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Abstract

Pterocarpus officinalis (Jacq.) is the dominant wetland legume tree species of the seasonally flooded swamp forests in Guadeloupe, Lesser Antilles. This tree is periodically exposed to saline and flooded soil conditions. We examined mycorrhizal soil infectivity (most probable number (MPN) values) and arbuscular mycorrhizal (AM) colonization of *P. officinalis* along the salinity gradient where the salt levels ranged from 26 to 2‰ and from 22 to 5‰ at the end of the dry and wet season, respectively. MPN values were higher in the dry season than in the wet season. They decreased when the salt levels increased whatever the season. AM colonization of *P. officinalis* was well developed (up to 50%) only within the low salinity levels (below 10‰) whatever the season. No spores were found in soil cores, suggesting that propagules were only mycelium pieces and/or root fragments of colonized *Pterocarpus* roots. These AM fungi may be adapted to salt stress and explain the maintenance of the high mycorrhizal inoculum potential in the *P. officinalis* swamp forest.

Keywords: Arbuscular mycorrhizal colonization; Swamp forest; Salinity; Flooding; Guadeloupe

1. Introduction

Arbuscular mycorrhizal fungi (AMF) in wetland habitats are exposed periodically to anaerobic soils and high soil salinity (Bohrer et al., 2004; Carvalho et al., 2004). Since AMF require oxygen to thrive, stressful environments regularly flooded with salt water may be detrimental for their survival and infectivity. Nevertheless, some AMF are able to persist in flooded soils and to colonize wetland plants (Khan, 1993; Miller and Bever, 1999; Turner et al., 2000; Landwehr et al., 2002). More than 50% of the plant's population were colonized by AMF in some wetlands conditions (Ragupathy et al., 1990). One species, *Glomus geosporum*, is usually dominant in European salt marshes (Landwehr et al., 2002; Carvalho et al., 2004). These studies suggest fungal adaptation to salt and flood conditions. In this respect, AMF possess propagules allowing long-term survival in soils and immediate opportunistic root colonization (Smith and Read, 1997). Spores, infected root fragments and extraradical mycelia are the main sources of inoculum potential in soils contributing to the infectivity of plants. The relative contribution of each type of propagules to plant root colonization is difficult to establish. Depending on fungal species, spore production and germination were affected by salinity and soil water levels (Miller and Bever, 1999; Carvalho et al., 2004). Le Tacon et al. (1986) reported that anaerobic conditions inhibited the germination of spores of *Glomus mosseae*, but that this effect was reversible upon exposure of the spores to air. The identity of the host plant was also known to be an important factor affecting density of AM propagules and sporulation (Smith and Read, 1997). However, extraradical mycelia and root fragments seemed to be relatively more important than spores for the initiation of plant colonization in wetlands, salt mashes and aquatic systems (Brown and Bledsoe, 1996; Carvalho et al., 2004).

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In the present work, we studied the AM inoculum potential in swamp soils from a monospecific stand of the wetland legume tree *Pterocarpus officinalis*. This tree is useful for the study of AM soil infectivity because it grows along a gradient of salinity. This allowed us also to determine the effect of salinity on AM colonization, while keeping the host plant constant.

2. Materials and methods

2.1. Study site

The *Pterocarpus* swamp forest covers 2600 ha of freshwater, coastal wetlands in Guadeloupe (Imbert et al., 2000). It is found mainly around the bay of the Grand cul-de-sac Marin, behind mangrove areas. The study site is located on the eastern side of the bay, in the Abymes coastal plain (61°30′N, 16°10′W). At this site, the swamp forest is separated from the mangrove forest by a narrow strip (ca. 50 m-wide) of mainly herbaceous vegetation, dominated by the fern *Acrostichum danaefolium* and the liana *Rhabdadenia biflora*. Some stunted *Pterocarpus* trees, mostly dead, occur inside this ecotope zone. From the swamp forest edge inwards, tree height gradually rises from about 5 to 20 m and over. As the canopy rises, stem density decreases, whereas basal area increases. *P. officinalis* is the only tree species contributing to the forest canopy. Understorey species, like *Coccoloba venosa* and *Montrichardia arborescens*, are few and far between. The forest is flooded most of the year, depending on the duration of the rainy season. Water level may vary from place to place, due to *Pterocarpus* buttresses that create low mounds. The soil is clayey, soft and brownish in the upper 30 cm, denser and greyer below.

2.2. Sampling and measurements

The investigations were conducted along a transect starting from the seaward edge toward the inner part of the swamp forest. Sampling was made on three mature trees of *P. officinalis* in six plots (3 m × 3 m) along a part of the transect that spanned an existing salinity gradient. The length of this part of the transect was approximately 170 m. Water-table level was assessed around each sampled tree by measuring water depth in comparison with ground surface. Pore-water salinity at 20 cm below ground level and salinity of above-ground standing water were measured using an ATAGO ATC-S, temperature-compensated hand refractometer (ATAGO Inc., Bellevue, WA, USA). Salinity and water depth measurements were made at the end of the dry and wet season.

