

Combined effects of arbuscular mycorrhizas and light on water uptake of the neotropical understory shrubs, *Piper* and *Psychotria*

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Summary

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- Root hydraulic conductance (K_r) was measured for five understory shrub species of the neotropical moist forest to determine the effects of arbuscular mycorrhizas (AM) for both carbon-rich and carbon-limited host plants.
- K_r was measured using a high pressure flow meter (HPFM) for potted plants grown in a factorial combination of AM fungi (presence/absence) and light (3.5 and 30% of full sun, low/high).
- AM colonization improved K_r for the more shade-tolerant species plants when growing in low light. By contrast, water uptake efficiency of the light-demanding species was significantly decreased by AM fungi in high light. Regardless of AM colonization, light-demanding species had a lower capacity than shade-tolerant species to meet transpirational demands, and they allocated substantially more to fine root production relative to leaf area when colonized.
- The differential effects of AM colonization and light on a species' root hydraulic conductance in relation to phylogeny and light adaptation demonstrate that AM fungi may be critical in determining early plant succession and community composition not only due to effects on nutrient uptake, but on water uptake as well.

Key words: arbuscular mycorrhiza (AM), high pressure flow meter (HPFM), hydraulic conductance, *Piper*, *Psychotria*, tropical forest.

Abbreviations

AM, arbuscular mycorrhiza; K_r , root hydraulic conductance ($\text{kg MPa}^{-1} \text{s}^{-1}$); A_r , root surface area (m^2); A_L , leaf area (m^2); K_r/A_L : leaf specific conductance; RDW, root dry weight (g); SRA (A_r/RDW), specific root area ($\text{m}^2 \text{kg}^{-1}$).

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Introduction

Although arbuscular mycorrhizas (AM) are most frequently cited for their importance in nutrient uptake, particularly phosphorus (P), the ubiquitous fungus-root symbiosis can also have beneficial effects on plant water relations. Irrespective of an impact on P nutrition, mycorrhizal plants have been shown to increase transpiration (Hardie, 1985), conductance (Augé *et al.*, 1986b), and drought resistance (Davies *et al.*, 1993). AM can improve plant water relations through accumulation

of solutes in AM roots (Buwalda *et al.*, 1983; Augé & Stodola, 1990) and increased osmotic potential (Augé *et al.*, 1986a), adjustments in root turgor or plant hormone levels (Augé & Duan, 1991), changes to root morphology, and improved soil aggregation (Miller & Jastrow, 1990; Rillig & Steinberg, 2002). Evidence suggests that direct uptake of water through AM hyphae can increase transpiration (Faber *et al.*, 1991) and conductance (Ruiz-Lozano & Azcón, 1995), but other empirical evidence (George *et al.*, 1992; Tarafdar, 1995) as well as theoretical limitation due to hyphal internal diameter

(Fitter, 1985) suggests that there is no significant direct water transport by AM hyphae to plants. The degree and method by which AM influence water relations is likely to vary among host species, depending both on host root architecture and mycorrhizal responsiveness (dependence). The architecture of a plant's root system potentially limits root hydraulic conductance (K_r) and possibly gas exchange and growth (Tyree & Ewers, 1991). Optimal hydraulic architecture would support maximum transpiration, but conducting elements that permit high water flow are also more susceptible to breakage of the xylem water column (embolism) during drought conditions. Based on this proposed evolutionary trade-off (Tyree *et al.*, 1994), light-demanding species should maximize K_r to support higher transpirational demands while shade-tolerant species will have denser roots that resist drought-induced embolisms. The capacity of AM to increase a plant's water flow as well as foraging ability in drying soils could increase maximum K_r under moist conditions while reducing the likelihood of embolisms during drought, thereby expanding a species habitat range and reducing the differences between light-demanding and shade-tolerant species.

In this study, we quantified the interactive impact of AM and light on K_r for two conspicuous shrub genera of Barro Colorado Island (BCI), *Psychotria* (Rubiaceae) and *Piper* (Piperaceae), using species ranging from light-demanding (requiring high light at some stage in the life cycle) to shade-tolerant (ability to germinate, grow, and reproduce in the shaded understory). Based on habitat preferences, the genus *Piper* generally appears to be more light-demanding than *Psychotria* (Croat, 1978). In addition, *Piper* and *Psychotria* are potentially different in their mycorrhizal dependency with *Piper* being more 'facultative' in their AM dependency according to family records (Piperaceae: Garcia & Vázquez-Yanes, 1985; Maffia *et al.*, 1993; Rubiaceae: Alwis & Abeynayake, 1980; Redhead, 1980). The range of life history strategies within each genus and the varying degree of AM responsiveness provide an opportunity to compare and contrast the effect of light and AM on water uptake within a phylogenetic context. Root conductance of seedlings was measured with a high-pressure flow meter (HPFM), a method shown to be both accurate and rapid (Tyree *et al.*, 1995).

We measured K_r to determine whether it is affected by: AM (directly and indirectly through plant P nutrition); and/or light environment. The results were considered in relation to a plant species' light adaptation. K_r was standardized for variability in plant size by each of three size variables: root surface area (A_r), root dry weight (RDW), and leaf area (A_L). The variable used for standardization may provide different results and insights according to their physiological and ecological relationship to K_r (Tyree *et al.*, 1998), as well as the differential response of the size variables to AM and light.

As outlined in Tyree *et al.* (1998), water flows from the root surface (fine roots usually account for > 90% of total root surface area) to the xylem vessels through nonvascular tissue and

represents the primary resistance to root water uptake. K_r relative to A_r (or fine roots) therefore provides a physiological estimate of root conductive efficiency. Extraradical AM hyphae are analogous to very fine roots and may increase the absorptive potential of AM roots without increasing the measured value of A_r . We hypothesized therefore that mycorrhizal plants would have greater K_r/A_r relative to controls. In addition, AM should reduce the need for fine roots while improving mineral nutrition, further improving K_r/A_r . A nonmycorrhizal control treatment with additional P, was included to help determine the proportion of water uptake due to improved nutrient status.

RDW is a measure of total carbon investment for both structural and functional components of roots (fine roots). Scaling K_r by RDW provides a measure of water uptake relative to the costs of resource allocation below ground. Light strongly affects carbon allocation, with shaded plants allocating more to assimilatory organs (leaves) than roots; therefore, we predicted that K_r /RDW would be strongly affected by light intensity. A different response for K_r /RDW and K_r/A_r would depend on a species' response to the treatments with regard to production of fine roots relative to root biomass, or specific root area (SRA: A_r /RDW). It is not known whether SRA is a fixed trait in these species or will be differentially affected by AM and/or light.

Finally, we scaled K_r by leaf area [(K_r/A_L) , also termed leaf specific conductance] because of the interaction between K_r and transpiration. This provides an indication of the 'sufficiency' of the root system to provide water to leaves. Light-demanding species should have higher K_r/A_L to support higher transpiration, hence net assimilation. AM colonization has been shown to affect plant morphology (e.g. leaf area:biomass ratio), but more commonly influences plant physiology. It is not clear therefore whether changes in K_r due to AM will be responsive to leaf area.

Materials and Methods

Site and species

The study was conducted at the Smithsonian Tropical Research Institute on Barro Colorado Island (BCI), Republic of Panama (9–10° N, 79–51° W). The lowland (165 m elevation) tropical moist forest is characterized by a distinct dry season (early January–late April). Mean annual precipitation is 2600 mm (Windsor, 1990), but soil water potentials during the dry season may fall to –2.0 MPa (Becker *et al.*, 1988). Nevertheless, 90% of plants on BCI, including all shrub species, are evergreen (Croat, 1978). Further details of the site and species can be found in Leigh *et al.* (1982) and Croat (1978).

