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Arbuscular Mycorrhizas in *Dicorynia guianensis* and *Eperua falcata* Trees from Primary Tropical Rain Forest of French Guiana

J. MARTIN[1], M. BEREAU[2], E. LOUISANNA[2], and J.A. OCAMPO[1*]
[1]*Dept. Microbiología, Estación Experimental del Zaidín, C.S.I.C., Prof. Albareda 1, E-18008 Granada, Spain, Tel. +34-958-121011, Fax. +34-958-129600, E-mail. jocampo@eez.csic.es; and*
[2]*INRA, Station de Recherches Forestières, BP 709, 97387 Kourou Cedex, France, Tel. +33-594-329282, Fax. +33-594-322318, E-mail. bereau_m@kourou.cirad.fr*

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Abstract

The most probable number of arbuscular mycorrhizal (AM) fungal propagules, spore density and mycorrhizal colonization were studied in *Dicorynia guianensis* and *Eperua falcata* trees in three sites of the primary rain forest of French Guiana. A high number of propagules (17–230 per g soil) and number of spores (50–154 per g of soil) were found in the rhizosphere soil of both trees. The number of propagules and spores were similar in the rhizosphere soil of *D. guianensis* trees in all the three sites tested. In the rhizosphere soil of *E. falcata* from the Grand Plateau site, the highest number of propagules and spores were found. There were similar numbers of propagules and spores in the rhizosphere of *E. falcata* from the Petit Plateau and Paracou sites; in these sites the number of propagules and spores in the rhizosphere soil of *D. guianensis* was higher than in the *E. falcata* rhizosphere soil. The number of propagules was correlated with the number of spores but not with the level of AM colonization. The percentage of AM root length colonization was high (>60%) and similar in both trees from all the sites tested. No arbuscules were observed in roots of trees from any of the soil samples tested, but soybean grown in these soils developed many arbuscules.

Keywords: *Dicorynia guianensis*, *Eperua falcata*, *Glycine max*, rainforest, arbuscular mycorrhizas

*The author to whom correspondence should be sent.

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1. Introduction

The importance and world-wide distribution of the arbuscular mycorrhizal (AM) symbiosis is well documented. Most studies of rain forest mycorrhizas have recorded a dominance of AM (Janos, 1980; Bereau et al., 1997a; Torti et al., 1997). The nutrient uptake processes in tropical rainforests are usually concentrated in the fine networks in the surface soil horizons (Stark and Jordan, 1978). AM associations which occur in this zone have been postulated to have significant effects on nutrient uptake and the essential processes of forest growth, regeneration and dynamics (Alexander, 1989; Janos, 1983; Allen et al., 1998). Bereau and Garbaye (1994) reported for the first time the presence and prevalence of AM symbiosis on 21 tree species in a tropical rain forest in French Guiana.

The Caesalpinioïdeae sub-family is the dominant group of trees in the French Guiana, in diversity, density and biomass (Puig et al., 1990). The family Caesalpiniaceae is considered frequently to be ectomycorrhizal in other regions of the world. However, in French Guiana rain forest, AM are very dominant and arbuscules are rare or absent as in other tropical trees (Janos, 1984) and replaced by hyphal coils. *Dicorynia guianensis* Amshoff and *Eperua falcata* Aublet are economically important Caesalpiniaceae species, and are widely studied by the scientific community. Only AM fungi have been found in the roots of both tree species (Bereau et al., 1997a). It is established that seedlings of *D. guianensis* are dependent on endomycorrhizal colonization for optimal growth (Bereau et al., 1997b). From forest observations and experimental results, the endomycorrhizas seem to be a critical factor controlling *D. guianensis* regeneration in the primary tropical rain forest of French Guiana (Bereau et al., 1997b). A general ecological approach was therefore necessary to gain information about the soil-AM community from their natural forest environment.

The aim of the present investigation was to study soil inoculum of AM fungi, counting spore density, determining most probable number of propagules and root mycorrhizal colonization in these two tree species in the rain forest of French Guiana.

