

Dual mycorrhizal colonization of forest-dominating tropical trees and the mycorrhizal status of non-dominant tree and liana species

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Abstract The contribution of mycorrhizal associations to maintaining tree diversity patterns in tropical rain forests is poorly known. Many tropical monodominant trees form ectomycorrhizal (EM) associations, and there is evidence that the EM mutualism contributes to the maintenance of

monodominance. It is assumed that most other tropical tree species form arbuscular mycorrhizal (AM) associations, and while many mycorrhizal surveys have been done, the mycorrhizal status of numerous tropical tree taxa remains undocumented. In this study, we tested the assumption that most tropical trees form AM associations by sampling root vouchers from tree and liana species in monodominant *Dicymbe corymbosa* forest and an adjacent mixed rain forest in Guyana. Roots were assessed for the presence/absence of AM and EM structures. Of the 142 species of trees and lianas surveyed, three tree species (the monodominant *D. corymbosa*, the grove-forming *D. altsonii*, and the non-dominant *Aldina insignis*) were EM, 137 were exclusively AM, and two were non-mycorrhizal. Both EM and AM structures were observed in *D. corymbosa* and *D. altsonii*. These results provide empirical data supporting the assumption that most tropical trees form AM associations for this region in the Guiana Shield and provide the first report of dual EM/AM colonization in *Dicymbe* species. Dual colonization of the *Dicymbe* species should be further explored to determine if this ability contributes to the establishment and maintenance of site dominance.

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Introduction

Mycorrhizal associations are thought to have a significant influence on patterns of plant community diversity (Dhillion 1994; Klironomos et al. 2000; van der Heijden et al. 2003), but the magnitude of this influence in tropical rain forests is

unknown. It is widely accepted that most tropical trees form arbuscular mycorrhizal (AM) associations (Alexander 1989; Janos 1983, 1985; St. John 1980), and while many surveys of mycorrhizal associations in tropical rain forests exist (Bakarr and Janos 1996; Bereau and Garbaye 1994; Hopkins et al. 1996; Reddell and Milnes 1992; Smith and Smith 1997), there are still many tree species for which mycorrhizal status is undocumented. The relative paucity, or absence, of boreal/temperate ectomycorrhizal (EM) woody plant families (e.g., Fagaceae, Betulaceae, Pinaceae, etc.) from lowland tropical forests has contributed to the assumption that most trees in lowland tropical rain forests are AM (Janos 1983, 1985). EM associations have been observed in selected genera in the humid tropics, primarily from the families Caesalpiniaceae, Fabaceae, Gnetaceae, Euphorbiaceae, Myrtaceae subfam. Leptospermoideae, Nyctaginaceae, Araucariaceae, and Polygonaceae (Alexander 1989; Alexander and Hogberg 1986; Henkel et al. 2002; Wang and Qiu 2006). There has been extensive documentation of EM associations in the Dipterocarpaceae of Asia (Alexander et al. 1992; Alexander 1989; Lee et al. 1997), Caesalpiniaceae and the Uapacaceae of Africa (Hogberg and Pearce 1986; Newbery et al. 1988, 1997; Torti and Coley 1999), but most species outside of these families are thought to exclusively form AM associations.

Mycorrhizal status, as well as the influence of mycorrhizal mutualisms on rain forest diversity and tree assemblages, are poorly known compared to the wealth of botanical and ecological studies in these ecosystems (Alexander and Lee 2005). Tropical monodominance, where ~50–100% of the canopy is dominated by one tree species, is one example of a tropical forest where mycorrhizal associations may directly affect tree diversity patterns (Connell and Lowman 1989; Hart et al. 1989; McGuire 2007a, b; Torti et al. 2001). Tropical monodominant tree species are frequently EM, and this correlation has led to the suggestion that EM tree species attain local dominance by having survival and establishment advantages over neighboring AM associates. This is partly based on the supposition that EM fungi are better than AM fungi at foraging in nutrient-deficient soils (Alexander and Hogberg 1986; Halling 2001; Janos 1985) and also because unlike AM fungi, EM fungi have the enzymatic capabilities to access limiting nutrients bound in organic layers (Abuzinadah and Read 1986; Buscot et al. 2000; Trojanowski et al. 1984).

