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The mycorrhizal status of vascular epiphytes in Bale Mountains National Park, Ethiopia

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Abstract. Roots of 28 species of epiphytic vascular plants were collected on tree trunks and branches at six afromontane forest sites between 1700 and 3300 m above sea level in Bale Mountains National Park, Ethiopia. Seven of the 28 epiphyte species were colonized by vesicular-arbuscular mycorrhizal fungi (VAM). Mycorrhizal colonization only occurred at two of the six sites examined, at 2900 m and 3300 m, but more than one type of VAM endophyte was present in each case. Three facultative epiphytic species were all highly colonized by VAM on the forest floor, whereas roots from epiphytic habitats were weakly colonized. No correlations were found between VAM colonization, fine root diameter and root hair length, but VAM colonization and root hair abundance were negatively correlated. The lack of VAM colonization of potential, epiphytic host species at the majority of the sites examined points to the dispersal of VAM propagules as the factor limiting mycorrhizal colonization of epiphytic habitats. It is suggested that root systems of hemiepiphytic tree species serve as corridors between forest floor and tree trunks through which VAM may spread via hyphal growth.

Key words: Afromontane forest – Epiphytic plants – Fine root – Tropics – Vesicular-arbuscular mycorrhizal fungi

Introduction

Although epiphytic vascular plants contribute considerably to species richness and mineral nutrient capital in tropical and subtropical forest ecosystems (Benzing 1990), very little research has been concerned with mycorrhizal associations of these plants (Bermudes and Benzing 1989; Lesica and Antibus 1990). Mycorrhizal fungi influence the growth of terrestrial plants by facilitating the uptake of nutrient elements, particularly phosphorus, and increasing the plant's resistance to drought and root pathogens (Janos 1987; Michelsen and Rosendahl 1990). Both nutrient and moisture deficiencies may limit the growth of epiphytes (Benzing 1990), but recent observations from neotropical forests suggest that mycorrhizal fungi often do not colonize epiphytes (Bermudes and Benzing 1989; Lesica and Antibus 1990). However, much more basic information on the mycorrhizal status of epiphytic plants, and of taxa common to forests of the African continent, is needed before generalizations can be made about epiphytic dependence on mycorrhizal fungi.

The present study is a survey of mycorrhizal fungi in roots of epiphytic vascular plants sampled from trunks and branches of trees growing along an altitudinal gradient from 1700–3300 m above sea level in the Bale Mountains of Southeast Ethiopia.

Materials and methods

The sampling was carried out from 1700 to 3300 m above sea level in Bale Mountains National Park, Southeast Ethiopia, south of the summit Tullu Deemtu (4377 m) in October 1988. The climate of the area is montane subtropical, with recurring mists in the upper part of the altitudinal range. No rainfall data for the area are available, but the rainfall is estimated to exceed 1400 mm per year and to fall mainly during two wet seasons (Zerihun et al. 1989).

Roots samples of all epiphytic vascular plant species encountered at six sites, each of 400 m^2, were collected along the altitudinal gradient (Table 1). The roots were collected from plants growing on trunks and branches of trees from 2 to 6 m above ground. No attempt was made to extract epiphytes from the canopy of tall trees. The root samples of the epiphytes were obtained by tracing the distal rootlets from the base of the plants. Within each site, a minimum of five subsamples of each species of epiphyte present were taken. Each subsample consisted of roots of epiphyte specimens growing on different trees. If the sampled epiphytic species was also present on the forest floor, five root subsamples of these facultative epiphytes were collected from five separately growing specimens. Roots of the two hemiepiphytic species were only collected from trees, not from the forest floor.

For each site, the five subsamples of each plant species were bulked and the sample subsequently fixed in formalin/acetic acid. In the laboratory, the roots were cleared in KOH and stained with trypan blue (Michelsen 1992). The roots were cut into 1-cm pieces. After mixing, 30–100 pieces of unlignified fine roots were mounted in lactoglycerol on a microscope slide and analysed for the presence of mycorrhizal fungi. Colonization was quantified by the grid-line intersect method by examining 100–200 intersects. The presence of arbuscules in the root cortex was the diagnostic character of vesicular-arbuscular mycorrhizal fungi (VAM) colonizations. Furthermore, the relative abundance of VAM vesicles was recorded, together with the type of VAM endophyte roughly categorized by the diameter of intercellular VAM hyphae in the root cortex (fine: <1 μm; medium: 2–5 μm; coarse: >5 μm).

