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Aspects of Early Growth and Host Relationships in the Hemi-Parasitic *Santalum album*: *Alternanthera* Taxa as Primary Hosts and Growth in Response to Foliar Feeding

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ABSTRACT

Species of *Alternanthera* (Family Amaranthaceae) are efficient primary hosts for the obligate root hemi-parasite *Santalum album* (Family Santalaceae). The first experiment presented in this paper examines whether four closely related *Alternanthera* taxa differ in their performance as host. *S. album* seedlings were grown in pots with *Alternanthera dentata* cv 'Ruby', *Alternanthera dentata* cv 'Tricolor', *Alternanthera amoena* cv 'Aurea', and *Alternanthera angustifolia* respectively. Seedlings were between 5 and 11 weeks old at the beginning of the experiment. Haustorial connections had been formed with the roots of *A. dentata* cv "Tricolor" and *Alternanthera amoena* cv "Aurea" after 10 weeks. The variety *A. dentata* cv "Ruby" formed haustorial connections after 4 weeks. Sandal seedlings did not form any haustorial connections with *A. angustifolia* during the trial period. Shoot and root growth of *S. album* seedlings were similar in the presence of the four host taxa and did not change significantly throughout the experimental time period, probably due to the low number of haustorial connections between host and parasite. Of the four taxa tested, *A. angustifolium* showed the least potential as primary host because of poor root growth, early mortality and a complete lack of haustorial connections between host and parasite.

The second experiment (1) examined whether young sandalwood seedlings can utilise essential minerals when administered directly to their own leaves, and (2) compared the growth response to foliar feeding with the growth observed in the presence of *A. dentata* var. "Ruby", which is likely due to a transfer of nutrients from host to parasite. The absorption of nutrients was determined as an increase in the growth of sandal seedlings after 20 weeks. The data indicate that *S. album* seedlings are able to utilise foliar nutrients. However, the presence of *A. dentata* var. "Ruby" leads to a larger increase in growth than the application of foliar nutrients.

Key Words: *Santalum album*, *Alternanthera dentata* cv 'Ruby', *A. dentata* cv 'Tricolor', *A. amoena* cv 'Aurea', *A. angustifolia,* Host, Parasite, Pot (Primary) Host, Haustoria, Foliar Nutrients.

INTRODUCTION

Santalum album is an obligate root hemi-parasite. In the field seedlings can survive without host plants for up to 3 years (Nagaveni and Srimathi 1985, Widiarti 1989, Rai 1990). In cultivation, *S. album se*edlings show moderate, but healthy growth without host attachment for 3 months, but soon afterwards growth declines and a considerable number of seedlings die (Barrett and Fox 1997).

Initially a two-stage host system was recommended, the primary host being a small low-growing species, and the long-term host being a large *Acacia* or other tree species (McKinnell 1990). However, recently three stages of parasitism have been identified as required in order to successfully establish *S. album* plantations. These are: a) in-pot hosts (initial or primary); the primary host is planted into a *S. album* seedling container during nursery propagation (Ehrhart and Fox 1995); b) intermediate hosts (bridging nursery and field) (Fox et al. 1996, Radomiljac et al. 1998); and c) long-term (secondary) hosts (Ehrhart and Fox 1995, Fox et al. 1995 b, Radomiljac and McComb 1998).

The suitability of a pot host species is dependent on its ability to supply nutrients and moisture to the hemi-parasite (Radomiljak 1998). Early parasitic relationships are required for optimum survival in the nursery and successful field establishment. The use of an effective primary host reduces out-planting stress when transferred to the field and increases survival and growth of *S. album* in the field (Rai and Kulkarmi 1986, Kagy 1987, Ehrhart and Fox 1995, Radomiljak 1998, Radomiljak et al. 1998). Very young seedlings rely on seed reserves as nutrient resources, but essential nutrients can also be directly taken up from the soil. Sandalwood is able to obtain minerals via its root system without supplementary nutrition from hosts and maintain healthy (albeit stunted) growth for at least 13 weeks (Barrett et al. 1993, Barrett and Fox 1997).

The pot host is ideally a small, low growing species that lives only 1 or 2 years and does not compete with *S. album* (McKinnell 1990, Rai 1990, Barrett and Fox 1994, Fox et al. 1996). Other desirable characteristics include ease of cultivation; well distributed, fine root growth that allows easy attachment of the sandalwood root haustoria (McKinnell 1990, Fox et al. 1996); an ability to withstand top pruning; and finally persistence in the field after out-planting (Fox et al. 1996) since the pot host: parasite relationship should persist until the intermediate host plant is parasitised (Radomiljac 1998).

S. album has a wide range of primary hosts, but primary hosts differ in their efficiency, and the suitability of a particular pot host is also dependent on the locality (Rai 1990, Fox et al. 1995 b, Fox et al. 1996, Radomiljak 1998).

Numerous *Alternanthera* sp. have been used as successful pot hosts. *Alternanthera* species have a vigorous root system providing a large surface for haustorial connections, good air flow and pot drainage (Kagy 1987). *Alternanthera* taxa are easy to maintain in the nursery and have excellent rates of attachment to sandalwood (Kagy 1987). *A. sessilis* (L.) DC, first identified by CIRAD-Forêt in New Caledonia promotes good growth in *Santalum austrocaledonicum* Vieillard seedlings (Kagy 1987). *A. nodiflora* and *A. bettzickiana* were found to be good primary host for *S. album* (McKinnell 1990, Ehrhart and Fox 1995, Fox et al. 1995 b, Fox et al. 1996). *A. nana* as pot host positively affects survival and growth of *S. album* in the field (Radomiljak 1998).