To begin assessing the ecological significance and specific mechanisms by which EM fungi may be functioning in these systems, it is first necessary to test the underlying assumption that most other trees are AM associates. To do this, we conducted a study in central Guyana where large patches of dominant, EM *Dicymbe corymbosa* Spruce ex Benth. (Caesalpiniaceae), and

Dicymbe altsonii Sandwith (Caesalpiniaceae) exist within matrices of mixed, presumably AM, forest. We surveyed the mycorrhizal status of all tree species occurring within 12 ha of permanent plots established from 1999 to 2003 (Henkel 2003; McGuire 2008). Although *D. altsonii* does not occur in these particular plots, we included it in the mycorrhizal survey, as it is common nearby.

Materials and methods

Study site

This study was conducted in central-western Guyana in the Potaro and Ireng River watersheds (Henkel 2003; McGuire 2008). *D. corymbosa* forms extensive monodominant patches throughout these two watersheds, with abrupt ecotones separating the low-diversity, monodominant forest from the mixed forest. *D. altsonii* forms large groves, but does not reach the dominance levels of *D. corymbosa*. Tree diversity in the mixed forest is substantially higher than in the *Dicymbe* forests, and neither of the *Dicymbe* species occur there. The first site in the Potaro River watershed is located at approximately 5°18' N, 59°54' W (720-m altitude), and the second site within the Ireng River watershed is at approximately 5°23' N, 59°61' W (700-m altitude).

Root sampling

Permanent tree plots were established in both sites, and all trees ≥ 10 cm diameter at breast height were tagged, measured, and identified (Henkel 2003; McGuire 2008). Root samples were collected from one to three individuals of each species in the permanent plots by tracing lateral roots from the bases of adult trees and collecting fine roots occurring in the top 10 cm of soil. Roots were collected from the four cardinal directions of each selected tree. Root samples were taken during June 2001, June 2002, and December 2002. Roots were cleaned of soil and organic matter with water and placed in formalin acetic acid the same day they were collected for later analysis at the University of Michigan. Roots were cleared with 10% KOH and alkaline H₂O₂ and subsequently stained with trypan blue according to the protocol of Giovannetti and Mosse (1980). This protocol was occasionally modified for roots high in tannins by extending incubation times of the roots in 10% KOH. For the darkest roots, a pretreatment wash with 10% bleach was also added. The presence/absence of AM and EM structures was noted for each species. In the field, EM structures were identified by the presence of a mantle, visible with the naked eye. Cross-

sections in the laboratory were made to confirm the presence of a Hartig net. Positive AM colonization was recorded only if arbuscules and/or hyphal coils were observed. Approximately 50 cm of root segments were examined for each tree species. Preserved root samples are archived at the University of Michigan. Herbarium vouchers of tree and liana specimens were deposited in the University of Michigan herbarium and the University of Guyana herbarium.

Results

Of the 142 woody species surveyed, AM structures were detected in 133 tree species and six lianas (see [Supplementary documents](#), Fig. 1). This represents 41 families and 81 identified genera. We were unable to identify seven taxa to family. No mycorrhizal structures were detected in two tree species: *Pagamea coriaceae* (Rubiaceae) and one of the above unidentified taxa. The roots of these two species were high in tannins, which may have obscured the detection of AM structures. EM structures were detected in the monodominant *D. corymbosa*, the grove-forming *D. altsonii* (Fig. 2), and in the non-dominant *Aldina insignis* (Fabaceae). Dual EM and AM colonization was observed in *D. corymbosa* and *D. altsonii* (Fig. 2), sometimes within the same rootlet. More than three quarters of all *Dicymbe* rootlets were EM, but fewer than one fourth showed AM or dual AM and EM. A quantitative study of colonization levels, however, was not conducted in this survey. No root nodules were found on either of the *Dicymbe* species.

Discussion

Mycorrhizal survey

The results of this study support the assumption that most tropical tree species form arbuscular mycorrhizae. Root samples for all species other than *Dicymbe* spp. collected within monodominant forest were highly colonized by AM fungi, suggesting that AM inoculum is not limiting in this forest despite the high levels of EM dominance. This ubiquity of AM associations can also be extended to neotropical lianas in this system, as all six liana species surveyed were AM. Mycorrhizal associations in lianas have rarely been detected (Wanek et al. 2002), but this may be due to the fact that they are often excluded from mycorrhizal surveys. Lianas of the genus *Gnetum* have been found to be EM in Cameroon (Onguene and Kuyper 2001) and the neotropics (Singer et al. 1983), but EM structures have not been documented for other liana genera. Considering that lianas can comprise a significant proportion of the woody biomass in tropical rain forests (DeWalt and Chave 2004; Perez-Salicrup et al. 2001) and that their abundance appears to be increasing as a result of global change (Phillips et al. 2002), they should be included in future mycorrhizal and forest diversity studies to increase our knowledge of their ecologies.

