Journal of Tropical Ecology (1997) 13:623–629.

SHORT COMMUNICATION

Vesicular-arbuscular mycorrhizae in two tropical monodominant trees

SYLVIA D. TORTI*, P. D. COLEY* *and* DAVID P. JANOS†

**Department of Biology, University of Utah, Salt Lake City, UT 84112, USA.*
†Department of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124, USA.

KEY WORDS: ectomycorrhizae, flooding, monodominance, neotropics, vesicular-arbuscular mycorrhizae.

Mycorrhizae, symbioses between plant roots and fungi, are found in virtually all terrestrial plant ecosystems. Substantial evidence indicates that mycorrhizal associations are beneficial to both partners. In most systems, the fungus benefits the host plant by effectively scavenging nutrients and in return, utilizes carbohydrates produced by the plant (Allen 1991, Harley & Smith 1983). Moreover, both major types of mycorrhizae, vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae (EM), can alter the outcome of competitive interactions between host plants (Allen & Allen 1984, Hartnett *et al.* 1993, Hetrick *et al.* 1989, Newman *et al.* 1992, Perry *et al.* 1989).

Early studies focused primarily on temperate mycorrhizae, but more recently, ecologists have turned toward the tropics. In contrast to the temperate zone, where trees tend to be EM, the majority of tropical tree species surveyed thus far form VAM (Janos 1983). Some notable exceptions of tropical trees forming EM occur in the plant families Dipterocarpaceae, Caesalpiniaceae, Euphorbiaceae, Fagaceae and Myrtaceae. Although tropical forests are typically diverse, many tree species in these five families grow in persistent, monodominant stands which can extend for hundreds of hectares (Connell & Lowman 1989). Janos (1983, 1985) has suggested that under low nutrient or seasonal conditions, EM may be more efficient than VAM at scavenging nutrients. Furthermore, EM may be able to absorb organic nitrogen (Alexander 1983), decompose organic litter (Trojanowski *et al.* 1984) and/or protect the host's roots from herbivory or pathogen attack (Marx 1972). Hence, the ability of some tropical trees to associate with EM has been hypothesized to give them a competitive advantage by which they can achieve local or regional dominance over species with VAM (Connell & Lowman 1989, Gartlan *et al.* 1986; Janos 1983, 1985). Corroboration of this EM hypothesis for monodominance depends upon tropical monodominant species forming EM and the subsequent demonstration that EM provide a competitive advantage to the host.

We ask whether EM are critical to monodominance by surveying the roots of two tropical, Caesalpiniaceae monodominant species for mycorrhizae. One species, *Mora excelsa* Benth. (Caesalpiniaceae), forms extensive monodominant forests on well-drained soils in Trinidad and Guyana (Rankin 1978, ter Steege 1994). Johnston (1949) reported *M. excelsa* as forming VAM, but did not comment on whether it also formed EM. The dominance of *M. excelsa* is curious because large stands occur on similar soils and topography as those of the immediately adjacent mixed-species forest, suggesting edaphic factors are not sufficient to explain its dominance (Rankin 1978). The second species, *Prioria copaifera* Griseb. (Caesalpiniaceae), grows in single-species stands in lowland swamps and also in mixed-species forests on well-drained soils (Condit *et al.* 1993). In an extension of the EM hypothesis for monodominance, we ask whether *P. copaifera*, a swamp monodominant species, is EM, VAM or non-mycorrhizal, and if there are patterns of mycorrhizal infection across varying conditions of flooding and light. We anticipate that *M. excelsa* will at least form VAM, as Johnston reported, and because it is a monodominant, caesapinoid tree, it may form EM as well. Similarly, we expect that like most vascular plants, *P. copaifera* will form mycorrhizal associations. Moreover, we predict that the degree to which *P. copaifera* plants are mycorrhizal will be negatively correlated with the degree of flooding and positively correlated with light availability. Mycorrhizal fungi are aerobic, hence, flooding should decrease the amount of oxygen available to the fungus and might limit its growth (Khan & Belik 1995). Trees in light gaps are predicted to have higher levels of infection because they photosynthesize more than shaded plants and presumably would have more carbohydrates available to the fungus (Smith & Smith 1996, Whitbeck 1994).

