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Vesicular-arbuscular mycorrhizae of epiphytic and terrestrial Piperaceae under field and greenhouse conditions

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Abstract. We examined the roots of 27 epiphytic and terrestrial species of Piperaceae collected in primary and secondary habitats in Monteverde, Costa Rica. Terrestrial roots of only two of the nine *Peperomia* species, two of eight *Piper* species, and of *Pothomorphe umbellatum* contained internal vesicles and/or arbuscules. We did not find internal vesicles and/or arbuscules in 3024 cm of fine roots of epiphytic Piperaceae, even though 15% of these root segments had associated external typical glomalean hyphae. *Glomus* and *Acaulospora* spores, and *Gigaspora* auxiliary cells occurred in both canopy and terrestrial habitats. After inoculation of a low nutrient substrate, the facultatively epiphytic *Peperomia costaricensis* averaged 23% mycorrhizal root length. Relatively high atmospheric inputs of dissolved inorganic nutrients that alleviate the requirement for mycorrhizae, and heterogeneity of mycorrhiza inocula in the canopy may explain the absence of mycorrhizae from epiphytic Piperaceae. We suggest that the Piperaceae comprises predominantly facultatively mycotrophic species, and that facultative mycotrophism facilitates their radiation to the canopy.

Key words: Vesicular-arbuscular mycorrhizae – Piperaceae – Epiphytes – Facultative mycotrophy – Cloud forest

Introduction

In order to survive under oligotrophic conditions presumed to exist in forest canopies (Benzing 1981, 1984), epiphytes have developed morphological and physiological adaptations for garnering and retaining nutrients (Benzing 1987, 1990). Epiphytes derive all of their nutrients, or nearly all, from atmospheric sources such as rain and mist (Nadkarni and Matelson 1991) which are generally dilute, especially in phosphorus (Vitousek and Sanford 1986). Vesicular-arbuscular mycorrhizal fungi (VAM) are one means by which epiphytes might increase nutrient uptake. The ability of VAM to reach the canopy is uncertain, however, because wind is unlikely to move VAM spores in forested regions (Hetrick 1984; Allen 1991).

We surveyed the occurrence of VAM among 27 species of Piperaceae. We focused on the Piperaceae because it is speciose (Burger 1971) and includes many epiphytes. Some of these are facultatively epiphytic – found in both epiphytic and terrestrial habitats – which allows for comparison of VAM between the canopy and the ground. Preliminary work (Maffia 1990) documented low levels of VAM of terrestrial *Pothomorphe umbellatum* L. (Piperaceae), and revealed VAM (i.e., internal and external hyphae, vesicles and spores) in randomly collected root samples from canopy root mats.

Several authors have examined members of the Piperaceae for VAM. St. John (1980) noted VAM colonization in terrestrial black pepper *Piper nigrum* L. in Brazil. Garcia and Vazquez-Yanes (1985) found two terrestrial *Pothomorphe* Miq. and five terrestrial *Piper* L. species to have VAM in Veracruz, Mexico. Mohankumar and Mahadevan (1987) found one terrestrial *Pothomorphe* and two terrestrial *Piper* species to contain VAM in India. Bermudes and Benzing (1989) examined three species of epiphytic *Peperomia* Ruiz & Pavon. from two Ecuadorian rain forest sites. They found 10% colonization for *Peperomia macrostachya* (Link) Trel. & Yuncker and an unidentified *Peperomia* species, but failed to find colonization in *Peperomia tropaeolifolia* Sodiro. Lesica and Antibus (1990) did not find VAM in nine species of *Peperomia* from Costa Rica. They collected five of these species from tree trunks in a montane cloud forest, and the other four from trunks, canopy organic mats, and bare limbs of trees in a lowland rain forest. Michelsen (1993) did not find VAM in two epiphytic *Peperomia* species in

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Ethiopia, but Nadarajah and Nawawi (1993) did find VAM in both epiphytic and terrestrial *Peperomia pellucida* (L.) H.B.K.

In addition to examining field collections for VAM, we investigated mycorrhiza formation by a single species, the facultatively epiphytic *Peperomia costaricensis* C.DC., by growing it in a low nutrient substrate with two types of inoculum.

