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Mycorrhizae in Hawaiian Epiphytes[1]

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ABSTRACT: In surveys in the Hawaiian Islands, mycorrhizae occurred frequently in epiphytic, nonorchidaceous angiosperms and pteridophytes. Both vesicular-arbuscular (VA) and ericoid mycorrhizae were present in epiphytes growing 1–3 m above the forest floor on dead and living tree trunks and on living tree ferns in montane wet forest sites. All eight angiosperm species were mycorrhizal, and 13 of 22 pteridophytes possessed VA mycorrhizae. The high frequency of mycorrhizae in epiphytic species suggests that propagules of mycorrhizal fungi routinely are dispersed to these microsites. Possible means of dispersal are discussed.

IN THE MONTANE WET forests of the Hawaiian Islands a dense epiphytic flora of angiosperms and pteridophytes flourishes. Intensive studies of Hawaiian angiosperms and pteridophytes growing in a variety of habitats (Gemma et al. 1992, Koske et al. 1992) revealed that a high percentage of these species form vesicular-arbuscular mycorrhizae (VAM). In those surveys mycorrhizae were found to be common among the epiphytes. Because VA and ericoid mycorrhizae seldom are reported to occur in epiphytic, nonorchidaceous angiosperms and pteridophytes (Boullard 1957, Cooper 1976, Nadkarni 1981, Berch and Kendrick 1982, Trappe 1987, Lesica and Antibus 1990, Maffia et al. 1990), we were prompted to sample a greater diversity of species to better assess the prevalence of mycotrophy among Hawaiian epiphytes.

MATERIALS AND METHODS

Root samples (10–40 cm long) were collected from plants growing on living and dead trunks of trees (mostly of *Metrosideros polymorpha* Gaud.) and tree ferns (*Cibotium glaucum* [J. E. Sm.] Hook. & Arnott) in montane wet forests between July 1987 and June 1990. Roots of the epiphytes typically were growing in thick mats of moss that covered the trunks. Sampled plants were attached to their substrate at elevations of 1–3 m above ground level. Care was taken during collecting to sample individual plants for which roots could be positively identified as belonging to the particular plant. Most collections were made in the Kōke'e and Alaka'i Swamp areas (Kōke'e State Park) at elevations of ca. 1100 m above sea level on the island of Kaua'i. A few samples were collected from similar sites on the islands of Maui and Moloka'i.

Roots were preserved in 50% isopropyl alcohol and later were cleared in hot 2.5% KOH and stained with 0.05% trypan blue in an acidified glycerol solution (Koske and Gemma 1989). Stained roots were mounted on slides in a polyvinyl alcohol-based solution (PVLG) (Koske and Tessier 1983). Entire root systems were examined at 40–60 × with a dissecting microscope, and portions of each root system were examined at 400 × with a compound microscope. The presence of vesicles, arbuscules, hyphal coils, and internal hyphae was noted for each specimen. Only those species in which arbuscules were found were considered to have functional VAM. Extent of VAM infection was quantified by assigning a mycorrhizal index (MI) value of 0–3 to stained root samples, where 0 indicates no VAM infection, 1 = up to 25% of root length infected (i.e., containing any

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combination of the VAM fungal structures listed above), 2 = 25–50% infected, and 3 = >50% infected. The MI of each species was calculated by averaging the MI of each sample of the species examined.

Differences between intensity of infection of Anthophyta and Pterophyta were analyzed using a *t* test, and differences in incidence of mycorrhizae in members of the two groups were assessed using the chi-square test, adjusted for small sample size. For both tests, significance was indicated when $P < 0.05$.

When ericoid mycorrhizae were found in roots, their presence was noted, but no rating of intensity of infection was assigned.

Voucher slides of roots have been preserved in the authors' collection. Voucher specimens of most plant materials are lodged in the herbarium at the National Tropical Botanical Garden, Lāwa'i, Kaua'i. Nomenclature for angiosperms is based on Wagner et al. (1990) and for pteridophytes on Wagner (1981) and Takhtajan (1986). Some data for the pteridophytes that are included in the results section have been reported previously (Gemma et al. 1992). They are cited here for completeness.