For each plot, five soil cores (20 cm in depth; 7 cm in diameter) were taken around each mature tree, pooled per soil salinity level and stored at room temperature (20 °C). The method chosen for mycorrhizal soil infectivity determination was the most probable number (MPN) method (Porter, 1979). The method involves cultivation of a test plant having a high mycorrhizal dependency (Plenchette et al., 1983) on a range of successive dilutions of the soil to be tested. Soil samples were air dried and sieved (2 mm) prior to disinfect a sub sample of each by autoclaving (115 °C, 45 min). The remaining humidity was determined. Then, for each sample, non-disinfected (ND) and disinfected (D) soils were mixed together to obtain the following range of dilutions: 1, 1/4, 1/16, 1/64, 1/256 and 1/1024 (ND/D; w/w). Five replicates were made for each dilution. Soil (100 g) were placed in small pots then planted with a 2-week-old seedling of millet (*Pennisetum americanum* L.) for a 6-week growing period. Pots were placed in a controlled environment (24 °C day/18 °C night; relative humidity 80%; light intensity 112 μmol m^{-2} s^{-1}). After 6 weeks the entire root system of each plant was gently washed, cleared and stained (Phillips and Hayman, 1970). Observation of colonization was made under a dissecting microscope 50×. One infection point was considered as sufficient to state that the plant was colonized. Calculation of MPN values were made as described by Sieverding (1991) according to Fisher and Yates (1948) and expressed per 100 g of dried soil.

The fine roots were randomly collected for each mature tree of *P. officinalis* (three replicates per tree), gently washed, cleared and stained (Phillips and Hayman, 1970). Roots were then cut into 1 cm pieces, mixed and placed on slides for microscopic observations at 250× magnification (Brundrett et al., 1985). One hundred root pieces were observed per plant. The extent of AM colonization was expressed as a percentage of the number of mycorrhizal root pieces/number of non-mycorrhizal root pieces. Data of mycorrhizal colonization were subjected to one-way analysis of variance, and mean values were compared using Newman–Keuls multiple range tests (Gagnon et al., 1989). Data of mycorrhizal colonization were transformed by arcsin (square root) before analysis.

3. Results

The salinity gradient ranged from 26 to 2‰ and from 22 to 5‰ at the end of the dry and wet season, respectively (Tables 1

Table 1
Soil salinity, mycorrhizal soil infectivity (MPN and confident limits) and mycorrhizal colonization of *P. officinalis* at the end of the dry season

Plots	Salinity ‰ (0–20 cm)	Water depth (cm)	MPN* (100 g)	Confident limits $p < 0.05$	Colonization** (%)
1	26	4	75 a	35–160	14.5 a
2	20	3	150 a	70–320	29.4 b
3	15.5	21	560 b	262–1196	47.5 c
4	10	−12	2400 bc	1123–5126	66.0 d
5	3	−3	3402 c	1592–7266	76.9 e
6	2	−7	3800 c	1779–8166	82.8 e

* Values followed by the same letters are not significantly different (confident limits, $p < 0.05$).
** Values followed by the same letters are not significantly different (Newman–Keuls, $p < 0.05$).

Table 2
Soil salinity, mycorrhizal soil infectivity (MPN and confident limits) and mycorrhizal colonization of *P. officinalis* at the end of the wet season

Plots	Salinity (‰) (0–20 cm)	Water depth (cm)	MPN* (100 g)	Confident limits $p < 0.05$	Colonization** (%)
1	22	16	20 a	9–42	22.1 a
2	15	15	31 a	15–67	40.3 b
3	13.5	33	90 bc	42–191	48.8 b
4	8	0	1157 d	542–2472	65.1 c
5	3	9	688 d	322–1469	68.9 c
6	5	5	301 cd	141–644	75.1 c

* Values followed by the same letters are not significantly different (confident limits, $p < 0.05$).
** Values followed by the same letters are not significantly different (Newman–Keuls, $p < 0.05$).

Table 3
Correlation coefficients (r) between mycorrhizal soil infectivity (MPN values), soil salinity (S‰) and mycorrhizal colonization of roots (% AM colonization)

	Dry season			Wet season		
	MPN	S	AM (%)	MPN	S	AM (%)
MPN		−0.96*	0.95*		−0.86*	0.75*
S			−0.99*			−0.97*

* Significant ($p < 0.05$).

and 2). There was not an evident flooding gradient in wet and dry seasons in the *P. officinalis* swamp forest (Tables 1 and 2).

