

Host microtubules in the Hartig net region of ectomycorrhizas, ectendomycorrhizas, and monotropoid mycorrhizas

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Abstract: Various categories of mycorrhizas are recognized primarily by the structural changes that occur between fungi and roots. In all mycorrhiza categories, cytological modifications of root cells accompany the establishment of the functional symbiosis, and among these are alterations in the organization of the cytoskeleton. Using immunolabelling combined with confocal scanning laser microscopy, this study documents changes in microtubules (MTs) in root cells of ectendomycorrhizas and monotropoid mycorrhizas; in addition, ectomycorrhizas were reinvestigated to determine the effect of fungal colonization on host root cells. In *Pinus banksiana* L. – *Laccaria bicolor* (Maire) Orton ectomycorrhizas, MTs were present in epidermal and cortical cells adjacent to the Hartig net. The remaining cortical MTs had a different organization when compared with those of cortical cells of control roots. MTs were present in Hartig net hyphae. In ectendomycorrhizas formed when roots of *P. banksiana* were colonized by the ascomycete, *Wilcoxina mikolae* var. *mikolae* Yang & Korf, MTs were present adjacent to intracellular hyphae and host nuclei, but few cortical MTs were present. MTs were present within Hartig net and intracellular hyphae. In field-collected roots of *Monotropa uniflora* L., MTs were associated with fungal pegs, intracellular extensions of inner mantle hyphae within epidermal cells. The close association between MTs and fungal pegs may be related to the formation of the highly branched host-derived wall that envelops each fungal peg. The development of exchange interfaces in the three systems studied involve changes in the organization of microtubules.

Key words: cytoskeleton, microtubules, Hartig net, mycorrhizas, immunolocalization, confocal microscopy.

Résumé : On reconnaît diverses catégories de mycorrhizes, surtout par les modifications structurales qui surviennent entre le champignon et les racines. Dans toutes les catégories de racines, les modifications cytologiques des cellules racinaires accompagnent l'établissement d'une symbiose fonctionnelle, et parmi celles-ci on note des altérations de l'organisation du cytosquelette. À l'aide de l'immunomarquage combiné avec la microscopie confocale par balayage au laser, les auteurs décrivent les changements qui surviennent dans les microtubules (MTs) des cellules racinaires d'ectendomycorrhizes et de mycorrhizes monotropoïdes; ils ont également observé de nouveau des ectomycorrhizes, afin de déterminer l'effet de la colonisation fongique sur les cellules racinaires de l'hôte. Chez les ectomycorrhizes *Pinus banksiana* L. – *Laccaria bicolor* (Maire) Orton, les MTs sont présents dans les cellules épidermiques et corticales adjacentes au réseau de Hartig. Les MTs des autres cellules corticales montrent une organisation différente, lorsqu'on les compare à ceux des cellules corticales de racines témoins. On retrouve des MTs dans les hyphes du réseau de Hartig. Chez les ectendomycorrhizes formées par l'ascomycète *Wilcoxina mikolae* var. *mikolae* Yang & Korf sur les racines du *P. banksiana*, les MTs se retrouvent en position adjacente aux hyphes intracellulaires et aux noyaux de l'hôte, mais il y a peu de MTs dans les cellules corticales. Les MTs sont présents dans le réseau de Hartig et les hyphes intracellulaires. Chez des racines du *Monotropa uniflora* L. récoltées en nature, les MTs sont associés avec les hyphes de pénétration et les extensions intracellulaires des hyphes du manteau interne, à l'intérieur des cellules épidermiques. L'association étroite entre les MTs et les hyphes de pénétration fongique pourrait être reliée à la formation de la paroi de l'hôte fortement ramifiée qui enveloppe chaque hyphe de pénétration. Le développement des surfaces d'échange, dans les trois systèmes étudiés, implique des changements dans l'organisation des microtubules.

Mots clés : cytosquelette, microtubules, réseau de Hartig, mycorrhizes, immunolocalisation, microscopie confocale.

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Introduction

Mycorrhizal fungi alter root development at the organ, cellular, and subcellular levels (Peterson and Farquhar 1994; Bonfante and Perotto 1995; Smith and Read 1997). Subcellular changes are often more pronounced in endomycorrhizas than ectomycorrhizas in that in the former, the host cell wall is breached, and the cell responds by elaborating a perifungal membrane and interfacial matrix material separating the fungal cell wall from the host cytoplasm (Bonfante and Perotto 1995; Peterson et al. 1996, 1998; Armstrong and Peterson 2002). Along with these modifications, changes in the cytoskeleton of host cells have been demonstrated first in arbuscular mycorrhizas (AMs) (Genre and Bonfante 1997, 1998, 2002) and orchid mycorrhizas (Uetake et al. 1997; Uetake and Peterson 1997, 1998; see also reviews by Peterson et al. 2000; Timonen and Peterson 2002). In AMs, both microtubules (MTs) and actin filaments (AFs), although modified in position compared with noncolonized cells, remain in cortical cells containing arbuscules in *Arum*-type associations (Genre and Bonfante 1997, 1998; Matsubara et al. 1999) and hyphal coils and arbusculate coils in *Paris*-type associations (Armstrong and Peterson 2002). In *Medicago truncatula*, the presence of arbuscules in cortical cells triggers changes in MTs in adjacent, uncolonized cortical cells, suggesting that the fungus produces a chemical signal that alters the cytoskeleton (Blancaflor et al. 2001).

