Journal of Tropical Ecology (2004) **20**:525–534. Copyright © 2004 Cambridge University Press
DOI: 10.1017/S0266467404001592 Printed in the United Kingdom

Effects of light and fertilization on arbuscular mycorrhizal colonization and growth of tropical rain-forest *Syzygium* tree seedlings

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(*Accepted 28th September 2003*)

Abstract: This study investigated the effects of light and soil fertility, on arbuscular mycorrhizal fungi (AMF) colonization, and the growth responses (height and dry mass) of *Syzygium* seedlings. Seedlings of four *Syzygium* spp. were grown for 2 y in six different light treatments at the research station of the Sinharaja Forest, Sri Lanka. The light treatments exposed seedlings to: (1) 3%; (2) 16%; (3) 50%; (4) 100% of full sun (control); (5) short periods (2 h d^{-1}) of direct sunlight; and (6) long periods (6 h d^{-1}) of direct sunlight. In the 16% of full sun treatment five sets of fertilizer applications supplied: (1) magnesium; (2) potassium; (3) phosphorus; (4) all three nutrients combined; and (5) no fertilizer (control). The *Syzygium* species had the greatest mycorrhizal colonization in brighter treatments that provided direct light. Comparison across species revealed *S. firmum* to have moderate mycorrhizal colonization but high total dry mass. *Syzygium operculatum* had high percentages of mycorrhizal colonization while *S. rubicundum* had low percentages of mycorrhizal colonization especially in deep shade. *Syzygium makul* showed moderate levels of mycorrhizal colonization and dry mass, but low height growth. Among fertilizer applications, phosphorus enhanced seedling growth and mycorrhizal colonization for all species. However, species showed decreased growth with high amounts of potassium and combined fertilizer applications. Results suggest that AMF colonization will be highest, and *Syzygium* spp. growth greatest, beneath canopy openings large enough to receive direct sun in phosphorus-rich soils.

Key Words: arbuscular mycorrhiza, light, magnesium, mixed dipterocarp forest, Myrtaceae, phosphorus, photosynthetic photon flux, potassium, regeneration, soil nutrients, Sri Lanka, *Syzygium*

INTRODUCTION

Mycorrhizal colonization is associated with most tropical trees (Alexander 1989, Alexander *et al.* 1992, Högberg 1989, Janos 1983, 1996). Mycorrhizas increase the root access to a greater volume of soil than can be exploited by the plant roots themselves. This greater exploitation of soil increases the nutrient uptake to the host plant (Reinhard *et al.* 1994). Therefore, mycorrhizal colonization improves the nutrition and growth of the host plant (Smith & Smith 1996). The fungi in turn depend upon host-plant photosynthate as their energy source (Harley & Smith 1983). This symbiotic relationship affects the competitive success of plants and can influence plant community composition in the forest (Janos 1983).

Both ecto- and arbuscular mycorrhizal fungi occur in tropical forests though arbuscular mycorrhizal fungi (AMF) are more common (Turner 2001). Ectomycorrhizal fungi (EcMF) are more important under nitrogen limitation while AMF are more common under phosphorus limitation in the soil (Harley & Smith 1983, Smith & Read 1999), though Newbery *et al.* (1997) could not find evidence for differential use of nitrogen and phosphorus by plants of either mycorrhizal type in lowland African rain forest.

The degree of mycorrhizal colonization in the host plant is affected by several factors, such as the light environment in which the host plant is growing (Bethlenfalvay & Pacovsky 1983, Hayman 1974, Tester *et al.* 1986), atmospheric CO_2 concentration (Louche-Tessandier *et al.* 1999, Staddon *et al.* 1999), soil nutrient availability (Son & Smith 1988), soil pH (Marx & Zak 1965, Soedarjo & Habte 1995, Yawney *et al.* 1982), soil temperature (Hayman 1974, Schenck *et al.* 1975, Smith & Bowen 1979), and the hormonal balance between the

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symbionts (Rudawska 1989). However, most studies have concerned differences in mycorrhizal colonization due to varying levels of soil nutrients (Boerner 1990, Habte & Musoko 1994, Habte & Soedarjo 1996, Johnson *et al.* 1980, Moyersoen *et al.* 1998, Onguene & Habte 1995, Soedarjo & Habte 1995) and light intensity (Becker 1983, Guadarrama & Alvarez-Sanchez 1999, Louche-Tessandier *et al.* 1999). The effects of light quality and fertilizer application have only been studied for ecto-mycorrhizas (de la Rosa *et al.* 1998, 1999). No studies have examined tropical rain-forest tree seedling growth and arbuscular mycorrhizal colonization under differing light quantity, quality and fertilizers.