2. Materials and Methods

The study was carried out with *Dicorynia guianensis* and *Eperua falcata* trees (Cesalpiniaceae), in the primary rain forest of Paracou Forest experimental site and Nouragues Natural Reserve of French Guiana, with a canopy height of 30–40 m.

The Paracou Forest Site is located on the coast 110 km west of Cayenne

(5° 20' N, 52° 5' W). The soils are latosols developed on migmatite and shales (Boulet and Brunet, 1983). Average annual precipitation is 2200 mm. Mean temperature is 25°C with low seasonal changes (Huc et al., 1994). Eight adult *D. guianensis* and *E. falcata* trees each were randomly sampled.

The Nouragues Natural Reserve is located in the inland part of French Guiana 100 km south of Cayenne (4° 3' N, 52° 42' W). The soils are ferralsol type. The annual rainfall is 3250 mm and the mean annual temperature is 26°C (J.P. Dominique and M. Grimaldi, personal communication). Samples were taken from the two hills on both sides of the Nouragues creek, termed "Plateau" (Grand and Petit Plateau) due to their wide flat summits. The primary forest is overhung by an inselberg (rock-savannas), culminating at 411 m above see level.

D. guianensis forms dense mats of thin and highly branched roots intercepting water and nutrient fluxes of low intensity on large areas, whereas *E. falcata* sends long poorly branched roots with polymorphous short elements to microsites where these fluxes are concentrated, for instance around the base of other trees (Béreau and Garbaye, 1994). Nine adult *D. guianensis* and 7 adult *E. falcata* trees were randomly sampled in the Grand Plateau and 8 adult *D. guianensis* and 8 adult *E. falcata* trees were randomly sampled in the Petit Plateau. Soil and roots were collected during the dry season, July 1999. Fine roots and approximately 250 g of adjacent soil were collected from each sample tree by tracing thick roots from the base of the trunk to their ultimate branching. Each sample consisted of five bulked subsamples taken from the top 10 cm of soil of each tree.

The pH and total N, P, K, Mg, Ca and Na of soil samples were determined by the method of Lachica et al. (1965).

The spores were isolated by wet-sieving 5 g of soil from each sample through 50–700 mm sieves and centrifuging in a 50% (w:v) sucrose gradient (Walter et al., 1982). We counted cytoplasm-filled, viable-looking individual spores in a Doncaster dish (Doncaster, 1962), using a dissecting microscope. We did not count floating spores which looked empty or parasitized.

Roots were rinsed in distilled water to remove any trace of soil, cleared and stained according to the Kormanik and Mc Graw method (1982) with some modifications. The bleaching time with 10% KOH at 90°C was of 3–4 h for *D. guianensis* and 4–6 h for *E. falcata*. The KOH solution had to be changed at 1.5 h intervals because the roots of the two species were darkly pigmented. After the KOH treatment, roots were rinsed in water and bleached with 30% H_2O_2 for 3–4 min for *D. guianensis* and 20–30 min for *E. falcata*, then treated as in the original method. Percentage root length with AM colonization was measured by the line intersect method (Giovannetti and Mosse, 1980).

The number of effective propagules in the soil samples was estimated by the most probable number (MPN) method (Porter, 1979; Woomer, 1994) using

Table 1. Soil pH and total N, P, K, Ca, Mg and Na (expressed as mg g^{-1}) of soils around the roots of *Dicorynia guianensis* and *Eperua falcata* trees.

Site	Tree species	pH	N	P	K	Ca	Mg	Na
Paracou								
	D. guianensis	4.6a	2.2b	0.7a	1.8b	17.7a	0.3a	0.008a
	E. falcata	4.4a	4.5c	0.6a	1.1b	20.7a	0.1a	0.007a
Les Nouragues								
Grand Plateau								
	D. guianensis	4.2a	2.7b	0.6a	0.2a	22.3a	0.3a	0.008a
	E. falcata	4.2a	4.1c	0.8a	0.2a	16.1a	0.2a	0.005a
Petit Plateau								
	D. guianensis	4.8a	1.2a	0.7a	0.2a	16.1a	0.1a	0.006a
	E. falcata	4.3a	2.1b	0.6a	0.3a	15.4a	0.1a	0.005a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0. 05).