In ectomycorrhizas, the fungus develops a mantle on the root surface and a Hartig net, an intercellular branched complex of hyphae (Smith and Read 1997). In this case, MTs and AFs have been localized in the fungal hyphae but not in host cells adjacent to the Hartig net (Timonen et al. 1993; Timonen and Peterson 2002).

A third mycorrhizal category, ectendomycorrhiza, represents a very specialized association between a few ascomycete fungi and roots of *Pinus* and *Larix* species (see review by Yu et al. 2001). Interestingly, these same fungi form typical ectomycorrhizas with other tree species (Scales and Peterson 1991a; Yu et al. 2001). In ectendomycorrhizas, a mantle and Hartig net form but, in addition, intracellular hyphae develop from branches of Hartig net hyphae (Scales and Peterson 1991b; Yu et al. 2001). Although some ultrastructural observations of host cells and intracellular hyphae have been published (Piché et al. 1986; Scales and Peterson 1991b), there is no information concerning changes in the cytoskeleton in ectendomycorrhizas.

A similar situation exists for monotropoid mycorrhizas, associations between a limited group of basidiomycete fungi and the roots of myco-heterotrophic genera belonging to the subfamily Monotropoideae in the family Ericaceae (Biddartendo and Bruns 2001, 2002). Ultrastructural details of the interface between fungi and root epidermal cells of *Monotropa uniflora* L. (Lutz and Sjolund 1973) and *Monotropa hypopitys* (Duddridge and Read 1982) have been published, but the effect of fungal colonization on the host cell cytoskeleton has not been examined.

It is important to investigate the changes in the cytoskeleton of root cells during mycorrhiza formation to gain a better understanding of the role that MTs and AFs play in the changes in structure of both symbionts and the establishment of the nutrient exchange interface.

The objectives of this study were, therefore, to (1) determine the effect of the basidiomycete fungus, *Laccaria bicolor* (Maire) Orton, on the organization of MTs in ectomycorrhizas formed with *Pinus banksiana* L., (2) determine the effect of the ascomycete fungus, *Wilcoxina mikolae* var. *mikolae* Yang & Korf, on the organization of MTs in *P. banksiana* ectendomycorrhizas, and (3) determine the MT arrangement in epidermal cells of field-collected *M. uniflora* mycorrhizas.

Materials and methods

Plant material

Seeds of *P. banksiana* (jack pine; lot 8960049) were obtained from the National Tree Seed Centre, Natural Resources Canada, Fredericton, New Brunswick. Although two additional pine species (*Pinus resinosa* Ait. and *Pinus strobus* L.) were studied, only results with *P. banksiana* are reported here. Seeds were sterilized by a 1-min treatment in 70% ethanol, three rinses in sterile distilled water, 8 min in a 10% commercial bleach solution, followed by three rinses in sterile distilled water. They were germinated on sterilized quartz sand and seedlings allowed to grow until the shoot reached a minimum of 3 cm height before being transplanted to plastic Petri plates 11.5 cm in diameter containing a 1:1 mix of sterile Pro-mix "BX" (Plant Products Co. Ltd., Brampton, Ontario) and Turface (calcined montmorillonite clay; Applied Industrial Materials, Buffalo Grove, Illinois). Each Petri plate had a hole cut into both the bottom and lid, through which the seedling was inserted to allow the shoot system to grow into the air; 30 plates were established for each pine species.

Plants of *M. uniflora* were collected in mid-June 2002 from Little Tract, a regional Agreement Forest containing a mixture of hardwood and conifer species, located in Wellington County, Ontario. Plants with inflorescences were harvested and immediately transferred to the laboratory; plants had large root clusters consisting of numerous small mycorrhizal roots, and these were randomly sampled for microscopy and immunolabelling.

Fungal material and inoculation of seedlings

Isolates of *W. mikolae* var. *mikolae* (UAMH 6696) and *L. bicolor* (UAMH 8232) were obtained from the University of Alberta Microfungus Collection and Herbarium (UAMH), Edmonton, Alberta, and cultured on either full-strength (*L. bicolor*) or one-third-strength (*W. mikolae* var. *mikolae*) potato dextrose agar at room temperature in the dark. Plugs 8 mm in diameter were cut from the growing front of mycelium with a sterile cork borer, and two plugs were used as inoculum per seedling of *P. banksiana* at the time they were placed into the Petri plates. For each treatment, 10 seedlings were inoculated either with *W. mikolae* var. *mikolae* or *L. bicolor* or with plugs of potato dextrose agar as controls. After inoculation, seedlings were grown up to 6 months in a growth room on a 12 h light (75 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-2}$ photosynthetically active radiation; 24 °C) : 12 h dark cycle (20 °C). Root tips were collected at various times for microscopy from seedlings that had shoots approximately 12–15 cm in height and that had several long roots and numerous short mycorrhizal roots.

