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Mycorrhiza

Vesicular-arbuscular mycorrhizal fungi, particularly *Glomus tenue*, in Venezuelan bromeliad epiphytes

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Abstract. The mycorrhizal status of water-impounding "tank" bromeliad epiphytes from three locales differing in altitude and moisture regime within Venezuelan cloud forest was examined. Species of vesicular-arbuscular mycorrhizal (VAM) fungi found in arboreal soils were compared to VAM fungi found in terrestrial soils. Sixteen of the 19 epiphytes examined for the presence of VAM fungi had roots with infection stages; 14 of these specimens showed growth of the fine endophyte *Glomus tenue*. Fine endophyte was the only VAM fungus found associated with epiphytes in the driest locale studied, while coarse VAM fungi (*Gigaspora* and *Scutellospora* spp.) were found at sampling locales receiving more moisture. Root infection was usually composed of intercellular hyphae and peletons; few arbuscules were observed. However, abundant extracellular hyphae were often observed tangled about roots in arboreal soil. It is concluded that epiphytic bromeliads probably benefit, at least periodically, from VAM fungi scavenging for sporadically available nutrients in arboreal soils. *Glomus tenue* may be particularly important as a colonizing VAM fungus in drier sites of Venezuelan cloud forest. The species composition of VAM fungi in arboreal soils was different to that of terrestrial soils sampled directly under epiphytic bromeliad perches, suggesting that VAM fungi species associated with bromeliads are dispersed to their hosts by vagile animal vectors.

Key words: *Glomus tenue* – Epiphytic tank bromeliads – Arboreal soils – Vesicular-arbuscular mycorrhizal fungus dispersal – Animal vectors

Introduction

Bromeliads (Bromeliaceae) occupy diverse habitats throughout the New World (Reitz 1983). Since many of these habitats are poor in mineral nutrients, such as epiphytic perches, members of this plant family show a range of adaptations for nutrient acquisition, including water impoundment in "tanks" among their overlapping leaf bases from which soluble nutrients, particularly nitrogen, are absorbed by leaves. Roots also absorb nutrients from suspended soil and detritus that lodges among leaves and around the bases of plants (Benzing 1980). This study was undertaken to examine the mycorrhizal status of water-impounding "tank" bromeliad epiphytes collected from Venezuelan cloud forest, and to compare mycorrhizal associates observed to those in plant roots from the forest floor.

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Materials and methods

Site description

Research was carried out within the vicinity of Estacion Biologica Rancho Grande, Parque Nacional Henri Pittier, Aragua, Venezuela (10° 21′ N, 65° 41′ W) at elevations ranging from 1000 to 1200 m. Rancho Grande is an area of primary tropical premontane wet forest (Ewel and Madriz 1968), commonly characterized as cloud forest (Beebe and Crane 1947). The area has a dry season from December through May. Clouds cover the forest during the late afternoons of the dry season, although rain seldom falls. A botanical description of the park is given in Huber (1986). Three locales were studied: Rancho Grande and La Cumbre, both within several kilometers of the Rancho Grande Biological Headquarters, and Portacheulo. Portacheulo is on the watershed between the Caribbean Sea and Lake Valencia, northwest of the Rancho Grande Biological Station, and is representative of high and very moist elevations. Rancho Grande is representative of lower and drier elevations in the national park. La Cumbre is intermediate in altitude and precipitation between Rancho Grande and Portacheulo.

Sampling

Sampling of bromeliads for mycorrhizal colonization was done as part of a larger study designed to address the density and diversity of soil fauna in suspended soils associated with epiphytes, as compared to terrestrial soil-dwelling fauna (Paoletti et al. 1991). To sample bromeliads, nine trees were climbed with the aid of ropes and ladders at each of the locales. One bromeliad and associated roots, soil and bark were removed with a machete from each tree trunk at between 3 and 23 m above the ground. Specimens about 70 cm in diameter were selected, removed, sealed in large plastic bags and lowered to the ground. Beneath each tree, samples of forest floor litter and soil were taken to a depth of

10 cm. The dominant epiphytes of the area are the bromeliads *Aechmea lasseri*, *Vriesia splendens*, and *V. platynema.* The first two species were predominantly sampled at Portachuelo, while the latter was most commonly sampled at Rancho Grande. Roots and attached organic matter subsampled from bromeliads were transferred to vials of formalin/acetic acid (FAA) for preservation until they could be examined for the presence of VAM fungi.

