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SHORT NOTE

Occurrence of arbuscular mycorrhizal fungi in bromeliad species from the tropical Atlantic forest biome in Brazil

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Abstract The mycorrhizal status of epiphytic, rupicolous, and terrestrial bromeliad species from the Brazilian Atlantic Rain Forest has been examined. Roots of 13 species of bromeliads were analyzed for the presence of mycorrhizal structures such as arbuscules, hyphae, and vesicles as well as other fungal structures. Rhizosphere soil was sampled to identify arbuscular mycorrhizal fungal (AMF) species associated only with terrestrial bromeliad species. Most specimens collected were epiphytic bromeliads in the genera *Aechmea*, *Bilbergia*, *Nidularium*, *Tillandsia*, and *Vriesea*. Differentiating structures of AMF were found in only three species of bromeliads. The pattern of mycorrhizal colonization was mainly internal, and external mycelium and arbuscules were observed only in the terrestrial *Nidularium procerum*. Root endophytes with dark brown septate mycelium, thin external hyphae, and *Rhizoctonia*-like sclerotia were also detected in some root segments. A total of ten spore morphotypes were recovered from the rhizosphere of *N. procerum*, with *Acaulospora mellea*, *A. foveata*, and *Glomus* sp. being the most common species recovered. Our study demonstrated that most of the epiphytic species are not associated with AMF. We attribute this mainly to the exposed bare root conditions found in epiphytic bromeliads.

Keywords Arbuscular mycorrhizal fungi · Brazilian Atlantic Rain Forest · Bromeliads · Root colonization · Root endophytes

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Introduction

Bromeliads (Family Bromeliaceae) are distributed in most ecosystems between the states of Virginia and Texas in the USA (latitude, 37°N) and the central area of Argentina and Chile (latitude, 44°S), with only one species occurring in Africa (Leme and Marigo 1993; Benzing 1994; Zomlefer 1994). The family is divided into three subfamilies, Bromelioideae, Pitcairnioideae, and Tillandsioideae, encompassing about 2,700 species in 46 genera (Judd et al. 1999). Approximately 40% of the bromeliads (1,200 species) occur in Brazil distributed mainly in the Atlantic Rain Forest and high-altitudes grasslands (Leme 1997), the former harboring the highest diversity and number of endemic bromeliad species on the planet (Cogliatti-Carvalho et al. 2001). In the southern part of Brazil, Santa Catarina State harbors 114 species of bromeliads, mainly in the Atlantic Forest ecosystem (Reitz 1983) where *Aechmea*, *Ananas*, *Nidularium*, *Tillandsia*, and *Vriesea* are the most frequent genera (Klein 1979).

The critical environmental conditions where members of this family evolved resulted in specializations not observed in any other plant group (Leme and Marigo 1993), especially considering the habitat of bromeliads, which ranges from strictly terrestrial (growing in the ground) to rupicolous (attaching themselves to rocks) and epiphytic (living on tree branches, tree crowns, and bark). The

epiphytic life form is prevalent within the family, and epiphytic bromeliads usually have a leaf architecture that allows them to accumulate water among leaves (forming the tank bromeliads) and organic material below the plant rosette, creating microclimatic conditions that can sustain a diverse biota (Leme and Marigo 1993). Bromeliad roots are structurally and functionally reduced. In terrestrial species, they are used in the uptake of water and nutrients, whereas in epiphytic and rupicolous species, they primarily serve to attach the organism to the substrate (Benzing 1980). However, the roots of epiphytic and rupicolous plants might also become absorptive when contacting a nutrient rich substrate (Reitz 1967). Taking into account that mycorrhiza is the rule in vascular plants, the reduced root condition of bromeliads as well as their occurrence in aberrant habitats (dry with low nutrient availability canopy or tree trunks) may elucidate our understanding of the dependence of plant species on mycorrhizal association (Janos 1993).

