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Catherine A. Gehring · Joseph H. Connell

Arbuscular mycorrhizal fungi in the tree seedlings of two Australian rain forests: occurrence, colonization, and relationships with plant performance

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Abstract The roots of rain forest plants are frequently colonized by arbuscular mycorrhizal fungi (AMF) that can promote plant growth in the nutrient poor soils characteristic of these forests. However, recent studies suggest that both the occurrence of AMF on rain forest plants and the dependence of rain forest plants on AMF can be highly variable. We examined the occurrence and levels of AMF colonization of some common seedling species in a tropical and a subtropical rain forest site in Queensland, Australia. We also used a long-term database to compare the growth and mortality rates of seedling species that rarely formed AMF with those that regularly formed AMF. In both forests, more than one-third of the seedling species rarely formed AMF associations, while 40% of species consistently formed AMF in the tropical site compared to 27% in the subtropical site. Consistent patterns of AMF occurrence were observed among plant families at the two sites. Variation among seedling species in AMF occurrence or colonization was not associated with differences in seed mass among species, variation in seedling size and putative age within a species, or lack of AMF inoculum in the soil. Comparisons of four seedling species growing both in the shaded understory and in small canopy gaps revealed an increase in AMF colonization in two of the four species in gaps, suggesting that light limitation partially explains the low occurrence of AMF. Seedling survival was significantly positively associated with seed biomass but not with AMF colonization. Furthermore, seedling species that regularly formed AMF and those that did not had similar rates of growth and survival, suggesting that mycorrhizal and nonmycorrhizal strategies were equivalent in these forests. Furthermore, the high numbers of seedlings that lacked AMF and the overall low rate of seedling growth (the average seedling required 6 years to double its height) suggest that most seedlings did not receive significant indirect benefits from AMF through connection to canopy trees via a common mycorrhizal network.

Keywords Arbuscular mycorrhizal fungi · Canopy gap · Common mycorrhizal network · Rain forest · Seedlings

C. A. Gehring (✉)
Department of Biological Sciences and the Merriam-Powell Center for Environmental Research,
Northern Arizona University,
Flagstaff, AZ 86011-5640, USA
e-mail: Catherine.Gehring@nau.edu
Tel.: +1-928-5239158
Fax: +1-928-5237500

J. H. Connell
Department of Ecology, Evolution and Marine Biology,
University of California,
Santa Barbara,
Santa Barbara, CA 93106-9610, USA

Introduction

The low nutrient concentrations of many tropical rain forest soils provide the appropriate conditions for the development of mycorrhizas that assist plants in nutrient uptake. Studies examining the mycorrhizal status of rain forest plants have frequently shown that most of the plant species sampled are colonized by arbuscular mycorrhizal fungi (AMF) (e.g., St. John 1980; Bereau et al. 1997; Metcalfe et al. 1998; Onguene and Kuyper 2001). A smaller number of studies have shown that rain forest plants can be dependent upon mycorrhizal fungi for growth (e.g., Janos 1980; Zangaro et al. 2000). However, recent studies suggest that the incidence of AMF colonization can be low to moderate in tropical forest. Zangaro et al. (2000) observed average levels of AMF colonization of 2% in Brazilian climax forest, while only 56% of the plant species surveyed in Chinese rain forest were colonized by AM fungi (Zhao et al. 2001).

In addition to the substantial variation in rates of AMF colonization in tropical rain forest, recent studies suggest that the dependence of tropical rain forest seedlings on AMF also may be highly variable. Janos (1980) observed that 23 of 28 species of Costa Rican rain forest tree seedling were dependent on AMF for growth. In contrast, only 14% of Brazilian climax tropical forest species (Zangaro et al. 2000) and only one of four highly colonized Australian rain

forest seedling species were dependent on AMF for growth (Gehring 2003).

Because of light limitation in understory seedlings, seedlings and adult trees may have different relationships with AMF in tropical forests where understory light intensities can be 1–3% of full sun. Seedling growth and mycorrhizal development may be limited when light intensity is low (e.g., Bereau et al. 2000; Whitbeck 2001; Gehring 2003, 2004). Tree seedlings may derive benefits from hyphal connections to adult trees under these conditions as has been demonstrated for species sharing a common ectomycorrhizal network (Simard et al. 1997; Dickie et al. 2002; Onguene and Kuyper 2002; Kennedy et al. 2003). The magnitude of carbon exchange among plants connected by AMF has been questioned as little or no carbon may leave fungal tissues (Robinson and Fitter 1999), but other nutrients that contribute to seedling performance, such as phosphorus, may pass through a mycelial network (Simard et al. 2002). However, if seedlings are not colonized by AMF, they cannot benefit directly from mycorrhizal networks. Data on occurrence of AMF in the roots of seedlings, accompanied by performance measurements, are a first step in elucidating the importance of AMF to seedlings in the field. Our study had three goals: (1) to document the occurrence and levels of AMF colonization of common rain forest seedling species in a tropical and a subtropical rain forest; (2) to determine if inoculum availability, light intensity, or seed mass explained the variability in AMF colonization; and (3) to relate occurrence of AMF to seedling growth and mortality to determine if AMF provided a performance advantage to seedlings directly or through connection to neighbors.