The diameters of 20 1-cm fine root pieces were measured for each sample, together with the lengths of fully developed root hairs. The abundance of root hairs was estimated on a scale from 0 to 3 (0: absent, 1: few, 2: common, 3: abundant). Correlations between root variables of the VAM-colonized species were analysed with the SAS statistical package (SAS Institute 1988) using Kendall's correlation coefficient.

Results

Epiphytic plant species were found on tree trunks in all six investigated types of afromontane forests (Friis 1992) between 1700 and 3300 m above sea level (Table 1). However, they showed a peak abundance at 2900 m and above, where the trunks of the trees were completely covered by moss and lichen mats in which epiphytic ferns and flowering plants rooted. The upper limit of vascular epiphytes was 3300–3500 m, corresponding to the level at which the forest changed to a low ericaceous shrubland. Two epiphytes, *Asplenium aethiopicum* and *Arthropteris monocarpa*, occurred at both extremes of the altitudinal range but the majority had a more limited range of 800 m or less (Table 2).

Table 1. Description of sites of epiphytic sampling in Bale Mountains National Park, Ethiopia

Height above sea level (m)	Maximum tree height (m)	Dominant trees species
1700	40	Seasonal rain forest with *Podocarpus falcatus*, *Ocotea kenyensis* and *Warburgia ugandensis* (6°31′ N 39°44′ E)
2300	30	Seasonal rain forest with *Erythrina brucei*, *Allophylus abyssinicus* and *Lepidotrichilia volkensii* (6°45′ N 39°44′ E)
2700	25	Seasonal rain forest with *Hagenia abyssinica* and *Dombeya torrida* (6°45′ N 39°44′ E)
2900	20	Seasonal rain forest with *Hagenia abyssinica*, *Hypericum revolutum* and *Schefflera volkensii* (6°45′ N 39°44′ E)
3100	15	Open forest with *Schefflera volkensii*, *Hypericum revolutum* and *Hagenia abyssinica* (6°46′ N 39°44′ E)
3300	9	Ericaceous woodland-forest with *Erica arborea*, *Rapanea melanophloëos*, *Hypericum revolutum* and *Hagenia abyssinica* (6°47′ N 39°45′ E)

Of the 28 epiphytic plant species investigated, only seven (25%) were colonized by mycorrhizal fungi, and in some cases the degree of colonization was very low (Table 2). All colonizations were of the VAM type; none of the orchid species were colonized by orchidaceous mycorrhizal fungi. Various other fungal infections (Bermudes and Benzing 1989) occurred sparsely in some root samples, but these were considered to be nonmycorrhizal. Judging from the diameter of VAM hyphae in the root cortex, some of the colonized epiphyte species hosted more than one species of VAM endophyte. Vesicles of VAM were rare, occurring only in epiphytes at the 2900 m site, and in terrestrially rooted plants. No spores of VAM were observed on external mycelium still attached to roots.

A strong heterogeneity in the occurrence of mycorrhizal epiphytes was found between sites. Five out of ten species sampled in the site at 2900 m and three out of 12 at 3300 m were mycorrhizal, whereas no mycorrhizal epiphytes were found at the other sites, at 1700, 2300, 2700 and 3100 m.

The three facultative epiphytic species, *Geranium arabicum*, *Impatiens hochstetteri* and *Parochetus communis*, which were collected on the forest floor as well as on tree trunks, were all highly colonized when terrestrial, whereas all roots taken from epiphytic habitats, except those from the site at 2900 m, were weakly colonized. The two hemiepiphytic tree species *Rapanea melanophloëos* and *Schefflera volkensii* were medium to highly colonized in epiphytic habitats.

No significant correlations were found between VAM colonization, root diameter and root hair length for VAM-colonized epiphytes (Table 3). This was also the case when non-mycorrhizal epiphytes were included in the analyses (no results presented). VAM colonization and root hair abundance were negatively correlated, whereas root hair length and abundance were positively correlated (Table 3).