This study assesses the effect of these varying factors (light and fertilizer) on mycorrhizal colonization and seedling growth of *Syzygium* spp. (Myrtaceae), an economically important rain-forest genus of South and South-East Asia. Dual colonization has been reported in *Syzygium kurnda* (EcMF and AMF) by Reddell *et al.* (1996); however, other *Syzygium* spp. (*S. aqueum* and *S. makul*) have been reported as colonized solely by arbuscular mycorrhizal fungi (De Alwis & Abeynayake 1980). We investigated four *Syzygium* spp. that are colonized only by arbuscular mycorrhizal fungi. We recorded the presence of vesicles and intracellular coils but no arbuscules.

We hypothesized that differing irradiance levels and fertilizer treatments would influence the degree of AMF colonization in the host seedling roots. Specifically we tested four main predictions: (1) the increase in irradiance (quantity) would increase seedling growth and total dry mass gain. Then higher availability of carbohydrate would increase the mycorrhizal colonization of seedlings; (2) The increase of red : far red ratio (quality) increases the photosynthetic carbon gain of seedlings (see Turnbull 1991). Therefore, the increase in red : far red ratio under similar amounts of photosynthetic photon flux densities (PFD) per day would increase the mycorrhizal colonization; (3) the addition of fertilizer to the host plant would reduce carbon allocation to root growth of *Syzygium* seedlings. This would decrease AMF colonization under partial-shade conditions; and (4) the growth and mycorrhizal colonization of different *Syzygium* species tested, would differ among each other to light and fertilizer treatments.

METHODS

Study site and species

Twenty-four well-ventilated environmental shelters were constructed in a fully open area at the research station of Sinharaja Forest, Sri Lanka in January 1996. Sinharaja is an ever-wet mixed dipterocarp forest (no dry season) with a canopy dominated by species of *Shorea* section *Doona* (Dipterocarpaceae) and *Mesua* (Clusiaceae) (Ashton & Gunatilleke 1987, De Rosayro 1942). The forest receives an annual rainfall of 5000 mm with mean monthly temperatures varying between 25 and 27 °C throughout the year (Ashton 1992). The topography is undulating between valley and ridge with a mean elevation about 600 m above sea level. Soils are classified as ultisols following the USDA (1975) terminology, or red yellow latosols using the FAO (Moorman & Panabokke 1961).

The four species in this study were *Syzygium firmum* Thw., *S. makul* Gaertn., *S. rubicundum* Wight & Arn. and *S. operculatum* (Roxb.) Nied. (Myrtaceae). All are common in the wet climatic zone of Sri Lanka (Ashton & Gunatilleke 1987). *Syzygium firmum* and *S. makul* are endemic to South-West Sri Lanka. Both species occupy deep soils of valleys and mid-slopes of late-successional forest but differ in their altitudinal ranges with *S. firmum* at low elevation (10–350 m asl) and *S. makul* at middle elevations (300–700 m asl). *Syzygium operculatum* occurs in disturbed areas of lower slopes and valleys and within the riparian areas of rivers and streams. *Syzygium rubicundum* occurs on mid-slopes of mid-successional forest at middle elevations (300–700 m) (Ashton 1981). All four species are dominant in the canopy of mixed dipterocarp forest, provide important timbers for housing, and have edible fruits desired by people and frugivorous birds and bats.

Experimental design

The 24 environmental shelters were designed to create light treatments that represented a range of photo-synthetic photon flux densities (PFD) and red : far red ratios (R : FR) found within the Sinharaja forest (Ashton 1992). Seedlings were exposed to six combinations of light and spectral quality: (1) 3% of full sun; (2) 16% of full sun; (3) 50% of full sun; (4) 100% of light - full open; (5) short periods of direct sun (direct light for 2 h of the day); and (6) long periods of direct sun (direct light for 6 h of the day) (see Singhakumara *et al.* 2003, for more details of the set-up of shelters). A spectro-radiometer (Li-Cor 1800, LI-COR, Lincoln, NB) verified the spectral quality of the light treatments (Table 1). Quantum sensors (Li-Cor 190SA) verified amounts of PFD for each light treatment. All shelters allowed for adequate ventilation without using electrical power (see Ashton 1995 for details).