Three species of both *Piper* (*Piper culebranum* L.C. Rich, *Piper aequale* Jacq., and *Piper cordulatum* Sw.) and *Psychotria* (*Psychotria acuminata* Benth, *Psychotria furcata* DC., and *Psychotria marginata* Sw.) were chosen to represent a range of life

histories from early mid successional to understory specialist species (as ordered above). *Psychotria furcata* was physically too small for the HPFM and excluded from the study. *Psychotria marginata* and *Pi. cordulatum* are deep shade-tolerant species that occur in drier sites (Croat, 1978). *Piper aequale* is an understory specialist (Freeden & Field, 1996; Valladares *et al.*, 2000), but requires higher light for germination and early growth (J. Wright, pers. comm.; Orozco-Segovia & Vázquez-Yanes, 1989). By contrast, *Pi. culebratum* and *P. acuminata* are more light-demanding species typically associated with small light gaps (Croat, 1978, pers. obs.) and the former is commonly found in moist ravines (Croat, 1978). *Psychotria marginata* has a root morphology (limited branching and few root hairs, pers. obs.) more reminiscent of families that are strongly AM dependent (Baylis, 1975). The three *Piper* spp. all had root hairs; fine roots of *Pi. aequale* were densely covered.

General

Study plants without AM colonization were created by growing cuttings of each species in flats of sterile vermiculite until at least one new leaf had flushed (*c.* 4–6 wk). Individuals were then randomly chosen and transplanted individually into 2.5 litre pots. Pots were watered to saturation daily and amended weekly (after watering) with 50 ml of a one-fifth strength Hoagland nutrient solution containing both macro- and micronutrients (Machlis & Torrey, 1956), but lacking phosphorus (P). The standard Hoagland's containing P was created using KH_2PO_4 as the phosphate source.

Soil used was a nutrient-poor mixture of red clay (*c.* 0.2 g kg⁻¹, 1.1 g kg⁻¹ N, and 3.0 g kg⁻¹ C (Yavitt *et al.*, 1993)) from 30 to 100 cm below the soil surface (avoiding the thin layer of humus) and washed river sand in a 2 : 1 ratio. Sand provided thorough drainage and facilitated easier removal of roots at harvest. All soil was mixed well, spread out to reduce moisture content, and then sterilized using 'Dowfume' (methyl-bromide gas with 2% chloropicrin) following the methods of Janos (1985).

AM fungal inoculum was acquired from field-collected, feeder roots of a locally abundant palm, *Oenocarpus panamensis* (Arecaceae), commonly colonized by several species of AM fungi (Janos, 1985, including, but not restricted to, *Acaulospora foveata*, *A. tuberculata*, and a copious amount of an unidentified *Glomus* sp.; D. Janos, pers. comm.). Growth of each plant species may fall within a range of responses according to the particular fungal species inoculum (Streitwolf-Engel *et al.*, 1997); therefore, a mixed-species inoculum is appropriate for ecological queries. To maximize the likelihood of colonization, colonized feeder roots and soil were collected the same day (Powell, 1976, finely chopped with a machete) and *c.* 15 g of the root/soil mixture was stirred into the upper layer of the soil (Plenchette *et al.*, 1989). The non-mycorrhizal treatment (AM–) received autoclaved feeder roots and a live filtrate

(Whatman #1 paper) excluding AM spores and hyphae (Koide, 1988).

Experimental design

Pots for each species were replicated in a 2 × 2 factorial design in order to test the fixed effects: AM inoculation (AM+/AM–) and light (high/low). Five individuals of each species were randomly chosen for each treatment combination. The two light levels, 3.5% PPFD (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 35% PPFD (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$), approximate the levels in shaded understory and along gap edges where the shrub species commonly grow. The two light environments were established on two growth benches using a shade cloth roof. A fan circulated the air and reduced differences in air temperature and humidity between and among treatments. It is acknowledged that the light treatment is not defined only by light intensity, but we maintain that treatment differences in associated factors are ecologically representative. Access to a single growing house and limitation of space on BCI made it impossible to replicate the light treatment across benches. Pots were arranged randomly on each bench and rearranged weekly.

Physiological comparisons between mycorrhizal and control plants are best done with plants of similar size (see Snellgrove *et al.*, 1982), so we created a second AM– control in which P was included in the Hoagland's nutrient solution ('P-amended'). The soil used in this experiment was low in P (0.2 ppm; Yavitt *et al.*, 1993), so we predicted that the addition of a standard Hoagland's concentration would be sufficient to increase P availability to AM– plants. The addition of P, however, generally had no significant effect on growth parameters (relative growth rate, total leaf area, root area or dry weight) of P-amended controls relative to AM– controls without additional P. Unfortunately, plant material was lost in shipping and unavailable for direct measures of plant P concentrations. Any small increases in P-amended relative to AM– plants had little effect on the strong differences between AM+ and the AM– treatments. AM– plants with and without P amendment therefore were pooled for analysis and presentation of data. All significant exceptions will be discussed in the text.

Measurements and harvesting

Five plants were randomly chosen for root conductance measures and harvest (after *c.* 4 months of growth). Root hydraulic conductance was measured with a high pressure flow meter (HPFM; Dynamax, Houston, Texas, USA) as described in detail in Tyree *et al.* (1993). The theory of HPFM as a measurement tool for K_r is discussed in Tyree *et al.* (1995). The HPFM is attached to a severed stem just above the soil and water is forced into the roots through the stem in the opposite direction of flow during transpiration. Pressure and flow are measured simultaneously with K_r estimated as the slope between the two variables in the linear region. Plants

with large fluctuations in pressure and/or flow were excluded. This could occur if the seal on the stem was leaky or if a primary root was damaged.

Following measurement of K_r , leaf area was determined with a Li-Cor 3200 leaf area meter (Li-Cor, Lincoln, Nebraska, USA). Roots were gently washed to remove soil and root surface areas and lengths were measured with a Delta-T root scanner system (Dynamax). Root surface area was adjusted as the included software underestimates root surface area by a factor of π (M. T. Tyree, pers. comm.). It is unlikely that other researchers are making this same adjustment, therefore this should be taken into account when making any comparisons with results from other studies using the Delta-T scanner system. Shoots (leaves and stems) and roots were subsequently dried at 60°C for > 48 h and weighed.

Conductance measures (K_r , kg MPa⁻¹ s⁻¹) were standardized by root area (m²), root dry weight (g), and leaf area (m²). The relationship of root surface area (fine roots) relative to total root biomass was defined as specific root area (SRA). Average relative growth rate (RGR) of new plant dry mass was calculated as $(\ln W_2 - \ln W_1)/t_2 - t_1$ where W_2 and W_1 were the dry masses at the beginning and end of the experiment. Mycorrhizal colonization levels were measured for individuals not used in the experiment. Roots were cleared and stained with aniline blue following the modified version by Grace & Stribley (1991) and analyzed using the simplified presence/absence version of the slide method (Giovanetti & Mosse, 1980). To estimate percentage colonization, each root piece ($N = 50$ or 100, controls and inoculated pots, respectively) was scored as colonized or not by presence of appresoria and typical AM hyphae, hyphal coils, arbuscules, or vesicles in the cortex (Giovanetti & Mosse, 1980).

