Mycorrhiza (1993) 2:153-156

Mycorrhiza

Vesicular endophytes in roots of the Pinaceae

E. Cázares[1]*, **J. M. Trappe**[1,2]

[1] Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA
[2] Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

Abstract. Vesicles and hyphae typical of vesicular-arbuscular mycorrhizae (VAM) were common in seedlings of *Pseudotsuga menziesii*, *Abies lasiocarpa* and *Tsuga mertensiana* growing in openings where herbaceous hosts of these fungi were common. Seedlings of *A. lasiocarpa*, *Tsuga heterophylla*, and *T. mertensiana* growing under closed forest canopies also had vesicles but at a much lower incidence than seedlings in the openings. The Pinaceae are generally assumed to be ectomycorrhizal, but *Glomus*-type colonizations occurred on the same seedlings as the ectomycorrhizae. The ecological significance of abundant VAM-type endophytes in otherwise ectomycorrhizal hosts deserves comprehensive study.

Key words: Vesicular-arbuscular mycorrhizae - *Abies lasiocarpa* - *Pseudotsuga menziesii* - *Tsuga heterophylla* - *Tsuga mertensiana*

Introduction

Vesicular-arbuscular mycorrhizae (VAM) are characteristic of most herbaceous plant species (Harley and Harley 1987; Trappe 1987). In the Coniferophyta, VAM characteristically form on all families except the Pinaceae, which usually have been assumed to have ectomycorrhizae (EM) (Harley and Smith 1983). However, "endotrophic mycorrhizae" on Pinaceae have been occasionally reported, for example on *Pinus monophylla* Torr. and Frém. and *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco (as *Pseudotsuga mucronata* Sudw.) by McDougall and Jacobs (1927). Their drawing of the *Pseudotsuga* mycorrhiza, though not detailed, shows hyphal coils similar to those common in VAM of the Cupressaceae. Henry (1933, 1934), Asai (1934), Dominik (1951) and Shvartsman (1955) recorded "endotrophic mycorrhizae" on various species of Pinaceae, but they neither differentiated between VAM and other intracellular forms such as ericoid or ectendomycorrhizae nor illustrated the structures they observed. Consequently, their reports cannot be evaluated.

The first unequivocal record of VAM-type endophytes in the Pinaceae was by Golubinskaya (1967), who presented a convincing drawing of *Glomus* type hyphae and vesicles in rootlets of *Picea obovata* Led. She also found typical VAM vesicles in *Larix sibirica* (DuRoi) Koch, *Pinus sibirica* DuTour and *Pinus sylvestris* L. Similar hyphae were observed in *Abies sibirica* Led., but no vesicles were observed. Dowgiallo and Rambelli (1972) subsequently described vesicles on *Pinus halepensis* Mill., and Malloch and Malloch (1981) reported them in rootlets of *Abies balsamea* (L.) Mill. and *Larix laricina* (DuRoi) Koch.

Mycorrhiza researchers, ourselves included, either ignored these findings or dismissed them as atavistic curiosities with little relevance to the biology of the Pinaceae. Recently, however, Douglas fir [*P. menziesii* (Mirb.) Franco var. *menziesii*] and western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] were discovered to form VAM in a soil bioassay study (Cázares and Smith 1991). This led us to examine field-grown members of these species for VAM to determine whether or not the phenomenon was common in natural habitats. Subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.] and mountain hemlock [*Tsuga mertensiana* (Bong.) Carr.] were also examined because of our studies on mycorrhizal ecology of those species. These studies had no financial support and consequently were pursued as opportunity permitted. We present them here not because we have definitive answers but rather to call this common but overlooked phenomenon to the attention of colleagues. Its meaning to the ecology of fungus or host deserves research.

---

* *Present address:* Instituto Tecnológico de Cd. Victoria, Apartado Postal 175, Cd. Victoria, Tamaulipas, México 87010

Correspondence to: J. M. Trappe, Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA

Materials and methods

Study area

Douglas fir seedlings were collected along the side of the Higley Peak road in a clearcut (lat. 47°31′ N, long. 123°54′ W, elevation ca. 1000 m), Jefferson County, Washington. Western hemlock seedlings were collected near Lake Quinault (lat. 47°30′ N, long. 123°58′ W, elevation ca. 500 m), Grays Harbor County, Washington in an old growth stand. Both sites are in the *T. heterophylla* zone (Franklin and Dyrness 1973). Annual precipitation at these sites averages over 3000 mm, nearly all in rainfall distributed throughout the year. Subalpine fir and mountain hemlock seedlings were collected near Lyman Lake (lat. 48°12′ N, long. 120°551′ W, elevation 1708 m), Glacier Peak Wilderness Area, Wenatchee National Forest, Chelan County, Washington. The vegetation is typical for the mountain hemlock-subalpine fir forests of the *T. mertensiana* zone (Franklin and Dyrness 1973). Annual precipitation averages 2750 mm, mostly as snow which accumulates from November through April (Mann and Dull 1979).