RESULTS AND DISCUSSION

VAM occurred in 62 of the 91 root systems examined. They were present in every angiosperm specimen examined (31 samples encompassing eight species) and in 13 of the 22 fern species examined (Tables 1, 2). Among the angiosperms, a high MI was recorded for *Astelia menziesiana* Sm., *Cheirodendron* sp., *Coprosma kauensis* (A. Gray) A. Heller, *Dianella sandwicensis* Hook. & Arnott, *M. polymorpha*, and *Peperomia hesperomannii* Wawra (Table 2). All 13 samples of *M. polymorpha* were densely colonized by VAM fungi. Both VA and ericoid mycorrhizae were present in all collections of *Vaccinium calycinum* Sm. and *Styphelia tameiameiae* (Cham. & Schlec.) F. v. Muell., members of families whose members typically form only ericoid mycorrhizae (Harley and Smith 1983). VAM previously have been reported to occur in these two species in Hawai'i (Koske et al. 1990).

Among the pteridophytes, *Vittaria elongata* Sw., *Mecodium recurvum* (Gaud.) Copel., *Elaphoglossum aemulum* (Kaulf.) Brack, and *E. crassifolium* (Gaud.) Anderson & Crosby had the highest MI values (Table 2). Rhizome scales of *E. wawrae* (Luerss.) C. Chr. often contained hyphae and vesicles of VAM fungi. Mycorrhizae were present in the epiphytic lycopod *Huperzia phyllantha* (Hook. & Arnott) Holub. and absent from *Psilotum complanatum* Sw.

The average MI of all specimens was significantly higher for the angiosperms (2.3) than for the pteridophytes (1.1), as was the frequency of mycorrhizae in the specimens examined (Table 1). Mycorrhizae were more common in the endemic species (18 of 23) than in the indigenous species (three of seven), but the difference was not significant.

TABLE 1

SUMMARY OF OCCURRENCE OF MYCORRHIZAE IN HAWAIIAN EPIPHYTES

PARAMETERS	ANTHOPHYTA	PTEROPHYTA[a]	TOTAL
No. species examined	8	22	30
No. species mycorrhizal[b]	8a	13a	21
Average MI of species[c]	2.3a (±0.6)	1.1b (±1.1)	1.6 (±1.1)
No. specimens examined	31	60	91
No. specimens mycorrhizal	31a	31b	62

[a] Also includes Lycopodophyta and Psilophyta.
[b] VA and ericoid mycorrhizae; values in rows followed by the same letter did not differ significantly ($P = 0.05$).
[c] MI, mycorrhizal index (see text). SD in parentheses.

TABLE 2

MYCORRHIZAE IN HAWAIIAN EPIPHYTES

TAXA	M/n[a]	MI[b]	STATUS[c]
Anthophyta			
Araliaceae			
Cheirodendron sp.	1/1	3.0	E
Epacridaceae			
Styphelia tameiameiae (Cham. & Schlec.) F. v. Muell.	2/2[d]	1.5	I
Ericaceae			
Vaccinium calycinum Sm.	2/2[d]	1.5	E
Liliaceae			
Astelia menziesiana Sm.	5/5	3.0	E
Dianella sandwicensis Hook. & Arnott	2/2	2.5	E
Myrtaceae			
Metrosideros polymorpha Gaud.	13/13	2.3	E
Piperaceae			
Peperomia hesperomannii Wawra	4/4	2.3	E
Rubiaceae			
Coprosma kauensis (A. Gray) A. Heller	2/2	2.5	E
Psilophyta			
Psilotaceae			
Psilotum complanatum Sw.	2/0	0	I
Lycopodophyta			
Lycopodiaceae			
Huperzia phyllantha (Hook. & Arnott) Holub.	1/1	1.0	E
Pterophyta			
Ophioglossaceae			
Ophioglossum pendulum L.	3/4	2.3	I
Vittariaceae			
Vittaria elongata Sw.	2/2	3.0	I
Gramittidaceae			
Adenophorus abietinus (D.C. Eaton) K. A. Wilson	1/2	1.5	E
A. pinnatifidus Gaud.	0/3	0	E
A. tamariscinus (Kaulf.) Hook & Grev.	4/5	1.2	E
Grammitis tenella Kaulf.	1/5	0.4	E
G. baldwinii (Baker) Copel.	0/1	0	E
Xiphiopteris saffordii (Maxon) Copel.	0/2	0	E
Polypodiaceae			
Pleopeltis thundbergiana Kaulf.	0/1	0	I
Hymenophyllaceae			
Mecodium recurvum (Gaud.) Copel.	2/2	3.0	E
Sphaerocionium lanceolatum (Hook. & Arnott) Copel.	3/5	1.0	E
Vandenboscia davalliodes (Gaud.) Copel.	0/1	0	E
V. cyrtotheca (Hillebr.) Copel.	0/1	0	E
Aspleniacae			
Asplenium nidus L.	0/1	0	I
Elaphoglossaceae			
Elaphoglossum aemulum (Kaulf.) Brack	4/4	2.5	E
E. alatum Gaud.	3/4	1.2	E
E. crassifolium (Gaud.) Anderson & Crosby	2/2	3.0	E
E. hirtum (Sw.) C. Chr.	0/4	0	I
E. pellucidum Gaud.	2/3	0.9	E
E. wawrae (Luerss.) C. Chr.	2/3	1.7	E