Bromeliads have been investigated according to spatial distribution (Dejean and Olmsted 1997), use of substrate (Zotz and Vollrath 2002), choice of habitat (Freitas et al. 1998), and diversity (Reitz 1983), but little is known on their mycorrhizal. Although widespread in all terrestrial ecosystems, in bromeliads of natural ecosystems, arbuscular mycorrhizal has barely been recorded in the literature. In a tropical forest of Mexico, Allen et al. (1993) found no evidence of arbuscular mycorrhizal fungal (AMF) colonization or ectomycorrhiza structures in three specimens of *Tillandsia bartramii*, *T. balbisiana*, and *Catopsis nutans* each. In Venezuela, Rabatin et al. (1993) sampled 19 specimens of *Aechmea lasseri*, *Vriesia splendens*, and *V. platynema* from a primary tropical premontane wet forest and detected growth of the fine endophyte *Glomus tenue* as the main AMF species colonizing epiphytic bromeliads. Camargo-Ricalde et al. (2003) observed low to medium levels of mycorrhizal colonization in three species of *Hechtia* occurring in a "matorral xerófilo" in south-central Mexico. Utilizing morphological and molecular approaches, Rowe and Pringle (2005) demonstrated that the epiphytic bromeliad *Vriesea werkleana* is associated with several AMF species, mainly pertaining to genus *Glomus*.

Despite the high diversity of this plant family in Brazilian ecosystems, especially in the Atlantic Forest biome, no systematic study has been carried out to analyze root colonization and AMF species associated with native bromeliads. In a tropical forest in the state of São Paulo, Trufem and Viriato (1990) detected 1–30% of root colonization in *Nidularium rubens* and found very few spores of *Acaulospora foveata* and *A. scrobiculata* associated with its rhizosphere. In this work, we quantify the presence of arbuscular mycorrhizal association in bromeliads occurring in three distinct habitats and identify AMF species occurring within their substrate.

Materials and methods

Study sites

Samples were collected from a secondary forest in Morro Geisler, Indaial, and in Parque Natural Municipal São Francisco de Assis, Blumenau, in the state of Santa Catarina in the south region of Brazil. Morro Geisler (63 m above sea level, 26°54′02.04″S, 49°13′14.13″W) has an area of 20 ha with an average annual precipitation of 1,660 mm and average annual temperature of 20.2°C. Parque São Francisco de Assis (54 m above sea level, 26° 55′15.9″S, 49°04′16.8″W) is located in the downtown area of Blumenau and comprises an area of 43 ha with an average annual precipitation and temperature of 1,456 mm and 20.4°C, respectively (GAPLAN 1986). Vegetation in both areas is covered by the Brazilian Atlantic Rain Forest (Veloso et al. 1991) with more than 116 tree species recorded.

Sampling

Epiphytic and rupicolous specimens were randomly collected in Morro Geisler, whereas terrestrial species were randomly sampled in Parque São Francisco de Assis between March 2003 and February 2004. For epiphytes, only about 30% of the root system (including root tips) was collected, and care was taken not to detach the entire plant from the phorophyte. Epiphytic specimens were growing at 1 to 4 m above the ground. Flowering individuals of plant species were lodged in Herbarium Dr. Roberto Miguel Klein at Universidade Regional de Blumenau. Roots were washed in running tap water and stored at 4°C until the processing. Roots were cleared in hot 10% KOH for 5 min, washed to remove excess of KOH, and further bleached in 3% of H_2O_2 for 5 min. After washing, roots were immersed in 1% HCl for 5 min and stained with acidic glycerin solution containing 0.05% of trypan blue (Koske and Gemma 1989). For each sample, roots were cut in 1-cm pieces, and 10 to 30 randomly selected segments were mounted on slides with polyvinyl lacto glycerol (PVLG). Roots were scored with a microscope for the presence of the following mycorrhizal structures: arbuscules, vesicles, intraradical, and extraradical hyphae. Presence of non-AMF fungal endophytes was also recorded and categorized in three main groups: dark septate endophytes (DSE), thin external blue hyphae, and *Rhizoctonia*-like sclerotia. Percentage of mycorrhizal root colonization and presence of endophytes was estimated as the number of segments

containing AMF or endophytic structures relative to the total number of segments analyzed for each species.