Table 2. Most probable number of arbuscular mycorrhizal fungal propagules, number of spores and percentage of root length colonization of *Dicorynia guianensis* and *Eperua falcata* trees.

Site	Tree species	MPN of propagules per g of soil	Number of spores per g of soil	% root length colonization
Paracou				
	D. guianensis	115b	77b	70.8a
	E. falcata	21a	49a	66.2a
Les Nouragues				
Grand Plateau				
	D. guianensis	123b	89b	71.2a
	E. falcata	230c	154c	65.1a
Petit Plateau				
	D. guianensis	107b	79b	72.2a
	E. falcata	17a	50a	61.4a

Column values followed by the same letter are not significantly different as determined by Duncan's multiple range test (P = 0. 05).

Glycine max (Linnaeus Merrill) with rapid root growth as the test plant. To determine MPN, 50 ml from the soil sample of each tree of the three plots was used. From each sample 5 dilutions (10^{-1}–10^{-5}) in sand:vermiculite (1:1, v:v)

were prepared and each dilution was tested in five pots. One *G. max* seedling was grown in each pot with 100 ml of soil. Five ml of 1/2 strength Long Ashton nutrient solution with 50 mg phosphate l^{-1} (Hewitt, 1952) were added weekly to each pot. *G. max* seedlings were harvested after 6 weeks to record the presence or absence of mycorrhizal colonization.

Data were subjected to one way ANOVA and Duncan tests (P = 0.05) to detect significant differences between treatment means. Percentage data were subjected to arcsin transformation before analysis by linear regression.

3. Results

No significant differences in pH values of soils from the different sites were found. Nitrogen content was always significantly higher in the soil around *E. falcata* than around *D. guianensis* roots. The K content of soil from Paracou was higher than from Les Nouragues. The P, Ca, Mg and Na contents were similar in all soils tested (Table 1).

Spores belonging to the Glomaceae and Acaulosporaceae families were the most frequent (sessile spores); spores belonging to the Gigasporaceae family (spores with bulbous subtending hyphae) were not found. Black spores, probably belonging to the Acaulosporaceae family, and lobed-shape spores were also abundant.

Table 2 shows that the highest number of AM fungal propagules and spores were found in the rhizosphere of *E. falcata* grown in the Grand Plateau site. No significant differences in the number of propagules and spores in the rhizosphere of *E. falcata* were found in Paracou and in Petit Plateau. In these sites, fewer propagules and spores were found in the rhizosphere of *E. falcata* than in that of *D. guianensis*. No significant differences in the number of propagules and spores in the rhizosphere of *D. guianensis* trees were observed in the three sites. The correlation between number of propagules and number of spores was significant (r = 0.9, P = 0.0001).

The root samples of *D. guianensis* and *E. falcata* from the three sites tested showed a similar percentage of AM root length colonisation (Table 2). The AM colonization found in *D. guianensis* and *E. falcata* was characterized by the presence of vesicles, intracellular coils, but no arbuscules. However, the AM colonization of soybean grown in the soil samples of each tree showed abundant arbuscules.

4. Discussion

Although an increasing number of studies have been carried out on AM fungi in rain forests, information about the diversity of this symbiosis is scarce

(Hopkins et al., 1996; Janos, 1996; Bereau et al., 1997a; Torti et al., 1997; Allen et al., 1998). With a few exceptions, AM symbioses are dominant in tropical forests (Allen et al., 1998). It has also been shown that there are few viable AM fungal spores in the soils of lowland tropical rain forest and that colonization is assumed to pass from living root to living roots (Janos, 1983). In contrast, we found here a very high number of viable spores in the tropical rainforest of French Guiana (50 to 150 spores per g soil). In tropical forests from other parts of the world, spores were counted as less numerous: in India only 3–20/g of soil (Sharma et al., 1984), and in the Catinga Amazonica of Venezuela only 1.1–1.8/g soil (Moyersoen, 1993).