Materials and methods

Study sites

This study was conducted at two sites, a primary tropical rain forest in northern Queensland (Davies Creek: 17° 05′, 145° 34′E, 850-m elevation) and a subtropical rain forest site (O'Reilly's: 28° 14′S, 153° 10′E, 900-m elevation) in southern Queensland, Australia. Seedling dynamics (recruitment, growth, and mortality) have been studied at these sites since 1965 by Connell and colleagues, providing an unparalleled database on rain forest seedling performance over time (Connell et al. 1984; Connell and Green 2000; Connell et al. 2005). The primary tropical forest site consists of a 1.7-ha plot located on the western slopes of the Lamb Range on the northeastern edge of the Atherton tableland. The soil is derived from deeply weathered biotite granite with low levels of nitrogen (0.47% total Kjehldahl N) and phosphorus (6.8 mg P/kg soil) and a pH of ~4 (Gehring et al. 2002). Annual rainfall is approximately 300 cm, most of which falls during a December–April summer wet season (Connell and Green 2000). The study site likely has never been logged and has been protected as a research site by the Queensland Department of Forestry since 1955 (Connell and Green 2000). The plot and surrounding area are now part of the Wet Tropics World Heritage Area in Queensland. Previous studies based on soil cores indicate that primary tropical rain forests in northern Australia are dominated by AMF (Hopkins et al. 1996).

The subtropical rain forest study site consists of a 1.94-ha plot characterized by well-drained basaltic krasnozem soils (Connell et al. 1984). The site also likely has never been logged and is currently designated as World Heritage Area. Rainfall at the site averages 191 cm/year with most of it falling during the warm summer months. Both sites have a dense, closed evergreen canopy with an average height of ~30 m and a basal area of 61.5 m^2/ha for the tropical site and 63.4 m^2/ha for the subtropical site (Connell et al. 1984).

Measurements of occurrence and colonization by AMF

To examine the occurrence of AMF in seedlings at the two study sites, we collected seedlings of 25 tree species at the tropical site (21 canopy and 4 understory species) and 11 common tree species (10 canopy and 1 understory species) at the subtropical sites from 1997 to 1999. These seedlings represented the majority (72.6% in the tropical site and 67.4% in the subtropical site) of the newly germinated seedlings on the two study plots as indicated by periodic censuses from 1965 to 1996 (Tables 1 and 2). Seedlings of varying sizes and estimated ages were collected for each species ranging from new germinants (1–6 months old) to seedlings at least 10 years of age. The lower age boundary was determined by direct observation of germination during regular censuses while the upper age boundary was estimated using germination records and height measurements of seedlings marked by Connell and followed for >30 years. While we did not excavate seedlings actually marked by Connell and his colleagues due to the consequent loss of long-term data on growth and survivorship, we used seedlings of similar size immediately outside the census lines and thus likely to be from the same germination cohort. Seedling recruitment at these sites is episodic with the more common species that are the focus of this study having particularly pronounced temporal variation in seedling recruitment (Connell et al. 2005). For example, for the most common species on the tropical plot, *Chrysophyllum* sp. nov., 98% of the seedlings observed in the long-term census from 1965 to 1996 appeared in five peaks spaced 4 to 11 years apart (Connell and Green 2000). Our recruitment and age estimates are thus more reliable than would be expected for seedlings with annual patterns of recruitment.

Seedlings were collected using a trowel or shovel to dig to sufficient depth (generally <50 cm) to obtain the majority of the root system. Root systems were examined for evidence of breakage to ensure that most of the roots were collected. All seedlings were collected from the shaded understory where investment in root tissue was comparatively small (average root to shoot ratio for the 25 tropical

species=1:5). To account for spatial and temporal variation in AMF colonization, seedlings of each species were collected in several locations throughout the forest and were sampled during at least two seasons over the 2-year period. A minimum of five seedlings were collected per species, but higher numbers were sampled for most species (Table 1). Seedlings were stored in plastic bags and kept cool until they were transported to the laboratory. In the laboratory, seedlings were separated into roots and shoots, and a fresh weight of each was obtained along with seedling height and the number of leaves. Due to its small size, the entire root system of the seedlings was used to estimate AMF colonization. Additional seedlings of each species were collected to calculate a fresh weight/dry weight conversion that could be used for the study seedlings.

To determine the occurrence and extent of colonization by AMF, seedling roots were cleared in 10% potassium hydroxide at 90°C for 1–9 h depending on root thickness and degree of pigmentation. Care was taken to ensure that the root cortex remained intact even after prolonged heating. Root samples were then bleached in alkaline hydrogen peroxide for 15–20 min, acidified in 1% hydrochloric acid, and stained overnight in trypan blue in lactoglycerol as modified from Phillips and Hayman (1970). Root samples were destained in 50% glycerol and multiple (>15), 1-cm segments of root from each plant were mounted on slides and viewed under a compound microscope at 200× magnification (McGonigle et al. 1990). The presence of AM fungal structures was scored for 100 intersections of root and reticle line per plant. An intersection was considered mycorrhizal if the reticle intersected an arbuscule, a coil, a vesicle, or an internal hypha attached to one of these structures. For species that showed no evidence of AM colonization using trypan blue, root samples also were stained using Chorazol Black E and acid fuchsin to reduce the likelihood that evidence of AMF colonization was overlooked (Brundrett et al. 1996).