The growth habit of a potential host can have a substantial effect on its performance in relationship to the parasitising plant. The four *Alternanthera* taxa examined here display variation in both, leaf and growth form, and it is not known how different taxa influence sandal growth parameters.

The aims of the present study are to (1) compare early growth of *S. album* seedlings in the presence of the following four *Alternanthera* taxa: *Alternanthera dentata* cv 'Ruby', *Alternanthera dentata* cv 'Tricolor', *Alternanthera amoena* cv 'Aurea' (synonym: *A. bettzichiana*) and *Alternanthera angustifolia* and (2) determine whether young sandalwood seedlings can utilise essential minerals, which have not been obtained from a host plant but administered directly to their own leaves. We then compared the growth response to foliar feeding to growth observed in the presence of *A. dentata* var. "Ruby".

MATERIALS AND METHODS

Germination of Sandal Seed

For experiment 1, freshly collected *S. album* seed (Kununurra, Western Australia, September 2000), was pre-treated with 0.05% gibberellic acid for 16 hours, surface sterilised with 3-5% NaOCl, and then sown into punnets (5.0×8.5×14.0 cm) containing sterilised coarse sand on 24 Nov. 2000. Punnets were placed in seedling trays (6.0×29.5×35.5 cm) standing in trays (6.0×31.0×44.5 cm) filled with rainwater and placed on 25ºC heat beds. Seedlings were individually transplanted into rectangular pots (9×9×15 cm) filled with one part coarse sand: one part fine sand: one part peat, with fertilisers added at mixing (fertilisers in grams per cubic metre: 86.7 g potassium nitrate, 86.7 g potassium sulphate, 1.134 kg, single superphosphate, 1.134 kg calcium carbonate lime and 3.42 kg dolomite lime)

For experiment 2, seed of *S. album* was collected at the Bangalore Forest Research Centre in India in 1992 and stored at 4 ºC until the beginning of the experiment in 1998. Seeds were sown in seed trays on heated beds (thermal growth panels) in the glasshouse. Seed trays were filled with sterilised, coarse sand and maintained at 25 ºC on the heated beds.

Description of the *Alternanthera* Taxa Examined

Alternanthera dentata (Moench) Scheygr cv 'Ruby' is an ornamental from South America and the West Indies with a form 'Rubiginosa' (known as indoor clover) with red to purple leaves (Huxley et al. 1992). It is a low growing (up to 12 cm high and 35 cm wide) hardy perennial. *A. dentata* (Moench) Scheygr cv 'Tricolor' is one of the larger varieties of *A. dentata*, and can grow up to 30 cm high and 50 cm wide. *Alternanthera amoena* (Lem.) Voss cv 'Aurea' is an ornamental from Brazil (Wiersema and Leon 1999). It is synonymous with *Alternanthera ficoidea* (L.) P. Beauv. var. *amoena* (Lem.) L.B. Sm. & Downs and *Alternanthera bettzickiana* (Regel) Voss (known as Calico plant). In Huxley et al. (1992), this plant is named as *Alternanthera bettzickiana* (Reg.) Nichols, and two forms known as 'Aurea Nana' dwarf, leaves yellow green; and 'Brilliantissima' leaves bright red. The "Aurea" variety is a small, low growing shrub (up to 12 cm high and 35 cm wide).

A. angustifolia is a facultative annual herb, native to the northwest of Western Australia, and seed was collected in the Pilbara District (Ethel Creek Station, Pilbara District on 21 July 1997).

Propagation of *Alternanthera*

Alternanthera angustifolia was propagated by seed (see Fox et al. 1995 a). *Alternanthera dentata* cv 'Ruby', *A. dentata* cv 'Tricolor', and *A. amoena* cv 'Aurea' were propagated by cuttings. All cuttings were initially apical, 60 mm in stem length with two nodes and five leaves, and were pre-treated with IBA (1.5 g L^{-1}). *A. dentata* roots easily within 3 to 7 days under glasshouse conditions during summer, and cuttings appear to grow best in UC Mix. Cuttings of 60 mm length established the best root systems, basal cuttings had highest dry root weights and more shoot length than apical cuttings, and leafy cuttings were better than cuttings without leaves (Luong and Fox, personal communication).

Experiment 1: Early Growth of *S. album* Seedlings in the Presence of Four *Alternanthera* Taxa

The following *Alternanthera* taxa were used: *A. dentata* cv 'Ruby', *A.dentata* cv 'Tricolor', *A. amoena* cv 'Aurea', and *A. angustifolia* respectively.