Given limited time and manpower we used only one light treatment, the 16% full sun, for studying effects of five kinds of fertilizer application on mycorrhizal colonization of seedlings. We selected the 16% light treatment for the fertilizer part of the study because previous work demonstrated this to be a threshold light environment for regeneration release and growth in the

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Table 1. Measures of the amount and quality of PFD for the six light treatments (see Ashton 1995 for details).

	Light treatments					
	3%	16%	50%	2-h	6-h	100%
Maximum PFD recorded on clear sunny days (μmol m^{-2} s^{-1})	50	350	800	1600	1600	1600
Total daily PFD recorded on clear sunny days (mol m^{-2} d^{-1})	3.1	15.7	42.8	19.4	34.6	100
Red : far red ratio	0.23	0.97	1.05	1.27	1.27	1.27

Table 2. Soil pH and available soil nutrients (mg g^{-1}) in the 16% light treatment. Measurements were made at the start (pre-treatment), and at the end (post-treatment) of the experiment.

		Post-treatment				
	Pre-treatment	Mg	K	P	MgKP	Control
Soil pH	4.38	4.65	4.10	4.80	4.86	3.86
Avail. P	0.018	0.004	0.003	0.021	0.018	0.003
Avail. K	0.034	0.040	0.490	0.040	0.330	0.010
Avail. Ca	0.049	0.039	0.032	0.044	0.050	0.031
Avail. Mg	0.058	0.103	0.015	0.034	0.102	0.015

forest (Ashton 1992, Ashton *et al.* 1995). This light treatment also demonstrated greatest differentiation in growth performance among *Syzygium* spp. (Singhakumara *et al.* 2003), strongly suggesting this treatment to be an appropriate level of light for testing fertilizer effects on mycorrhizal colonization of seedling fine roots.

Three of the fertilizer treatments comprised: (1) murate of potash (K_2O), 0.5 g per poly bag; (2) triple super phosphate ($3Ca(H_2PO_4)_2$), 0.83 g per poly bag; and (3) kieserite ($MgSO_4.7H_2O$), 5 g per poly bag. The fourth treatment had all three fertilizers combined; and the fifth was a control without any addition of fertilizer. Fertilizers were sprinkled as solids on the soil surface within the poly bags in equal amounts every 3 mo over the 2-y duration of the project, such that the sum of the nutrients added was equal to the total application amount. The total fertilizer application was calculated to be approximately double that recorded in the untreated topsoil (control). The amount of fertilizer added was determined by an analysis of available nutrients in the original topsoil used for planting the seedlings (Table 2).

Soil samples were taken for nutrient analysis from three seedling poly bags per shade × fertilizer combination before treatments were started and at the end of the experiment. Soil pH was measured using a water extract. Available soil phosphorus was extracted using NH_4F/HCl solution and measured using an auto-analyser. Available Ca, Mg and K were extracted using $SrCl_2$ and then measured using an Atomic Absorption Spectrophotometer (Table 2). Soils were maintained at field moisture capacity throughout the experiment by hand watering.

Over the 2-y duration of the experiment soil pH and soil nutrients (except available Ca) significantly declined ($P < 0.05$) for the control, presumably because nutrients were taken up by the growth of the seedlings and no nutrients were added (Table 2). However, the periodic fertilizer applications of the other treatments maintained the soil pH over the 2-y period. Available Ca remained consistently the same across all the treatments over the 2-y period. The P treatment and MgKP treatment maintained available soil P at about the same amount as at the start of the experiment. The Mg treatment and MgKP treatment doubled available Mg. The other fertilizer treatments showed over a three-fold decline in available Mg. The K treatment and MgKP combined treatment increased available K by more than ten-fold, making the soil in these treatments over-fertilized.

Seedling establishment in the shelters

Seed from each species was collected from six different parent trees located within the Sinharaja, with the exception of *S. firmum*, which was collected at lower elevations from the nearby Kanneliya forest reserve. Twenty-four seedlings of each species were used in four blocks per light and fertilizer combination. The total number of seedlings in the light experiment was 576 (6 light treatments × 4 species × 4 blocks × 6 seedlings per block); whereas in the fertilizer experiment the number of seedlings was 480 (5 fertilizer applications × 4 species × 4 blocks × 6 seedlings per block). Seedlings were rotated among the four blocks every 3 mo (see Singhakumara *et al.* 2003 for details).