Seedlings might show low or variable levels of AMF because of limited inoculum in the soil. Established vegetation that is colonized by AMF can be a significant source of inoculum through both hyphae and spores (Janos 1980). Previous studies have shown that spores are moderately abundant at the tropical site, and bioassays indicate high rates of AMF formation at this site (Gehring et al. 2002). Similar studies have not been conducted at the subtropical site. We further explored the hypothesis that inoculum might be spatially patchy, and thus limited for some seedlings, by examining roots collected at random for evidence of AMF. Random soil cores (15 cm length×6 cm diameter) were taken from both the tropical (n=25) and subtropical (n=15) sites in order to assess the occurrence of AMF on the roots of vegetation surrounding seedlings. Roots were separated from the soil, cleared, and stained as described above. Slides were scored for the presence of AMF using the criteria described above. A sample was considered to be a potentially significant source of AMF inoculum and given a positive score if greater than 33% of the roots examined were colonized by AMF.

Influence of seed size and light intensity

Because variation in seed size has been shown to correlate with mycorrhizal dependency and colonization in other systems (e.g., Janos 1980; Siqueira et al. 1998), a regression analysis was performed to determine if seed mass was significantly correlated with the occurrence or percentage root colonization by AMF for the 17 seedlings species at the tropical site for which seed mass data were available. Seed mass data were obtained by direct measurement of the dry mass of >25 seeds of a species collected at the study site or from data published in Grubb et al. (1998). In addition, seedling dry mass was regressed against percentage AMF colonization for all species showing evidence of colonization to determine if variability in rates of colonization was associated with seedling size and putative age.

Variation in light intensity also has been shown to significantly influence levels of AMF colonization in tree seedlings (e.g., Bereau et al. 2000; Whitbeck 2001; Gehring 2003, 2004) and may contribute to low occurrence of colonization among seedling species that might more frequently form AMF associations in higher light. We tested this hypothesis for two species in the tropical forest (*Syzygium endophloium* and *Franciscodendron laurifolium*) and two species in the subtropical forest (*Actephila lindleyi* and *Baloghia inophylla*) by comparing the rates of AMF colonization of seedlings from the same germination cohort (1997) growing in the shaded understory and in nearby gaps in the canopy (distance between gap and understory sites was <10 m). Gaps were defined as continuous areas larger than 4 m^2 in which open sky was visible above a height of 2 m. Unfortunately, equipment for measuring light intensity was not available at the time the seedlings were sampled so that differences in light intensity could not be quantified. It was not possible to compare gap vs understory rates of AM colonization for the other seedling species because they could not be found in gaps, which have occurred infrequently over the past 30+ years at the two study sites, despite significant influence of cyclones and tropical storms (Connell et al. 2005). AMF colonization data from these collections were not included in the main seedling survey described above but represented a separate data set.

Estimates of seedling growth and mortality

In order to examine if rates of seedling mortality and growth through time were related to the occurrence or level of AMF colonization among seedling species, we used a long-term seedling database to estimate rates of mortality over a 10-year period for 13 species of seedling at the tropical site and eight species of seedling at the subtropical site. These species were selected to represent extremes of AMF incidence; 7 of the 13 focal seedling species at the tropical site and 4 of the 8 focal species at the subtropical site had high occurrence of AMF associations (>75% of seedlings sampled; average AMF occurrence=96.2% of

Table 1 Data on seedling abundance, AMF occurrence (% of seedlings sampled that were colonized), and AMF colonization (mean percentage root length colonized for those seedlings that were mycorrhizal) for the tropical rain forest study site