Materials and methods

Study area

We collected roots of both epiphytic and terrestrial Piperaceae in and adjacent to the Monteverde Cloud Forest Reserve (MVCFR) located in west-central Costa Rica (10° 12′ N, 84° 42′ W) on the Cordillera de Tilaran at elevations from 1400 m to 1850 m. Lawton and Dryer (1980) characterize MVCFR as a lower montane cloud forest with a "dry season" from January to May. Fog and clouds almost continually envelop the upper elevations in both the wet and dry seasons. Annual rainfall is 2450 mm.

Field survey

We sampled primary forest in a Leeward Cove Forest within MVCFR. We collected from three different types of secondary vegetation: trees in open pastures, secondary forest, and along roadsides. We collected roots of seventeen species of *Peperomia*, eight species of *Piper*, and one species each of *Pothomorphe* and *Sarcorachis* Trel. (Table 1) in May 1988. Dr. W. Burger of the Chicago Field Museum confirmed identifications. We deposited voucher specimens at the University of California, Santa Barbara (UCSB) and at the Field Museum of Natural History in Chicago (F).

We gathered roots of epiphytes from portions of fallen branches that were not touching the ground and from the canopy by using mountain climbing techniques (Perry 1978). We collected in the canopy of primary forest, 16–23 m above the forest floor and 0–3 m from trunks. We collected epiphytes from a variety of substrate types including bare branches, tree trunks, and interwoven "mats" of live roots and humus that occurred on horizontal branches. We collected as much of each plant's fine root system as possible.

Root processing

We separated apparently living roots <2 mm in diameter following the protocol of St. John and Uhl (1983). We preserved all samples in vials of formalin-propanol-acetic acid within 3 h of collection. Our procedure for clearing and staining preserved roots is similar to those of Phillips and Hayman (1970) and Kormanik et al. (1980). We determined the amount of mycorrhizal colonization of a plant by recording the presence or absence of VAM structures (e.g., external hyphae, internal hyphae, internal vesicles, or arbuscules) in 1-cm sections of root. We considered a root segment to be mycorrhizal when internal vesicles and/or arbuscules were present, as did St. John and Uhl (1983). We did not attempt to extract spores of VAM from substrates by sieving, but we noted extramatrical spores and auxiliary cells associated with root segments.

Greenhouse experiments

We investigated whether or not *Peperomia costaricensis* could become mycorrhizal under greenhouse conditions. Greenhouse work took place over a 4-month period from April to July, 1989. We took 45 *Peperomia costaricensis* cuttings, (10–15 cm tall with 3–5 leaves) from two adult plants that had been initially collected from the canopy in MVCFR and then maintained in pots at the UCSB greenhouse for approximately 2 years. The planting medium was a 1:1:2 peat-vermiculite-sand mixture with no added fertilizer which we sterilized by twice heating to 90° C for 40 min. The planting medium pH was 7.1. We placed cuttings in planting medium in 2″ pots, and watered them twice a week with distilled water.

We used two types of VAM inoculum: (1) a commercial inoculum ("Nutri-link," from Native Plant Institute, Salt Lake City, Utah) of *Glomus intraradix* Schenck and Smith spores embedded in a clay matrix, and (2) soil containing root fragments collected from a secondary forest floor site in MVCFR. The MVCFR inoculum included the roots and associated soil of various 20- to 30-cm-tall, light-gap seedlings. We used the MVCFR inoculum within 2 weeks of collection. One set of 20 cuttings received 5 g of "Nutri-link" per 2″-pot, equivalent to approximately 5000 live spores per pot. This is probably many more spores than would occur in a comparable volume of soil in the field (Janos 1980). A second set of 25 cuttings received 20 g of soil inoculum from MVCFR. To stimulate root growth, we trimmed root balls and dipped them in a rooting hormone prior to transplanting. After 9 weeks, we randomly selected six "Nutri-link" inoculated cuttings and 10 field inoculated cuttings for root examination.

Results

Field survey

Of the 27 species examined, two terrestrial *Peperomia* species, two terrestrial *Piper* species and *Pothomorphe umbellatum* contained internal vesicles and/or arbuscules (Table 1). Overall, colonization was extremely low with only 21 cm of 4867 cm of fine roots examined having internal colonization, a colonization rate of less than 1%. The terrestrial species, *Pothomorphe umbellatum* with 7% colonization, accounted for half of all internal colonization found.