Spore extraction and trap cultures

Rhizosphere soil (about 300 ml, 0- to 20-cm depth) was collected only from terrestrial species to extract and identify AMF species. Spores were extracted by wet sieving (Gerdemann and Nicolson 1963) followed by sucrose gradient centrifugation (20/60%). From each sample, 100 ml of soil was homogenized with 2 l of water in a plastic bucket, and the suspension passed through two nested sieves with 710 μm and 45 μm opening. Material retained at the bottom sieve was centrifuged in the sucrose gradient at 2,000 rpm for 1 min. The supernatant was placed back on the 45-μm sieve and washed with tap water to remove excess of sucrose. Spores were collected under a dissecting microscope, mounted in slides with PVLG and PVLG mixed with Melzer's reagent, and identified following reference culture description of the International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi—INVAM (http://invam.caf.wvu.edu) and original species descriptions.

Trap cultures were established only for rupicolous species. Root segments and a portion of the organic debris below the plant rosette were mixed with sterilized sand, and the mixture was placed in 270 ml plastic tubetes and sown with *Sorghum sudanense* Stapf. After 3–4 months, 80 ml of the substrate was sampled to detect spore production as explained above. Traps were not established for epipythic and terrestrial species because all roots were used to assess colonization.

Results

Thirteen species of the bromeliad genera *Aechmea*, *Bilbergia*, *Nidularium*, *Tillandsia*, and *Vriesea* were sampled (Table 1), which represents the most common genera found in our study sites. From the 59 individuals collected, 39 were epiphytic, 15 rupicolous, and only 5 terrestrial. *Nidularium procerum* Lindm. was the only species with terrestrial habitat. *Aechmea calyculata* (E. Morren) Baker, *A. caudata* Lindm., *Nidularium innocentii* Lem., and *Vriesea ensiformis* (Vell.) Beer were the only species found both epiphytic and rupicolous. Epiphytic bromeliads occurred on ten distinct phorophytes including *Euterpe edulis* Mart. (Arecaceae), *Gymnanthes concolor* (Spreng.) Müll. Arg. (Euphorbiaceae), and *Copaifera trapezifolia* Hayne (Leguminoseae-Caesalpinoideae; Table 1).

Thirty percent (4 of 13) of bromeliad species were colonized by arbuscular mycorrhizal fungi (Table 1). Terrestrial specimens of *N. procerum* and rupicolous *N. innocentii* were highly colonized by mycorrhizal fungi with 75–95% of analyzed root pieces containing AMF structures (Table 2). Internal and external hyphae accounted for most

Table 1 Species of bromeliads collected in Morro Geisler and Parque São Francisco in the epipythic (E), rupicolous (R) and terrestrial (T) habitats

Bromeliad species	Habitat	N/n	Root Total	Root Myc	Phorophytes
Aechmea calyculata	E	4/0	120	0	*Euterpe edulis*
(E. Morren) Baker	R	4/0	120	0	
Aechmea caudata Lindman	E	5/0	135	0	*Hieronyma alchorneoides*
	R	2/0	60	0	
Aechmea nudicaulis (L.) Grieseb.	E	3/0	90	0	*Cabralea canjerana*
Aechmea recurvata (Klotzsch) L.B. Sm.	E	3/0	90	0	*Erythrina falcata*
Aechmea sp. 1	R	2/0	60	0	
Bilbergia distachia (Vellozo) Mez	E	3/0	75	0	not determined
N. innocentii Lem.	E	5/2	150	6	*Euterpe edulis*, *Gymnanthes concolor*, *Guapira opposita*
	R	5/5	150	113	
N. procerum Lindm.	T	5/5	150	145	
T. gardneri Lindl.	E	5/0	120	0	*Copaifera trapezifolia*, *Myrsine* sp.
Tillandsia stricta Sol. Ex Sims	E	3/0	75	0	*Schizolobium parahyba*
V. ensiformis (Vell.) Beer	E	5/0	150	0	*Myrcia pubipetala*, *Gymnanthes concolor*
	R	2/1	60	6	
Vriesea flammea L.B. Sm.	E	2/0	60	0	not determined
V. vagans (L.B. Sm.) L.B. Sm.	E	1/0	8	0	not determined