Discussion

The observed scarcity of mycorrhizal colonization in epiphytes of the Bale Mountains, Ethiopia contrasts with the general prevalence of plant roots colonized by mycorrhizal fungi in terrestrial vegetation (Janos 1987). It also contrasts with the results of a survey of the terrestrial vegetation conducted in the same area as the present study (A. Michelsen et al., unpublished work), in which VAM colonizations prevailed in roots of almost all plant species along the altitudinal gradient from 1500–4300 m above sea level, except in the zone dominated by ericoids. The scarcity of mycorrhizal fungi in roots of the epiphytes of Bale Mountains is, however, in accordance with the reports of Bermudes and Benzing (1989) and Lesica and Antibus (1990) from the neotropics, which largely covered species in families other than those examined here.

Epiphytic orchids are infrequent in the Bale Mountains. Only two orchid species were collected and both lacked orchid mycorrhiza. Mycorrhizal colonizations

Table 2. Mycorrhizal status and fine root morphology of epiphytic plant species from Bale Mountains National Park, Ethiopia, 1700 to 4300 m above sea level. Percent mycorrhizal colonization of each species is based on five pooled subsamples from different trees at each site. E, Epiphytic form; T, terrestrial form; H, hemiepiphyte; F, fine; M, medium; C, coarse VAM endophyte. Root hairs: 0, absent; 1, few; 2, common; 3, abundant. Root diameter and root hair length are given ±SE ($n=20$)

Taxon	Life form	Altitude (m)	Colonized root length		VAM type	Root diameter (mm)	Root hair abundance	Root hair length (mm)
			Total (%)	Vesicles (%)				
Araliaceae								
Schefflera volkensii (Harms) Harms	E, H	2900	70	34	F, M, C	0.24±0.04	1	0.12±0.03
Asteraceae								
Cineraria abyssinica Oliv. & Hiern	E	3300	0	0	—	0.19±0.02	0	—
Balsaminaceae								
Impatiens hochstetteri Bak.	E	2300	0	0	—	0.15±0.02	2	0.19±0.05
	T	2700	36	18	M	0.12±0.01	1	0.16±0.04
	E	2900	37	0	F, M, C	0.19±0.02	3	0.20±0.02
Campanulaceae								
Canarina eminii Schweinf.	E	2300	0	0	—	0.38±0.03	0	—
	E	3100	0	0	—	0.39±0.04	0	—
Crassulaceae								
Umbilicus botryoides A. Rich.	E	3100	0	0	—	0.17±0.03	3	0.54±0.12
	E	3300	0	0	—	0.19±0.04	2	0.39±0.11
Cucurbitaceae								
Zehneria cfr. *scabra* (L.F.) Sond.	E	3300	0	0	—	0.16±0.02	2	0.12±0.03
Geraniaceae								
Geranium arabicum Forssk.	E	2900	6	0	M	0.12±0.01	2	0.14±0.03
	T	3100	65	7	F, M	0.13±0.01	0	—
Juncaceae								
Luzula johnstonii Buchenau	E	3300	0	0	—	0.10±0.01	3	0.51±0.13
Lamiaceae								
Stachys aculeolata Hook f.	E	3300	2	0	M	0.14±0.02	3	0.34±0.07
Leguminosae, Papilionoideae								
Parochetus communis D. Don	T	2900	68	8	F, M, C	0.17±0.01	2	0.09±0.03
	E	2900	40	3	F, M	0.18±0.02	2	0.27±0.04
	E	3300	5	0	M	0.15±0.01	3	0.14±0.02
Myrsinaceae								
Rapanea melanophloëos (L.) Mez	E, H	3300	24	0	F, M	0.29±0.02	3	0.26±0.04
Orchidaceae								
Liparis abyssinica A. Rich.	E	2300	0	0	—	0.70±0.08	3	0.18±0.06
Polystachya fusiformis (Thou.) Lindl.	E	1700	0	0	—	2.00±0.22	0	—
Piperaceae								
Peperomia abyssinica Miq.	E	2300	0	0	—	0.29±0.04	3	0.33±0.06
Peperomia tetraphylla (Forst.) Hook. & Arn	E	1700	0	0	—	0.14±0.01	3	0.38±0.06
Pteridophytae								
Arthropteris monocarpa (Cordem.) C. Chr.	E	1700	0	0	—	0.12±0.01	3	0.28±0.04
	E	2700	0	0	—	0.18±0.01	3	0.55±0.17
Asplenium aethiopicum (Burm. f.) Becherer	E	1700	0	0	—	0.11±0.01	3	0.20±0.06
	E	3300	0	0	—	0.12±0.01	3	0.24±0.06
Asplenium cfr. *elliottii* C. H. Wright	E	2700	0	0	—	0.17±0.02	3	0.43±0.09
Asplenium lunulatum Sw.	E	2300	0	0	—	0.14±0.02	3	0.39±0.07
	E	2900	0	0	—	0.19±0.02	3	0.42±0.06
Asplenium sandersonii Hook	E	1700	0	0	—	0.19±0.01	3	0.20±0.04
Loxogramme lanceolata (Sw.) A. Presl	E	1700	0	0	—	0.24±0.02	3	0.36±0.08
	E	2300	0	0	—	0.28±0.03	3	0.31±0.05
	E	2700	0	0	—	0.15±0.02	3	0.56±0.08
Pleopeltis cfr. *macrocarpa* (Willd.) Kaulf.	E	2900	0	0	—	0.10±0.01	3	0.53±0.05
	E	3300	0	0	—	0.15±0.02	3	0.42±0.08
Xiphopteris flabelliformis (Poir.) Schelpe	E	2900	0	0	—	0.12±0.01	3	0.51±0.12
	E	3300	0	0	—	0.15±0.01	3	0.42±0.09
Rubiaceae								
Galium aparinoides Forssk.	E	2900	0	0	—	0.09±0.01	3	0.12±0.03