Sets of eight randomly selected *S. album* seedlings were potted with each of the four *Alternanthera* pot hosts on 20 Feb. 2001. *S. album* seedlings were grown 5 cm apart from established *A. dentata, A. amoena* and *Alternanthera* sp cuttings and *A. angustifolia* seedlings respectively. The cuttings were 9 weeks old, 60 mm in stem length with 2 nodes and 5 leaves; the *A. angustifolia* seedlings were 5 weeks old when planted with *S. album*. Sandal seedlings differed in age at the beginning of the experiment. Seedlings planted with 'Tricolor' cuttings were 10.3 ($\pm$ 0.56) weeks old, those planted with cuttings of *A. dentata var.* Ruby 9.1 ($\pm$ 0.48) weeks, and seedlings grown with cuttings of *Alternanthera amoena* cv 'Aurea' were 7.7 ($\pm$ 0.7) weeks at the beginning of the experiment. *S. album* seedlings planted with *A. angustifolia* seedlings were 5.8 ($\pm$ 0.55) weeks old. The young plants ranged in height from 7.2 to 14.8 cm and had 6 to 15 leaves. There were no significant differences between the seedlings in height (ANOVA)($p = 0.395$), or leaf number ($p = 0.710$).

Weekly measurements were taken over a time period of 10 weeks (76 days). The trials were conducted under a shaded glasshouse at the Field Trial Area (FTA), Curtin University of Technology (Perth, Western Australia).

Our experiment was designed for five varieties. Three varieties of ornamental potted plants (*A. dentata cv "Ruby"* and cv *"Tricolor"*, *A. amoena*) were obtained from a commercial nursery in Perth (Waldecks, Bentley, W.A.). A fourth variety named *Alternanthera sp*. var. "FTA" was originally derived from a Bentley garden and has been grown at the FTA, Curtin University since 1999 (Mioduszewski, personal communication). However, plants of this variety were identified as *A. dentata* var. "Ruby" at the end of the experimental period.

Height (cm) and leaf number were recorded initially and weekly thereafter for ten weeks for all *S. album* seedlings. Seedlings were randomly selected from each treatment and harvested after four (n=2), eight (n=3) and ten weeks (n=3). Seedlings were dried at 60ºC for 72 hours. At each harvest, the following measurements were recorded for harvested *S. album*: dry root and shoot weight (g); leaf area (cm^2) using an electronic planimeter (Otfoto 1.1 and NIH Image 1.62); number of haustoria (at least 0.05 mm); and number of haustorial connections on host roots.

Experiment 2: Can Young Sandalwood Seedlings Utilise Essential Minerals when Administered Directly to their Leaves?

Sandal wood seedlings were approximately 12 weeks old when potted with (rooted cuttings of) *A. dentata* var. "Ruby in coarse sand. *S. album* seedlings were between 5.1 cm and 10.3 cm tall and leaf number ranged from 6.5 and 11.75 per seedling. A total of 56 sandal plants and 56 *A. dentata* plants were used (seventeen treatments, see Tables 5 and 6; sample size / treatment: n = 4). After planting nutrient solutions lacking in one of N, P, K; N, P, K + trace elements; Ca; Mg; Fe or trace elements were added to each pot. The missing nutrients (minerals) were supplied for the first time 4 1/2 weeks after planting (30/04/99) and from then onwards repeated weekly for a total of eleven times (last treatment: 10/07/99). Final harvest occurred 8 weeks after the last treatment (5/09/1999). The missing nutrients were applied by spraying the foliage of both, sandal seedlings and *Alternanthera* host. All nutrient solutions were applied as per manufacturer's recommendations {Mg (Mg micro: 60 mL per 100 L water); Fe (Sequestrene 138 Fe: 0.05 % solution); Ca: Librel liquid Ca, chelated calcium 1% solution; N, P, K; Aqua solution 16 mL/20 L water: N 23 %, P 4.0%, K 18 %; N, P, K + trace elements: Aqua solution: N 23 %, P 4.0%, K 18 %, Zn 0.05 %, Co 0.06 %, Molybdenum 0.0013 %, Manganese 0.15 %, Iron 0.06 %, and Boron 0.011 %; trace elements: Fetrilon combination 0.05 % solution or 10 mL per 20 L water contains: 4% Zinc, 4% Iron, 3% Manganese, 1.5% Boron, 0.5% Cobalt, 0.05% Molybdenum, 2% Magnesium and 2.8 % Sulphur}.

Since we assumed that an uptake of nutrients is reflected in the growth of the sandalwood seedlings, the following seven growth parameters were measured. Height (cm) and leaf number were recorded initially and fortnightly until four weeks and thereafter monthly until 20 weeks. At harvest dry shoot, dry root weight and the number of haustoria formed were determined. Haustorial connections were not examined. Leaf and root areas were obtained with an electronic planimeter. The relative growth rate (RGR) was calculated (see Table 5). Only those results obtained at final harvest are presented here, since, based on previous results (Luong, personal observation; Fox and Barrett 1995), it is unlikely that a sufficient number of haustorial connections between host and parasite would have developed before final harvest (20 weeks after the beginning of the trial).

In order to examine whether sandalwood seedlings absorb the added minerals via their leaves, we focussed on growth parameters of *S. album* in the absence of the host (pre-parasitic seedlings). We compared the results obtained for the control treatment (the particular mineral(s) was (were) missing) with those obtained when those minerals were supplied. In order to determine *whether the presence of the host enhances the uptake of nutrients* (via haustorial connections), we compared a particular nutrient treatment with (+) host with the same nutrient treatment without (-) host for each of the seven growth parameters. Our aim was to evaluate whether growth in response to foliar nutrients is comparable to growth, which results from the parasitic relationship between sandal and host. Sandal may utilise its host's nutrients as well as foliar nutrients, or may utilise only the nutrients transferred from the host.