Sampling

All seedlings were sampled as opportunity permitted in the course of other field work and were 2–4 years old. The eight Douglas fir were from a gravelly road bank among grasses and forbs; Douglas fir seedlings do not usually occur under a closed forest canopy. The 15 western hemlock were from under a closed forest canopy among understory shrubs and forbs, the usual habitat for seedlings of this species. Twenty-five subalpine fir and two mountain hemlock were from openings among grasses and forbs along the edge of a trail. Thirteen subalpine fir and ten mountain hemlock were from under a closed forest canopy with little ground vegetation.

Clearing and staining

Entire root systems were washed in running tap water, cut in pieces to fit in Tissue-Tek plastic capsules (Fisher Scientific Co., Pittsburgh, Pa.), cleared in a 15% H_2O_2 solution for 10 min, rinsed with tap water, and autoclaved for 3 min at 121° C in a 10% KOH solution. The KOH solution was decanted and root samples were rinsed with tap water, steamed for 30 min in a 10% KOH solution, rinsed again with tap water, placed in 1% HCL for 30 min and again rinsed with tap water. Cleared samples were steamed for 30 min in a staining solution of 0.5% trypan blue in lactoglycerol, rinsed with tap water and stored in lactoglycerol or water at 4° C until microscopic examination.

Assessing vesicular endophytic colonization

Glomus-type colonization was evaluated by stereo- and compound microscopy using the following categories: +, presence of more than ten vesicles per root system; ±, presence of less than ten vesicles per root system; −, complete absence of vesicles. In many cases, *Glomus*-type hyphae were apparently present but vesicles were absent, and to avoid overestimating these endophytes we chose vesicles as the criterion. No arbuscules were seen, although Cázares and Smith (1991) reported them from Pinaceae. Arbuscules are infrequent in the Cupressaceae and other gymnosperms even when forming mycorrhizae with VAM fungi that otherwise produce them in abundance in herbaceous hosts (Trappe, unpublished data).

Results

For seedlings grown in openings, ten or more *Glomus*-type vesicles were observed in 15 of the 25 subalpine firs, three of the eight Douglas firs, and both mountain hemlocks (Table 1). Western hemlock were not collected from openings. For seedlings growing under the closed forest canopy, ten or more vesicles were observed in one of the 13 subalpine firs, one of the 15 western hemlocks (Fig. 1), and none of the ten mountain hemlocks. No Douglas fir seedlings were collected from under a closed canopy forest (Table 1). Both long and short roots were colonized.

Table 1. Percentage of Pinaceae seedlings with *Glomus*-type vesicles in their root systems - open sites versus closed-canopy forests. +, Presence of more than 10 vesicles per root sample; ±, presence of less than 10 vesicles per root sample; −, complete absence of vesicles in the root sample

	Vesicle status (%)[a]					
	Open site			Closed canopy		
Taxa	(+)	(±)	(−)	(+)	(±)	(−)
Abies lasiocarpa	60	16	24	8	8	84
Pseudotsuga menziesii	37	50	12	—	—	—
Tsuga heterophylla	—	—	—	6	6	87
Tsuga mertensiana	100	—	—	0	20	80

[a] Percentage of seedlings of each species in each canopy type

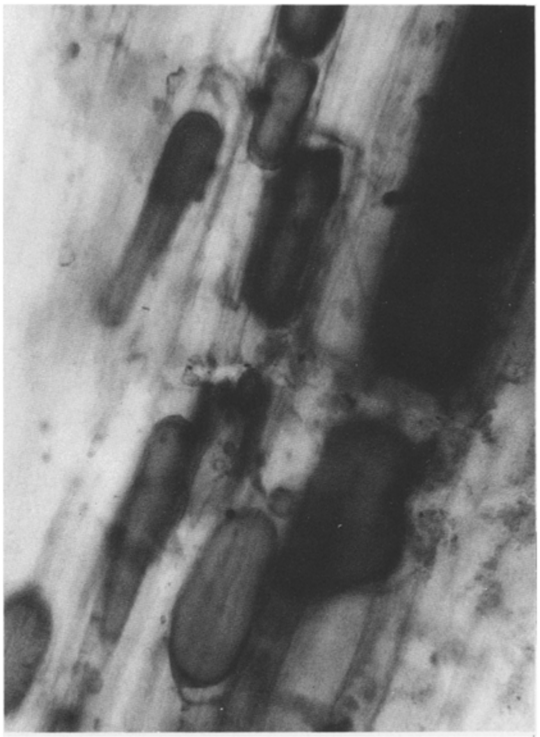

Fig. 1. Vesicles in a rootlet of a *Tsuga heterophylla* seedling growing under a closed-canopy forest (cleared and stained in trypan blue). ×250