Figs. 1–6. Microtubule organization in *Pinus banksiana* – *Laccaria bicolor* ectomycorrhizas. Fig. 1. Differential interference contrast microscopy of cortical cells of a control (uncolonized) root showing prominent nuclei (N) and numerous spherical deposits (arrowheads), likely phenolics. Figs. 2–6. Confocal scanning laser microscopy. Fig. 2. Arrangement of cortical microtubules (MTs) in the same cells shown in Fig. 1. Cortical MTs form an extensive network and are arranged transversely in reference to the longitudinal axis of the root in this cell located close to the root apical meristem. Fig. 3. MTs (arrowheads) in Hartig net hyphae adjacent to root cortical cells (*). Image is one of nine optical sections. Fig. 4. MTs in cortical cells adjacent to Hartig net (HN) hyphae. MTs are associated with cortical cell nuclei (N) as well as being present in the peripheral cell cytoplasm (arrowheads). One of 30 optical sections. Fig. 5. MTs associated with a cortical cell nucleus (N) and within the cytoplasm (arrowheads). One of 34 optical sections. Fig. 6. Numerous cortical MTs (arrowheads) in the same cortical cell illustrated in Fig. 4. Combination of 30 optical sections viewed by subtracting the fluorescence of the fungal hyphae. Scale bars = 25 μm .

Light and transmission electron microscopy

Lateral roots of *M. uniflora* were excised and fixed in 4% paraformaldehyde – 1% glutaraldehyde in 0.1 mol·L⁻¹ HEPES buffer at room temperature overnight, followed by three rinses with buffer. Half of these samples were postfixed for 2 h in 1.0% osmium tetroxide for transmission electron microscopy. For light microscopy, samples were dehydrated in a graded ethanol series and embedded in LR White resin (CANEMCO, Lachine, Quebec). For transmission electron microscopy, samples were rinsed in buffer, dehydrated in a graded series of acetone, followed by propylene oxide, and embedded in Spurr's resin (Spurr 1969). Sections of tissue embedded in LR White resin and monitor sections of Spurr-embedded tissue were cut at 1.0–1.5 μm thickness and stained with 0.5% toluidine blue O in 1% sodium borate. Photomicrographs were taken with a Nikon Cool Pix digital camera interfaced with a Leitz Orthoplan microscope. Thin sections of Spurr-embedded tissue were stained for 10 min in uranyl acetate followed by 5 min in lead citrate (Venable and Coggeshall 1965). Transmission electron microscopy micrographs were taken with a Philips CM 10 electron microscope with an acceleration voltage of 80 kV.

Confocal scanning laser microscopy

Approximately 50 root tips collected from five or more seedlings of *P. banksiana* colonized by either *L. bicolor* or *W. mikolae* var. *mikolae* (roots that were monopodial but had an obvious mantle or roots that had recently dichotomized were selected) as well as controls were fixed in a mixture of freshly prepared 2% (m/v) paraformaldehyde and 2% (v/v) glutaraldehyde in 50 mmol·L⁻¹ PIPES buffer with 1.0 mmol·L⁻¹ MgSO₄·7H₂O and 2.0 mmol·L⁻¹ EDTA (PME pH 6.9) for 1 h. After rinsing three times for 5 min each with PME buffer, roots were hand-sectioned longitudinally under a Zeiss stereomicroscope and treated for two 5-min periods with 0.1% (m/v) NaBH₄ in Na-phosphate buffer with 150 mmol·L⁻¹ NaCl (PBS, pH 7.0) to eliminate background fluorescence due to aldehyde fixation. Following three rinses in PBS, sections were blocked for 10 min in 1% (m/v) bovine serum albumin in PBS, then incubated overnight at 4 °C with monoclonal mouse anti- β -tubulin (Sigma-Aldrich Canada Ltd., Oakville, Ontario) as the primary antibody, diluted 1:500 in the blocking solution. After rinsing in PBS, sections of *P. banksiana* roots were incubated for 3 h

at room temperature in Alexa Fluor 488 F (ab')₂-goat anti-mouse IgG(H + L) A-11017 (Molecular Probes, Inc., Eugene, Oregon) as the secondary antibody, diluted 1:50 in the blocking solution. For *M. uniflora* roots, sections were incubated in the same manner but with Alexa Fluor 555 F (ab')₂-goat anti-mouse IgG(H + L) A-21425 (Molecular Probes, Inc.) as the secondary antibody. Sections were then treated with wheat germ agglutinin bound either to Texas Red[®]-X conjugate (*P. banksiana*) or Oregon Green[®] 488 conjugate (*M. uniflora*) to localize fungal hyphae. Both wheat germ agglutinin conjugates were obtained from Molecular Probes, Inc. All observations were made with a Leica TCS SP2 confocal scanning laser microscopy interfaced with a Leica up-right DM RXE microscope for transmitted light bright field, differential interference contrast, and incident light fluorescence (Leica Microsystems (Canada) Inc., Richmond Hill, Ontario). Serial optical sections were taken at intervals varying from 0.41 to 0.69 μm , and either single optical sections were used for figures or the Z-series was compiled for figures to view cellular organization and the localization of MTs. Details for each figure are found in the figure legends. A Leica confocal system was used for handling and combining optical sections. Images for the plates in this manuscript were prepared using Adobe Photoshop.⁵