Statistical analysis

A Type I 2 × 2 ANOVA was performed separately for each species using PROC GLM of SAS (SAS Institute, 1989) to measure the fixed effects of AM colonization and light environment on K_r and plant growth measures. Data were log transformed

because plants were in an exponential phase of growth and K_r has also been shown to increase exponentially in proportion to leaf and root area (Tyree *et al.*, 1998). An inspection of the residuals indicated that the transformation increased normality and improved R^2 values. As noted previously (see Experimental design above), data were pooled for the AM– treatments (with and without P). A Tukey posthoc test was used to compare AM+, AM–, and P-amended treatments before pooling all AM– plants. The ANOVA model was expanded to include the fixed effect of species in order to test for overall species differences across all treatments using a Tukey post hoc test. Interactions of species with AM fungi and light will be discussed. Percent colonization data were not statistically analyzed as only a few root fragments of AM– controls showed any sign of colonization (Table 1).

Results

The size of plants was highly variable among treatments with significant species × light and species–AM interactions in root biomass, leaf area, and RGR (ANOVA results not presented, but see Fig. 1a–c); in general, mycorrhizal plants were larger. Nonstandardized values of K_r were significantly higher in AM plants for all species (P -values ranged from 0.006 to 0.0003, data not shown). It has been shown that conductance increases in proportion to plant size (Tyree *et al.*, 1998), therefore all further comparisons across treatments and among species will be made on scaled data.

Relative root conductance

Root hydraulic conductance relative to root surface area (K_r/A_r) was significantly greater with AM colonization in the more shade-tolerant species (*Pi. aequale*, *Pi. cordulatum*, and *Ps. marginata*) and marginally so in *Pi. culebratum* due to similar results in low light (Table 2; Fig. 2a). The trend in high light indicated that AM colonization decreased K_r/A_r in the light-demanding species (Fig. 2a), particularly for *Ps. acuminata* ($P < 0.05$, Table 2). Only *Pi. cordulatum* had significantly

Table 1 Percentage of root pieces of *Piper* and *Psychotria* colonized by arbuscular mycorrhizas (AM) for plants grown in pots under high (35% PPFD) or low light levels (3.5% PPFD) with chopped root inoculum (AM+) or sterilized controls (AM–)

Species	High light		Low light	
	AM+	AM–	AM+	AM–
<i>Piper aequale</i>	32	0	28	0
<i>cordulatum</i>	30	2	42	0
<i>culebratum</i>	26	0	24	0
<i>psychotria</i>				
<i>acuminata</i>	32	2	22	0
<i>marginata</i>	10	1	6	0

Roots were cleared and stained with aniline blue and examined for colonization using the simplified presence/absence version of the slide method. Each root piece ($n = 50$ or 100, controls and inoculated pots, respectively) was scored as colonized or not by presence of any of the following elements: appresoria and typical AM hyphae, hyphal coils, arbuscules, or vesicles in the cortex.

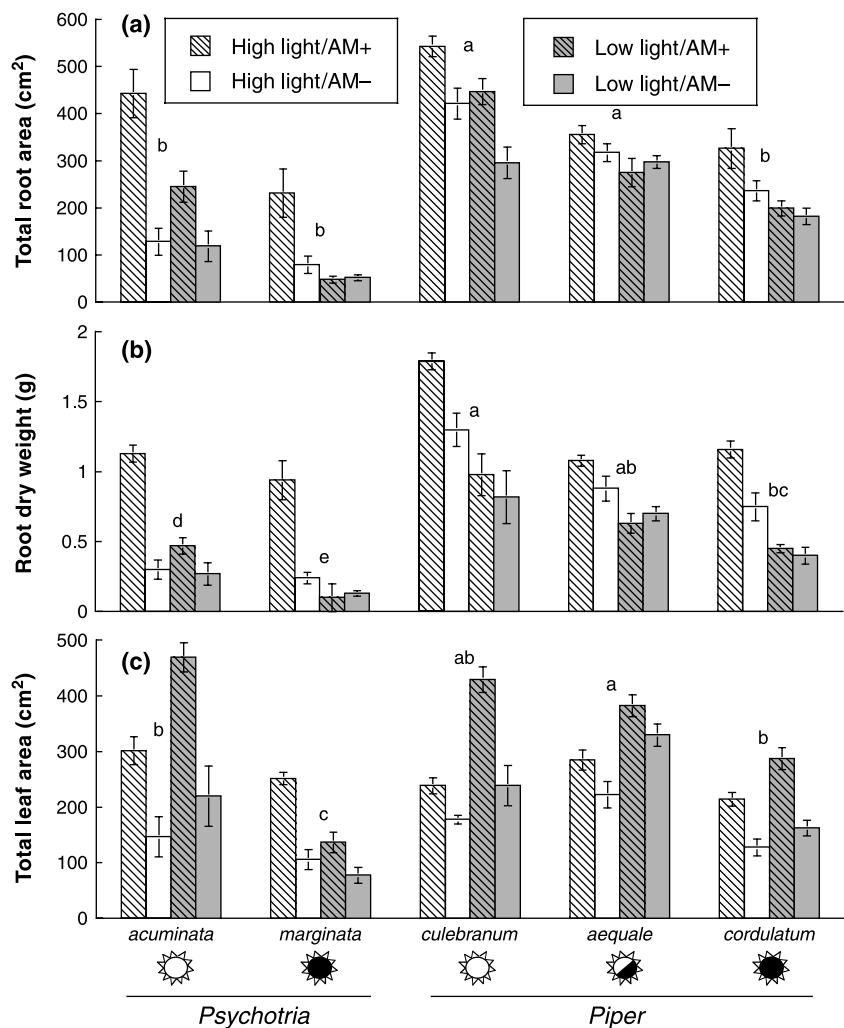


Fig. 1 Mean (\pm SE) values of (a) root area (A_r , cm²) (b) root dry weight (RDW, g), and (c) leaf area (A_L , cm²) for controls or plants inoculated with AM fungi grown from cuttings for 4 months in sun (35% PPFD) or shade (3.5% PPFD). Data from AM–controls with and without phosphorus fertilization were pooled for all species and variables. Symbol below each species indicates the range of light adaptation from light-demanding (open) to deep shade-tolerant (closed). Different letters above each species indicate significantly different overall means by a Tukey test ($P < 0.05$).

higher K_r/A_r in AM+ roots in high light (Fig. 2a). In general, K_r/A_r varied according to a species' light adaptation and the light in which it was growing.

Overall, the effects of AM on K_r/RDW were similar to those for K_r/A_r ; i.e. K_r/RDW was significantly greater in AM+ plants for the shade-tolerant species and significantly reduced (in high light) for the light-demanding species (Table 2; Fig. 2b). Unlike the results of K_r/A_r , light intensity also had a significant effect on K_r/RDW in all species (Table 2). This was due in part to significant increases in below-ground carbon allocation in high light (Table 2; Fig. 3a: R : S ratio; ranging from 44 to 110% higher) and consistent decreases in A_r/RDW or SRA. SRA was lower in high light for all species (significantly so except for *Ps. acuminata*, Table 2), and values for AM+ plants were 13–38% lower within high light (Fig. 3).

For four of the five species, K_r/A_L was unaffected by AM, but was reduced in low light (Tables 2, $P < 0.004$ – 0.0001 ; Fig. 2c). Except for *Ps. marginata*, smaller K_r/A_L at low light was due to greater A_L (Table 2; Fig. 1c). The exception to the above pattern was *Pi. culebratum* which showed no effect of

light on K_r/A_L , but a decrease when colonized by AM fungi. Comparing among treatments and overall, the roots of *Piper* spp. had greater K_r/A_L than the *Psychotria* species (Tukey, $P < 0.05$; Fig. 2c). This is explained by comparably higher allocation to root surface area relative to leaf surface area ($A_r:A_L$) for *Piper* than *Psychotria* (Fig. 3: Tukey, $P < 0.05$). Both K_r/A_L and the $A_r:A_L$ ratio were highly responsive to light, but not AM fungi (Table 2).