Table 2 (continued)

Taxon	Life form	Altitude (m)	Colonized root length		VAM type	Root diameter (mm)	Root hair abundance	Root hair length (mm)
			Total (%)	Vesicles (%)				
Solanaceae								
Solanum benderianum Dammer	E	3300	0	0	—	0.25 ± 0.02	3	0.41 ± 0.05
Urticaceae								
Parietaria debilis G. Forst.	E	2900	42	12	M, C	0.10 ± 0.01	0	—
	E	3100	0	0	—	0.10 ± 0.02	0	—
	E	3300	0	0	—	0.08 ± 0.01	1	0.07 ± 0.01
Pilea johnstonii Oliv.	E	2900	0	0	—	0.14 ± 0.01	0	—
	E	3100	0	0	—	0.10 ± 0.01	3	0.13 ± 0.03
Urera hypselodendron (A. Rich.) Wedell	E	3100	0	0	—	0.12 ± 0.02	2	0.10 ± 0.02

Table 3. Kendall correlation coefficients between fine root diameter, vesicular-arbuscular mycorrhizal fungus (VAM) colonization, root hair length and abundance of the mycorrhizal epiphytes. $n = 8$; * $P < 0.05$

	Root hair length	VAM colonization	Root hair abundance
Root diameter	0.214	0.143	0.161
Root hair length		−0.357	0.645*
VAM colonization			−0.725*

seem to occur inconsistently in roots of epiphytic orchids (Bermudes and Benzing 1989; Lesica and Antibus 1990). The seven epiphyte species colonized by VAM all belonged to different families. Those without mycorrhiza belonged to families in which the members generally have VAM (the exceptions are Juncaceae and Urticaceae) (Harley and Harley 1987; Tester et al. 1987).

A complete lack of mycorrhiza in epiphyte species was observed at four of the six sites in Bale, whereas some VAM were found at the two sites at 2900 and 3300 m. As the reason for the prevalent scarcity of mycorrhiza colonization can not be found in the taxonomic affiliations of the nonmycorrhizal epiphytes, it must probably be sought in properties of the epiphytic habitats. The data, including the observations that terrestrial forms of epiphytic species are highly mycorrhizal, suggest that the lack of significant VAM colonization of many of the epiphytic plant species is the result of a low VAM propagule density in the substrate. Such a correlation between degree of colonization and VAM propagule density in the substrate can be found in terrestrial habitats, e.g. in tree nurseries in the region (Michelsen 1992, 1993). In the present study, however, neither the epiphytic substrates nor the soils were examined for spores or other VAM propagules. The normal vectors for passive VAM propagule dispersal, such as wind and animal activity (Janos 1987), probably do not act efficiently between the soil of the forest floor and the arboreal habitats, but the impact of these vectors was not studied in this survey.