Spore extraction and root examination

In the laboratory, material preserved in FAA was placed in dishes and examined microscopically for the presence of mycorrhizal fungi. Because terrestrial members of the Bromeliaceae are reported to be associated with VAM fungi (Mosse 1981), roots were washed and stained using a modification of the method of Phillips and Hayman (1970), and subsamples of stained roots were mounted on slides in lactoglycerol for examination using the compound microscope. Spores, mycelia and auxillary vesicles were mounted on slides in polyvinyl alcohol. Taxonomic identifications of VAM fungi are based on Schenck and Perez (1987).

Results

Sixteen of the 19 epiphytes examined for the presence of VAM fungi had roots with infection stages; 14 of these showed growth of the fine endophyte (Table 1; Fig. 1b–e). *Glomus tenue* (Greenall) Hall was the only VAM fungus found associated with bromeliads at the drier Rancho Grande site. The roots of three plants showed significant internal infection and this was localized within the root system. Two of these plants were solely infected with *G. tenue*. Most *G. tenue* infection was composed of intercellular hyphae with vesicles and spores (Fig. 1d, e), and few arbuscules. External fine endophyte hyphae could often be traced to organic matter adhering to the root surface (Fig. 1b).

Table 1. Occurrence of vesicular-arbuscular mycorrhizal (VAM) fungi, notably the fine endophyte *Glomus tenue*, in epiphytic bromeliads from Venezuelan neotropical cloud forest. + + +, Arbuscular infection present; + +, hyphal and vesicular infection; +, low infection intensity; –, no infection

Sampling site	Bromeliad no.	*Glomus tenue*	Other VAM fungi
Rancho Grande	2	+ + +	–
	4	+	–
	5	–	–
	6	+ + +	–
Portacheulo	10	+	+ +
	11	+ +	–
	12	–	+
	13	–	+ +
	14	+ + +	+
	15	+ +	+
	16	+ +	–
	17	+ +	–
	18	+ +	–
La Cumbre	39	+	+
	40	–	–
	41	–	–
	43	–	+
	44	+	–
	45	+	–

Table 2. Nutrient content of arboreal soil associated with bromeliads and soil from the forest floor for two of the study sites. The data are the means of 9 samples; differences between paired samples are by the *t*-test; NS, not significant, * significantly different ($P=0.05$), ** highly significantly different ($P=0.01$), *** very highly significantly different ($P=0.001$)

Nutrient	Portachuelo		Rancho Grande	
	Arboreal soil	Terrestrial soil	Arboreal soil	Terrestrial soil
Organic matter (%)	76.7	19.1***	74.0	8.56***
Total nitrogen (%)	2.1	0.6***	2.3	0.5***
P (mg/g soil)	20.3	15.8 NS	27.1	9.56**
K (mg/g soil)	177.9	134.9 NS	302.9	17.8*
Ca (mg/g soil)	108.8	350.0***	1625.6	1939.9 NS
Mg (mg/g soil)	208.1	111.9***	238.3	441.7*

Coarse VAM fungi were found associated with roots of bromeliads sampled at Portacheulo, the most moist of the sites sampled. Auxiliary cells of species of *Gigaspora* and *Scutellospora* were found on abundant mycelial growth tangled in organic soil from the bases of Portacheulo bromeliads; however, few spores were found and those were decomposed and difficult to identify. The VAM fungus community from the forest floor at Portacheulo did not include species identified from bromeliads. Spores of *Acaulospora morrowae* and an unidentified *Sclerocystis* sp. were recovered.

Nutrient contents of arboreal and terrestrial soils are shown in Table 2. A much higher content of organic matter was found in soils associated with bromeliads, but phosphorus (P), at least at Portacheulo, is not significantly higher in suspended soils. P is generally low in all soils at both sites, suggesting that epiphytes as well as terrestrial hosts benefit from the mycorrhizal relationship.

