±)	3–4	Th	II	1–3	Thick, somewhat lignified Sr cortex
Fagus grandifolia	15	ECM, Ep	+	0.12–0.35	−	2	Scl	I–II	2–3	One thick, lignified cell layer in Sr
Quercus alba	3	ECM, Ep	+	0.15–0.35	+	2–4	Scl	II	3–4	Several thick, lignified cell layers in Sr Cortex
Quercus rubra	7	ECM, Ep	+	0.15–0.35	+	2–4	Scl	II	3–4	
Quercus velutina	5	ECM, Ep	+	0.15–0.35	+	2–4	Scl	II	3–4	
Ulmus americana	12	VAM, P	+	0.09–0.30	O	4–6	± Sb	I–II	2	Obclavate short roots with VAM
Prunus serotina	11	VAM, A	−	0.20–0.55	−	4–6	Phi	II	3	Supraendodermal phi thickenings
Acer rubrum	9	VAM, P	±	0.15–0.45	O	3–4	± Sb	II	2	Beaded roots with massive outer exodermal wall
Acer saccharinum	3	VAM, P	±	0.10–0.40	O	3–4	± Sb	II	2	
Acer saccharum	17	VAM, P	±	0.10–0.35	O	3–4	± Sb	II	2	
Fraxinus americana	10	VAM, A	−	0.25–0.50	D	6–11	Reg	II	4–5	Dimorphic exodermis and metacutinized root caps
Fraxinus nigra	4	VAM, A	−	0.12–0.35	D	5–9	Reg	II	2–3	
Fraxinus pennsylvanica	3	VAM, A	−	0.27–0.55	D	6–11	Reg	II	4–5	

[a]Number of cleared and stained root samples examined for mycorrhizae.
[b]ECM, ectomycorrhizal; VAM, vesicular–arbuscular mycorrhizae; C, cortical Hartig net; Ep, epidermal Hartig net; A, intercellular VAM; P, intracellular VAM.
[c]+, present; −, absent; ±, intermittent.
[d]Typical values for ultimate lateral roots.
[e]O, exodermis with thick outer wall; D, dimorphic exodermis with long and short cells.
[f]Includes hypodermis or exodermis but not endodermis.
[g]In, inner cell layer; Th, wall thickenings; Scl, lignified wall thickenings; Phi, phi thickenings; Drs, druse crystals; Sb, suberized walls; Reg, regular cell series.
[h]State I, with Casparian bands; state II, with Casparian bands and suberin lamellae.
[i]Lr, long root; Sr, short root.

Kendrick 1988). It is becoming increasingly apparent that many of the root features that involve lignified and suberized walls or secondary metabolite storage probably provide structural strength and defence against predatory soil animals and microbes. The correlation between root lifespan and complicated wall structures has already been noted (Pienaar 1968; Brundrett and Kendrick 1988). Metacutinization, the protection of root tips by suberin deposition in the root cap, commonly occurs in dormant tree roots (Romberger 1963), including most of those in the present study.

Aerenchyma was observed in some *Picea mariana* roots, and *Salix nigra* long roots had large air spaces in the primary cortex, as is typical of plants growing in anaerobic soils (Justin and Armstrong 1987). *Picea mariana, Fraxinus nigra*, and *Salix nigra* short roots were much narrower than those of closely related species growing in drier soils.

There are considerable differences in structure and function between nonwoody roots and woody roots that are encased in a periderm and primarily function as channels between nonwoody roots and the stem (Lyford and Wilson 1964). Roots in a secondary state of growth resulting in additional xylem, phloem, and periderm tissue were often found to be superficially similar. For example, it was difficult to distinguish between long roots of different angiosperm trees with ECM when cross sections were observed. These roots could probably be identified if fine details of periderm, xylem, and phloem anatomy were compared with descriptions in Cutler *et al.* (1987) and references on wood structure.

Angiosperm and gymnosperm trees with ECM are usually heterorhizic, with ultimate lateral roots differentiated into mycorrhizal short roots and coarser long roots (Noelle 1910; Kubíková 1967; Sen 1980). The long and short roots in het-

erorhizic root systems are fundamentally similar in structure (Clowes 1951; Wilcox 1964), but short roots are normally narrower and grow much more slowly than long roots (Wilcox 1964; Kubíková 1967; Sen 1980). The restricted growth of short roots may be necessary to allow ECM fungi time to form an association, since these fungi have difficulty colonizing more rapidly growing roots (Sohn 1981; Chilvers and Gust 1982). Thus trees with ECM would require slow growth of some of their lateral roots, and in time this process would result in the evolution of separate, genetically distinct (*sensu* Zobel 1986) long and short roots. In trees with heterorhizic root systems, the presence of an ECM association could often be deduced by examining superficial features, since mantle hyphae were often visible, and short roots of most species consistently had ECM.

Heterorhizy also occurs in *Ulmus* and to some extent in *Acer* root systems, which have VAM associations. However, these trees have *Paris*-series intracellular associations. Since VAM formation is generally slower than in *Arum*-series hosts (Brundrett and Kendrick 1989), it may be more compatible with short roots.

As expected from the many literature reports of mycorrhizae in related species, almost all the field-collected roots examined in this study contained high levels of mycorrhizae. The only exceptions were *Salix nigra* roots, of which the majority were nonmycorrhizal, and *Acer rubrum* and *A. spicatum* roots, which contained moderate levels of VAM colonization. These species are normally found in wet sites where the soil would be waterlogged, a habitat that also favours herbaceous plants with little or no mycorrhizal colonization (Trappe 1987). Ectendomycorrhizal associations, where ECM fungi penetrate within host cells (Harley and Smith 1983), were observed in *Populus* and *Carpinus* roots and may be fairly common in natural communities. In addition to the mycorrhizal fungi normally present in tree roots, there are other root phenomena that require further study, including the parasitic fungus observed in *Tsuga* roots, the chytrids in *Fraxinus nigra*, root-knot nematodes in *Fagus*, and the *Boletinellus*–aphid association of *F. americana*. Many more interesting root phenomena certainly await discovery.