Importance of P nutrition vs other AM effects

Conductance terms across treatments were significantly greater (or marginally so) for *Pi. cordulatum* than the other species (Fig. 2a–c, means comparison across treatments: Tukey, $P < 0.05$). Uncolonized roots of this deep shade-tolerant species had higher K_r/A_r than most species and increases by AM in both high and low light (49 and 64%, respectively) were highly significant (ANOVA, $P < 0.0001$; Table 2). Unlike the other species, P fertilization of AM– controls ('P-amended') significantly reduced the K_r/A_r advantages of AM+ plants by

Table 2 *F*-values of a 2 × 2 ANOVA for *Psychotria* and *Piper* root conductance (K_r) relative to root area (A_r), root dry weight (RDW), and leaf area (A_L); also: root:shoot ratio (R:S), root area:leaf area ratio ($A_r:A_L$), whole plant relative growth rate (RGR), and specific root area (SRA)

		<i>Psychotria</i>			<i>Piper</i>	
		<i>acuminata</i>	<i>marginata</i>	<i>culebratum</i>	<i>aequale</i>	<i>cordulatum</i>
K_r/A_r	AM	4.72*	4.92*	3.40†	11.12**	21.96***
	Light	3.76†	1.62	37.20***	0.80	0.49
	A × L	1.17	4.58*	4.24*	2.48	0.18
K_r/RDW	AM	6.49*	4.34*	0.01	14.04***	11.14**
	Light	18.77***	11.23**	84.05***	10.56**	22.65***
	A × L	8.58**	25.27***	4.30†	7.22*	0.90
K_r/A_L	AM	0.23	0.01	5.53*	2.87	0.61
	Light	14.92***	29.51***	0.43	10.38**	28.87***
	A × L	0.99	2.36	0.01	1.43	0.29
A_r	AM	19.82***	6.59*	7.64**	5.23*	3.91†
	Light	1.81	18.94***	6.69*	0.03	10.54**
	A × L	0.78	8.93**	0.28	2.28	0.93
RDW	AM	18.04***	12.63**	3.58†	0.36	6.77*
	Light	3.30†	69.07***	13.83***	12.79**	35.62***
	A × L	1.89	26.18***	0.09	2.85†	1.54
A_L	AM	11.61**	20.14***	21.72***	6.30*	16.86***
	Light	1.96	6.47*	16.37***	17.09***	4.02†
	A × L	0.01	1.04	3.15†	0.54	0.00
RGR	AM	14.37***	23.09***	10.76**	4.88*	14.75***
	Light	0.22	30.89***	2.59	0.29	4.12†
	A × L	0.27	3.85†	0.28	0.27	0.14
R:S	AM	1.00	7.39*	0.01	1.10	3.69†
	Light	7.61*	18.26***	61.34***	26.98***	31.56***
	A × L	1.48	27.42***	3.05†	0.68	0.03
$A_r:A_L$	AM	0.16	2.35	3.11†	2.78	5.19*
	Light	14.28***	5.08*	45.39***	19.73***	16.73***
	A × L	1.15	4.34*	0.43	0.00	0.04
SRA	AM	0.03	0.08	2.21	0.46	2.95†
	Light	1.46	29.44***	7.15**	6.90*	36.49***
	A × L	1.79	5.66*	0.16	0.75	0.22

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; † $P < 0.10$ (marginal significance). Controls or plants colonized by arbuscular mycorrhizas (AM) were grown in pots under high (35% PPFD) or low (3.5% PPFD) light in a screened growing house.

30% in high light and entirely accounted for the differential AM response in K_r/RDW and K_r/A_L (Fig. 4). By contrast, K_r/A_r for *Ps. acuminata* was 25% lower in AM+ plants than AM- controls (high light only) and 50% lower in AM+ plants than P-amended controls (overall AM effect, $P < 0.02$; Tukey $P < 0.05$; Fig. 4). The negative effects of AM fungi on K_r/A_r occurred despite the fact that mycorrhizae significantly increased RGR in all species (Table 2), and particularly for *Ps. acuminata* (35 and 48% increases in low and high light, respectively; Fig. 3). Phosphorus fertilization decreased the growth advantages of AM+ *Ps. acuminata* by c. 30% in both light levels (data not presented), confirming that P uptake is a critical component of the improved growth response by AM fungi.

The effect of AM and light on root growth and morphology

Changes in proportional carbon allocation and morphological adjustments to roots were primarily associated with light.

Light significantly affected all species for nearly all below-ground measures (Table 2). In high light, plants allocated more to total root production and in proportion to shoots (RDW and R : S ratio), allocated more to total root surface area and relative to leaf surface area (A_L and $A_r : A_L$ ratio), and reduced the quantity of root carbon used for fine roots (SRA; see Fig. 3). Overall, below-ground investment was larger for the light-demanding species within each genus for each of these measures.

The effect of AM on root allocation and morphology was less pronounced. When carbon may have been limiting (i.e. low light), colonization was associated with reductions in R : S and $A_r : A_L$ ratio for all species (Fig. 3a,c), but with significant effects in the most shade-tolerant species (Table 2). Although there was no pattern for R : S and $A_r : A_L$ ratio in high light, SRA was consistently lower for AM+ plants growing in high light (Fig. 3b). Again, lower values of SRA were only significant in the understory specialists: *Ps. marginata* (Table 2, AM × light: $P < 0.012$), and marginally so for *Pi.*