Data Analysis

All data were tested for normality and homogeneity of variance, and transformed using square root transformation if necessary. Data were analysed using either a one-way analysis of variance (a=0.05) followed by Tukey's tests if $p < 0.05$ or a Kruskal-Wallis single factor analysis of variance by ranks (number of samples >2) (non-parametric) (SPSS 11.0, Minitab). A Mann Whitney test was used to compare ranked data when a two sample hypothesis was tested.

RESULTS

Experiment 1: Early Growth of *S. album* Seedlings in the Presence of four *Alternanthera* Taxa.

Growth of S. album *seedlings*

During the experimental period of 10 weeks, no significant increase in growth was observed for *S. album* seedlings. No significant differences between the four host varieties existed in regard to shoot and root weight (Figure 1), height of seedlings (Figure 2), leaf area (Table 1), number of leaves (Table 2), number of formed haustoria and number of haustorial connections (Table 3) after four, eight and ten weeks. Although the statistical analysis did not reveal any significant differences, *S. album* seedlings grown with cv 'Ruby' produced the greatest number of haustoria and formed the most haustorial connections with this host plant (Table 3). Seedlings of *S. album* grown with cv 'Ruby' were also the first to form haustorial connections after four weeks. By eight weeks, sandal seedlings had formed haustoria in the presence of and haustorial connections with *A dentata* cv 'Ruby', *A. dentata* cv 'Tricolor' and *A. amoena* cv 'Aurea'. Although haustoria were present, no functional haustorial connections were observed for seedlings grown with *A. angustifolia*.

Growth of Alternanthera

At final harvest significant differences were observed between the four *Alternanthera* taxa in regard to the number of leaves, leaf area, root weight and shoot weight (Table 4). *A. amoena* var. "Aurea" produced the highest number of leaves, but leaf area was largest for "Tricolor". "Tricolor" also produced the heaviest plants (largest shoot and root weight), and individuals of this variety were taller than those of "Ruby" and *A. amoena*. The lowest shoot and root weight and the smallest leaf area were observed for *A. angustifolia*. Roots of *A. angustifolia* were generally shorter and with fewer lateral roots than those of the other tested taxa. Survival was poor for *A. angustifolia*. Four seedlings died, with three mortalities within the first week and one mortality during the fourth week of the experimental period.

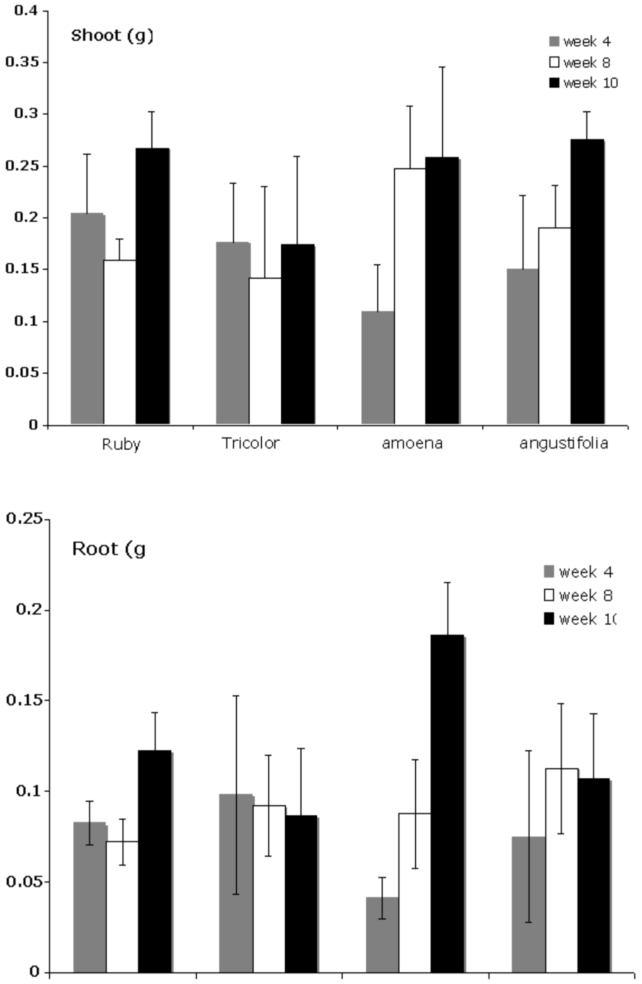

Figure 1. *ABOVE:* Dry shoot weight (g) (mean $\pm$ SE) of *S. album* seedlings grown with *A. dentata* cv 'Ruby', cv 'Tricolor', *A. amoena cv 'Aurea'*, and *A. angustifolia*, at four weeks (F = 0.442, p < 0.732, df = 9), eight weeks (F = 0.858, p <0. 491, df = 14) and ten weeks (F = 579, p < 0.641). *BELOW*: Dry root weight (g) (mean $\pm$ SE) of *S. album* seedlings grown with *A. dentata* cv 'Ruby', cv 'Tricolor', *A. amoena* cv 'Aurea', and *A. angustifolia*, at four weeks *(X^2 = 1.79, df = 2, p < 0.408)*, eight weeks (*X^2 = 0.231, df = 2, p < 0.891*) and ten weeks (*X2 = 4.115, p< 0.128, df = 2*).