Glomus-type endophytes were more common in seedlings from openings than from under the closed forest canopy (Table 1). In the openings, some vesicles were present in most of the subalpine firs and Douglas firs and both of the mountain hemlocks. In the closed forest canopy, vesicles were present in only a small proportion of the subalpine firs, western hemlocks and mountain hemlocks.

EM were always present on the seedlings examined, and dark-walled septate endophytes were commonly present in roots of the subalpine fir and mountain hemlock at the Lyman Lake site. Vesicles occasionally occurred within EM. Seedlings from under a closed forest canopy were more heavily colonized by *Cenococcum geophilum* Fr. than those from openings.

Differences in vegetation and soil characteristics were observed between openings and closed forest canopy. The openings were located along a trail in loose, exposed soil (Lyman Lake) or along a gravelly road bank, mainly colonized by forbs and grasses (Olympic Penninsula). The understory vegetation under the closed forest canopies in both localities was sparse and growing in a soil with well-developed humus among considerable, coarse, woody debris.

Discussion

VAM inoculum potential was not determined for the sites sampled, but we hypothesize that *Glomus*-type colonization of Pinaceae relates to the presence of higher levels of VAM fungal propagules in the openings than under the canopy. An additional influence might be the presence of typical VAM plants serving as inoculum companions to the Pinaceae in the openings, as reported for other associations by Hirrell et al. (1978). Whatever the causal factors, our data suggest that the proportion of species of Pinaceae with *Glomus*-type endophytes plus EM is much higher than the 2% indicated by Newman and Reddell (1978).

Succession from VAM to EM in the same root system has been described for *Helianthemum* (Read et al. 1977), *Eucalyptus* (Lapeyrie and Chilvers 1985; Chilvers et al. 1987) and *Alnus* (Lin et al. 1987). Malajczuk et al. (1981) demonstrated that eucalypts, usually strongly ectomycorrhizal, would readily form VAM when inoculated with suitable fungi and hypothesized that this ability could be important in early succession on disturbed soil.

EM occasionally occur in gymnosperms that are usually VAM, e.g. *Chamaecyparis lawsoniana* (Murr.) Parl. (Levisohn 1954), *Cryptomeria japonica* (L.) D. Don (Sharma and Mishra 1982), *Cupressus cashmeriana* Roy. ex Carr. (Bakshi 1974), *Juniperus communis* L. (Lihnell 1939), *Sequoiadendron giganteum* (Lindl.) Buchh. (Eropkin 1970) and *Taxodium distichum* (L.) Rich. (Eropkin 1970). It appears that a modest receptivity to ectomycorrhizal fungi has developed among many lines of the gymnosperms. Most likely the Pinaceae are derived from VAM ancestors and have evolved a strong receptivity to ectomycorrhizal endophytes without developing resistance to VAM endophytes.

We have no data on the effects of *Glomus*-type endophytes in the Pinaceae. Our intent was to better evaluate the degree to which it occurs in nature. More studies on the occurrence of this phenomenon among the members of the Pinaceae in their natural habitats are needed. VAM are important in revegetation of disturbed habitats (Allen et al. 1987). Once VAM hosts become established, they are a main source of inoculum for new host plants (Read et al. 1987). The ecological importance of *Glomus*-type endophytes in survival and growth of Pinaceae in open habitats with high VAM inoculum potential (such as clearcuts or road banks), as well as in forests where the overstory species are EM hosts, remains to be determined. It is clear to us at this stage, however, that study of the role of mycorrhizae in establishment of Pinaceae in clearcuts, burns, and other disturbed habitats is incomplete if only EM are considered. Staining root systems for VAM determination should be routine for such work.

Acknowledgements. These studies were supported by National Science Foundation Grant BSR-8717427, the U.S. Forest Service, Pacific Northwest Research Station and the Consejo Nacional de Ciencia y Tecnologia of México. Personnel of the U.S. Forest Service, Wenatchee National Forest, Chelan Ranger District expedited these studies in many helpful ways.