The results and literature citations presented above support the conclusion that trees within a genus usually have the same type of mycorrhiza, and these relationships are generally also consistent within a family (the Juglandaceae is an exception, since it contains *Carya* with ECM and *Juglans* with VAM). This high correlation between plant phylogeny and mycorrhizal relationships has been observed for families with ECM, as well as those containing species that are usually nonmycorrhizal, but there are also many exceptions (Harley and Smith 1983; Testier *et al.* 1987).

There are examples of trees which can form both ECM or VAM, such as the *Populus tremuloides* seedlings examined in this study, and the *Eucalyptus* seedlings studied by Chilvers *et al.* (1987), but the overall results of the present study and surveys of literature such as Harley and Harley (1987) suggest that this is an infrequent occurrence. Many of the reports of VAM in roots of species that normally form ECM report hyphae and vesicles but not arbuscules (Vozzo and Hacskaylo 1974; Malloch and Malloch 1981, 1982; Rothwell *et al.* 1983; Harley and Harley 1987). It is unlikely that these reports result from misidentification of the fungus, since VAM-fungus hyphae and vesicles have a characteristic appearance. However, VAM fungi also colonize a variety of substrates, including soil organic material and senescing roots of otherwise nonmycorrhizal species (St. John *et al.* 1983; Brundrett and Kendrick 1988). It is probable that most reports of these structures in roots that normally form ECM associations represent such endophytic but not symbiotic associations. Roots of nonhost plants may not be able to completely exclude VAM hyphae, given the nature of the activity of these fungi and their nearly universal presence in soils (Harley and Smith 1983). Many of the older reports of ECM or VAM in hosts that are now known to have another type of mycorrhizae should be disregarded if they do not include evidence that mycorrhizal definitions were rigorously applied or anatomical investigations were carefully conducted.

Many of the Pinaceae with ECM had an inner cortex layer with thick cell walls that prevented Hartig net hyphae from extending as far as the endodermis. This layer was absent in *Picea*, had highly refringent walls in *Larix, Pinus, Abies*, and *Tsuga*, but showed signs of lignification only in *Tsuga*. MacKenzie (1983) has also observed thick inner-cortex walls in *Picea sitchensis* roots, and he found them to contain densely packed cellulose microfibrils. Nylund (1987) has suggested that the host cell walls between which Hartig net hyphae grow contain acid polysaccharides (pectins) that provide the flexibility thought necessary to allow hyphal ingress. Thick walls predominantly composed of crystalline cellulose, as found in the inner cortex of many Pinaceae, might be too inflexible to allow further hyphal penetration.

A cortical Hartig net is typical of ECM in the Pinaceae, but most angiosperms with ECM have a Hartig net that is restricted to the epidermis (Alexander and Hogberg 1968; Massicotte *et al.* 1987). Ling-Lee *et al.* (1977) found phenolics in hypodermal cells of *Eucalyptus* roots and suggested that these wall properties could be responsible for restricting ECM fungi to the epidermis. Phenolic accumulation often occurs in host cells as a result of ECM formation (Duchesne *et al.* 1987), but the influence of these substances on Hartig net hyphae requires investigation. All of the ECM angiosperm genera examined in the present study had some degree of wall thickening in hypodermis cells subtending their epidermal Hartig net, and an exodermis with suberized Casparian bands was present in many *Betula* and *Quercus* short roots. Suberin can function as a barrier to fungus penetration (Kolattukudy 1984), but suberization of short-root hypodermal cells probably occurs after Hartig net formation in these species. The thick, inflexible nature of hypodermal cell walls, and not the secondary metabolites they contain, is most probably responsible for preventing Hartig net hyphal growth past the epidermis, but these wall structure – ECM fungus interactions require further investigation. Cortex layers between the hypodermis and endodermis also usually had thick walls that became extremely lignified in *Fagus* and *Quercus*. The cortex of most ECM angiosperms contains additional features that probably have structural or defensive roles. The endodermis and exodermis, if present, may help to regulate transport between the host and fungus. Cells in intervening cortex layers are probably relatively inactive, and this region is often reduced in short roots.

Within different hosts, VAM fungi form either predominantly intracellular (*Paris* series; Gallaud 1905) or intercellular (*Arum* series; Gallaud 1905) associations. It has been shown that these morphological differences, which influence the efficiency of VAM associations (the distribution and abundance of arbuscules within the cortex), result from the presence or absence of cortex air channels (Brundrett *et al.* 1985; Brundrett

and Kendrick 1988). Suberin in the exodermis apparently also influences VAM formation by restricting hyphal penetration to unsuberized short cells or young root regions. Some details of mycorrhizal morphology, such as the branching patterns of VAM fungi within roots or the mantle featrues produced by ECM fungi on roots, are associated with particular mycorrhizal fungi (Abbott 1982; Agerer 1986; Haug and Oberwinkler 1987).

Unfortunately, most recent studies of mycorrhizal morphology have used electron microscope procedures to provide high resolution subcellular information without first conducting histochemical investigation of root structure using light microscope staining procedures. Thus, many potential interactions between host wall structure and mycorrhiza formation have remained undiscovered.

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