M. excelsa roots were collected during the rainy season, July 1994, from the Mayaro Reserve in southeastern Trinidad, West Indies. The forest is dominated by *M. excelsa*, a tall canopy tree with a dense crown which inhibits light transmission to the understorey (Rankin 1978). In one monodominant stand, root samples from ten saplings (1–1.5 m height) were taken from the shaded understorey by carefully removing the soil around the saplings and uprooting them with minimal force so as not to break the roots. At a second, similar site, roots from six shaded trees (10–15 m height) were harvested by following roots to their distal ends and sampling a subset of the fine roots. Finally, roots from two *M. excelsa* saplings were collected in the mixed-species forest from below an adult *M. excelsa* tree. Terminal roots were clipped off, rinsed gently in water and stored in vials with FAA (90% formalin, 5% acetic acid, 5% ethanol) until analysis.

We collected *P. copaifera* roots from five sites in the Darien, Panama, during

Table 1. Summary of *P. copaifera* sites, number of samples and % (untransformed means and standard errors) of each root sample infected with vesicular-arbuscular mycorrhizae

Site (forest type)	Flooding	Light	No. of trees	% of sample infected (± SE)
Canglon (monodominant)	Lowland, flooded (0.7 m) July 1994	Understorey	18	73 ± 3
Sambu-high (mixed-species)	Upland, never flooded	Understorey	12	52 ± 10
Sambu-low (monodominant)	Lowland, occasionally flooded, not in 7/94	Understorey	7	42 ± 9
El Real (monodominant)	Lowland, seasonally flooded, not in 7/94	Light gap formed by logging	10	26 ± 3
Jesus (monodominant)	Lowland, occasionally flooded, not in 7/94	Light gap formed by treefall	5	44 ± 9

the rainy season, June 1994 (Table 1). Four of the five sites are monodominant, *P. copaifera* forests that flood seasonally, but only one of these four sites, Canglon, was inundated with water (to 0.7 m depth) when we sampled. The fifth site (Sambu-high) is an upland mixed-species forest (including *P. copaifera*) that does not flood. Within 50 m of this site is Sambu-low, a *P. copaifera* stand that floods periodically. Samples from the two remaining sites, Jesus and El Real, were collected in light gaps. The Jesus gap was created by a natural treefall and the El Real gap was the result of logging. We harvested a total of 52 *P. copaifera* root samples from saplings (1–1.5 m height) and small trees (10–12 m height). Roots were harvested, rinsed and stored as described for *M. excelsa*.

We initially inspected *M. excelsa* and *P. copaifera* roots for evidence of EM using a dissecting microscope. We found no evidence of a hyphal mantle, but some *P. copaifera* roots did have dark, stubby tips, often indicative of an EM mantle. We selected root tips of varying gross morphologies from both species and embedded them in an acrylic resin (LR White, medium grade, no. 14381 Electron Microscopy Sciences). We made cross sections varying from 3-5 microns on a microtome and then stained the sections (Wilcox 1982). Using a compound scope, we analyzed the cross-sections for a Hartig net, the characteristic structure of EM.

We then cleared and stained the remaining roots for VAM using a modification of the methods described by Kormanik & McGraw (1982). Roots were rinsed in distilled water to remove the fixative and cleared in 10% KOH at 90 °C for $1\frac{1}{2}$ to 2 h. The prolonged KOH treatment was necessary because the roots were darkly pigmented. After the KOH treatment, roots were bleached with alkaline H_2O_2 for 15–20 min and then placed in 1% HCl for 2 min. Roots were stained at room temperature for 10–15 h with trypan blue and then destained in lactoglycerol.

We estimated the level of infection (% of sample infected) for *P. copaifera* by systematically scanning a microscope slide and scoring the presence or absence of mycorrhizal infection at each intersection of root and reticle line (100–200 intersections/slide). An intersection was considered mycorrhizal if the reticle line intersected a hypha, an intracellular coil and/or a vesicle. Because root samples from *M. excelsa* were collected from two similar sites, we made no attempt to correlate the extent of infection with any environmental character.

No evidence of EM was found in either *M. excelsa* or *P. copaifera*, however, both species did form VAM. The finding that *M. excelsa* did not form EM cannot be taken to mean that across its distribution, *M. excelsa never* forms EM. Sixteen of seventeen trees sampled in the *M. excelsa* forests had typical *Arum*-type VAM structures and both saplings collected from the mixed-species forest also had VAM.

All root samples of *P. copaifera* were VAM (Table 1). Infection of the *P. copaifera* roots comprised predominantly hyphae, in the form of runner hyphae and intracellular coils. Vesicles, both inter- and intracellular, were common in all samples, but only one sample had a structure which we thought to be an arbuscule.