AMF colonization of roots

For comparisons of fine-root mycorrhizal colonization among shade treatments each species had six seedlings per treatment randomly selected from those growing in the control soil (no fertilizer application). For comparisons among fertilizer treatments seedlings were selected from the 16% full sun treatment in the same manner as for the light treatments (six seedlings per fertilizer application per species). Since the soil was collected from a single location for planting the seedlings and was added in equal amount to poly bags the initial mycorrhizal inocula should be same for all seedlings.

Obviously, plant size varied by light and fertilizer treatment, and such variations can complicate

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interpretation of mycorrhizal colonization patterns (see Staddon *et al*. 1999). We reduced such effects by ensuring that from each seedling a representative sample was taken from four or five different portions of the entire root system (Kormanik & McGraw 1982). Roots were then fixed in FAA (5 ml formaldehyde: 5 ml acetic acid: 90 ml 70% alcohol) for subsequent analysis. Each sample was immersed in water and cut into 1-cm segments with their attached side branches. Fifty to 100 segments from each seedling were cleared with 10% KOH and stained with cotton blue following Phillips & Hayman (1970). Fifty segments per seedling were examined using a light microscope under 400 × magnification. Colonization was expressed as the percentage of segments of each species that showed vesicles, intracellular coils or coarse intracellular hyphae.

Seedling growth and dry mass

Seedling height (from the top of the apical shoot to the soil level) was measured every 6 mo. After the 2-y experimental period, seedlings were destructively sampled in order to measure total dry mass. At the final sampling no evidence was recorded of pot-bound roots suggesting that growth was not affected by the volume limitations of the poly bag and the potting soil. Twelve seedlings (three seedlings per block) that had no disease were selected from each species per shade and fertilizer treatment combination. Sample seedlings were oven dried for 48 h at 80 °C in order to measure the dry mass of seedlings.

Data analysis

A split-plot design was used to investigate differences in mycorrhizal colonization and growth following an analysis of variance (S-plus Version 4). Analysis was performed for shade and fertilizer treatments separately where shade (four replicates) and fertilizer (two replicates) were the main plots while species were the subplots. Fertilizer treatments in the 16% full sun treatment were used for analysis. All data were log-transformed prior to analysis. Per cent mycorrhizal colonization which was not normally distributed was arcsine square-root transformed. Differences among species were evaluated at the 5% significance level using Tukey's studentized range test.

We used a phenotypic plasticity index $\{P = [1 - (x/X)]\}$ to measure the variation of growth and mycorrhizal colonization that included comparisons between mean values of the deep shade (x) and the highest value (X) among light treatments (Ashton *et al*. 1999).

Table 3. F-statistics for analysis of variance on arcsine square root-transformed data for per cent mycorrhizal colonization, and log-transformed data for height (cm) and total dry mass (g) using a split-plot design separately for (a) light and (b) fertilizer treatments. Light (six treatments, four replicates per each treatment) and fertilizer were the main treatments (16% of full sun treatment = five fertilizer treatments, two replicates per each treatment); subplots were the species.

	df	Mycorrhizal colonization	Height	Total dry mass
(a)				
Light	5	61.1***	253***	418***
Residual	9			
Species	3	14.6***	3.1*	22.3***
Light × species	15	NS	7.2***	8.9***
Subplot residual	36			
Error	95			
(b)				
Fertilizer	4	9.5***	24***	36***
Residual	3			
Species	3	8.4***	13.7***	8.8***
Fertilizer × species	12	NS	10.2***	6.2***
Subplot residual	16			
Error	39			

Degree of significance: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

RESULTS

Mycorrhizal colonization

Differences were evident among light and fertilizer treatments (16% of full sun treatment) for mycorrhizal colonization. Analysis of variance showed that light treatments contributed higher differences ($F = 61$, $P < 0.001$) in mycorrhizal colonization than for fertilizer application ($F = 10$, $P < 0.001$). Both increasing light (quantity and quality) and addition of fertilizer increased mycorrhizal colonization to *Syzygium* seedlings (Tables 4 and 5). *Syzygium* species did not differ in mycorrhizal colonization within a light or fertilizer treatment (Table 3).