Spores were not found in the soil samples collected during the dry and wet seasons (Tables 1 and 2). Nevertheless, AMF were present in the root samples of *P. officinalis,* whatever the salt level (Tables 1 and 2). The higher AM colonization (82–75%) was recorded for the lowest salt level (2–5‰) (Tables 1 and 2). AM colonization was greater to 50% until the salt levels of 8 and 10‰ were reached in wet and dry seasons, respectively. MPN values due to mycelium and root fragments also decreased as salt level increased (Tables 1 and 2). However, MPN values were higher in the dry season than in the wet season.

Significant negative correlations were obtained between the salt levels and the MPN values both in wet and dry seasons (Table 3). MPN values and AMF colonization were positively correlated in dry season (Table 3). Whatever the season, colonization percentage was negatively correlated with salinity level (Table 3, Fig. 1).

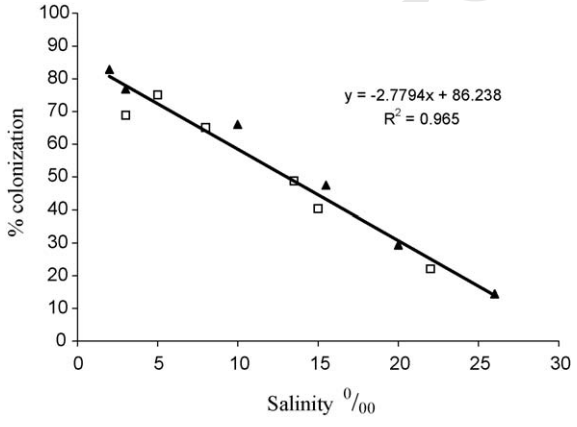

Fig. 1. Relationship between salinity level and development of AM colonization. (□) Dry season; (▲) wet season.

4. Discussion

Salinity has been shown to be an important factor influencing AM colonization of *P. officinalis* and fungal inoculum potential in swamp soils. AM colonization was indicated by the presence of aseptate hyphal coils and vesicles as *Paris*-type mycorrhizas (Bâ et al., 2004). Surprisingly, no spores were found in sieved soil samples collected during the dry and wet seasons. Since spores were absent, we conclude that the infection units and colonization of roots were due to mycelium and/or root fragments. We do not know the relative proportion of infection units and AM colonization derived from mycelium or root fragments. Furthermore, it is not clear whether the absence of spores in swamp soils was due to the toxic levels of minerals and/or to variations of depth water in dry and wet seasons. Some studies indicate that water depth is an important factor determining the distribution of spore species along a dry to wet gradient (Miller and Bever, 1999). The overall trend was for fewer spore species in wetter sites than in drier sites. Other studies suggest that soils from salt marshes contain spores of AMF in high numbers, whereas reduction of spore germination at water levels above field capacity may be related to the low tolerance of AMF to hypoxic conditions (Landwehr et al., 2002; Carvalho et al., 2004).

We found evidence for potential adaptation of native AMF to salt swamp soils and for the ability of AM propagules to spread into the roots. Indeed, AMF colonization was greater to 50% until the salt level of 8‰ was reached in dry and wet seasons and decreased in both seasons as salt level increased. Since AM colonization was already recorded on wetlands plants (Bohrer et al., 2004), some results were unexpected at very high salinity levels (Brown and Bledsoe, 1996). Our results suggest that AMF were well adapted to stressful salt swamp soils. The absence of spore in sieved swamp soils did not permit conclusions as to the taxonomy of these AMF. PCR with taxon-specific primers have been used to identify AMF within colonized roots (Landwehr et al., 2002). We plan to incorporate such techniques in our future work.

AM colonization did not vary significantly from wet to dry season, whereas the MPN values did. One possible explanation is that fungi may endure prolonged exposure to salt and flooding by spreading inside the *Pterocarpus* roots, whereas extraradical mycelium and root fragments in soils may not. In this respect, roots of *P. officinalis* would be well aerated due to aerenchymatous tissue, adventive roots and buttress lenticels.

Brown and Bledsoe (1996) observed AMF in the aerenchymatous tissue of salt marsh plants, suggesting that AMF are adapted to life in oxygen-deficient soils. MPN values were mainly higher than those recorded in other tropical soils, i.e., 10 propagules/100 g for a lowland wet forest in Costa Rica (Fisher et al., 1994) or 1–100 propagules/100 g for soils used for intensive banana cultivation in Martinique (Declerck et al., 1999). This suggests that *P. officinalis* may be considered as a high mycorrhizal dependent tree which favor AM fungal development. The high mycorrhizal soil infectivity could be also a real potential for the traditional culture of taro in the *Pterocarpus* swamp forests (Saur and Imbert, 2003). From an ecological point of view, AM soil infectivity and seasonal dynamics of AM colonization should be considered further to better assess the role and the distribution of AMF in the *Pterocarpus* swamp forests.

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