Results

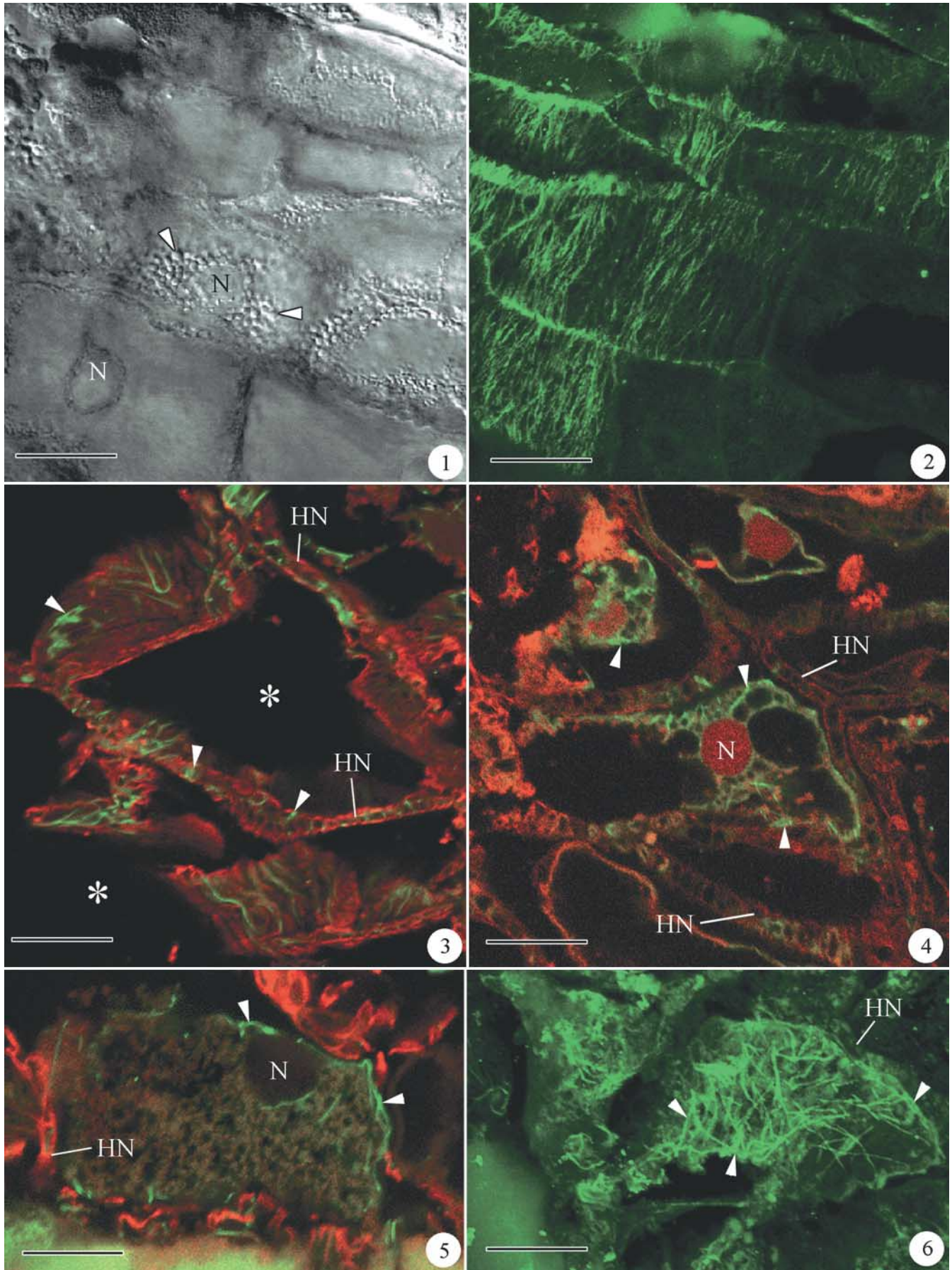
Controls

Cortical cells of uncolonized young lateral roots of *P. banksiana* had prominent nuclei and many spherical bodies, likely phenolic deposits (Fig. 1). Cortical MTs (the population of MTs located in the peripheral cytoplasm in these cells) were oriented transversely in relation to the long axis of the root (Fig. 2). This did vary somewhat depending on the position along the axis of the root. Older roots that showed considerable browning often showed few MTs (data not included).