Light treatments. Analysis of variance showed significant differences in mycorrhizal colonization among species across light treatments (Table 3). Comparing among light treatments, mycorrhizal colonization increased with increasing light in all species. In general, highest per cent colonization was recorded in the treatment that provided full sun (100% light), or periods of direct sun (2-h, 6-h). Lowest per cent colonization was in the deepest shade (3% of full sun) (Table 4). Light treatments (16%, 50% of full sun) impoverished in red : far red ratio consistently showed lower mycorrhizal colonization for most species as compared with treatments that altered periodicity of direct light (2-h, 6-h); even though total

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Table 4. Differences in per cent mycorrhizal colonization, height and total dry mass across light treatments. Treatments are arranged in ascending order of amount of PFD for the four species (SF – *S. firmum* SM – *S. makul*, SO – *S. operculatum*, SR – *S. rubicundum*) after 2 y of growth. Across light treatments data followed by different letters are statistically different ($P < 0.05$) according to Tukey's studentized test. Species Plasticity (P) = [1 − (x/X)] where x is the deep shade value and X is the maximum value among light treatments.

	Light treatments						
	3%	16%	2-h	50%	6-h	100%	*P*
Per cent mycorrhizal colonization							
SF	40.8b	73.3a	84.5a	65.7ab	72.0a	77.0a	0.52b
SM	48.6b	66.6b	82.5a	56.2b	86.5a	74.6ab	0.44c
SO	53.8c	82.5a	80.7a	71.6bc	95.8a	78.8ab	0.44c
SR	28.7c	53.3bc	66.3a	49.1bc	79.0a	64.8ab	0.64a
Height (cm)							
SF	2.8d	16.9c	38.6b	35.8b	43.4a	37.3b	0.89b
SM	2.0c	17.0b	35.8a	32.0a	38.3a	33.8a	0.95a
SO	2.7d	27.6c	44.6a	34.5bc	39.6ab	33.1c	0.91ab
SR	3.8c	25.1b	40.2a	40.8a	43.1a	38.5a	0.87b
Total dry mass (g)							
SF	0.72e	6.5d	19.7c	24.2b	25.3b	32.4a	0.98a
SM	0.18e	4.4d	15.5c	21.1b	20.8b	33.7a	0.99a
SO	0.26d	8.8c	20.4ab	17.8b	18.3b	22.4a	0.99a
SR	0.10e	3.9d	11.6c	14.2bc	16.5ab	19.1a	0.99a

amounts of PFD received per day were comparable among the respective treatments (16% vs. 2-h; 50% vs. 6-h; see Table 1). *Syzygium rubicundum* showed the greatest variation across light treatments in mycorrhizal colonization (as measured by plasticity); *S. firmum* showed the least variation (Table 4). *Syzygium operculatum* had high mycorrhizal colonization across all light treatments while *S. rubicundum* had relatively lower mycorrhizal colonization compared to other *Syzygium* species.

Fertilizer treatments. Addition of fertilizer had a significant effect ($F = 8$, $P < 0.001$) on mycorrhizal colonization among species (Table 3). All studied *Syzygium* species had higher mycorrhizal colonization with addition of fertilizer as compared with the control. No differences were found in colonization percentage among fertilizer treatments for any of the species (Table 5).

Table 5. Differences in per cent mycorrhizal colonization, height and total dry mass across the different fertilizer applications (Mg, K, P, MgKP, Control) of the 16% of full sun treatment. Differences are shown by species (SF – *S. firmum*, SM – *S. makul*, SO – *S. operculatum*, SR – *S. rubicundum*) after 2 y of growth. Letters denote significant differences across fertilizer treatment for each species according to Tukey's studentized least significance difference test ($P < 0.05$).