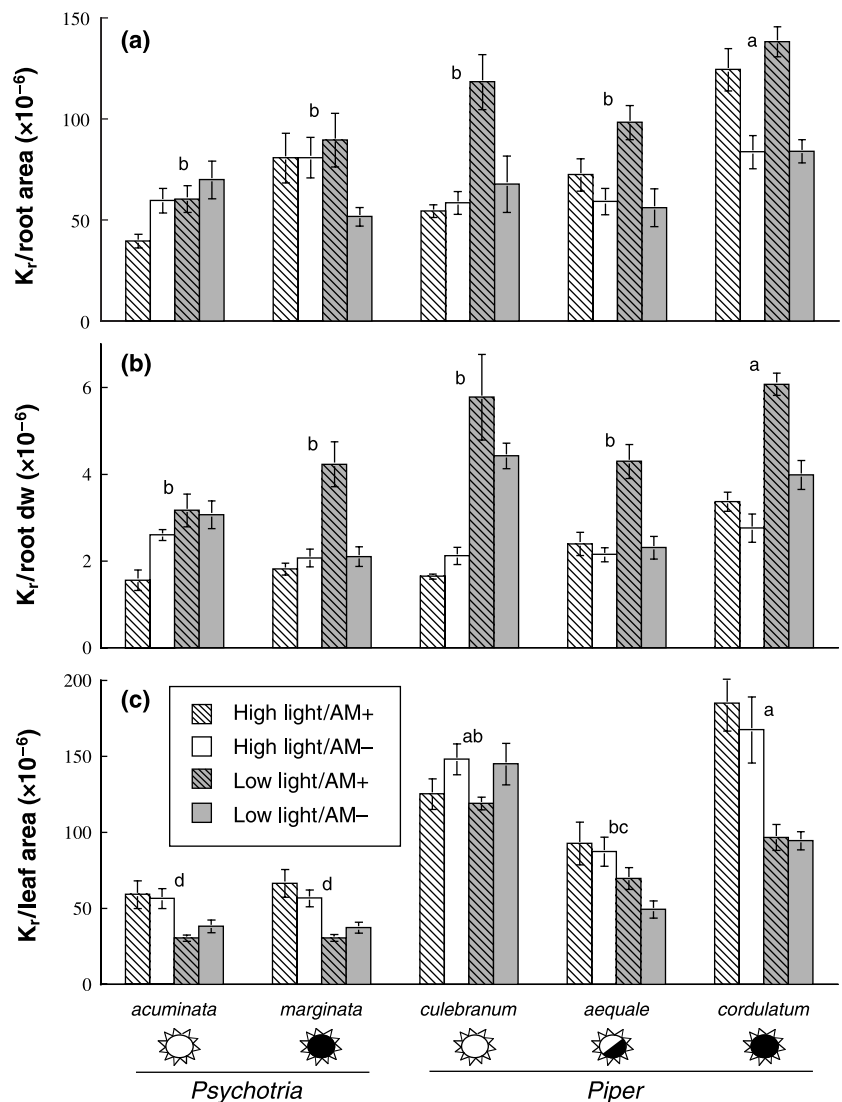


Fig. 2 Mean (\pm SE) values of *Psychotria* and *Piper* root conductance (K_r , $\text{kg s}^{-1} \text{MPa}$) relative to (a) root area (A_r , m^2) (b) root dry weight (RDW, g), and leaf area (A_L , m^2) for controls or plants inoculated with AM fungi grown from cuttings for 4 months in sun (35% PPFD) or shade (3.5% PPFD). Data from AM- controls with and without phosphorus fertilization were pooled for all species and variables. Symbol below each species indicates the range of light adaptation from light-demanding (open) to deep shade-tolerant (closed). Different letters above each species indicate significantly different overall means by a Tukey test ($P < 0.05$).

cordulatum (Table 2 AM: $P < 0.10$). A reduction in proportional root biomass and fine root production is not unexpected given that AM hyphae are acting as the fine absorption area essentially long root hairs. The below-ground response to AM colonization of the two most shade-tolerant species was consistent across both light levels for *Pi. cordulatum*, but interacted with light for *Ps. marginata* (Table 2; Fig. 3).

Similar to results for the same species grown in laboratory microcosms, but opposite to those of field gardens (Kyllo, 2001), the main effect on RGR was AM colonization rather than light. The lone exception was *Ps. marginata*, in which the combination of high light and AM colonization increased RGR by *c.* 30%. Plants growing in high light allocated a lower proportion of total plant biomass to leaf area (leaf area ratio, LAR; $P < 0.0001$ for all species, data not presented). For both *Psychotria* species, mycorrhizal plants allocated even less. The 50% increase in RGRs of AM+ *Ps. marginata* and *Ps. acuminata* in conjunction with the 25–50% decrease in LAR,

respectively, indicates that AM fungi improved carbon fixation by physiological increases in net assimilation using relatively less leaf area. There was no AM effect on LAR for any of the *Piper* species.

Discussion

Positive AM effects on K_r efficiency

Arbuscular mycorrhizae had a clear impact on root conductance in both *Piper* and *Psychotria* species. For shade-tolerant species growing at light intensities in which these species would commonly grow beneath the closed canopy of a neotropical forest, mycorrhizae reduced the total and proportional production of fine roots, but improved their water uptake efficiency by 60–70% (Fig. 2a). Tropical understory shrubs have shallow root systems with no access to deeper water, and greater proportional allocation to root surface area relative to tree

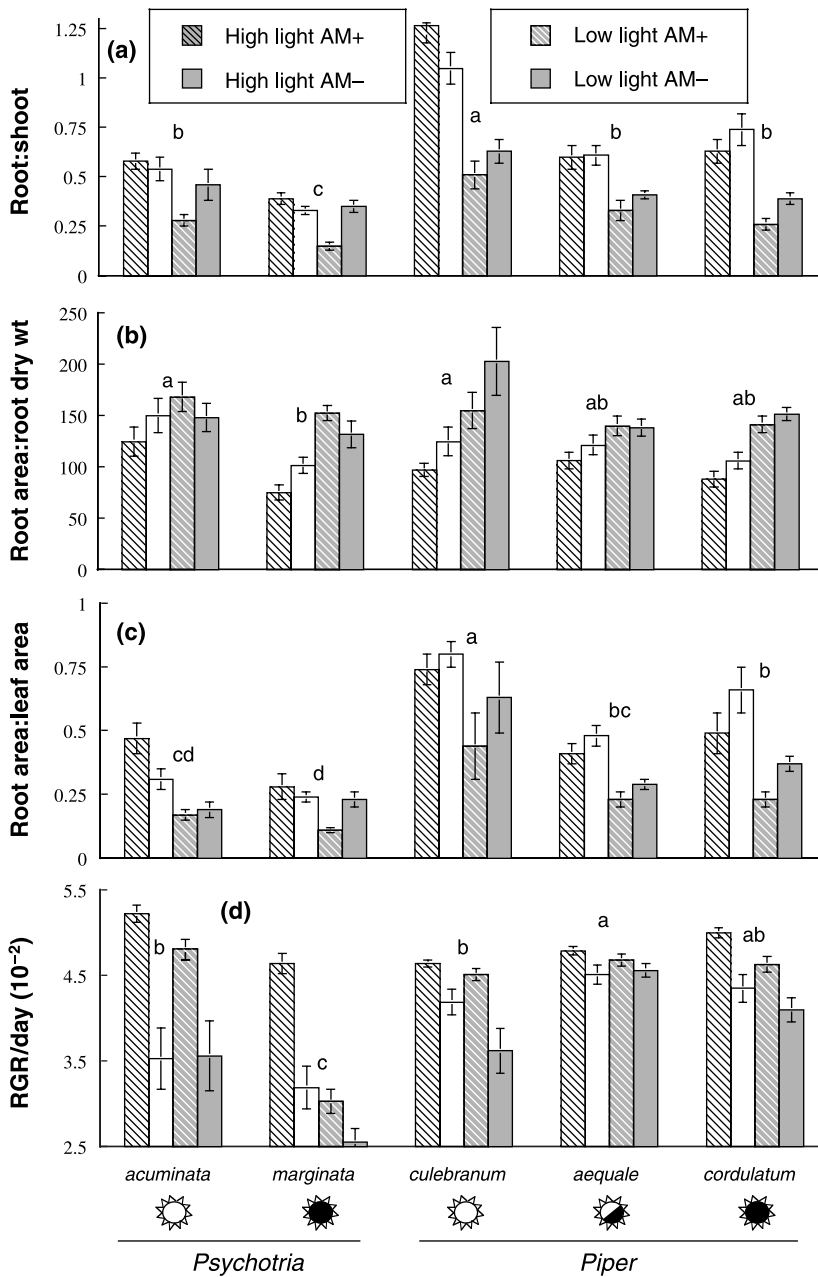


Fig. 3 Means (\pm SE) of *Psychotria* and *Piper* relative growth rate (RGR/day $\times 10^{-2}$), root:shoot ratio (R:S), root area:leaf area ratio ($A_r:A_l$), and specific root area (SRA m^2/kg) for controls or plants inoculated with AM fungi grown from cuttings for 4 months in sun (35% PPFD) or shade (3.5% PPFD). Data from AM- controls with and without phosphorus fertilization were pooled for all species and variables. Symbol below each species indicates the range of light adaptation from light-demanding (open) to deep shade-tolerant (closed). Different letters above each species indicate significantly different overall means by a Tukey test ($P < 0.05$).