[a] M, no. of specimens with vesicular-arbuscular mycorrhizae; n, no. of specimens examined.
[b] MI, average mycorrhizal index for the species (see text).
[c] E, endemic; I, indigenous.
[d] All specimens bore both ericoid and VA mycorrhizae.

The frequent occurrence of VAM in Hawaiian epiphytes suggests that the fungi are easily dispersed to the mossy mats that blanket the dead and living vegetation. Elevation of the substrate above the soil surface did not appear to correlate with MI, and MI values of 3.0 were recorded from specimens collected at all sampling heights.

The means by which propagules of mycorrhizal fungi reach epiphytic habitats are not known. However, Hawaiian birds collect and transport fern rhizomes and roots, mosses, and other plant materials for nest building (van Riper and Scott 1979, Carlquist 1980). Because propagules of VAM fungi (e.g., hyphae, vesicles, spores) may be found on or in such nesting material and in adherent soil (Parke and Linderman 1980, Gemma and Koske 1990, Gemma et al. 1992), it is possible that birds disperse the fungi to habitats suitable for epiphytic plants. Other animals (e.g., arthropods and small mammals) also may be similarly involved in transport of the fungi (McIlveen and Cole 1976, Trappe and Maser 1976, Rabatin and Stinner 1988).

At a potential epiphytic microsite, plant and fungus could arrive independently of each other or may co-disperse (Koske and Gemma 1990). The latter would result if a vegetative fragment of a plant (e.g., a rhizome) or a young seedling/sporophyte bearing mycorrhizal fungi were deposited at a site. As the young plant began to grow, newly formed roots would become infected with mycorrhizal fungi growing from old roots or from soil adhering to the plant. Such co-dispersal has been documented in rhizomatous species that are primary colonizers of sand dunes (Gemma and Koske 1989, Koske and Gemma 1990).

The high frequency of mycorrhizae in the collections indicates that VAM fungi are constant, predictable inhabitants of the epiphytic community in Hawaiian wet forests. Similar results were observed among terrestrial species in Hawai'i, where >90% of the angiosperms and 75% of the pteridophytes were mycorrhizal (Gemma et al. 1992, Koske et al. 1992).

Until now, VAM generally have been thought to be rare or absent in epiphytic pteridophytes (Boullard 1957, Cooper 1976, Berch and Kendrick 1982). The mycorrhizal status of epiphytic ferns and nonorchidaceous angiosperms in tropical sites has only recently received much attention (Trappe 1987, Lesica and Antibus 1990, Maffia et al. 1990). Of 12 species of epiphytic pteridophytes examined in Costa Rican rain forests, none was mycorrhizal (Lesica and Antibus 1990). In that same survey, of 56 angiosperm species, only eight formed VAM, four formed ericoid mycorrhizae, and six of the 14 orchid species were mycorrhizal. Similar findings were reported from another Costa Rican site by Maffia et al. (1990).

The Costa Rican results are in sharp contrast to the findings of our survey, and the differences may reflect the unusual biogeography and flora of the Hawaiian Islands (Carlquist 1974, Wagner et al. 1990, Gemma et al. 1992, Koske et al. 1992). However, not all non-Hawaiian epiphytic sites are characterized by the absence or infrequency of mycorrhizae. Epiphytic bromeliad species from South America are routinely mycorrhizal (S. Rabatin, pers. comm.).

Information on the mycorrhizal status of endemic Hawaiian epiphytes may be valuable in attempts to propagate these species in greenhouse conditions. With nearly 40% of the native angiosperm flora of Hawai'i threatened or endangered to some degree (Wagner et al. 1990), temporary conservation in botanical gardens may be necessary (Theobald 1989). Because standard greenhouse practices (e.g., sterile soilless mixes, fungicides, and heavy fertilization) routinely exclude or inhibit VAM fungi, it may be necessary to modify cultivation methods to encourage the fungi for those plant species that are obligate mycotrophs (Gemma and Koske 1988).

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