Species	Family	% of total seedlings (1965–1996)[a]	No. of seedlings sampled	%AM occurrence	%AM colonization (mean±1SE)
Cryptocarya corrugata[b] C.T. White and W.D. Francis	Lauraceae	1.47	10	100	61.4±7.56
Cryptocarya mackinnoniana[b] F. Muell.	Lauraceae	2.03	11	100	32.0±4.61
Beilschmiedia collina[b] B. Hyland	Lauraceae	2.22	7	100	31.6+3.39
Cryptocarya angulata C.T. White	Lauraceae	6.17	13	46	21.7+2.53
Syzygium cormiflorum (F. Muell.) B. Hyland	Myrtaceae	0.05	5	100	10.5±2.04
Syzygium endophloium[b] B. Hyland	Myrtaceae	2.16	19	100	37.0+3.98
Syzygium wilsoni[b] (F. Muell.) B. Hyland	Myrtaceae	0.96	8	100	34.1±4.27
Garcina sp. nov.[bc]	Clusiaceae	0.99	17	100	37.8±3.29
Flindersia bourjotiana F. Muell.	Rutaceae	0.74	7	86	45.0±6.95
Flindersia brayleana F. Muell.	Rutaceae	0.15	10	66	29.1±5.07
Halfordia scleroxlya F. Muell.	Rutaceae	0.14	7	100	50.0±3.25
Apodytes brachystylis F. Muell.	Icacinaceae	0.77	9	100	45.7±3.70
Ceratopetalum succirubrum C.T. White	Cunoniaceae	13.11	12	75	33.4±3.52
Doryphora aromatica (Bailey) L.S. Sm.	Monimiaceae	1.20	10	50	36.2±3.76
Franciscodendron laurifolium (F. Muell.) B. Hyland and Steenis	Sterculiaceae	3.29	29	37	17.4±6.82
Chrysophyllum sp. nov.[bc]	Sapotaceae	28.69	12	23	19.3±2.80
Castanospora alphandii[b] (F. Muell.) F. Muell.	Sapindaceae	1.44	11	18	13.3±1.02
Alyxia ilicifolia F. Muell. subsp. *ilicifolia*	Apocynaceae	0.38	7	0	–
Brackenridgea nitida[b] A. Gray	Ochnaceae	0.53	12	0	–
Randia fitzalanii (F. Muell.) Benth.	Rubiaceae	0.65	10	0	–
Rockinghamia angustifolia (Benth.) Airy Shaw	Euphorbiaceae	0.01	25	0	–
Cardwellia sublimis[b] F. Muell.	Proteaceae	2.43	12	0	–
Darlingia darlingiana[b] (F. Muell.) L.A.S. Johnson	Proteaceae	0.83	12	0	–
Placospermum coriaceum[b] C.T. White and W.D. Francis	Proteaceae	1.67	10	0	–
Lomatia fraxinifolia[b] F. Muell. ex. Benth.	Proteaceae	0.56	10	0	–

[a]The percentage of 37,726 seedlings that germinated at the site from 1965 to 1996; sampled species contributed 72.6% of seedlings

[b]Seedling species used for mortality and growth analyses

[c]These species are undescribed, but this notation indicates their reference in Hyland and Whiffin (1993)

sampled seedlings), while the remaining species at both sites had low occurrence of AMF (<25% of seedlings sampled; average AMF occurrence=3.7% of sampled seedlings). This dichotomy allowed us to compare growth and survivorship of seedling species that formed AMF associations with those that either did not form AMF asso-

Table 2 Data on seedling abundance, AMF occurrence (% of seedlings sampled that were colonized), and AMF colonization (mean percentage root length colonized for those seedlings that were mycorrhizal) for the subtropical rain forest study site

Species	Family	% of total seedlings (1965–1996)[a]	No. of seedlings sampled	%AM occurrence	%AM colonization (mean±1SE)
Cryptocarya obovata[b] R. Br.	Lauraceae	1.54	20	100	35.2±2.91
Acmena ingens[b] (C. Moore) Guymer and B. Hyland	Myrtaceae	1.98	10	90	32.6±5.55
Argyrodendron actinophyllum[b] (Bailey) Edlin.	Sterculiaceae	27.29	11	90	38.9±6.59
Argyrodendron trifoliolatum[b] F. Muell.	Sterculiaceae	7.14	10	73	22.8±6.59
Synoum glandulosum (Sm.) A. Juss.	Meliaceae	1.17	5	20	40.0 (*n*=1)
Randia benthamiana F. Muell.	Rubiaceae	2.39	12	8	22.2 (*n*=1)
Baloghia inophylla[b] (G. Forst) P.S. Green	Euphorbiaceae	1.52	15	0	–
Actephila lindleyi Airy Shaw	Euphorbiaceae	4.76	24	0	–
Ellatostachys nervosa[b] (F. Muell.) Radlk.	Sapindaceae	3.58	10	0	–
Sarcopteryx stipitata[b] (F. Muell.) Radlk.	Sapindaceae	4.09	10	0	–
Orites excelsa[b] R. Br.	Proteaceae	11.90	11	0	–

[a]The percentage of 21,175 seedlings that germinated along seedling lines from 1965 to 1996; sampled species contributed 67.4% of seedlings
[b]Seedling species used for mortality and growth analyses

ciations or did so only rarely. We also performed a multiple regression analysis to compare rates of 10-year seedling survivorship with percentage AM colonization and seed biomass. Average AMF colonization for a species included zero values. All three data sets were available for 15 species across both sites. These comparisons allowed a further test of the importance of variation in AMF on survivorship and compared its effect to variation in seed biomass which contributes to variation in plant performance in tropical seedlings (Foster 1986).

The seedling census methods used to estimate seedling mortality and growth were described in detail in Connell et al. (1984) and Connell and Green (2000). Briefly, in 1963, all trees >10 cm DBH on the two study plots (1.7-ha tropical plot, 2-ha subtropical plot) were mapped, tagged, measured, and identified. To obtain samples of smaller trees, on permanently marked belt transects comprising 30% of each plot and extending throughout the mapped area, the same census was done for all trees between 2.5 and 10 cm DBH. In 1965, all saplings and seedlings <2.5 cm DBH, including newly germinated seedlings, were mapped and tagged on permanently marked narrower strips nested within those for the small trees. The saplings and seedlings were mapped on 9.6% and 16.8% of the total plot areas at the tropical and subtropical sites, respectively. At intervals of 1 to 4 years thereafter, all previously mapped and tagged individuals were re-censused to estimate mortality. Censuses were done at least every other year between 1986 and 1999, the years of greatest interest in this study. In addition, all seedlings that had germinated and survived in the interval since the previous census were mapped, tagged, measured (height to the nearest cm), and identified on the same permanently marked areas used in the original mapping. These censuses were done 16 times between 1965 and 1999.