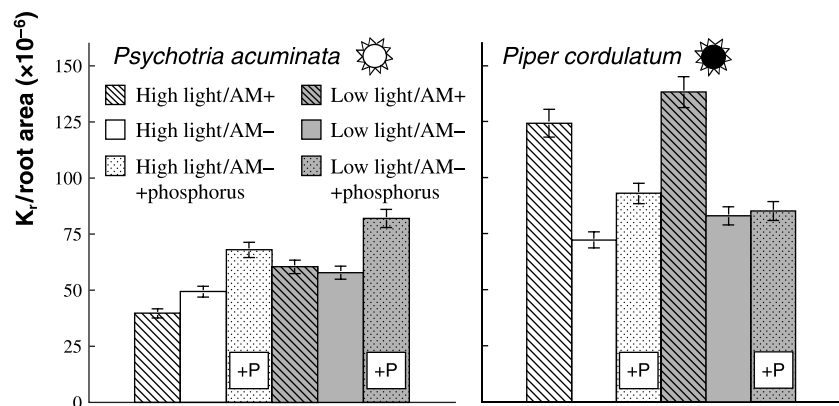
saplings (Becker & Castillo, 1990). The higher proportion of root surface area to mass in roots may be critical to survival throughout the dry season in this forest.

Piper cordulatum, a deep shade-tolerant, had the highest efficiency of K_r across all species and was the only species that showed similar AM improvements for K_r/A_r in both low and high light. For *Pi. cordulatum*, AM also increased K_r in both light levels relative to below-ground carbon allocation, and leaf area. Thus, AM colonization in this species increased the physiological efficiency of the hydraulic system and effectively allocated the plant's carbon to maintain sufficient water for transpiration. This shade-tolerant species is known to be well

buffered against dry season water stress (Rundel & Becker, 1987), and a large component of this advantage may be due to the benefits of the AM mutualism.

The results of P fertilization for *Pi. cordulatum* indicate that one-third of the increase in physiological water uptake efficiency in high light was due to indirect effects of P nutrition. The remaining 70% was due to other effects of AM fungi on the soil/root interface including the possibility of direct uptake and transport by extraradical hyphae. AM hyphae play a critical role in nutrient scavenging, but with regard to water, the magnitude of their role is still unclear. Although *Pi. cordulatum* is an understory specialist, it is commonly found

Fig. 4 Mean (\pm SE) values of root conductance (K_r , $\text{kg s}^{-1} \text{MPa}$) relative to (a) root area (A_r , m^2) of *Psychotria acuminata* (light demanding) and *Piper cordulatum* (shade tolerant). Data for both the AM– control and a ‘phosphorus-amended’ control (+P) are presented for controls or plants inoculated with AM fungi grown from cuttings for 4 months in sun (35% PPFD) or shade (3.5% PPFD).



thriving along paths where light levels are much higher. A more conductive root system due to AM colonization may provide a growth advantage in both low and high light environments of wet forests. The lack of a fertilization effect for *Pi. cordulatum* in low light was likely due to lower P needs associated with lower growth rates.

Negative AM effects on K_r efficiency

Although AM fungi improved the efficiency of root conductance for the shade-tolerant species, AM had no high light effect on K_r/A_r for *Pi. culebratum* and a clear negative effect for *Ps. acuminata*. In both light levels, *Ps. acuminata* colonized by AM fungi had lower K_r/A_r than nonmycorrhizal plants. The likelihood that AM negatively affected water uptake of both light-demanding species in high light is reinforced by the significantly lower K_r for *Pi. culebratum* relative to total root biomass (RDW) and leaf area (A_L). Roots of the light-demanding species had lower efficiency in response to high light as well. In other words, the efficiency of their K_r was decreased when transpirational demands would have been elevated.

Under high light conditions, carbon would not limit plant growth or AM fungal demands if water and nutrients are available. Lower water uptake efficiency, however, may represent a critical limitation to the competitive growth advantages of pioneer species. The light-demanding species had much lower K_r/A_r than the more shade-tolerant congeners when colonized by AM fungi, but there were no clear differences in the nonmycorrhizal state. Although their root conductive efficiency was lower, these species compensated by allocating a greater proportion of their total biomass below ground (R : S ratio), and producing more fine roots in total and relative to leaf area ($A_r : A_L$ ratio). This suggests that these species would be better competitors for water and nutrients than their deep shade-tolerant neighbors when mycorrhizae are reduced or absent (e.g. highly disturbed sites). Increases in RGR due to P fertilization for AM– controls of *Ps. acuminata* demonstrate that AM hyphae improved growth through nutrient uptake despite reduced water uptake.

Leaf specific conductance

Assimilation and transpirational demands per leaf area vary considerably depending on light, AM colonization, and a species' light adaptation. Leaf specific conductance is a rough measure of the sufficiency of water uptake for transpiration, hence photosynthesis. Not surprisingly, K_r/A_L was highly responsive to light, but for the more shade-tolerant *Piper* species, K_r did not respond to changes in leaf area. Instead, greater K_r/A_L in high light was due entirely to decreases in the scaling factor, leaf area. AM colonization did not affect K_r/A_L for any species except *Pi. culebratum*, despite the fact that all species in both light intensities had much higher leaf area when mycorrhizal. In other words, root conductance was closely adjusted to match the changes in leaf surface area due to AM colonization and to support the greater transpirational demands in high light. For *Pi. culebratum*, increases in leaf area due to AM colonization directly corresponded to lower conductance, demonstrating that K_r in this species is less responsive to AM fungi than above-ground carbon distribution.

For *Ps. marginata*, K_r/A_L was significantly greater in high light despite larger leaf areas, particularly for AM+ plants. This indicates that this species would have a greater capacity to supply sufficient water for unpredictable changes in light intensity (e.g. light flecks). The greater potential for transpiration in AM+ plants in high light directly supports the 45% increase in RGR for this species and may be essential for its unique leaf and flower phenology. The roots of *Ps. marginata* in the field grow down no further than 40 cm, have few or no root hairs (pers. obs.), and limited tertiary branching (J. Wright, pers. comm.); yet, this species is highly drought tolerant compared to other *Psychotria* species (Wright *et al.*, 1992). *Psychotria marginata* not only continues to assimilate carbon during the dry season, but produces two leaf types, one of which is adapted to the dry season; furthermore, it is the only common *Psychotria* to flower throughout the year rather than just following the first rains (Mulkey *et al.*, 1992). For the *Psychotria* species, the combined results of RGR and LAR indicate that AM colonization greatly increased their net carbon assimilation rates using relatively little leaf area.

Previous research suggests that light and water adaptations are linked in the *Piper* and *Psychotria* species of this study: each species may be categorized as either light-demanding/drought-avoiding or shade-tolerant/drought-tolerant (Croft, 1978; Wright, 1991; Wright *et al.*, 1992) with the former having higher leaf specific conductance (neotropical tree saplings: Tyree *et al.*, 1998, *Quercus* species: Nardini & Tyree, 1999). Engelbrecht *et al.* (2000) demonstrated this correlation specifically for two *Piper* species, *Pi. cordulatum* and *Piper trigonum* C. DC. (*Piper arieanum*), a species restricted to wet rainforests or very moist microsites. In low light of our study, *Pi. culebranum* (a drought-avoiding species common to moist ravines) followed the same pattern and had higher K_r/A_L than the shade-/drought tolerant, *Pi. cordulatum*. In light levels higher than those used by Engelbrecht *et al.* (2000), however, K_r/A_L was greater in *Pi. cordulatum* with the main difference (c. 50% increase) due to AM. In fact, *Pi. culebranum* was the only species with a significantly lower K_r/A_L due to AM and it occurred in both light levels. In contrast to the negative effects of AM colonization on *Pi. culebranum*, root conductance for *Pi. cordulatum* in our study was about two times higher for AM+ individuals than whole plant conductance reported by Engelbrecht *et al.* (2000; before the comparison, K_r was adjusted to reflect that root conductances are twice as large as whole plant conductance. Individuals between the two studies had the same leaf area and the comparison was made for those plants grown at a similar light level.). The large increase for this understory specialist was due to the addition of mycorrhizal fungi. The results for *Piper*, demonstrate the potential for very different effects of AM colonization on water uptake directly related to photosynthesis and how it might vary according to life history.