At the two sites where mycorrhizal epiphytes were found in arboreal habitats, the VAM in the roots of the epiphytes probably belonged to different taxa, judging from the variation in diameter of the hyphae present in the root cortex of each plant species. Thus, when VAM propagules in rare cases are present in epiphytic habitats, as at the forest sites at 2900 and 3300 m, the means of dispersal seem to work successfully for more than a single VAM species. One factor of importance for introduction of VAM to arboreal habitats could be the dense root systems of hemiepiphytic tree seedlings which germinate in moss mats of tree crowns and then extend their roots to the forest floor. This was common for *R. melanophloëos* and *S. volkensii* at the sites at 2900 and 3300 m. These hemiepiphytes produce a large system of persistently corticated fine roots which descend to the ground and possibly serve as corridors through which VAM, via hyphal growth in the root cortex, spread actively from the forest floor to the epiphytic habitats. The roots of *R. melanophloëos* and *S. volkensii* were medium to highly colonized and they, moreover, had the highest diversity of endophyte types found in epiphyte roots at their respective sites. This supports the idea that hemiepiphytic roots on tree trunks and branches serve as centres from which VAM are dispersed. However, as the growth habits of hemiepiphytes range from huge, ultimately free-standing strangler trees to epiphytic plants with only a small root reaching the ground (Putz and Holbrook 1986), this does not apply to all hemiepiphytes. More detailed studies on the spatial distribution of roots of hemiepiphytes colonized by mycorrhizal fungi, both within single tree trunks and between individual trees, are obviously needed to test the hypothesis.

Abiotic factors such as water and nutrient availability may also influence the mycorrhizal colonization of epiphytes. Drought stress which inhibits VAM growth has been suggested to be one of the factors causing differences in the occurrence of VAM in epiphytes of two Costa Rican forests (Lesica and Antibus 1990). However, this probably does not apply as epiphyte zonation with respect to water availability does occur within trees (Johansson 1989; van Leerdam et al. 1990). Furthermore, in Bale, the site at 3100 m (with nonmycorrhizal epiphytes only) did not seem to differ, taking each site as a whole, in humidity from those sites where mycorrhizal epiphytes occurred. The same probably applies to nutrient availability.

The clear lack of correlation between the degree of mycorrhizal colonization and fine root diameter contradicts Baylis' hypothesis (1975) of a positive relation between the diameter of distal roots and mycorrhizal dependence. The hypothesis was confirmed for tropical trees by St. John (1980), but does not fit the VAM-colonized epiphytic plant species in the present study. Epiphytes with many root hairs, however, seem to be less dependent on VAM, as the degree of VAM colonization was negatively correlated with root hair abundance. In the material used for the analysis, roots of five separate specimens were bulked for each species and site; hence the overall degree of colonization may include considerable variation between specimens. It should also be kept in mind that the degree of root colonization may not accurately indicate dependence on mycorrhizas (Janos 1987). Nevertheless, the correlations obtained may prove to be general for mycorrhizal epiphytes when additional material is examined.

The fine roots of all epiphytes, with the exception of *Canarina eminii* and the two orchids, *Liparis abyssinica* and *Polystachya fusiformis*, were not 'magnolioid' (sensu St. John 1980), but were of the intermediate type with a diameter of 0.3 mm or less, commonly with abundant root hairs always less than 1 mm long. Thus in most cases, epiphytes seem to rely on nonmycorrhizal root systems with medium- to well-developed root hairs for their nutrient uptake. The highly variable degree of colonization of epiphytes (e.g. of *Impatiens hochstetteri* and *Parietaria debilis*) between the different sites in the Bale Mountains suggests furthermore that some epiphytes at least may be facultatively mycotrophic.

Nutritional insufficiency has been suggested as a major constraint on epiphyte success (Benzing 1990). However, the substrates of epiphytes are highly heterogenic and their life strategies may vary accordingly. The role of mycorrhizae in epiphytic habitats is probably very variable and related to the species. In terrestrial systems, it is often possible under stress conditions imposed by adverse cultivation practices to enhance plant growth by increasing the VAM propagule density through inoculation (Michelsen 1993). Similar long-term experiments with mycorrhizal inoculation of epiphytes in their natural environments could clarify whether naturally recurring rare events of mycorrhizal colonization in some cases affect the fitness of the colonized individual.

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