We estimated 10-year survival using individually marked seedlings that germinated in 1986 or 1988 and calculating

Table 3 The relationship between seedling biomass and percentage root length colonized by AMF for five tropical rain forest and five subtropical rain forest seedling species

Species	r^2	*df*	F	P
Tropical site				
Chrysophyllum sp.	0.002	1,10	0.024	0.881
Ceratopetalum succirubrum	0.096	1,10	1.059	0.327
Cryptocarya corrugata	0.103	1, 9	0.921	0.365
Syzygium endophloium	0.151	1,11	1.621	0.235
Cryptocarya angulata	0.079	1,6	0.860	0.375
Subtropical site				
Argyrodendron actinophyllum	0.195	1,9	2.185	0.173
Argyrodendron trifoliolatum	0.013	1,9	0.106	0.752
Cryptocarya obovata	0.061	1,18	1.112	0.306
Acmena ingens	0.067	1,9	0.577	0.469
Synoum glandulosum	0 054	1,5	0.214	0.661

Only species in which some individuals were colonized by AMF were included

Table 4 Comparison of seedling size and AMF colonization of seedling species in the shaded understory and small canopy gaps at the tropical and subtropical sites

Species	Seedling biomass (g)	Statistics	Root/shoot ratio	Statistics	AM colonization (%)	Statistics
Tropical						
Franciscodendron laurifolium Gap	0.156±0.006	$F_{1,19}$=68.4, P<0.001	0.513±0.033	$F_{1,19}$=38.1, P<0.001	9.06±3.28	t=2.09, P<0.026
F. laurifolium Understory	0.085±0.006		0.280±0.020		1.24±0.98	
Syzygium endophloium Gap	0.108±0.006	$F_{1,15}$=48.9, P<0.001	0.492±0.065	$F_{1,15}$=0.58, P=0.459	42.4±5.93	t=1.575, P=0.079
S. endophloium Understory	0.048±0.005		0.538±0.030		31.73±3.44	
Subtropical						
Actephila lindleyi Gap	0.209±0.022	$F_{1,47}$=5.4, P=0.025	0.371±0.024	$F_{1,47}$=11.8, P=0.001	0.0	Not done
A. lindleyi Understory	0.148±0.014		0.281±0.012		0.0	
Baloghia inophylla Gap	0.315±0.020	$F_{1,34}$=4.3, P=0.045	0.455±0.023	$F_{1,34}$=6.4, P=0.017	0.0	Not done
B. inophylla Understory	0.231±0.030		0.35±0.024		0.0	

Data are presented as means± one standard error

the percentage that survived over a 2-year interval to 1996 or 1998. Ten-year survival rates were compared between low AMF and high AMF seedling species separately for the two sites using a Student's t test. Species used in this analysis are indicated in Tables 1 and 2. These species were selected based on incidence of AMF colonization as indicated above and also because sufficient data were available for them to estimate a 10-year survival (a minimum of 50 germinated seedlings; range 50–325).

We also estimated seedling growth rates during their first 4 years using the long-term census data for the same species described for the mortality analyses. Species of seedlings varied substantially in initial seedling height, so we calculated the mean change from the initial height of a seedling per year for each seedling and then averaged this to generate a single value for each species. We compared the average annual growth rates over the 4-year period with a Student's t test for the same high and low AMF species used for the mortality comparisons.

Results

Patterns of AMF occurrence and colonization

As observed in several other tropical plant species (e.g., Alexander 1989; Bereau and Garbaye 1994; Bereau et al. 2000; Gehring 2003, 2004), all species of seedlings that formed AMF associations formed associations of the Paris-type that contained vesicles and intracellular coils but few arbuscules. No ectomycorrhizal associations were observed despite the presence of ectomycorrhizal fungi in adjacent eucalypt-dominated forests.

In the tropical forest, 40% of the seedling species consistently formed AMF associations (>75% of seedlings sampled were colonized), 36% rarely formed AMF associations (<25% of seedlings sampled were colonized), and 24% of the species were highly variable in the occurrence of AMF associations (25–74% of seedlings were colonized) (Table 1). If these data are combined with data on the abundance of these seedling species in the tropical forest over a 30-year period (Table 1), only 15.9% of the seedlings germinating during that time would be estimated to have high occurrence of AMF colonization, while the remainder would have low (51.2%) or variable (32.9%) occurrence. Even lower rates of AMF occurrence were observed among the subtropical seedlings with 63% of the species rarely forming AMF associations, 27% of species frequently forming AMF associations, and 9% showing high variability in AMF occurrence (Table 2). However, if these data are combined with data on the abundance of these seedling species at the subtropical sites (Table 2), 45.7% of the seedlings germinating from 1965 to 1996 have high occurrence of AMF, 43.7% low occurrence, and 10.6% variable occurrence.