	Fertilizer treatments				
	Mg	K	P	MgKP	Control
Per cent mycorrhizal colonization					
SF	85.9a	85.7a	90.8a	87.6a	60.5b
SM	86.2a	89.6a	85.2a	84.7a	57.3b
SO	94.6a	94.9a	91.7a	92.8a	67.4b
SR	80.8a	88.7a	81.3a	83.6a	47.9b
Height (cm)					
SF	29.4a	28.1a	30.8a	32.9a	23.8b
SM	23.4b	22.6b	33.5a	25.5b	24.6b
SO	23.4c	27.6c	40.5a	25.9c	32.2b
SR	28.1c	28.2c	37.8a	28.9c	32.4b
Total dry mass (g)					
SF	18.2b	14.8c	20.4a	21.8a	15.3c
SM	15.9bc	10.6d	20.3a	17.7b	14.2c
SO	13.0c	10.3c	19.6a	17.3b	13.1c
SR	10.1b	6.7c	12.1a	14.7a	10.4b

Height increment

Height of *Syzygium* seedlings increased with increasing light (Table 4) and with addition of fertilizer (Table 5). Analysis of variance for height increment (last measurement minus first) showed significant differences to differing light ($F = 253$, $P < 0.001$) and fertilizer applications ($F = 24$, $P < 0.001$). There were significant differences among species across light treatments and within a light treatment. Significant differences were also found among species across fertilizer treatments and within a fertilizer treatment (Table 3).

Light treatments. Height increment was greatest in the brightest treatments with the highest R : FR ratios (2-h; 6-h; 100% of light), intermediate in the 16% full sun light treatment, and lowest in the deep shade (Table 4). *Syzygium firmum* had the greatest height increment in the 6-h treatment; *Syzygium operculatum* had the greatest height increments in both 2-h and 6-h treatments; *S. makul* and *S. rubicundum* had the greatest

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height increments within all the brightest treatments. *Syzygium makul* exhibited the greatest variation among light treatments (as measured by plasticity) while *S. rubicundum* showed the least (Table 4). Similar findings have been reported for the *Syzygium* species by Singhakumara *et al.* (2003) in a study done, using the same experimental setup.

Fertilizer treatments. *Syzygium* spp. (except *S. firmum*) showed the greatest height growth in the phosphorus (P) fertilizer application. *Syzygium operculatum* and *S. rubicundum* showed lower height increments for the magnesium, potassium and combined fertilizer application treatments than the control (Table 5).

Total dry mass

Syzygium seedling dry mass increased with increasing light (Table 4) and addition of fertilizer (Table 5) similar to mycorrhizal colonization and height increment. Analysis of variance for dry mass showed significant differences among light treatments, species and the light × species interaction. Dry mass gain also had significant differences among fertilizer treatments, species and the fertilizer × species interaction (Table 3). However, differences in dry mass gain for *Syzygium* seedlings were higher due to changing light ($F = 418$, $P < 0.001$) than to fertilizer application ($F = 36$, $P < 0.001$). On average, *Syzygium* species increased in total dry mass from 0.3 to 28 g with increasing light from deep shade to full sun (Table 4). However, there was only 13–18 g increase of total dry mass from control to the phosphorus fertilizer treatment (Table 5).

Light treatments. Across light treatments total dry mass was highest in the full sun treatment (100%) and was lowest in the deep shade treatment (3%) for all species. *Syzygium firmum*, *S. makul* and *S. operculatum* showed greater dry mass than *S. rubicundum* over all light treatments. There was no difference in plasticity of dry mass gain among the four species (Table 4). These findings are consistent with the results of Singhakumara *et al.* (2003).

Fertilizer treatments. *Syzygium makul* and *S. operculatum* showed the greatest dry mass with the addition of phosphorus, intermediate dry mass values with the combined fertilizer application, and the least dry mass values with the other treatments, especially the potassium (K) application (Table 5). Differences were less pronounced between fertilizer treatments for *S. firmum* and *S. rubicundum*. As with the light treatments, *S. firmum*, *S. makul* and *S. operculatum* showed greater dry masses than *S. rubicundum* over all treatment applications (Table 5).

DISCUSSION

We tested the effects of differing light and fertilizer application on mycorrhizal colonization and growth of *Syzygium* seedlings. Overall we found that *Syzygium* species increase their growth and mycorrhizal colonization with increasing light and to addition of fertilizers. In this discussion we first examine the effects of light quantity and quality on mycorrhizal colonization. Then we discuss the effects of fertilizer application. Finally we conclude by discussing differential growth responses among the *Syzygium* species to light and fertilizer application.

Does light intensity affect the mycorrhizal colonization of *Syzygium* species?