N/n Number of plants sampled per species/number of plants with mycorrhizal colonization
Root Total The total number of root segments observed for each species
Root Myc The number of root segments containing mycorrhizal colonization

Table 2 Pattern of mycorrhizal colonization detected in three bromeliad species

Bromeliad species	Habitat	Vesicles (%)	Hyphae (%)	Arbuscule (%)	Total mycorrhizal colonization (%)
N. innocentii Lem.	E	0	10.6	0	10.6
	R	37.8	71	0	75.2
N. procerum Lindm.	T	57	97	92	96.4
V. ensiformis (Vell.) Beer	R	1.5	45	0	20

Habitat: epiphytic (E), rupicolous (R), and terrestrial (T)

of the structures detected, whereas arbuscules were only seen in the terrestrial species. Vesicles occurred in all specimens containing mycorrhizal colonization, except for epiphytic *N. innocentii*, which had roots colonized solely by hyphae (Table 2).

Root segments of some specimens were randomly sampled and analyzed for the presence of root endophytes. The most common type of root endophytes was a DSE with brown septate hyphae growing among the cortex cells. DSE was detected in most epiphytic bromeliads except for *Aechmea calyculata*, *A. caudata*, and *Vriesea vagans*. In *N. innocentii* and *T. gardneri*, a very thin mycelium staining faint blue with trypan blue was detected abundantly and attached to the roots. *Rhizoctonia*-like sclerotia were also found confined in individual cells in both *Nidularium* species and *V. ensiformis* (Table 3).

From the rhizosphere soil of *N. procerum*, ten spore morphotypes were recovered, seven of which were identified to species and three to genus. The most common AMF species recovered was *Acaulospora mellea* Spain and Schenck followed by *Glomus* sp.1 with frequency of occurrence of 80 and 60%, respectively. Other species associated with *N. procerum* were *A. foveata* Trappe and Janos, *A. morrowiae* Spain and Schenck, *A. rugosa* Morton, *A. spinosa* Walker and Trappe, *Glomus aggregatum* Schenck and Smith, *G. clarum* Nicol. and Schenck, *Glomus* sp.2, and *Gigaspora* sp.1. After 3–4 months under greenhouse conditions, no sporulation was detected from trap cultures established with roots from rupicolous bromeliads.

Discussion

This study analyzes arbuscular mycorrhizal colonization in roots of bromeliad species occurring in the Brazilian Atlantic Rain Forest. We found mycorrhizal structures mainly in terrestrial and rupicolous species. Except for *N. innocentii*, no epiphytic species showed evidence of mycorrhizal colonization. Similar results were described by Allen et al. (1993) in a tropical forest in Mexico where no evidence of AMF or ectomycorrhizal structures was observed in canopy bromeliads. However, Rabatin et al. (1993) detected arbuscular mycorrhizal colonization, attributed mainly to *G. tenue*, in three species of tank bromeliads in a cloud forest in Venezuela. Epiphytes sampled in our study were found with exposed bare roots surrounding the phorophyte stem without being associated with organic debris. This probably explains the absence of mycorrhizal colonization, whereas the specimen sampled by Rabatin et al. formed "suspended gardens" (Leme and Marigo 1993) by deposition of plant debris, dead animal skeletons, etc. under the plant rosettes. Accumulation of organic matter seems to be important to provide a substrate where some kind of mycorrhizal association could develop (Allen et al.