In contrast, we found moderate amounts of typical coarse VAM external hyphae associated with both epiphytic and terrestrial root segments. Approximately 15% of all epiphytic and 13% of all terrestrial Piperaceae root segments had associated external hyphae. For epiphytic *Peperomia*, we found external hyphae on 3% of root segments from bare branches, but on 9% of *Peperomia* root segments collected from organic "mats". The amounts of terrestrial external hyphae that we found may underestimate their presence, because we had to vigorously clean these roots. We occasionally noted the presence of unstained clear external hyphae (see Morton and Benny 1990), but did not include these in our counts, and may also have contributed to underestimation of external hyphae presence.

We found *Glomus* Tulasne & Tulasne and *Acaulospora* Gerdemann & Trappe emend. Berch spores, and *Gigaspora* Gerdemann & Trappe emend. Walker & Sanders auxiliary cells in both terrestrial and epiphytic habitats, although fewer than 1% of root segments had associated spores (Table 1).

Table 1. The occurrence of vesicular-arbuscular mycorrhiza structures in Piperaceae of Monteverde Cloud Forest Reserve in Costa Rica. *T*, Terrestrial; *E*, epiphytic; *No. plants*, number of plants examined; *No. segs*, number of 1-cm root sections examined; *InV/Arbs*, number of segments in which we found internal vesicles and/or arbuscules; *ExHy*, number of segments on which we observed external hyphae; *Spores*, number of segments with which spores were associated

	T/E	No. plants	No. segs.	InV/ Arbs	ExHy	Spores
Peperomia Ruiz & Pavon						
angularis-complex	E	1	52	0	39	0
angularis C.DC.	E	1	21	0	17	0
costaricensis C.DC.	E	17	564	0	73	10
	T	1	53	0	0	0
dotana Trel.	E	2	93	0	0	0
hernandifolia A. Dietr.	E	2	100	0	12	0
	T	4	212	1	42	5
hylophila C.DC.	E	10	390	0	97	3
	T	1	26	0	0	0
lancifolia Hook.	T	5	205	0	78	0
peltilimba C.DC.	E	15	618	0	19	3
pittieri C.DC.	E	1	13	0	0	0
poasana C.DC.	T	2	70	0	0	0
pseudo-alpina Trel.	E	3	195	0	72	5
	T	2	96	1	2	2
reptabunda Trel.	E	3	168	0	19	1
	T	1	37	0	0	0
rhombea Ruiz & Pavon	T	1	24	0	12	0
rotundifolia (L.) HBK	E	3	83	0	2	0
serpens (Sw.) Loud.	E	59	348	0	24	1
	T	1	75	0	0	0
tenella (Sw.) A. Dietr.	E	2	54	0	44	1
tetraphylla (G. Forst) Hook.	E	2	162	0	1	0
Peperomia Total	E	77	2861	0	419	24
	T	18	798	2	134	7
Piper L.						
biseriatum C.DC.	T	3	92	0	4	4
epigynum C.DC.	T	1	65	0	20	0
gibbosum C.DC.	T	1	28	0	0	0
glabrescens (Miq.) C.DC.	T	5	197	5	23	0
hispidium Sw.	T	5	123	3	8	1
lanceaefolium HBK	T	3	82	0	30	0
otophorum C.DC.	T	5	285	0	14	1
subsessilifolium C.DC.	E	3	130	0	9	0
	T	1	23	0	0	0
Piper Total	E	3	130	0	9	0
	T	24	895	8	99	6
Pothomorphe Miq.						
umbellatum L.	T	3	150	11	13	3
Sarcorachis Trel.						
naranjoana (C.DC.) Trel.	E	1	33	0	15	0
Piperaceae Total	E	81	3024	0	443	24
	T	45	1843	21	246	16

Greenhouse experiment

Our greenhouse inoculation experiment produced higher amounts of internal colonization with both "Nutri-link" and native root inocula than found in field-collected *Peperomia costaricensis*. All six plants sampled that we had inoculated with "Nutri-link" had VAM with colonization of individuals ranging from 10% to 35%. The average colonization rate was 24% (130 segments of 537 segments examined). Only four of the 10 plants that we had inoculated with native roots and soil produced mycorrhizae. Colonization values of these ranged from 2% to 28%. The average colonization rate for these plants with VAM was 19% (33 segments of 170 segments examined). Amounts of colonization produced by the two types of inocula did not significantly differ (Mann-Whitney U-test, $U=10$, $n_1=6$, $n_2=4$, $P=0.593$).