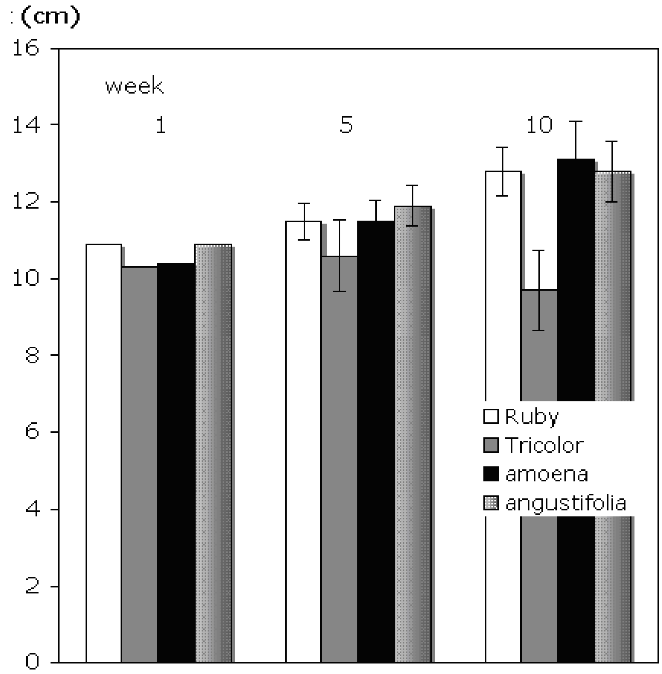

Figure 2. Height (mean $\pm$ SE) of *S. album* seedlings grown with *A. dentata* a) cv 'Ruby' b) cv 'Tricolor'; *A. amoena* cv 'Aurea', *Alternanthera* cv 'FTA' and *A. angustifolia* for week 1 (start of experiment) (*X^2 = 0.67, df = 2, p= 0.715*), 5 weeks (*X^2 = 0.755, df = 2, p = 0.685)* and 10 weeks (n=3) (*X^2 = 4.54, df = 2, p = 0.103*).

Experiment 2: Can young sandalwood seedlings utilise foliar nutrients?

The second experiment (1) examined whether young sandalwood seedlings can utilise essential minerals when administered directly to their own leaves, and (2) compared the growth response to foliar nutrients with the growth observed in the presence of *A. dentata* var. "Ruby", which is likely due to a transfer of nutrients from host to parasite.

Table 1. Leaf area (mean ± SE, cm^2) of *S. album* seedlings grown with *A. dentata* cv 'Ruby', cv 'Tricolor'; *A. amoena* cv 'Aurea'; and *A. angustifolia* at 4, 8 and 10 weeks. ANOVA on log-transformed data.

	Leaf area (cm^2)		
Week	4	8	10
Ruby	18.7 ± 3.6	20.6 ± 2.7	44.9 ± 8.8
Tricolor	25.5 ± 6.6	26.3 ± 7.2	22.2 ± 10.6
amoena	16.7 ± 8.7	25.8 ± 4.2	36.2 ± 9.8
angustifolia	17.3 ± 6.6	24.9 ± 4.9	29.8 ± 6.7
F	0.408	0.381	1.391
P	0.381	0.77	0.297
df	9	14	14

The absorption of nutrients was measured as an increase in the growth of sandal seedlings after 20 weeks. Sandal seedlings are able to absorb essential minerals via their leaves (see Table 5). When grown without a host, leaf number and number of haustoria were larger in plants which received N, P, K + trace elements than in seedlings which did not receive any additional minerals; height, leaf number and number of haustoria were greater in seedlings which were supplied with Ca; height, leaf number and leaf area increased in seedlings supplied with Fe, and height was greater in plants, which were grown with trace elements.

However, the presence of *A. dentata* var. "Ruby" led to a larger growth response than the application of foliar nutrients alone (Tables 5 and 6). Root number, root area and the number of haustoria were significantly greater in seedlings of *S. album* hosted to *Alternanthera dentata* var. "Ruby" than in plants without a host, when Mg, Fe, or trace elements were applied. Leaf number and area were significantly larger in seedlings of *S. album* hosted to *Alternanthera dentata* var. "Ruby" than in plants without a host when either Mg, or trace elements were provided. The growth of sandal seedlings was similar with and without host when N, P, K; N, P,

Table 3. Number of haustoria (mean ± SE) formed on *S. album* roots (ANOVA on square root transformed data) and number of haustorial connections with roots (mean± SE) (Kruskal-Wallis single factor analysis of variance) of *A. dentata* cv 'Ruby', cv 'Tricolor'; *A. amoena* cv 'Aurea' and *A. angustifolia*.

Haustoria Number			
Week	4	8	10
Ruby	3.0 ± 2.34	11.0 ± 4.4	23.8 ± 7.0
Tricolor	5.0 ± 5.0	14.7 ± 3.9	10.7 ± 9.7
amoena	1.5 ±1.5	24.7 ± 16.8	10.0 ± 4.7
angustifolia	10.0 ± 10.0	10.3 ± 4.3	19.0 ± 3.8
F	0.188	0.478	1.16
P	0.901	0.704	0.369
df	9	14	14
Haustoria connections			
Week	4	8	10
Ruby	0.25 ± 0.25	0.5 ± 0.02	1.7 ± 0.84
Tricolor	0.0	0	1.3 ± 1.3
amoena	0	0	0.3 ± 0.33
angustifolia	0	0	0
X^2	1.0	3.67	0.654
P	0.61	0.16	0.721
df	2	2	2

Table 2. Number of leaves (mean ± SE) of *S. album* seedlings grown with *A. dentata* cv 'Ruby', cv 'Tricolor', *A. amoena* cv 'Aurea', and *A. angustifolia* for week 1 (start of the experiment) (n = 2), after 4 (n = 3) and 10 weeks (n=3). (Kruskall Wallis single factor analysis of variance, H is considered to approximate X^2; df = 2).