Mycorrhizal colonization of *Syzygium* seedlings increased with increasing irradiance (deep shade vs. full sun). These findings are consistent with previous studies on other species from tropical rain forests (Becker 1983, Gunatilleke *et al.* 1996). Seedlings growing in high light treatments photosynthesize more and therefore more carbohydrate is available for fungal growth. Consequently, mycorrhizal colonization to host plant is increased (Hayman 1974). Another possible reason is increased growth of lateral roots since the initiation of new lateral roots depends on irradiance (Tester *et al.* 1986). The formation of new root tips favours increased mycorrhizal colonization (see Allen 2001).

Syzygium seedlings had lower mycorrhizal colonization in deep shade compared with the full sun treatment. When a plant is growing under low-light environments, it is advantageous for the plant to allocate a greater proportion of its resources to growing leaves. As a result plants allocate a smaller proportion of photosynthate to root growth, which would then reduce the mycorrhizal colonization (Son & Smith 1988). Low irradiance also has shown to be associated with both low root exudation and low concentration of soluble carbohydrates in the roots (Johnson *et al.* 1982). As a result mycorrhizal colonization to host plant is reduced.

Is there an effect of red : far red ratio on seedling growth and mycorrhizal colonization?

Syzygium species showed a decrease in seedling growth and the percentage of AMF colonization due to impoverished red : far red ratio in 16% (R : FR = 0.97) and 50% (R : FR = 1.05) of full sun light treatments when

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compared with the treatments that received direct sun light (2-h, 6-h) with high red : far red ratios (R : FR = 1.27) (Table 4). Previous studies have shown little effect of R : FR on EcMF growth (de la Rosa *et al.* 1998, 1999). However effects of R : FR ratio on plant growth and morphology are well documented by several other studies (Kwesiga & Grace 1986, Morgan & Smith 1979, Vazquez-Yanes *et al.* 1990). These studies have shown that a decrease in R : FR ratio produces increased stem elongation. However Lee *et al.* (1996) who studied the effect of light quantity and quality on the growth of seedlings of tropical rain-forest tree species have found a decrease in stem length under low R : FR. They interpreted this reduced growth as likely to be due to reduced leaf allocation and smaller leaf area per stem length. We also found lower seedling height and dry mass gain of *Syzygium* species in 16% (R : FR = 0.97) light treatment than in the 2-h treatment (R : FR = 1.27) that received similar amounts of PFD per day. Both leaf number and total leaf area were lower in 16% light treatment than to 2-h treatment although we could not find a significant difference in leaf mass allocation (Singhakumara *et al.* 2003). These findings suggest that increase in stem elongation due to reduced R : FR ratio is not universal.

The decrease of R : FR ratio decreases plant productivity via photosynthesis (Turnbull 1991). This is due to the effect of R : FR ratio on morphological (branch length and plant height), physiological (photosynthesis) and biochemical properties (chloroplast ultrastructure) of a plant (Lee *et al.* 1996, Turnbull 1991). Consistent with the findings of Singhakumara *et al.* (2003), we also found reduced growth and total dry mass for *Syzygium* seedlings in 16% light treatment compared with the 2-h treatment. Therefore, lower carbon allocation to roots would have reduced the AMF colonization in 16% light treatment compared with the 2-h treatment.

Does fertilization decrease AMF colonization?

We predicted that the addition of fertilizer would decrease mycorrhizal colonization. However both mycorrhizal colonization and seedling growth were greater in the fertilized treatments for all species as compared with the control, which had no fertilizer addition (Table 5). Most studies that have investigated response of mycorrhizal colonization to applications of fertilizer reported the reverse (Habte & Byappanahalli 1994, Habte & Musoko 1994, Reinhard *et al.* 1994, Son & Smith 1988). However, the degree of inhibition also depends on the host species involved (Habte & Turk 1991). We suggest several reasons for the deviation of our results from other studies.

(1) The intermediate light treatment (16% of full sun), within which the fertilization applications were conducted, provided low-light conditions compared with the full sun treatment. This could have resulted in low photosynthate allocation to roots since under low irradiance plants allocate more carbon for above-ground growth. This lower allocation of carbon to root growth could be compensated for, by the addition of fertilizer (Son & Smith 1988). Hence, *Syzygium* seedlings increased mycorrhizal colonization in the fertilized treatments compared with the control treatment. Turner *et al.* (1993) have also found an increased mycorrhizal colonization in dipterocarp seedlings with the addition of NPK in a Malaysian tropical rain forest. Alexander *et al.* (1992) also found heavily colonized roots of rain-forest trees in Malaysia within shade conditions, but the addition of P reduced such colonization.