Ectomycorrhizas

Pinus banksiana – *L. bicolor* ectomycorrhizas formed a multilayered mantle and a Hartig net around epidermal and cortical cells. The fungal hyphae constituting the Hartig net had MTs of various lengths and orientations (Fig. 3). MTs were also present in cortical cells adjacent to Hartig net hyphae (Figs. 4–6). These were often observed adjacent to

⁵Supplementary data in the form of animations of whole cells from which the optical section represented in Figs. 1, 3–11, and 14 have been taken are available on the *Canadian Journal of Botany* Web site or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, DUD 3592. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.



nuclei (Figs. 4, 5) as well as in the cytoplasm next to the cell wall, that is, cortical microtubules (Figs. 5, 6); these cortical MTs were less often observed compared with those associated with nuclei. In some cells, such as the one in Fig. 5, there was considerable background autofluorescence, which made it difficult to achieve clear images. Reconstruction of a series of images for MT labelling often showed host-cell MTs in more detail and indicated that the network of cortical MTs could be quite extensive in the region of the Hartig net (Fig. 6). Figure 6 is taken from the same cell illustrated in Fig. 4. Numerous cortical MTs were present within meristem cells and vascular parenchyma cells of mycorrhizal roots (data not shown).

Ectendomycorrhizas

Hand sections of *P. banksiana* – *W. mikolae* var. *mikolae* ectendomycorrhizas showed that short roots possessed a mantle, a Hartig net, and intracellular hyphae, normal features of this mycorrhiza category (data not shown). The alterations in host MTs were similar in some respects to those shown for ectomycorrhizas in that some cortical MTs remained in cells with intracellular hyphae (Figs. 7–9), but these had a different orientation compared with those in cortical cells of uncolonized roots (Fig. 2). MTs were also localized adjacent to cortical cell nuclei (Figs. 8, 9) and were closely associated with intracellular hyphae (Figs. 7–9). Hartig net hyphae and intracellular hyphae had MTs (Figs. 10, 11), and these could be distinguished easily from cortical cell MTs by examining reconstructions of series of images.

Monotropoid mycorrhizas

Field-collected roots of *M. uniflora* had well-developed mantles and a Hartig net confined to the epidermis (Fig. 12). Fungal pegs developed from inner mantle hyphae and penetrated the outer tangential wall of epidermal cells (Fig. 12). These were encased in host-derived wall material that was deposited as “finger-like” projections (Fig. 13). Epidermal cells with fungal pegs (Fig. 14) had MTs associated with these structures. MTs were also present in the cytoplasm surrounding the large vacuole in each epidermal cell (Fig. 14). Epidermal cells without obvious fungal pegs had an array of cortical MTs, as did adjacent cortical cells (Fig. 15). Optical sections through the cells shown in Fig. 15 did show that the MTs in the epidermal cell in the upper right were associated with a fungal peg.

Discussion

The plant-cell cytoskeleton can be altered by a number of abiotic and biotic factors, including invasion by pathogenic and symbiotic fungi (Hardham and Mitchell 1998; Peterson et al. 2000). This study extends the number of mycorrhizal categories that have been examined for the influence that fungal symbionts have on the MT component of the cytoskeleton in root cells, and the results strongly suggest that an association between host MTs and intracellular fungal structures may be a universal feature. In addition, it provides evidence that MTs are present in cortical cells adjacent to Hartig net hyphae in ectomycorrhizas.