Table 3 Number of root segments of bromeliad species colonized by root endophytes classified as DSE, thin external blue hyphae (TEBH) and *Rhizoctonia*-like sclerotia

Bromeliads species	Habitat	S[a]	DSE	TEBH	*Rhizoctonia*-like sclerotia
Aechmea calyculata (E. Morren) Baker	E	30			
	R	90			
Aechmea caudata Lindman	E	45			
Aechmea nudicaulis (L.) Grieseb.	E	30	10		
Aechmea recurvata (Klotzsch) L.B. Sm.	E	90	7		
B. distachia (Vellozo) Mez	E	75	21		
N. innocentii Lem.	E	150	6	3	
	R	120	10	1	9
N. procerum Lindm.	T	150	1		9
T. gardneri Lindl.	E	20	17	2	
V. ensiformis (Vell.) Beer	E	90	8		1
	R	60			2
V. vagans (L.B. Sm.) L.B. Sm.	E	8			

[a] *S* Number of root segments analysed

1993) and be of benefit to epiphytic bromeliads. Nevertheless, the presence of vesicles in *N. innocentii*, *N. procerum* and *V. ensiformis* suggest that AMF species can be associated potentially with Bromeliaceae.

The absence of mycorrhizal colonization in epiphytic bromeliads in our study can also be attributed to the low capacity of dispersion of AMF propagules (Allen 1991) and spatial heterogeneity in the epiphytic habitat (Janos 1993). In the study area, the soil representing the main source of AMF propagules was covered by litter and was not exposed, impairing the dispersion of spores or hyphae through soil movement to 1–4 m aboveground where epiphytic bromeliads were sampled. AMF spores have been found in fecal pellets of spiny rats (Mangan and Adler 2002), which may represent a mode of dispersion of spores from the soil to canopy areas. Spatial heterogeneity of the canopy in terms of nutrient availability, water conservation, and occurrence of light, as well as temperature and moisture gradients (Janos 1993) imposes strong selective pressure, possibly too strong for AMF. A third conjecture for the absence of AMF in epiphytic bromeliads is that their roots serve only for fixation to a given phorophyte, as nutrient and water uptake are carried out by leaf trichomes (Benzing 1976, 1980). In this scenario, carbon allocation to root system would be used for plant growth only rather than to maintenance of AMF growth. Rabatin et al. (1993) suggest the occurrence of mycorrhizal colonization in epiphytic bromeliads as an adaptation of some AMF species to the relatively dry canopy environment. We hypothesize that when epiphytic bromeliads have exposed roots, arbuscular mycorrhizal colonization is suppressed possibly due to very dry conditions of the microenvironment, which can favor growth and development of other kinds of fungi.

We attempted to quantify and identify non-AMF structures occurring in the roots of bromeliads including DSE. Allen et al. (1993) also detected coarse septate fungi in epiphytic bromeliads and considered them as non-pathogenic due to the lack of defense reaction of the roots. Jumpponen (2001) also concluded that dark septate endophytes can function similarly to mycorrhizal fungi under some environmental or experimental conditions. Hence, positive effects of DSE to epiphytic bromeliads should not be ruled out. Based on Jumpponen's assumption, we suggest that in the microenvironmental conditions experienced by the epiphytic bromeliads in this study, these root endophytes could be performing a role in exploring phorophyte bark, extracting nutrients through the production of enzymes and translocating these back to the host plant. According to this hypothesis, these endophytes would function as mycorrhizal fungi replacing the commonly found AMF as agents of nutrient uptake. We are aware that no experiment in controlled conditions was performed to test this, but further studies including molecular techniques and growth experiments in the greenhouse are required with epiphytic bromeliads and the endophytic component of their roots.