Discussion

Observations reported here on the incidence of VAM fungi associated with Venezuelan epiphytic bromeliads are similar to observations from other studies examining epiphytes for mycorrhizal associations. Epiphytes sampled from Costa Rican wet forest showed only low levels of root colonization, or lacked VAM. Epiphytes studied were from 14 plant families, including the Bromeliaceae, which are known to contain VAM fungus species (Lesica and Antibus 1990). Canopy soils associated with Costa Rican epiphytes were generally highly organic, acidic, and high in soluble P. Plant taxa which are facultatively mycotrophic can in all likelihood obtain nutrients without VAM fungal symbionts. In a separate study, Maffia et al. (1993), found low or no mycorrhizal infection in epiphytic Piperaceae collected from Costa Rican forest, despite the presence of VAM fungus spores and often abundant external hyphae in canopy soil associated with epiphytes. In this study, coarse VAM fungi infection appeared to be lim-

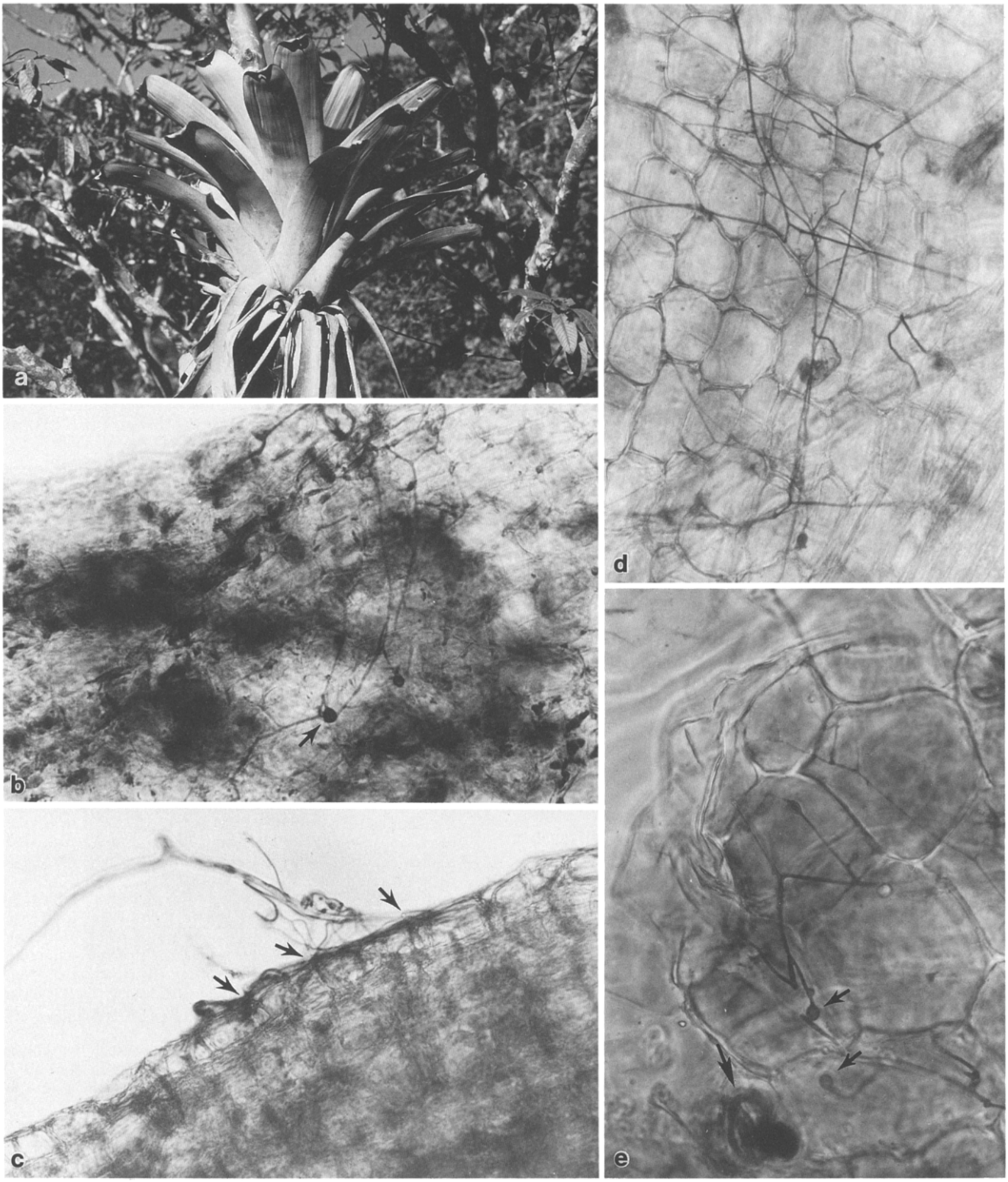

Fig. 1. **a** A specimen of the epiphytic bromeliad, *Vriesia playnema*, at Rancho Grande. **b** External hyphae and spore (*small arrow*) of the fine endophyte, *Glomus tenue*, tangled in organic matter adhering to the outer surface of a bromeliad root; ×160. **c** *G. tenue* external hyphae and penetration points (*small arrows*); ×160. **d** *G. tenue* intercellular hyphae; ×160. **e** *G. tenue* intercellular hyphae, vesicles and spores (*small arrows*); ×160

ited principally to hyphae and peletons and was often connected to an abundant external mycelium supporting spores and auxillary vesicles.

Terrestrial bromeliads have been little studied with regard to their response to VAM fungus infection. Mosse (1981) suggested that pineapple, *Ananas comosus*, was highly dependent on the VAM fungal symbiosis. However, more recent evidence indicates that growth stimulation of pineapple relatable to VAM fungus inoculation is only achieved at very low soil solution P (Aziz et al. 1990). These researchers suggested that the low dependency of pineapple on VAM fungus infection may be explained by its very low growth rate, a feature shared with epiphytic tank bromeliads. Slow growth rate and a facultative dependence on mycorrhizae are likely important adaptations for colonization of epiphytic habitats.