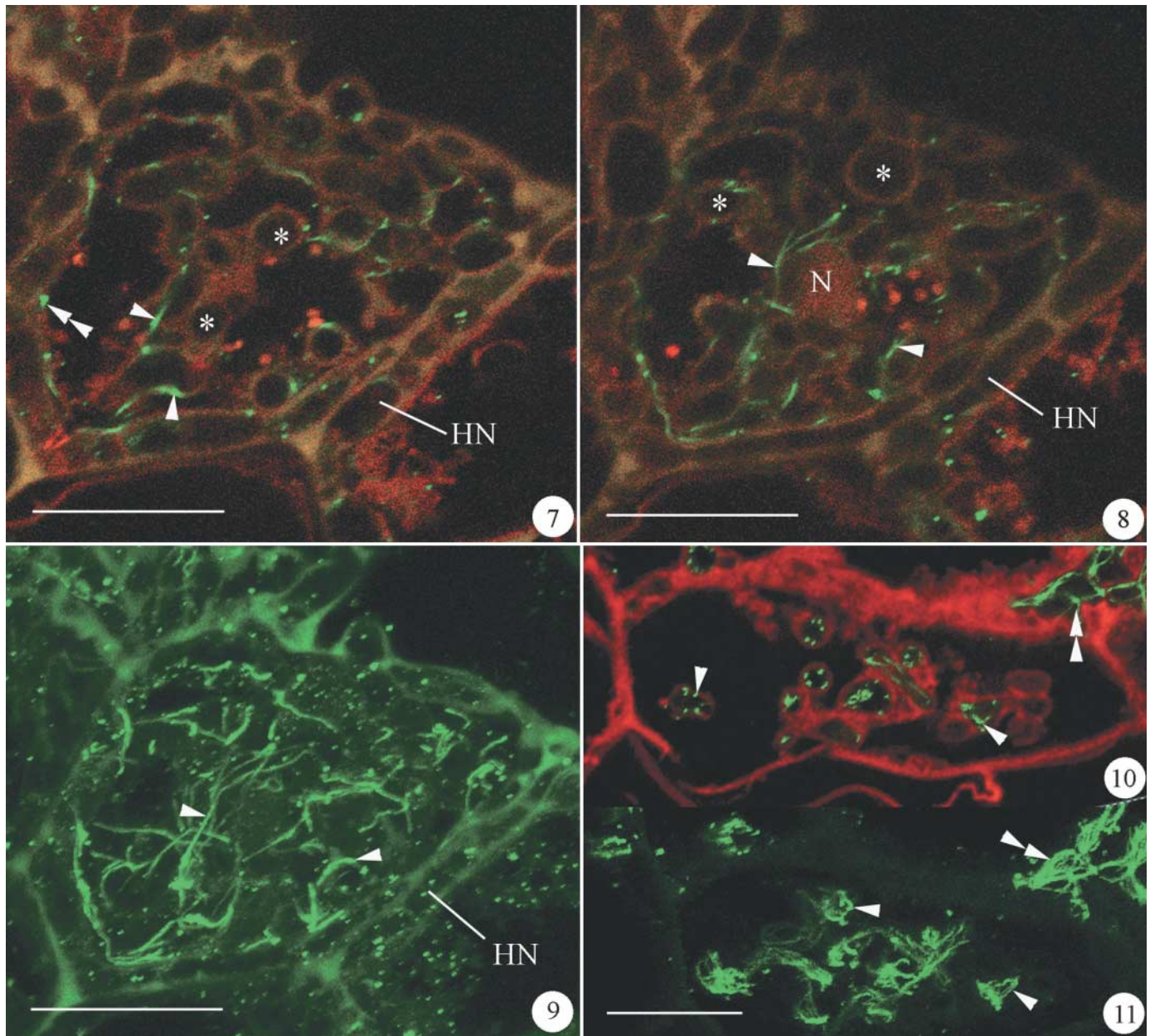
In an earlier study of *Pinus sylvestris* – *Suillus bovinus* ectomycorrhizas, MTs appeared to be absent in cortical cells adjacent to Hartig net hyphae (Timonen et al. 1993). Several reasons may account for this difference in results, including the combination of plant and fungal species used in the two studies. We found that of the three *Pinus* species used in our study, only *P. banksiana* gave clear images; root cells of this species contained fewer deposits of phenolics and other compounds than root cells of *P. strobus* and *P. resinosa*. Some of these compounds are known to emit autofluorescence and to interfere with immunolabelling (Timonen 1995). Also, in the present study, a range of ages of root tips, including root tips that had not undergone dichotomous branching, were prepared for immunolabelling. This is important in avoiding roots that may have senesced and therefore would likely have many dead epidermal and cortical cells. Another difference is that in the present study the labelled root and fungal tissues were examined by confocal scanning laser microscopy. This allowed for the collection of a Z-series of images for each sample and the reconstruction of three-dimensional images of MTs for entire cortical cells. In the study by Timonen et al. (1993), labelled roots were examined by epifluorescence microscopy; it is possible that MTs may have been present but not observed using this method.

The presence of MTs in Hartig net hyphae agrees with previous observations of *P. sylvestris* – *S. bovinus* ectomycorrhizas (Timonen et al. 1993; Timonen 1995; Timonen and Peterson 2002).

Since the nutrient exchange interface in ectomycorrhizas involves intercellular Hartig net hyphae as well as inner mantle hyphae located external to epidermal cell walls (Dexheimer and Pargney 1991), it is unlikely that host MTs are involved directly in nutrient exchange. Their presence, however, in cortical cells adjacent to Hartig net hyphae is evidence that these cells are alive and have a functioning plasma membrane with various associated transporters. The role of transporters in nutrient exchange in ectomycorrhizas is being explored, but further work is required in localizing these at the cellular level (Chalot et al. 2002). After this is accomplished, the relationship between MTs and modifications to the plasma membrane in root cells interfaced with Hartig net and mantle hyphae could be investigated.

The close association of MTs with intracellular hyphae in *P. banksiana* – *W. mikolae* var. *mikolae* ectendomycorrhizas is similar to what has been observed in orchid mycorrhizas that also have intracellular hyphal complexes (pelotons) in parenchyma cells (Uetake et al. 1997; Uetake and Peterson 1998). In both ectendomycorrhizas (Scales and Peterson 1991a) and orchid mycorrhizas (Peterson et al. 1996, 1998), the intracellular hyphae are surrounded by host-derived perifungal membrane and interfacial matrix material deposited between this membrane and the fungal wall. The nature of the interfacial matrix material in ectendomycorrhizas has not been investigated. In orchid mycorrhizas, various affinity methods showed that the matrix around intact pelotons differs from that around collapsed pelotons (Peterson et al. 1996). The interfacial matrix around collapsed pelotons consists of host-derived primary cell wall constituents, the precursors of which may be directed to this region by the cytoskeleton (Uetake et al. 1997). These authors also sug-