We found significant differences in the level of VAM for *P. copaifera* inidividuals across sites (percentages in Table 1 were arcsin transformed before analysis, one-way ANOVA; $F = 7.91$; $df = 4, 47$; $P = 0.0001$). Trees in the inundated site, Canglon, had significantly higher levels of VAM than the other four sites ($F = 22.77$; $df = 1, 47$; $P < 0.0001$). Plants in the three shaded sites experienced significantly greater VAM than did plants in the two gap sites, 63.1 versus 36.0% respectively ($F = 8.77$; $df = 1, 47$; $P = 0.005$).

The absence of EM on *M. excelsa* and *P. copaifera* roots suggests that EM are not critical to the dominance of these two species. This lack of EM is in contrast to other monodominant members of this family which are EM (Alexander 1989, Högberg & Piearce 1986, Newbery *et al.* 1988).

The VAM found in *P. copaifera* is characterized by the presence of vesicles and intracellular coils and an absence of arbuscules. This type of VAM (with coils and no arbuscules), called *Paris*-type VAM, has received less attention compared to the well-studied *Arum*-type VAM in which arbuscules are formed (Smith & Smith 1996). VAM with coils rather than arbuscules have been reported for other aquatic plants as well (Khan & Belik 1995) and recent evidence suggests that *Paris*-type VAM may be more widespread than previously thought (Smith & Smith 1996). Moreover, the type of VAM formed in a root is likely to be controlled genetically by the host plant (Smith & Smith 1996). We do not yet know what role the coils play in the fungal- plant symbiosis; they may perform the same putative, nutrient transfer function as arbuscules (see Smith & Smith 1996 for a review).

The finding that both well-drained and inundated *P. copaifera* trees form VAM is unprecedented, as no previous studies have investigated the mycorrhizal status of a tropical tree under varying conditions of flooding. Because fungi are aerobic, mycologists initially expected inundated plants to be devoid of mycorrhizae (Khan & Belik 1995), yet many temperate aquatic, swamp and marsh plants do form mycorrhizae (Keeley 1980, Khan & Belik 1995, Mejstrík

1965, Sondergaard & Laegaard 1977). However, there have been virtually no studies of tropical, aquatic trees (Khan & Belik 1995). Extrapolating from temperate studies, it appears that, in some cases, continually inundated roots are able to maintain mycorrhizae (Keeley 1980, Lodge 1989, Sondergaard & Laegaard 1977), while in others, seasonal flooding is correlated with a reduction in mycorrhizal infection (Coutts & Nicoll 1990, Mejstrík 1965). This variation is probably related either to differing abilities of plants to provide the fungus with sufficient oxygen, or the ability of the fungus to tolerate low-oxygen conditions (Khan & Belik 1995).

In the few studies of tropical trees and mycorrhizae, light has been reported to have both positive and negative effects on mycorrhizae. Working with EM in logged dipterocarp forests in Indonesia, Smits (1983) suggested that EM infection is lowest in logged sites because increased sunlight heats the soil to temperatures incompatible with EM fungal growth. Contrary to this, neotropical studies on VAM have found greater levels of infection in saplings growing in light gaps than those in the shade, presumably because carbohydrates of host plants are more abundant and therefore more available to the fungus (Whitbeck 1994). This disparity could be related to differences in the abilities of EM and VAM fungi to tolerate high temperatures. Presently, there is no evidence that high temperatures negatively affect VAM fungi (Janos 1996). Hence, our results of lower infection levels for the gap sites are not consistent with previous studies of VAM.

Alternatively, the low levels of VAM observed at the El Real and Jesus sites and the high level of VAM at Canglon might be explained by the onset of the rainy season and the subsequent inundation of the Canglon site. If, as Mejstrík (1965) found for temperate swamps, the infection of fungi builds up during the dry season, then roots ought to show their greatest levels of infection at the end of the dry season. The roots in this study were harvested 1 mo after the beginning of the rainy season. Therefore, we submit that the observed pattern of infection might reflect differences in the root growth of *P. copaifera* at sites with varying degrees of inundation. Possibly, at the high light sites, El Real and Jesus, root growth was stimulated with the onset of the rains and hence the roots quickly out-grew their VA fungi. Following this logic, the rapid and heavy inundation at the Canglon site would have lowered oxygen levels which in turn, may have suppressed root growth. Hence, the heavy VAM was observed because the roots had not experienced a growth spurt like the roots at the non-inundated, high light sites. This hypothesis is plausible given both the findings of Mejstrík (1965) and the fact that many temperate plants outgrow their ectomycorrhizal fungus at the beginning of the growing season each year.