Week	Ruby	Tricolor	amoena	angustifolia	X^2	p
1	10.2 ± 0.61	8.8 ± 0.84	9.6 ± 0.6	9.8 ± 0.45	2.025	0.36
4	9.8 ± 0.55	9.0 ± 1.1	11.0 ±1	10 ± 0.52	2.52	0.28
10	12.8 ± 1.05	10.7 ± 3.7	11.3 ± 1.8	11.3 ± 1.8	0.78	0.68

Table 4. Growth variables measured for the four host (*Alternanthera*) taxa measured at final harvest (10 weeks) (mean $\pm$ SE). One way ANOVA on log-transformed data.

Host species	No. leaves	Leaf area, cm^2	Height, cm	Root weight, g	Shoot weight, g
Ruby	22.2 $\pm$ 1.9	33.5 $\pm$ 3.2	6.7 $\pm$ 0.6	0.16 $\pm$ 0.03	0.18 $\pm$ 0.03
Tricolor	24 $\pm$ 6.5	64.2 $\pm$ 4.1	13.5 $\pm$ 3.5	0.3012$\pm$ 0.05	0.6840 $\pm$ 0.19
A. amoena	72.3 $\pm$ 9.2	52.18 $\pm$ 5	7.3 $\pm$ 0.59	0.1850 $\pm$ 0.04	0.3088 $\pm$ 0.1
A. angustifolia	43.5 $\pm$ 1.5	3.94 $\pm$ 1.5	17.6 $\pm$ 4.9	0.0091 $\pm$ 0.002	0.0649 $\pm$ 0.04
F	6.58	14.18	0.92	7.46	6.07
p	0.007	0.000	0.49	0.005	0.01

Table 5. The effect of essential minerals on selected growth parameters in seedlings of *S. album* grown with and without host (*A. dentata* var. "Ruby") for 20 weeks. Column means with same letters are not significantly different using the Tukey test at p = 0.05. RGR (Relative growth rate) (cm x cm^{-1} day^{-1} = log*e x* $height^2$ – log*e* height 1/ (t_2 - t_1).

Host/ no host	Nutrients	Height (cm)	No. of Leaves	Leaf Area (cm^2)	No. of Roots	Root Area (cm^2)	No. of Haustoria	Dry Weight (g)	RGR
(1) no	control	8.65^{cde}	9.25^{de}	3.54^{e}	15.75^{cde}	$1,775^{cd}$	2.50^{ef}	0.3257^{abc}	0.004
(2) no	N, P, K	8.0^{e}	12.75^{bcde}	6.81^{de}	13.75^{cde}	1.187^{cd}	3.25^{def}	0.1535^{c}	0.009
(3) no	N, P, K + trace elements	10.65^{abc}	20.75^{a}	8.18^{cde}	27.50^{bcd}	2.638^{cd}	7.50^{bcd}	0.2348^{bc}	0.019
(4) no	Ca	11.93^{a}	16.5^{abc}	19.35^{abc}	27.75^{bcd}	4.512^{abc}	6.50^{cde}	0.4108^{ab}	0.022
(5) no	Mg	9.41^{bcde}	8.00^{e}	3.43^{e}	12.75^{de}	0.978^{d}	2.50^{ef}	0.1322^{c}	0.005
(6) no	Fe	11.34^{ab}	17.75^{abc}	23.84^{ab}	24.50^{bcde}	4.227^{abcd}	5.75^{def}	0.4902^{a}	0.012
(7) no	Trace elements	11.03^{ab}	6.75^{e}	3.66^{e}	13.00^{de}	1.660^{cd}	1.75^{f}	0.2167^{bc}	0.00
(8) host	control	9.55^{bcde}	20.00^{a}	13.67^{bcde}	25.50^{bcde}	2.430^{cd}	5.25^{def}	0.3173^{abc}	0.012
(9) host	N, P, K	7.71^{e}	12.00^{cde}	3.80^{e}	11.50^{e}	1.530^{cd}	2.75^{ef}	0.1720^{c}	0.005
(10) host	N, P, K + trace elements	8.5^{de}	16.75^{abc}	12.69^{bcde}	12.50^{de}	3.213^{bcd}	3.50^{def}	0.2555^{bc}	0.010
(11) host	Ca	11.14^{ab}	18.75^{ab}	17.18^{abcd}	34.50^{ab}	6.633^{ab}	11.50^{ab}	0.4325^{ab}	0.026
(12) host	Mg	10.1^{abcd}	15.00^{abcd}	13.50^{bcde}	33.00^{ab}	3.577	8.75^{bcd}	0.3148^{abc}	0.023
(13) host	Fe	10.74	17.75^{abc}	27.01^{a}	44.50^{a}	7.293^{a}	14.50^{a}	0.5288^{a}	0.021
(14) host	Trace elements	10.36^{abcd}	15.00^{abcd}	20.07	28.75^{bc}	6.640^{ab}	11.00^{abc}	0.2475^{bc}	0.013

K + trace elements or Ca were applied. Relative growth rates were higher in the presence of the host than in the absence of *A. dentata* (see Table 5), however this result just missed significance at p= 0.05 (Mann–Whitney test, U = 36, p = 0.1). Only two treatments, when N, P, K + trace elements or N, P, K were provided, did not lead to an increase in the relative growth rate in the presence of *A. dentata* (Table 5).