G. tenue is an unusual and interesting VAM fungus, both on morphological and ecological grounds. It is identified by narrow, angular thread-like hyphae 0.5–1.5 μm in diameter, and a reticulate, characteristic infection pattern in stained roots. In addition, *G. tenue* forms small spores and vesicles 10–12 μm in diameter (Hall 1977). Ecologically the fine endophyte has been identified as a primary colonizer of particularly low nutrient sites such as mine spoils (Daft and Nicolson 1974), as well as a colonizer of uninoculated greenhouse pots (Baylis 1967; Johnson 1977; Powell and Daniel 1978). Johnson (1977) and Rabatin (1980) identified hyphae and spores of *G. tenue* growing in association with moss collected from tree branches in New Zealand forest and the soil surface of a nutrient depleted hay field in Pennsylvania, USA, respectively.

G. tenue may benefit growth of epiphytic bromeliads in Venezuelan cloud forest by growing rapidly and spreading throughout highly organic suspended soils as well as root cortical cells. The cost in terms of carbohydrate to the symbiont may be outweighed by the immediate benefit to the epiphyte in terms of absorption of only sporadically available nutrients. At Rancho Grande in particular, where moisture in suspended soil associated with bromeliads may often be minimal, the benefit associated with *G. tenue* scavenging of nutrients may be limited but significant.

The dissimilarity between the species of VAM fungi identified from epiphytic bromeliads as contrasted with those associated with terrestrial plant roots suggests that VAM fungi colonizing suspended soils possess adaptations to the fluctuating but relatively drier soil microenvironment of the canopy. Moreover, the differing VAM fungi species composition of arboreal versus terrestrial soil suggests that animal vectors may disperse VAM fungi propagules to epiphytes. Invertebrates, particularly soil-dwelling macroarthropods such as beetles and millipedes as well as earthworms, are known to ingest and excrete viable VAM fungi spores (Rabatin and Stinner 1988, 1991; Reddell and Spain 1991). Ants may be important dispersers of VAM fungi in these sites; they comprised the most numerically conspicuous macroinvertebrate taxa associated with epiphytic soils at Portacheulo; however, beetles were more dominant at Rancho Grande (Paoletti et al. 1991). *G. tenue*, because of its small spore size among VAM fungi and because it has been repeatedly reported as a primary colonizer, may be commonly spread by invertebrate vectors.

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