Aldina insignis was the only other species where EM structures were observed. For *A. insignis*, this is not surprising considering the documented EM status of congeneric *A. kunhardtiana*, *A. latifolia*, and *A. heterophylla* from Venezuela and Brazil (Moyersoen 1993; Singer et al. 1983).

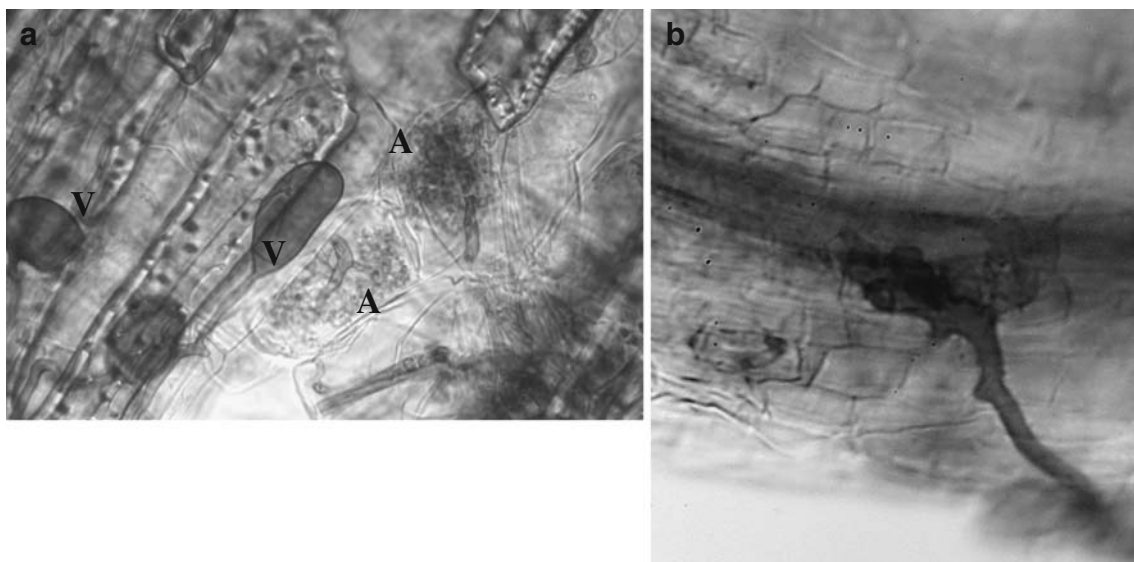
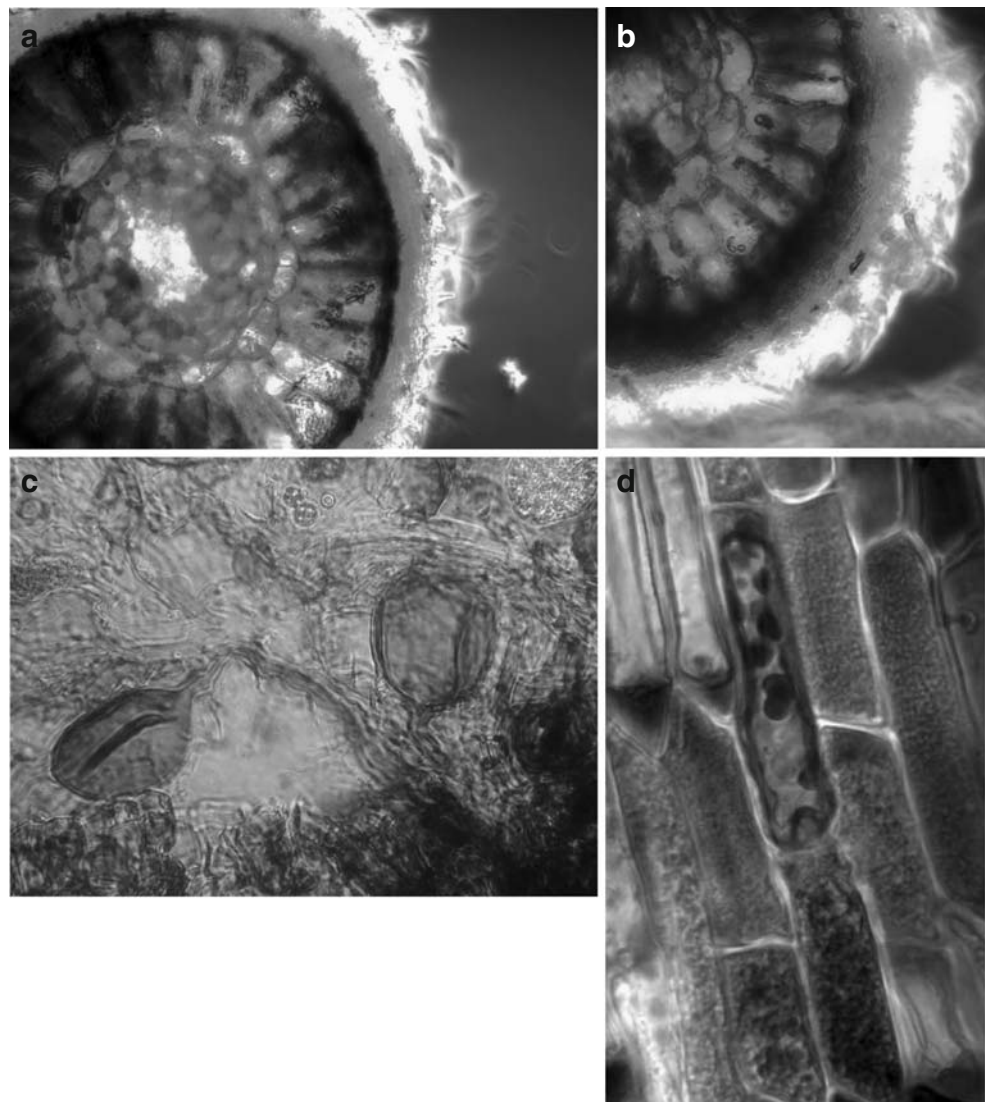