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References

Allen MF, Rincon E, Allen EB, Huante P, Dunn JJ (1993) Observations of canopy bromeliad roots compared with plants rooted in soils of a seasonal tropical forest, Chamela, Jalisco, Mexico. Mycorrhiza 4:27–28

Benzing DH (1976) Bromeliad trichomes: structure, function, and ecological significance. Selbyana 1:330–348

Benzing DH (1980) The biology of bromeliads. Mad River, Eureka, CA

Benzing DH (1994) How much is know about Bromeliaceae in 1994? Selbyana 15:1–7

Camargo-Ricalde SL, Dhillion SS, Jiménez-González C (2003) Mycorrhizal perennials of the "matorral xerófilo" and the "selva baja caducifolia" communities in the semiarid Tehuacán-Cuicatlán Valley, Mexico. Mycorrhiza 13:77–83

Cogliatti-Carvalho L, Rocha CFD, Freitas AFN, Rocha-Pêssoa TC (2001) As bromélias de Ilha Grande. Bromélia 6:7–11

Dejean A, Olmsted I (1997) Ecological studies on *Aechmea bracteata* (Swartz) (Bromeliaceae). J Nat Hist 31:1313–1334

Freitas CA, Scarano FR, Wendt T (1998) Habitat choice in two facultative epiphytes of the genus *Nidularium* (Bromeliaceae). Selbyana 19:236–239

GAPLAN (1986) Atlas de Santa Catarina. Rio de Janeiro: Aerofoto Cruzeiro (173 pp)

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Janos DP (1993) Vesicular-arbuscular mycorrhizae of epiphytes. Mycorrhiza 4:1–4

Judd WS, Campbell CS, Kellogg EA, Stevens, PF (1999) Plant systematics—a phylogenetic approach. Sinauer Assoc., Sunderland

Jumpponen A (2001) Dark septate endophytes—are they mycorrhizal? Mycorrhiza 11:207–211

Klein RM (1979) Ecologia da flora e vegetação do Vale do Itajaí. Sellowia (31):11–164

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488

Leme EM (1997) *Canistrum*. Bromélias da Mata Atlântica. Salamandra, Rio de Janeiro

Leme EMC, Marigo LC (1993) Bromélias na Natureza (Bromeliads in nature). Marigo Comunicação Visual, Rio de Janeiro

Mangan SA, Adler GH (2002) Seasonal dispersal of arbuscular mycorrhizal fungi by spiny rats in a neotropical forest. Oecologia 131:587–597

Rabatin SC, Stinner BR, Paoletti MG (1993) Vesicular–arbuscular mycorrhizal fungi, particularly *Glomus tenue*, in Venezuelan bromeliad epiphytes. Mycorrhiza 4:17–20

Reitz PR (1967) Raízes de *Tillandsia usneoides* (L.). Sellowia (19):99–100

Reitz PR (1983) Bromeliáceas e a malária-bromélia endêmica. Itajai: Herbario Barbosa Rodrigues (559p)

Rowe AR, Pringle A (2005) Morphological and molecular evidence of arbuscular mycorrhizal fungal association in Costa Rican epiphytic bromeliads. Biotropica 37:245–250

Trufem SFB, Viriato A (1990) Fungos micorrízicos vesículo–arbusculares da Reserva Biológica do Alto da Serra de Paranapiacaba, São Paulo, Brasil. Revista brasileira de Botânica 13:49–54

Veloso HP, Rangel Filho AL, Lima JCA (1991) Classificação da Vegetação Brasileira, adaptada a um sistema universal. Rio de Janeiro, Ed. IBGE

Zomlefer WB (1994) Flowering plant. Guide to families. University of North Carolina, Chapel Hill

Zotz G, Vollrath B (2002) Substrate preferences of epiphytic bromeliads: an experimental approach. Acta Oecol 23:99–102