Members of the same plant families had similar patterns of AMF occurrence in the two forest sites (Tables 1 and 2). For example, members of the Myrtaceae and Lauraceae had high occurrence of AMF colonization in both forests, while members of the Euphorbiacae and Sapindaceae rarely formed AMF associations in either forest. As previously reported in the literature (e.g., Lamont 1982; Skene 1998), members of the Proteaceae showed no evidence of AMF colonization in either forest (four species in the tropical site and one species in the subtropical site). Only the

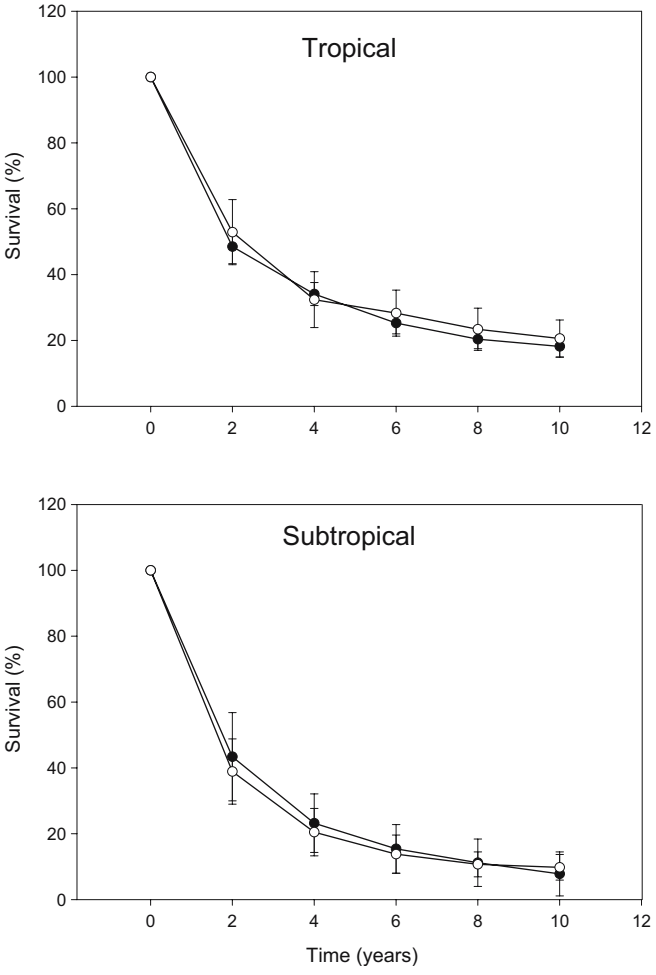

Fig. 1 Mean (±SE) survival rates of seedling species with high occurrence of AMF (*closed circles*) and with low occurrence of AMF (*open circles*) in a tropical and subtropical study site over a 10-year period. All seedlings germinated in either 1986 or 1988 and were individually marked and followed for the next 10 years

largest, oldest seedlings of each species had formed the cluster roots characteristic of that family.

Seedling size, seed size, and light intensity

Seedling biomass was not significantly correlated with levels of AMF colonization within any of the plant species at either of the two sites (all P values >0.05). Representative regression statistics are provided for five species at the tropical site and five species at the subtropical site in Table 3. These data argue that the presence or extent of AMF colonization is unrelated to the size of the seedling. Because the long-term database allowed us to sample seedlings whose germination date, and thus age could be inferred, these data also suggest that age may not be an important contributor to variation in AMF colonization within a seedling species. Seed size also was not significantly correlated with either the occurrence of AMF or with levels of AMF colonization, suggesting that seed size does not explain the observed variation in AMF colonization (r^2=0.041, $F_{1,17}$=0.668, P=0.440 for occurrence and r^2=0.070, $F_{1,14}$=1.008, P=0.335 with AMF colonization).

Seedlings growing in canopy gaps were significantly larger and had higher root to shoot ratios than seedlings in the shaded understory (Table 4), patterns consistent with responses to variation in light intensity in other studies of rain forest seedlings (e.g., Osunkoya et al. 1994; Gehring 2003, 2004). Variation in light intensity contributed to variation in AMF colonization for two of the four species. The two species from the subtropical site showed no evidence of AMF colonization in either the gap or understory. In contrast, one of the species at the tropical site showed levels of colonization below 2% in the understory and nearly sevenfold higher values in small canopy gaps (Table 4). The second tropical species was the most highly colonized of the four seedling species examined and showed a trend towards a significant difference in levels of colonization in the gaps compared with the shaded understory (Table 4).

At least one-third of the roots obtained from each of the soil cores were colonized by AMF (50 positive results from 50 cores), suggesting that a seedling germinating in the tropical or subtropical site is likely to encounter AMF inoculum. This is particularly true for the tropical site where the top layers of the soil have a 6- to 9-cm mat of roots.