References

Allen EB, Chambers JC, Connor KF, Allen MF, Brown RW (1987) Natural reestablishment of mycorrhizae in disturbed alpine ecosystems. Arct Alp Res 19:11–20

Asai T (1934) Über das Vorkommen und die Bedeutung der Wurzelpilze in den Landpflanzen. Jpn J Bot 7:107–150

Bakshi BK (1974) Mycorrhiza and its role in forestry. Forest Research Institute and Colleges, Dehra Dun

Cázares E, Smith JE (1991) Occurrence of vesicular-arbuscular mycorrhizae in Douglas-fir and western hemlock seedlings (abstract). Third European Symposium on Mycorrhizas, Sheffield 1991. University of Sheffield, Sheffield, p 97

Chilvers GA, Lapeyrie FF, Horan DP (1987) Ectomycorrhizal vs endomycorrhizal fungi within the same root system. New Phytol 107:441–448

Dominik T (1951) Research on mycotrophy of plant associations of coastal and inland dunes (in Polish). Acta Soc Bot Pol 21:125–164

Dowgiallo MG, Rambelli A (1972) Ricerche sullo stato micorrizico de alcune popolazioni a pino d'Aleppo dell'Italia centrale. Ann Bot (Rome) 31:19–31

Eropkin KI (1970) Mycorrhizae of gymnosperms. Communication II. Family Taxodiaceae (in Russian). Uch Zap Permsk Gos Pedagog Inst 80:50–56

Franklin JF, Dyrness CT (1973) Natural vegetation of Oregon and Washington. USDA For Serv Gen Tech Rep PNW-8

Golubinskaya NS (1967) Endotrophic mycorrhizae of trees and shrubs in western Siberia (in Russian). Mikroorg Rast 1967:13–22

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British flora. New Phytol 105 [Suppl]:1–102

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic Press, London

Henry LK (1933) Mycorrhizas of trees and shrubs. Bot Gaz 94:791–800

Henry LK (1934) Mycorrhizae of Pymatuning swamp. Proc Pa Acad Sci 8:9–16

Hirrell MC, Mehravaran H, Gerdemann JW (1978) Vesicular-arbuscular mycorrhiza in Chenopodiaceae and Cruciferae, do they occur? Can J Bot 56:2813–2817

Lapeyrie FF, Chilvers GA (1985) An endomycorrhiza-ectomycorrhiza succession associated with enhanced growth by *Eucalyptus dumosa* seedlings planted in a calcareous soil. New Phytol 100:93–104

Levisohn I (1954) Occurrence of ectotrophic and endotrophic mycorrhizas in forest trees. Forestry 27:145–146

Lihnell D (1939) Untersuchungen über die Mykorrhizen und die Wurzelpilze von *Juniperus communis*. Symb Bot Ups 3:1–141

Lin LP, Cheng CH, Song SC, Su H, Li CY (1987) Presence of both vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae on *Alnus formosana* (abstract). Chin J Microbiol (reprint)

Malajczuk N, Linderman RG, Kough J, Trappe JM (1981) Presence of vesicular-arbuscular mycorrhizae in *Eucalyptus* spp. and *Acacia* sp., and their absence in *Banksia* sp. after inoculation with *Glomus fasciculatum*. New Phytol 87:567–572

Malloch D, Malloch B (1981) The mycorrhizal status of boreal plants: species from northeastern Ontario. Can J Bot 59:2167–2172

Mann DL, Dull K (1979) Six years of site restoration at Lyman lake. In: Ittner R, et al (eds) Recreational impact on wildlands. USDA Forest Service, USDI National Park Service, Seattle, pp 276–279

McDougall WB, Jacobs MC (1927) Tree mycorrhizas from the central Rocky Mountain Region. Am J Bot 14:258–265

Newman EI, Reddell P (1987) The distribution of mycorrhizas among families of vascular plants. New Phytol 106:745–751

Phillips JM, Hayman DS (1970) Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. New Phytol 77:641–653

Read DJ, Kianmehr H, Malibari A (1977) The biology of mycorrhiza in *Helianthemum* Mill. New Phytol 78:305–312

Sharma GD, Mishra RR (1982) Mycorrhizal association in gymnosperms of Meghalaya (India). Acta Bot Ind 10:43–49

Shvartsman SR (1955) Mycorrhizas of wild and cultivated herbaceous and woody plants of the Bol'shie Barsuki sands. In: Imshenetskii AA (ed) USDA-NSF transl TT 6751290, 1967. Tr Konf Mikotrofii Rastenii, Moskva, pp 232–237

Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR (ed) Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, Fla, pp 2–25