A positive relationship existed between root number, root area and number of haustoria (Table 5). Measurements were paired and tested for linear regression. Root area increased by 0.17 cm^2 for each additional root (R^2= 0.738, y = 0.218 + 0.165 x), and the number of haustoria increased by 1 for each additional 2.61 roots (R^2 = 0.764, y = 0.409 + 0.383 x). More important, the number of haustoria depended

Table 6. Comparison between *S. album* seedlings grown in the presence of *A. dentata* (trials 8-14) with those that grew without host (1 – 7). A particular nutrient treatment with (+) host was compared with the same nutrient treatment without (-) host for each of the seven growth parameters. (see Table 5 for mean values); >: a particular treatment (1 – 14) is doing better than another one (1 – 14) which with it is compared; significance at p <0.05; *ns* *: means differ greatly (hosted > non-hosted) but *not* statistically different (ANOVAs, Tukey tests).

Comparison +host vs -host	Nutrients	Dry weight (g)	Height	Number of Roots	Root area (cm^2)	Number of haustoria	Leaf area (cm^2)	Number of Leaves
8 vs 1	none	ns	ns	ns	ns	Ns	8 >1	8 >1
9 vs 2	N, P, K	ns	ns	ns	ns	Ns	ns	ns
10 vs 3	N, P, K + trace elements	ns	10<3	ns	10<3	ns *	ns	ns
11 vs 4	Ca	ns*	ns	ns	ns	11> 4	ns	ns
12 vs 5	Mg	ns	ns	12 > 5	ns *	12 > 5	ns *	12 > 5
13 vs 6	Fe	ns*	ns	13 > 6	ns *	13 > 6	ns	ns
14 vs 7	Trace elements	ns	ns	14 > 7	ns *	14 > 7	14>7	14 > 7

strongly on the number of roots, but was associated less strongly with root area in the absence of the host. The presence of *A. dentata* led to a strong correlation between the number of haustoria with each of root number, root area and leaf area in *S. album*.

The presence of *S. album* did neither affect height nor leaf number of *A. dantata*, but had a strong tendency to reduce dry weight (Mann Whitney test, U = 36, p = 0.1). Only when N, P, K and trace elements were applied *Alternanthera* grew taller and produced more leaves in the absence than in the presence of the parasite.

DISCUSSION

Experiment 1: Early growth of *S. album* seedlings in the presence of four *Alternanthera* taxa

After ten weeks of association between host and parasite, no significant differences in leaf size and number, shoot and root weight existed between treatments in the presence of the four tested taxa of *Alternanthera*. The lack of statistically significant differences in the growth of *S. album* appears to be related to the small number of variable samples collected during this study. The age of the seedlings at the time of planting, the length of the experimental time period, variation in the size of the seeds/ seedlings and the parasitic nature of the species could have led to the variable growth response observed for the individuals.

Very young seedlings may be unable to form haustoria and haustorial connections. Seedlings were between 5 and 11 weeks old at the beginning of the present experiment and therefore of an age similar to that of sandal plants tested by other researchers (Fox and Doronilla 1993, Fox and Barrett 1995, Radomiljak et al. 1998). Fox and Barrett (1995) found haustoria in some individuals 6 weeks after germination. During the present study, sandal seedlings had formed haustoria and a small but very variable number of haustorial connections in the presence of *A. dentata* cv 'Ruby', *A. dentata* cv 'Tricolor' and *A. amoena* cv 'Aurea' after eight weeks. The number of haustoria (between an average of 10 and 24 haustoria depending on the taxon, see Results) was similar to that recorded by other authors (Barrett and Fox 1997 found between 4 and 16 haustoria after 15 weeks depending on the presence of fertiliser; Luong {personal observation} counted 4.3 haustoria after 19 weeks). However, the length of the time period during which host and parasite are associated with, is more important for the formation of haustoria and haustorial connections than the initial age of the seedling, since the number of haustoria and haustorial connections respectively increase with time.

During the present study we examined growth during a time period of ten weeks; a statistically more meaningful evaluation of the data in regard to differences between the host varieties would have required a longer experimental time period involving a larger number of replicates.

In general, seed size and growth are positively related in *S. album* (Luong personal observation, Brand et al. 1993, Effendi and Sinaga 1994). Seed size also affects the parasitic relationship between *Santalum* and its host. Haustorial connections were found for seedlings derived from very large seeds (0.25 – 0.3g) eight weeks after germination, but small seeds (0.1 – 0.14g) produced seedlings, which had not formed haustorial connections 16 weeks after germination (Luong unpublished observations).