AMF and seedling performance

Seedling species that either did or did not form AMF associations had similar rates of survival over a 10-year period in both forests. On average, 20.6% of seedlings that rarely formed AMF association survived in the tropical site compared to 18.2% for seedlings that tended to form AMF associations (t=0.371, P=0.718) (Fig. 1). Ten-year survival

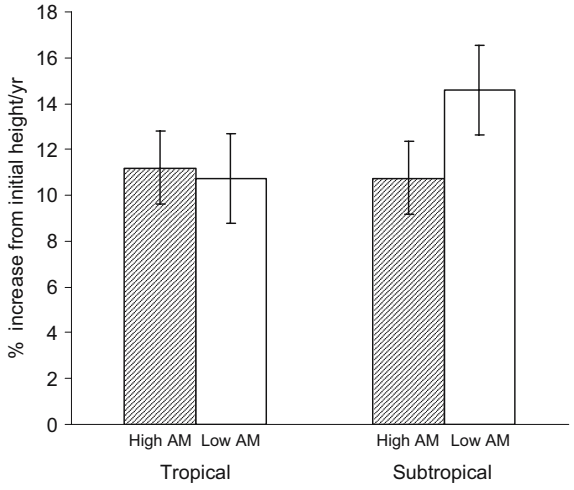

Fig. 2 Mean (±SE) growth rates of seedling species with high vs low occurrence of AMF in a subtropical and tropical study site. Growth rates are expressed as the percentage increase from initial seedling size per year and are estimated from the 4-year period between 1988 and 1992

rates were lower in the subtropical site, but seedling species that formed AMF associations and those that did not had similar survival (t=0.259, P=0.403). On average, 9.8% of seedlings that rarely formed AMF in the subtropical site survived 10 years compared with 7.8% for the high AMF seedlings (Fig. 1).

Seedling species with high vs low occurrence of AMF colonization also had similar growth rates in both forests (Fig. 2) (t=−0.969, P=0.387 for the subtropical site and t=0.134, P=0.897 for the tropical site). Growth rates were very low with most seedling species adding 10–15% to their height each year. At these rates of growth, a seedling that germinates at a height of 10 cm will reach an average height of 30 cm in 10 years.

A multiple regression analysis of a 10-years survival for the 15 seedling species, for which both AMF colonization and seed biomass data were available, was significant ($F_{1,13}$=5.10, P=0.043). Seed mass contributed significantly to the multiple regression results (β=0.548, t=2.260, P=0.043), while AMF colonization did not (β=−0.277, t=−0.774, P=0.455).

Discussion

Variation in AMF occurrence among species

In both rain forests examined, a third or more of the seedling species studied were rarely or never observed to form mycorrhizal associations. When these data are combined with the abundance of the seedling species at the study sites from 1965 to 1996 (Table 1), 43% of the seedlings encountered during that 30-year period would have been unlikely to be colonized by AMF at the tropical site compared with 52% at the subtropical site. These results contrast with several studies on the incidence of AM fungi in tropical forests that observed high occurrence of AMF (e.g., St. John 1980; Bereau et al. 1997; Allen et al. 1998; Onguene and Kuyper 2002) but are consistent with the results of Zangaro et al. (2000) and Zhao et al. (2001) who also found low occurrence of AMF in climax rain forest communities.

There was consistency among plant families in patterns of AMF occurrence at the tropical and subtropical sites; six of the seven plant families observed at both sites showed similar responses. The single exception to the pattern was in one member of the Sterculiaceae at the subtropical site which was consistently colonized by AMF, while the other two species examined showed variable occurrence of AMF. These results suggest that lack of AMF colonization may be a trait inherent to some plant families. While this consistent lack of AMF formation is not surprising for members of the Proteaceae that generally form cluster roots rather than mycorrhizas (Lamont 1982), it is unexpected for families such as the Euphorbiaceae and Sapindaceae that have been observed to form AMF associations in other tropical forests (e.g., Metcalfe et al. 1998; Zhao et al. 2001).

Potential mechanisms to explain the variability in AMF occurrence and colonization

Mycorrhizal inoculum can be spatially patchy which could contribute to variable rates of AMF colonization among plants (e.g., Janos 1992; Lovelock and Miller 2002). However, lack of inoculum was unlikely to have contributed to the low occurrence and colonization by AMF observed in many seedling species in this study. Random collections of soil cores from both sites revealed the presence of AMF colonization and thus potential inoculum, in the roots of all cores. Furthermore, at the tropical site, AMF spores were moderately abundant (mean of 54 spores/g soil) and bioassays used to assess mycorrhizal inoculum resulted in values of percentage root length colonized of about 20% except in areas where terrestrial vertebrates had been excluded for several years (Gehring et al. 2002). These results argue that seedlings are likely to encounter AMF inoculum upon germination and that other mechanisms are necessary to explain the lack of AMF colonization observed on seedling roots at these sites.