Mycorrhizae undoubtedly play important roles in the tropics, mediating the success of individual hosts and influencing forest dynamics and potentially, diversity. In fact, a common explanation for the existence of monodominance in the tropics is the EM hypothesis. Here we have shown that EM do not appear to be critical to the monodominance of *M. excelsa* or *P. copaifera*, as both species are VAM. The importance of EM to the dominance of other Caesalpiniaceae species remains to be established. Furthermore, this study reports the first well-documented record of a tropical swamp tree forming VAM. The next step would be to understand the role that VAM play in the ecology of *P. copaifera* during times of seasonal flooding and dry spells. And finally, this survey adds to the literature on the occurrence of *Paris*-type VAM and suggests that the structure and function of *Paris*-type VAM deserves further investigation.

ACKNOWLEDGEMENTS

We acknowledge INRENARE of Panama for their cooperation and support of this project. Many thanks to T. Kursar and O. Lopez for their enthusiastic help in collecting samples and to D. Feener for statistical advice. S.D.T. is grateful to E. King, B. Kropp, J. Gurr for their patience and willingness to teach techniques of mycorrhizal research. D. M. Newbery, E. I. Newman and J. Garbaye provided helpful comments on the manuscript. This research was supported by a NSF Predoctoral Fellowship to S.D.T. and NSF Grant BSR 9119619 to P.D.C.

LITERATURE CITED

ALEXANDER, I. J. 1983. The significance of ectomycorrhizas in the nitrogen cycle. Pp. 69–93 in Lee, J. A., McNeill, S. & Rorison, I. H. (eds). *Nitrogen as an ecological factor*. Blackwell Scientific Publications, Oxford, UK.

ALEXANDER, I. J. 1989. Mycorrhizas in tropical forests. Pp. 169–188 in Proctor, J. (ed.). *Mineral nutrients in tropical forest and savanna ecosystems*. Special Publications No. 9 of British Ecological Society, Blackwell Scientific Publications, Oxford, UK.

ALLEN, E.B & ALLEN, M.F. 1984. Competition between plants of different successional stages: mycorrhizae as regulators. *Canadian Journal of Botany* 62:2625–2629.

ALLEN, M. F. 1991. *The ecology of mycorrhizae*. Cambridge University Press, Cambridge, UK. 184 pp.

CONDIT, R., HUBBELL, S. P. & FOSTER, R. B. 1993. Mortality and growth of a commercial hardwood 'el cativo,' *Prioria copaifera*, in Panama. *Forest Ecology and Management* 63:107–122.

CONNELL, J. H. & LOWMAN, M. D. 1989. Low-diversity tropical rain forests: some possible mechanisms for their existence. *American Naturalist* 134:88–119.

COUTTS, M. P. & NICOLL, B. C. 1990. Waterlogging tolerance of roots of Sitka spruce clones and of strands from *Thelephora terrestris* mycorrhizas. *Canadian Journal of Forestry Research* 20:1894–1899.

GARTLAN, J. S., NEWBERY, D. McC., THOMAS, D. W. & WATERMAN, P. G. 1986. The influence of topography and soil phosphorus on the vegetation of Korup Forest Reserve, Cameroon. *Vegetatio* 65:131–148.

HARLEY, J. L. & SMITH, S. E. 1983. *Mycorrhizal symbiosis*. Academic Press, New York. 483 pp.

HARTNETT, D. C., HETRICK, B. A. D., WILSON, G. W. T. & GIBSON, D. J. 1993. Mycorrhizal influence of intra- and interspecific neighbor interactions among co-occurring prairie grasses. *Journal of Ecology* 81:787–795.

HETRICK, B. A. D., WILSON, G. W. T. & HARTNETT, D. C. 1989. Relationship between mycorrhizal dependence and competitive ability of two tallgrass prairie grasses. *Canadian Journal of Botany* 67:2608–2615.

HÖGBERG, P. & PIEARCE, G. D. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. *Journal of Ecology* 74:775–785.

JANOS, D. P. 1983. Tropical mycorrhizas, nutrient cycles and plant growth. Pp. 327–345 in Sutton, S. L., Whitmore, T. C. & Chadwick, A. C. (eds). *Tropical rain forest: ecology and management*. Blackwell Scientific Publications, Oxford, UK.