Each *Piper* species conducted more water relative to leaf area than the *Psychotria* species regardless of light adaptation. Their greater capacity to maintain transpirational demands is supported by an irrigation experiment showing that *Pi. aequale* and *Pi. cordulatum* had predawn leaf water potentials much greater than their turgor loss points and did not wilt as easily as *Ps. marginata* and *Psychotria furcata* under drought conditions (Wright, 1991). Greater drought tolerance for *Piper* is likely due in part to a greater $A_r:A_L$ ratio. Given that the differences in the $A_r:A_L$ ratio between genera were about two times larger for AM- controls of *Piper* than between mycorrhizal plants, *Piper* species should have a competitive advantage when non-mycorrhizal and therefore, a greater capacity to maintain the water uptake necessary to supply transpirational demands in early to mid-successional environments where light is high and AM colonization is presumably lower due to soil disturbance. Previous field results of the $A_r:A_L$ ratio for tropical shrubs (including one *Psychotria* species and *Pi. cordulatum*) ranged from 0.05 to 0.18 (0.09 for *Pi. cordulatum*; Becker & Castillo, 1990). The lower field estimates demonstrate the need for more experiments in natural conditions.

Light adaptation, root morphology, and AM dependence

For neotropical tree saplings, Tyree *et al.* (1998) found that K_r/A_r did not correlate with light adaptation, but pioneer species clearly had higher SRA than shade-tolerant species. Scaling K_r by RDW indicates the water uptake efficiency relative to carbon investment below ground rather than the physiological efficiency of fine roots. SRA (A_r/RDW) is a direct link between these two measures. If AM hyphae function as more cost-effective fine roots, then lower SRA would be predicted for shade-tolerant species when colonized (Janos, 1980a). In our study, however, treatment differences in SRA were relatively consistent in all species. It is unclear whether this trait would show a response with much greater extremes of light.

Only a few plant families do not form AM symbioses, the majority of which are pioneer colonizers or opportunistic invaders of disturbed sites. Species adapted to high light have greater transpirational demands than understory species. If AM reduce water uptake in light-demanding species, then we might expect a lower infection response or an increased allocation to roots, particularly fine roots (A_r), in order to compensate for this negative effect of the symbiosis. In this study, the light-demanding species showed the latter response, producing substantially more fine roots (A_r) when mycorrhizal (50 and 100% increases; *Pi. culebranum* and *Ps. acuminata*, respectively) in total and relative to leaf area ($A_r:A_L$). A positive nutrient effect, but a negative impact on K_r by mycorrhizae, corresponds to results for *Quercus ilex* and an ectomycorrhizal symbiont (Nardini *et al.*, 2000). Both *Quercus ilex* and *Ps. acuminata*, increased fine root production for mycorrhizal plants to yield sufficient water supply to shoots and compensate for the less efficient mycorrhizal roots.

Pot trials (Janos, 1980a) verify the theoretical predictions (Janos, 1980b) that pioneer species should be less dependent on AM for growth and survival. Mycorrhizal dependence (or responsiveness), calculated as the difference in mean total biomass between AM+ and AM- controls relative to AM+ plants (Hetrick *et al.*, 1991), was much higher in the *Psychotria* species (55–67%; *Ps. acuminata* and *Ps. marginata*, respectively) than in *Piper* species (8, 26, and 40%; *Pi. aequale*, *Pi. culebranum*, and *Pi. cordulatum*, respectively). This corresponds with lower dependence of Piperaceae vs. the Rubiaceae. Inconsistently low colonization levels for *Ps. marginata* may have been due to difficulty in staining these relatively woody roots. The greater capacity of more AM dependent species (*Pi. cordulatum* and *Ps. marginata*) to reduce carbon investment to plant structures whose functions are more efficiently performed by AM fungi is critical for an AM growth advantage, and has been demonstrated in other species (Hetrick *et al.*, 1991).

Conclusions

Tropical soils are generally low in P (2 ppm reported for the local soils; Cavellier, 1992), so AM are critical foragers of this

limiting nutrient. The increased RGR in AM+ plants of all species (including those amended with P) demonstrates that mycorrhizal fungi were improving nutrient uptake for the same species that had reduced water uptake efficiency. Increased fine root production may be a necessary tradeoff to obtain sufficient water in AM roots for species in which the symbiosis lowers water uptake efficiency. All plants in this experiment had constant access to water. Under field conditions of seasonal drought, my results indicate that the light-demanding species may suffer lower transpiration, hence lower photosynthesis. By contrast, the shade-tolerant species may have a greater competitive advantage due to AM colonization. An irrigation experiment demonstrated that growth and leaf production in *Piper* and *Psychotria* are not normally limited by water stress and they remain photosynthetically active throughout the dry season (Wright, 1991). More species need to be sampled to determine the generality of our patterns with regard to light adaptation and AM. Experiments simulating drought conditions, as well as field experiments during the dry season are necessary to determine the potential effects of AM on root hydraulic conductance and growth during this stressful period.