Fig. 1 Mycorrhizal structures observed in cleared and stained roots at 400 \times . **a** Arbuscules indicated by *A* and vesicles indicated by *V* of *Dugetia cuspidata* (Annonaceae) and **b** an appressorium is shown from *Inga nobilis* (Mimosaceae) roots

Fig. 2 Root tips of *Dicymbe altsonii* and *D. corymbosa*. A cross-section of ectomycorrhizal root tips reveals the mantle and Hartig net in *D. corymbosa* (a) at 200 \times and in *D. altsonii* (b) at 400 \times . Cleared and stained root tips show vesicles from arbuscular mycorrhizal colonization (400X) in *D. corymbosa* (c) and vesicles and arbuscules (200 \times) in *D. altsonii* (d)



However, no AM structures were detected in the cleared and stained roots of this tree species.

Dual infection

The presence of both EM and AM structures in *D. corymbosa* and *D. altsonii* is noteworthy as dual mycorrhizal colonization has also been observed in other tropical monodominant trees from the genera *Gilbertiodendron* and *Julbernardia* of the Caesalpiniaceae (Torti and Coley 1999), *Uapaca* of the Uapacaceae (Moyersoen and Fitter 1999; Ramanankierana et al. 2007), and *Leptospermum* and *Eucalyptus* of the Myrtaceae (Chen et al. 2000; Moyersoen and Fitter 1999). The ability for a dominant EM tree in the tropical rain forest to retain the capacity for AM association may be a critical factor facilitating the expansion of EM trees into surrounding AM communities. A temporary association with AM fungi may be beneficial for a predominantly EM seedling

established away from the parental grove, especially in nutrient-poor soils (Chilvers et al. 1987).

This is the first study to report dual AM and EM colonization in tree species of *Dicymbe*. Dual infection may also be more common than reported in monodominant, EM trees, but may not be adequately studied due to the microscopic nature of AM mycorrhizal structures. Future studies should investigate the role of the AM association in *Dicymbe* spp. seedling establishment and the change in AM vs. EM colonization rates with tree age.

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