Figs. 7–11. *Pinus banksiana* – *Wilcoxina mikolae* var. *mikolae* ectendomycorrhizas. All images obtained by confocal scanning laser microscopy. Figs. 7 and 8 are single images of 31 optical sections collected. Fig. 7. Cortical cell surrounded by a Hartig net (HN) and with an intracellular hyphal complex (*). MTs (arrowheads) are mostly associated with the intracellular hyphal complex with a few remaining in the peripheral cytoplasm (double arrowheads). Fig. 8. Cortical cell associated with a Hartig net (HN) and with an intracellular hyphal complex (*). Microtubules (arrowheads) are associated with the cortical cell nucleus (N), the intracellular hyphal complex, with a few in the peripheral cytoplasm. Fig. 9. An array of MTs (arrowheads) in the same cortical cell as in Fig. 8. Combination of 31 optical sections viewed with subtracting fluorescence of fungal hyphae. Fig. 10. MTs within intracellular hyphae (arrowheads) and Hartig net hyphae (double arrowheads). Most MTs are located close to the fungal cell wall. Single optical section of 31 collected. Fig. 11. Combination of 31 optical sections of same cell as in Fig. 10 viewed with subtraction of fluorescence of fungal hyphae. MTs are evident in intracellular hyphae (arrowheads) and Hartig net hyphae (double arrowheads). Scale bars = 25 μ m.

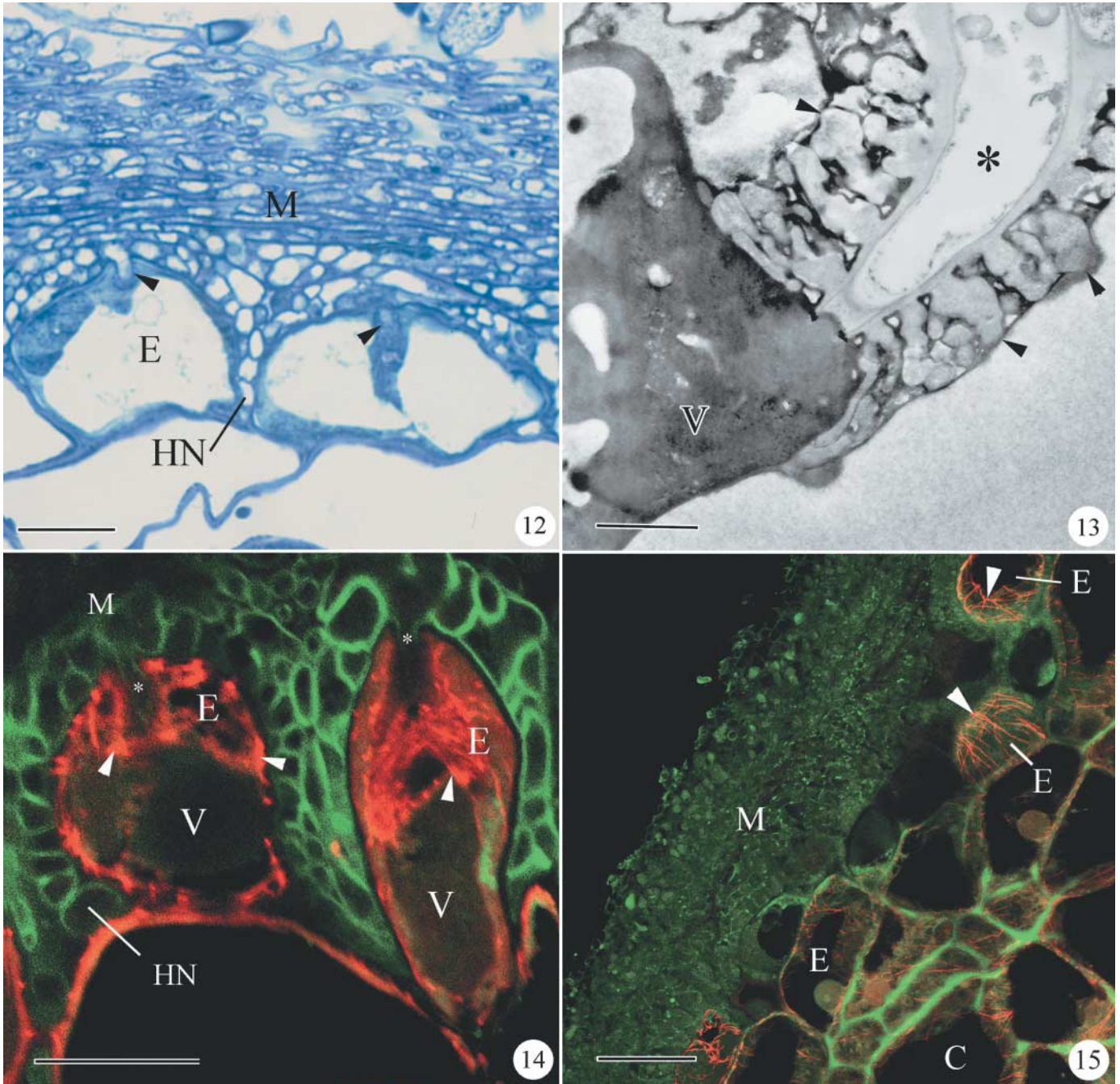


gested that the close association of MTs with intact pelotons may be related to the presence of MT-associated proteins in the perifungal membrane.