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References

- Alwis DP, Abeynayake K. 1980. A survey of mycorrhizae in some forest trees of Sri Lanka. In: Mikola P, ed. *Tropical mycorrhiza research*. New York, NY, USA: Oxford University Press, 146–151.
- Augé RM, Duan X. 1991. Mycorrhizal fungi and nonhydraulic root signals of soil drying. *Plant-Physiology* 97: 821–824.
- Augé RM, Schekel KA, Wample RL. 1986a. Osmotic adjustment in leaves of VA mycorrhizal and nonmycorrhizal rose rosa-hybrida cultivar samantha plants in response to drought stress. *Plant Physiology* 82: 765–770.
- Augé RM, Schekel KA, Wample RL. 1986b. Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. *New Phytologist* 104: 107–116.
- Augé RM, Stodola AJW. 1990. An apparent increase in symplastic water contributes to greater turgor in mycorrhizal roots of droughted rose plants. *New Phytologist* 115: 285–296.
- Baylis GTS. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PT, eds. *Endomycorrhizas*. London, UK: Academic Press, 241–260.
- Becker P, Castillo A. 1990. Root architecture of shrubs and saplings in the understory of a tropical moist forest in lowland Panama. *Biotropica* 22: 242–249.
- Becker P, Rabenold PE, Idol JR, Smith AP. 1988. Gap and slope gradients of soil and plant water potentials during the dry season in a tropical moist forest. *Journal of Tropical Ecology* 4: 173–184.
- Buwalda JG, Stribley DP, Tinker PB. 1983. Increased uptake of anions by plants with vesicular–arbuscular mycorrhiza. *Plant and Soil* 71: 463–467.
- Cavelier J. 1992. Fine-root biomass and soil properties in a semideciduous and a lower montane rain forest in Panama. *Plant and Soil* 142: 187–201.
- Croat TB. 1978. *Flora of Barro Colorado Island*. Stanford, CA, USA: Stanford University Press.
- Davies FT Jr, Potter JR, Linderman RG. 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration—response in gas exchange and water relations. *Physiologia Plantarum* 87: 45–53.
- Engelbrecht BMJ, Velez V, Tyree MT. 2000. Hydraulic conductance of two co-occurring neotropical understory shrubs with different habitat preferences. *Annals of Forest Science* 57: 201–208.
- Faber BA, Zasoski RJ, Munns DN. 1991. A method for measuring nutrient and water uptake in mycorrhizal plants. *Canadian Journal of Botany* 69: 87–94.
- Fitter AH. 1985. Functioning of VA mycorrhizas under field conditions. *New Phytologist* 99: 257–265.
- Freedman AL, Field CB. 1996. Ecophysiological constraints on the distribution of *Piper* species. In: Mulkey SS, Chazdon RL, Smith AP, eds. *Tropical forest plant ecophysiology*. New York, NY, USA: Chapman & Hall, 597–618.
- García MPV, Vázquez-Yanes C. 1985. Presencia de micorrizas vesículo–arbusculares en especies de *Piper* de los Tuxtalas, Veracruz, Mexico. *Biotica* 10: 223–228.
- George E, Haussler K, Vetterlein D, Gorgus E, Marschner F. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Canadian Journal of Botany* 70: 2130–2137.
- Giovanetti M, Mosse B. 1980. An evaluation of techniques for measuring V–A mycorrhizal infection in roots. *New Phytologist* 84: 489–500.
- Grace C, Stribley DP. 1991. A safer procedure for routine staining of vesicular–arbuscular mycorrhizal fungi. *Mycological Research* 95: 1160–1162.
- Hardie K. 1985. The effect of removal of extraradical hyphae on water uptake by vesicular–arbuscular mycorrhizal plants. *New Phytologist* 101: 677–684.
- Hetrick BAD, Wilson GWT, Leslie JF. 1991. Root architecture of warm-season and cool-season grasses relationship to mycorrhizal dependence. *Canadian Journal of Botany* 69: 112–118.
- Janos DP. 1980a. Vesicular–arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* 61: 151–162.
- Janos DP. 1980b. Mycorrhiza influence tropical succession. *Biotropica* 12: 56–64.
- Janos DP. 1985. Methods for V–A mycorrhiza research in the lowland wet tropics. In: Medina E, Mooney HA, Vazquez Yánes C, eds. *Physiological ecology of plants of the wet tropics*. Boston, MA, USA: Kluwer Academic Press, 173–187.
- Koide R. 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. *Oecologia* 77: 537–543.
- Kyllo D. 2001. *Effects of a common mycorrhizal network and light on growth and community structure of understory shrubs, Piper and Psychotria, in a moist neotropical forest*. PhD thesis. St. Louis, MO, USA: University of Missouri.
- Leigh EG, Rand AS, Windsor DM. 1982. *Ecology of a tropical forest: seasonal rhythms and long term changes*. Washington DC, USA: Smithsonian Institution Press.
- Machlis L, Torrey JG. 1956. *Plants in action*. San Francisco, CA, USA: Freeman.
- Maffia B, Nadkarni NM, Janos DP. 1993. Vesicular–arbuscular mycorrhizae of epiphytic and terrestrial Piperaceae under field and greenhouse conditions. *Mycorrhiza* 4: 5–9.
- Miller RM, Jastrow JD. 1990. Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry* 22: 579–584.
- Mulkey SS, Smith AP, Wright SJ, Machado JL, Dudley R. 1992. Contrasting leaf phenotypes control seasonal variation in water loss in a tropical forest shrub. *Proceedings of the National Academy of Sciences, USA* 89: 9084–9088.
- Nardini A, Salleo S, Tyree MT. 2000. Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. On hydraulic conductance and

- water relations of *Quercus ilex* L. Seedlings. *Annals of Forestry Science* 57: 305–312.
- Nardini A, Tyree MT. 1999. Root and shoot hydraulic conductance of seven *Quercus* species. *Annals of Forestry Science* 56: 371–377.
- Orozco-Segovia AV, Zquez-Yanes C. 1989. Light effect on seed germination in *Piper* L. Acta. *Oecologia* 10: 123–146.
- Plenchette C, Perrin R, Dwert P. 1989. The concept of soil infectivity and a method for its determination as applied to Endomycorrhizas. *Canadian Journal of Botany* 67: 112–115.
- Powell CL. 1976. Development of mycorrhizal infections from Endogone spores and infected root segments. *Transactions of the British Mycological Society* 66: 439–445.
- Redhead JF. 1980. Mycorrhiza in natural tropical forests. In: Mikola P, ed. *Tropical mycorrhiza research*. Oxford, UK: Oxford University Press, 127–142.
- Rillig MC, Steinberg PD. 2002. Glomalin production by an arbuscular mycorrhizal fungus: a mechanism of habitat modification? *Soil Biology and Biochemistry* 34: 1371–1374.
- Ruiz-Lozano JM, Azcon R. 1995. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. *Physiologia Plantarum* 95: 472–478.
- Rundel PW, Becker PF. 1987. Cambios estacionales en las relaciones hidricas y en la fenologia vegetativa de plantas del estrato bajo del bosque tropical de la isla Barro Colorado, Panama. *Revista E Biologia Tropical* 35: 71–84.
- Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB. 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular–arbuscular mycorrhizas. *New Phytologist* 92: 75–87.
- Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1997. Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. *Journal of Ecology* 85: 181–191.
- Tarafdar JC. 1995. Role of a VA mycorrhizal fungus on growth and water relations in wheat in presence of organic and inorganic phosphates. *Journal of the Indian Society of Soil Science*. 43: 200–204.
- Tyree MT, Davis SD, Cocard H. 1994. Biophysical Perspectives of Xylem Evolution: Is There a Tradeoff of Hydraulic Efficiency for Vulnerability to Dysfunction? *Iawa Bulletin* 15: 335–360.
- Tyree MT, Ewers FW. 1991. The hydraulic architecture of trees and other woody plants. *New Phytologist* 119: 345–360.
- Tyree MT, Patiño S, Bennink J, Alexander J. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flow meter in the laboratory and the field. *Journal of Experimental Botany* 46: 83–94.
- Tyree MT, Sinclair B, Liu P, Granier A. 1993. Whole shoot hydraulic resistance in *Quercus* species measured with a new high-pressure flow meter. *Annals of Forest Science* 50: 417–423.
- Tyree MT, Velez V, Dalling JW. 1998. Growth dynamics of root and shoot hydraulic conductance of five neotropical tree species: scaling to show possible adaptation to differing light regimes. *Oecologia* 114: 293–298.
- Valladares F, Wright SJ, Lasso E, Kitajima K, Percy RW. 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a panamanian rainforest. *Ecology* 81: 1925–1936.
- Windsor DM. 1990. Climate and moisture availability in a tropical forest, long-term records for Barro Colorado Island, Panama. *Smithsonian Contributions to Earth Sciences* 29: 1–145.
- Wright JS. 1991. Seasonal drought and the phenology of understory shrubs in a tropical moist forest. *Ecology* 72: 1643–1657.
- Wright JS, Machado JL, Mulkey SS, Smith AP. 1992. Drought acclimation among tropical forest shrubs (*Psychotria*, Rubiaceae). *Oecologia* 89: 457–463.
- Yavitt JB, Wieder RK, Wright JS. 1993. Soil nutrient dynamics in response to irrigation of a Panamanian tropical moist forest. *Biogeochemistry* 19: 1–25.