Soil modifications, natural or man-made, influence the number of spores and AM symbioses (Abbott and Robson, 1991). Under the close tree canopy of undisturbed lowland rain forest of French Guiana, there is a fairly continuous cover of leaf litter or floor vegetation and a stable microclimate at the soil surface. The soils are relatively poor in mineral nutrients, especially phosphorus. In our sample, the chemical properties of the soils do not vary between sites except for the higher K content in Paracou soils. In contrast to disturbed habitats, these factors could have contributed to the absence of variations in the mycorrhizal fungal spore population, number of propagules and plant colonization level between the different sites. The number of spores and number of propagules seem to be more related to the tree species: more spores we found in *D. guianensis* than in *E. falcata* rhizosphere soil except for the Nouragues Grand Plateau. It is known that root functioning influences the mycorrhizosphere (Linderman, 1992). Both species have different root morphologies and do not form rhizobial nodules (Béreau and Garbaye, 1994), but *E. falcata* can fix atmospheric nitrogen (Roggy et al., 1999). Rhizospheric N-fixation by free-living bacteria, phyllospheric fixation by cyanobacteriae and the ability to use specific nitrogen sources either biochemically or spatially are some of the proposed hypotheses to explain N-fixation by this tree species (Guehl et al., 1998). The different concentration of total N in the soil around the roots of the two tree species indicates that these trees may have different root physiology and different AM fungal community populations. However, the highest number of AM propagules found in the rhizosphere soil of *E. falcata* in the "Grand Plateau" suggests that other environmental parameters may also affect the AM fungal community populations.

It is interesting to note that spores with bulbous subtending hyphae, which are characteristic of the Gigasporaceae family (Morton and Benny, 1990), were never observed. The absence of *Gigaspora* from other tropical rainforest sites has also been observed (Louis and Lim, 1987).

The AM colonization found in *D. guianensis* and *E. falcata* was characterized by the presence of vesicles and intracellular coils and an absence of arbuscules (Béreau and Garbaye, 1994). This type of AM (with coils and no arbuscules) has

been reported for aquatic plants (Khan and Belik, 1995) and in some tropical forest trees (Torti et al., 1997). However, soybean cultivated in soils sampled from the rhizosphere of *D. guianensis* and *E. falcata* showed abundant arbuscules. Thus, the host plant influences the morphology of the symbiotic structure (Lackie et al., 1987).

The correlation between number of propagules and number of spores found in soils around *D. guianensis* and *E. falcata* trees suggests that the spores were the principal forms of AM inoculum in these soils. Correlation between number of propagules and number of spores has been found in other tropical forest soils (Fischer et al., 1994). However, no correlation has been found between the number of propagules and the level of AM colonization of roots, just as Koske and Halvorson (1981) remarked that colonization levels of AM are not necessarily correlated with sporulation. The lowest number of AM propagules (ca. 50 per g of soil) was enough to result in more than 60% root length colonization of *D. guianensis* and *E. falcata* trees. A density of 1–5 propagules g^{-1} soil was found to produce a high level of AM colonization (McGee et al., 1999). Thus, under undisturbed rain forest of French Guiana, lack of AM inoculum will not limit or prevent successful regeneration or growth of tree species.

The two trees species studied, with their exclusive endomycorrhizal symbiosis as observed in previous reports, are typical of the poor acid soils of the two distant sites of French Guiana chosen for this study. Considering the new information provided on soil inoculum and the apparent weak role of their density on root colonization, as well as on the contrasting root habit of the two species, further research is needed in spore type identification in order to determine whether some fungal specificity occurs in the symbiotic status of the two tree species.

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