In *Arum*-type arbuscular mycorrhizas in which fine arbuscule branches are separated from cortical cell cytoplasm by the periarbuscular membrane and interfacial matrix, the latter consisting of primary cell wall components

(Bonfante and Perotto 1995), Genre and Bonfante (1997) suggested that MTs associated closely with this interface may be involved in the deposition of the interfacial matrix. A similar suggestion has been made for interface matrix deposition adjacent to hyphal coils and arbusculate coils in *Paris*-type arbuscular mycorrhizas (Armstrong and Peterson 2002). This role for MTs is possible in ectendomycorrhizas

Figs. 12–15. *Monotropia uniflora* mycorrhizas. Fig. 12. Portion of root embedded in LR White resin and viewed with bright field microscopy. A thick mantle (M), a narrow Hartig net (HN) confined to the epidermis (E), and fungal pegs (arrowheads) that have breached the tangential cell wall are evident. Scale bar = 25 μm . Fig. 13. Transmission electron microscopy. Fungal peg (*) surrounded by finger-like projections of host-derived cell wall material (arrowheads). A large vacuole (V) is filled with electron-dense material. Scale bar = 5.0 μm . Fig. 14. Epidermal cells showing the localization of MTs (arrowheads) around both fungal pegs (*) and the vacuole (V). Mantle (M) and Hartig net (HN) hyphae are evident. Single optical section of 21 collected. Scale bar = 25 μm . Fig. 15. A glancing section of root showing epidermal cells (E) with MTs (arrowheads). Hyphal pegs are not obvious in these cells, but optical sections taken at various intervals did show that MTs in the epidermal cell at the top right were associated with a fungal peg. Single optical section of 21 collected. C, cortical cell; M, mantle. Scale bar = 15 μm . Note: MTs appear red and fungal tissue appears green in Figs. 14 and 15 because of the selection of fluorescent conjugates used.



as well, although the nature of the interfacial matrix has not been determined.

The association of MTs with the hyphal pegs in *M. uniflora* mycorrhizas may also be related to the deposition of

the complex host-derived wall material that encases the hyphal peg in finger-like projections typical of transfer cells (Dexheimer and Gérard 1993). This wall material contains polysaccharides that are synthesized within dictyosomes

(Dexheimer and Gérard 1993); it is probable that the cytoskeleton plays a role in directing dictyosome vesicles to the site of wall deposition.

Results of this study, when considered together with previous publications, suggest that there is a consistent association between MTs and intracellular hyphae in those mycorrhiza categories in which the cell wall is breached but hyphae are separated from the plant cell cytoplasm by a perifungal membrane and interfacial matrix material. Also, in all published reports, fungal hyphae trigger changes in cortical MTs in that they become fewer in number and appear to be shorter. The close association between host MTs and fungal hyphae occurs regardless of the nutritional relationship between the symbionts. For example, in developing protocorms and seedlings in orchids, and in monotropoid mycorrhizas, the host receives carbon compounds via fungal hyphae, whereas in arbuscular mycorrhizas and ectendomycorrhizas, bidirectional transport occurs, with the host receiving inorganic nutrients via the fungus in exchange for carbon compounds taken up by the fungus (Smith and Read 1997). Regardless of the direction of nutrient flow, the perifungal membrane is an integral part of the exchange interface (Ferrol et al. 2002) and therefore understanding the characteristics of this membrane is essential in understanding nutrient exchange processes. It is possible that the cytoskeleton is involved in configuring this membrane (Harrison 1999) and establishing transporter sites within it.

Host MTs and AFs may play roles in addition to the deposition of host-derived wall components and the establishment of perifungal membranes in mycorrhizal systems characterized by the development of intracellular hyphae. Attention needs to be directed towards the relationship between cytoskeletal structures and nuclear migration and alterations in other cellular organelles, such as mitochondria and endoplasmic reticulum. In *Arum*-type AM, the nucleus shifts from a peripheral position to a more central position in cortical cells containing arbuscules (Balestrini et al. 1992). A similar situation occurs in *Paris*-type AM as hyphal coils and arbusculate coils develop within cortical cells (Cavagnaro et al. 2001) and in ectendomycorrhizas in which intracellular hyphae often envelop the nucleus (Piché et al. 1986). In orchid mycorrhizas, the nucleus also shifts its position in protocorm cells and becomes closely associated with the developing fungal peloton (Uetake et al. 1997).

Alterations in the position of endoplasmic reticulum and mitochondria in orchid protocorm cells colonized by symbiotic fungi and the association of these organelles with AFs has been shown (Uetake and Peterson 2000), but there is considerable scope for further work with this and other mycorrhizal systems.

The present study has only considered the effect of fungal colonization on one component of the cytoskeleton, MTs, in ectendomycorrhizas, ectendomycorrhizas, and monotropoid mycorrhizas. Future work could determine changes in AFs and the relationship between both components of the cytoskeleton and changes in the overall cytology of root cells during mycorrhiza establishment.

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