(2) Excess fertilization in limiting light conditions may turn the relationship of mycorrhizas from one that is symbiotic to one that is parasitic (Bethlenfalvay & Pacovsky 1983, Tester *et al.* 1986). Evidence from this study suggests that under partial light high amounts of K and Mg fertilization increased mycorrhizal colonization but decreased seedling growth. If the fungi do not contribute to the nutrient acquisition of the plant, AMF colonization may depress host plant growth (Koide 1985). Jakobsen & Nielsen (1983) have also found extensive colonization of AMF when pea crops were grown in highly fertilized soils. Bolan *et al.* (1984) reported that an increase in nutrients increases mycorrhizal colonization to an optimum, beyond which colonization is depressed.

Do *Syzygium* species behave differently from one another in mycorrhizal colonization and growth?

Syzygium species differed in mycorrhizal colonization and growth across light and fertilizer treatments. Among the four species, *S. operculatum* had the greatest colonization in all light treatments compared with the other *Syzygium* spp. *Syzygium operculatum* had the greatest height growth and total dry mass gain in the P treatment. The high mycorrhizal colonization and responsivity to increasing light suggest *S. operculatum* to be sensitive to nutrient limitation. These traits conform to the known site affinity of *S. operculatum* for disturbed areas along fertile riparian zones of rivers and streams (Ashton 1981).

Syzygium rubicundum had the greatest height increment in the brighter treatments (6-h, 2-h, 50%, 100% of light) and are consistent with the findings of Singhakumara *et al.* (2003). Height growth is an indicator of fitness because it is usually correlated with biomass increase. Height is also a useful measure of seedling response to light competition (Fetcher *et al.* 1983). However, *S. rubicundum* had a lower dry mass as compared with the other *Syzygium* spp., and therefore lower carbohydrate storage. The relatively lower level of mycorrhizal colonization and rapid height growth

of *S. rubicundum* compared with the other *Syzygium* spp. confirms its successional status as a relatively fast-growing tree of open environments.

Syzygium firmum exhibited intermediate levels of mycorrhizal colonization and growth, but usually had the greatest total dry mass among *Syzygium* spp., suggesting it has greater carbohydrate storage relative to height increment. *Syzygium firmum*, the most shade-tolerant of the *Syzygium* spp. studied (Singhakumara *et al.* 2003), is also more conservative in its growth attributes (more dry mass storage compared with height increment), as compared with the other *Syzygium* spp. These conform to its known distribution as a subcanopy to canopy tree of late-successional forests.

Syzygium makul also exhibited intermediate levels of mycorrhizal colonization and total dry mass across light and fertilizer treatments compared with the other species. Across light treatments, *S. makul* had the lowest height increment of all species and agree with the findings of Singhakumara *et al.* (2003). *Syzygium makul* appears similar in growth habit to *S. firmum*. This again conforms to the known site and successional status of *S. makul*, which has a similar successional and site status to *S. firmum* but occupying higher elevation forests.

In summary, results of this study suggest that direct light with high R : FR ratio can have beneficial effects for *Syzygium* spp. seedling growth and mycorrhizal colonization. Among fertilizer applications, phosphorus enhanced growth (height and total dry mass). All species showed decreased growth with high potassium and combined fertilizer applications. Also, contrary to some other studies, mycorrhizal colonization for all species increased with addition of all fertilizers, lowest mycorrhizal colonization was recorded in the control treatment. However, amounts of light appear to be the most important factor determining mycorrhizal colonization and growth of *Syzygium* spp. Degree of mycorrhizal colonization appears to be associated with higher growth and dry mass of *Syzygium* seedlings when grown under higher irradiance but not under lower fertilizer levels.

ACKNOWLEDGEMENTS

The MacArthur Foundation Environment Program, USA and the National Science Foundation, Sri Lanka funded this study. We are grateful to the Forest Department for providing permission to carry out this work and for the Department for Forestry and Environmental Science, University of Sri Jayewardenapura, Sri Lanka for use of their laboratories. Thanks are also due to Dr R. Jayerathna, Rubber Research Institute of Sri Lanka for training on mycorrhizal identification and to Chaminda Kumara and B. W. Gunasoma for their technical and field assistance. Drs Linley K. Jesson and Margaret Gordon, Victoria University, New Zealand, provided helpful comments on the manuscript.

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