The stage of forest succession and associated variation in seed size has been suggested as an explanation for variation in AMF colonization among tropical forests. Janos (1980, 1983) hypothesized that seed reserves were important for mycorrhiza formation and seedling growth, particularly later in succession. In contrast, Allsop and Stock (1995) proposed that seedlings with large seed reserves would have lower dependency on mycorrhizas because they have sufficient nutrient reserves for early growth without mycorrhizas. The work of Siqueira et al. (1998) supports the latter hypothesis as they observed that pioneer plant species in Brazilian tropical forest have small seeds, high rates of AMF occurrence, high AMF colonization, and high dependency on AMF for growth. The opposite patterns were observed among climax forest species. Zangaro et al. (2000) found similar relationships in another Brazilian forest. In the Australian forests we studied, there was no relationship between AMF occurrence or colonization and seed size, despite seed sizes that ranged from 25 to 3,900 mg. In addition, no relationship between AMF and seed size was observed within families from which multiple species were sampled. However, all of the seedling species we examined were climax forest species. The variation in the relationship between seed size, successional status, and AMF development observed among studies suggests that further research is necessary, particularly mechanistic studies that disentangle seed size from other characteristics of successional stage.

Because AMF are obligately dependent on plants for energy (Smith and Read 1997), the low-light levels of the rain forest understory may result in negligible colonization of plant species that might be more highly colonized by AMF in higher light. We tested this hypothesis by comparing rates of AMF colonization for four plant species in the shaded understory and small canopy gaps. Based on the survey work reported in Tables 1 and 2, two of the seedling species did not form AMF in the shaded under-

story, one consistently formed AMF in the understory, and one showed variable occurrence of AMF in the understory. Levels of AMF colonization increased in small canopy gaps in the two species that showed some evidence of colonization in the understory. This result suggests the hypothesis that light intensity is an important predictor of AMF colonization in species that are obligately or facultatively mycotrophic. In support of this hypothesis, two additional species that showed consistently low or variable AMF colonization in the field had significantly higher levels of colonization with increasing light intensity in the greenhouse (Gehring 2003, 2004). Also, given that most of the roots in the randomly collected cores likely came from canopy trees, the high occurrence of AMF colonization in these cores further suggests that rain forest plants are more likely to be colonized by AMF when exposed to higher light intensity. The contrast between seedling roots and the core roots likely to have come from adult trees suggests that AMF occurrence and colonization are likely to increase in some rain forest tree species as they reach higher strata of the forest with increasing age.

AMF and seedling performance

We found that seedlings of species that consistently formed AMF and those that did not had similar rates of growth and survival. Furthermore, seedling survival was not significantly correlated with AMF colonization. These data argue that formation of AMF during early life does not allow species that form symbioses with AMF to outperform seedlings that do not form mycorrhizal associations. At least in seedlings, investment in mycorrhizas appears to represent an equivalent adaptation to the formation of cluster roots or the lack of an apparent specialized mechanism for nutrient uptake. In contrast, investment in larger seeds resulted in significantly higher survival among species.

Given the strongly light-limited environment of the rainforest understory, it might be more surprising that any plant species forms mycorrhizas given the low plant growth rates and the significant carbon cost of AM fungi (Smith and Read 1997). Formation of AMF might be inevitable for some plant species if the fungus rather than the plant is in control of the symbiosis, although understory seedlings would likely represent a poor carbon resource for AMF relative to canopy trees. Alternatively, seedlings that are colonized by AMF might benefit from these fungi when compared to conspecifics that are not colonized. In that case, AMF inoculum limitation, through loss of vertebrate spore dispersers, for example (Gehring et al. 2002), could favor nonmycorrhizal species. Finally, even if seedlings do not benefit from AMF colonization under low-light conditions, acquisition of AMF could be a bet-hedging strategy in which rain forest seedlings with AMF could take advantage of gaps in the canopy to acquire resources and grow more rapidly compared to nonmycorrhizal neighbors (Janos 1980, 1983; Whitbeck 2001).

Implications for common mycorrhizal networks

Our measures of colonization and seedling recruitment in the two forests suggest that 43–52% of the seedlings germinating over a 30-year period were not colonized by mycorrhizal fungi. These seedlings would not have been linked to canopy trees or other seedlings through a common mycorrhizal network (CMN). Linkage to a CMN could allow seedling access to a larger pool of soil nutrients and water, increased resource acquisition via transfer from other plants, or lowered costs of AMF because of carbon inputs from neighboring plants (Simard and Durall 2004). For example, Onguene and Kuyper (2002) observed that ectomycorrhiza formation and seedling survival were higher in seedlings near adult trees than in isolated seedlings in a Cameroon rain forest, presumably due to the presence of a CMN. Our findings suggest that these potential benefits could not have been received by a large portion of the seedling community. Furthermore, the remarkably low growth rates of the seedling species we examined (6 years was required for a doubling in height) suggest that competition with canopy trees may be more important in these forests than the potentially facilitative effects of connection to a CMN, which may be more important at low tree densities (Simard and Durall 2004). Our results and those of other recent studies showing high variability in AMF occurrence and dependency in tropical forests (e.g., Zangaro et al. 2000; Zhao et al. 2001) suggest that the relationships between rain forest seedlings and mycorrhizal fungi are complicated and argue for further field and greenhouse studies that mimic the range of natural conditions encountered by seedlings.

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