Discussion

Although spores of VAM and their external hyphae are present in MVCFR in both terrestrial and canopy sites, VAM are not prevalent in the Piperaceae. The extremely low colonization of roots of Piperaceae suggests no consistent pattern of usage of VAM by terrestrial or epiphytic plants, in primary or secondary vegetation. Only *Pothomorphe umbellatum*, a terrestrial treelet which we collected in secondary vegetation, had a low percentage (7%) of VAM. Our greenhouse study shows, however, that *Peperomia costaricensis*, which did not have VAM in our field samples, can form moderate amounts of VAM when inoculated in an infertile substrate.

Two non-exclusive hypotheses may explain the paucity of VAM on Piperaceae in the field. First, low colonization levels could be a consequence of relatively high site fertility. High atmospheric inputs of dissolved inorganic nutrients, characteristic of tropical cloud forests during certain times of the year (Kellman et al. 1982; Clark and Nadkarni 1992), may make VAM unnecessary to meet nutrient requirements. MVCFR canopy mat $CaCl_2$ – extractable phosphate (PO_4) values range from 57 to 111 ppm (Lesica and Antibus 1990), well above typical tropical forest floor phosphate values (Vitousek 1984). Second, VAM formation in the canopy might be limited by a lack of inoculum, as has been suggested by Benzing (1983) and Lesica and Antibus (1990). Extensive colonization (30–70%) of *Anthurium*, *Begonia*, and *Columnea* species (Lesica and Antibus 1990; Maffia 1990), however, contravenes this suggestion.

We hypothesize that the canopy comprises a "habitat mosaic" (Janos 1993). Developing canopy mats accruing vegetation might progressively accumulate VAM inocula dispersed by any of several potential vectors [e.g., birds, ants, small mammals (McIlveen and Cole 1976; Allen 1991)]. The greatest inoculum potentials in the canopy should occur in old growth forests with low tree and branch turnover. Chance deposition

of large numbers of VAM propagules, however, would cause a "young" canopy mat to have a high inoculum potential, but we predict that this occurs less often than gradual accretion of inoculum. Thick canopy root mats in MVCFR take years to develop, and may last for 20–30 years.

Although spatially patchy, canopy mats with abundant VAM might serve as inoculum sources through one-way, downward transfer by stemflow and throughfall. Spores or hyphae might be dislodged during rain events and swept down branches and trunks in stemflow, inoculating mats or epiphytes below. This mechanism might explain the presence of VAM on isolated trunk and lower limb epiphytes not rooted in canopy mats (Lesica and Antibus 1990; Maffia 1990). The trapping of even one spore (see Daft and Nicolson 1969; Sieverding 1991) on the moist underside of a root might be sufficient to form mycorrhizae, and to begin the inoculum accretion process. This postulated movement and establishment via stemflow is analogous to the *Glomus*-root slurry technique (Sylvia and Jarsfter 1990) used to inoculate aeroponically grown roots.

That some epiphytes have moderate to high numbers of VAM suggests that the inoculum potential of some canopy mats is adequate for mycorrhizal formation. In ecosystems with stable canopy mats, epiphyte species that specialize on such mats could be obligately mycotrophic (see Baylis 1975; Janos 1980). Consequently, a canopy inoculum mosaic could influence the distribution of epiphytic species in a way similar to that suggested for terrestrial habitats by Janos (1980).

This study has shown that VAM are rare or absent on both epiphytic and terrestrial Piperaceae in MVCFR, although many species of Piperaceae are capable of forming VAM as shown by our greenhouse work and other investigators (St. John 1980; Garcia and Vasquez-Yanes 1985; Mohankumar and Mahadevan 1987; Bermudes and Benzing 1989; Nadarajah and Nawawi 1993). This suggests that the Piperaceae predominately comprises facultatively mycotrophic species. Radiation to the canopy by members of the Piperaceae may be facilitated by facultative mycotrophism which allows them to inhabit sites such as bare branches, tree trunks or newly formed root mats that may lack VAM inoculum.

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