JANOS, D. P. 1985. Mycorrhizal fungi: agents or symptoms of tropical community composition? Pp. 98–103 in Molinas, R. (ed.). *Proceedings of the 6th North American Conference on Mycorrhizae*. Forest Research Laboratory, Corvallis, OR, USA.

JANOS, D. P. 1996. Mycorrhizas, succession and the rehabilitation of deforested lands in the humid tropics. Pp. 129–162 in Frankland, J. C., Magan, N. & Gadd, G. M. (eds). *Fungi and environmental change*. British Mycological Society Symposium Vol. 20. Cambridge University Press, Cambridge, UK.

JOHNSTON, A. 1949. Vesicular-arbuscular mycorrhiza in Sea Island cotton and other tropical plants. *Tropical Agriculture* 26:118–121.

KEELEY, J. E. 1980. Endomycorrhizae influence growth of blackgum seedlings in flooded soils. *American Journal of Botany* 67:6–9.

KHAN, A. G. & BELIK, M. 1995. Occurrence and ecological significance of mycorrhizal symbiosis in aquatic plants. Pp. 627–666 in Varma, A. & Hock, B. (eds). *Mycorrhiza: structure, function, molecular biology and biotechnology*. Springer-Verlag, Berlin.

KORMANIK, P. P. & MCGRAW, A-C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. Pp. 37–46 in Schenck, N. C. (ed.). *Methods and principles of mycorrhizal research*. American Phytopathological Society, St. Paul, USA.

LODGE, D. J. 1989. The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in *Populus* and *Salix*. *Plant and Soil* 117:243–253.

MARX, D. H. 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. *Annual Review of Phytopathology* 10:429–454.

MEJSTRÍK, V. 1965. Study on the development of endotrophic mycorrhiza in the association of *Cladietum marisci*. Pp. 283–290 in Macura, J. & Vencura, V. (eds). *Plant microbes relationships*. Publishing House of the Czechoslovak Academy of Sciences.

NEWBERY, D. M., ALEXANDER, I. J., THOMAS, D. W. & GARTLAN, J. S. 1988. Ectomycorrhizal rain-forest legumes and soil phosphorus in Korup National Park, Cameroon. *New Phytologist* 109:433–450.

NEWMAN, E. I., EASON, W. R., EISSENSTAT, D. M. & RAMOS, M. I. R. F. 1992. Interactions between plants: the role of mycorrhizae. *Mycorrhiza* 1:47–53.

PERRY, P. A., MARGOLIS, H., CHOQUETTE, C., MOLINA, R. & TRAPPE, J. M. 1989. Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist* 112:501–511.

RANKIN, J. 1978. *The influence of seed predation and plant competition on tree species abundance in two adjacent tropical rain forests*. PhD thesis, University of Michigan, Ann Arbor, USA.

SMITH, F. A. & SMITH, S. E. 1996. Mutualism and parasitism: diversity in function and structure in the 'arbuscular'(VA) mycorrhizal symbiosis. *Advances in Botanical Research* 22:1–43.

SMITS, W. Th. M. 1983. Dipterocarps and mycorrhiza. *Flora Malesiana Bulletin* 36:3926–3937.

SONDERGAARD, M. & LAEGAARD, S. 1977. Vesicular arbuscular mycorrhizae in some aquatic vascular plants. *Nature* 268:232–233.

TER STEEGE, H. 1994. Flooding and drought tolerance in seeds and seedlings of two *Mora* species segregated along a soil hydrological gradient in the tropical rain forest of Guyana. *Oecologia* 100:356–367.

TROJANOWSKI, J., HAIDER, K. & HUTTERMANN, A. 1984. Decomposition of ^{14}C-labeled lignin, holocellulose and lignocellulose by mycorrhizal fungi. *Archives of Microbiology* 139:202–206.

WHITBECK, J. 1994. *Effects of above-ground and below-ground resource distribution on the ecology of vesicular-arbuscular mycorrhizas*. PhD thesis, Stanford University, Standford, CA, USA.

WILCOX, H. E. 1982. Morphology and development of ecto- and ectendomycorrhizae. Pp. 103–114 in Schenck, N. C. (ed.). *Methods and principles of mycorrhizal research*. American Phytopathological Society, St. Paul, USA.

Accepted 28 February 1997