The observed variation in growth parameters may be related to the parasitic nature of *S. album*, that is the absence or presence of haustorial connections between host and parasite. Hence the more vigorous growth found in some young *S. album* seedlings may have been due to the haustorial attachment to a host, whilst the growth of other seedlings may have only relied on seed reserves (and a possible uptake of nutrients from the soil, see below). The time of *haustorial development* on sandalwood roots greatly varies (see above) and appears to be influenced by locality. *S. album* seedlings produced haustoria 33 weeks (Barrett unpublished observation) and 6 weeks respectively (Fox and Barrett 1995) after germination in Perth, Western Australia and at the age of 4 weeks in India (Nagaveni and Srimathi 1985). However, for *S. album*, it is poorly understood at which age haustorial connections are truly functional. Fox and Doronila (1993) estimate that in most cases haustorial connections do not lead to significant increase in growth before 106 days (15 weeks) after germination. Haustorial initiation is sometimes triggered by contact with small objects like stones or wooden sticks or the wall of the pot (see also Barrett and Fox 1997), but invasion of the penetration peg only occurs when the intimate connections are made with compatible, mature host roots (Tennakoon and Cameron 2006).

Of the four taxa tested, the Australian native *A. angustifolia* showed the least potential as primary host because of poor root growth, early mortality, a complete lack of haustorial connections and its annual life cycle. Plants produced flowers and began to senesce during the later stages of the experiment. Roots were generally shorter and with fewer lateral roots than those of the other tested varieties. It is well known that the root system (size and shape) is related to haustoria formation. Some species are therefore poor hosts for *S. album* due to their root type. The relatively thick and fleshy roots in Asteraceae species (*Erigeron linifolius; Elephantopus scaber*) and grasses (*Andropogon timoriensis*) appear to deter haustorial formation (Fox et al. 1996). However, more detailed research over a longer time period is required to examine whether the results of the present study were due to unfavourable conditions, or whether *A. angustifolia* is indeed a less suitable primary host than the other three taxa investigated. *A. dentata* var. "Tricolor" produced heavier shoots and roots and displayed larger leaves than the other tested varieties. However, vigorous shoot growth could lead to possible competition between "Tricolor" and *S. album* seedlings during later stages of the host parasite relationship.

The presence of functional haustorial connections characterises an efficient host-parasite relationship. *S. album* seedlings grown with 'Ruby' produced the greatest number of haustoria and of haustorial connections respectively, and seedlings of this taxon were the first to form haustorial connections after four weeks. Hence more detailed experiments should explore whether *A. dentata* var. 'Ruby' is indeed a better primary host to *S. album* than var. Tricolor', *A. amoena* cv 'Aurea', and *A. a angustifolia* respectively. One interesting aspect of further research could focus on a possible local adaptation of the hemi-parasite to a sympatric host (see Mutikainen et al. 2000).

Experiment 2: Can Young Sandalwood Seedlings Utilise Essential Minerals, if Administered Directly to Their Own Leaves?

At the nursery stage nutrient resources are mainly provided by seed reserves and later also from the soil (Barrett et al 1985, Fox et al. 1990, Barrett and Fox 1997). As seed reserves are exhausted, haustorial attachment to an efficient host becomes critical for survival. *A. dentata* var. "Ruby" displays characteristics desirable of a successful primary host: small, low growing, perennial species, ease of cultivation and well distributed, fine root growth that allows easy attachment of the sandalwood root haustoria. The first experiment conducted during the present study demonstrated that *S. album* seedlings grown with *A. dentata* var. 'Ruby' produced the greatest number of haustoria and haustorial connections respectively and formed haustorial connections after four weeks. We therefore selected the variety "Ruby" as primary host for the second experiment, which examined whether young sandal seedlings utilise essential minerals, if administered directly to their own leaves.

Radomiljac (1998) has shown that survival, height and diameter of sandal seedlings, when attached to a primary host were increased with supplementary nursery nutrition (1998). A symbiotic association with nitrogen - fixing bacteria and VAM fungi has also been observed and positively affects growth (Subba Rao et al, 1990, Nagaveni et al. 1998). The effect of mineral nutrition on growth has been examined in omission trials (uptake via roots from the soil). Sandalwood is able to obtain minerals via its root system without supplementary nutrition from hosts and maintain healthy (albeit stunted) growth for at least 13 weeks (Barrett et al. 1993, Barrett and Fox 1997).

The present study indicates that *S album* is also able to uptake minerals via its leaf system. The application of either of the following minerals: Ca; Fe; N, P, K + trace elements and trace elements respectively, resulted in an increase in the growth of pre-parasitic sandal seedlings after 20 weeks. However, the uptake of some minerals – as reflected in the measured growth variables - is greater in the presence of *Alternanthera*, and therefore haustorial connections appear to be a more efficient way to absorb these minerals. Magnesium and iron as well as trace elements affected growth parameters in the presence of the host and may be of limited availability in pre-parasitic seedlings. Nutrient omission trials have demonstrated a particular demand for iron and calcium in growing sandal plants (Barrett et al 1993, Barrett and Fox 1997). Seedlings resumed growth when provided with chelated iron, (Barrett and Fox 1997). *S. paniculatum*, *S. album* and *S. haleakalae* produced small chlorotic leaves with each new flush of growth (Hirano 1977). The importance of Ca, N, trace elements and Fe in maintaining healthy growth has been also documented for *S. spicatum* (Struthers et al. 1986). K and Na are expected to play a major part in the water relations of sandalwood once attached to host (Struthers et al. 1986). Multiple hosts have been observed to be of benefit in the field in order to increase the uptake of a large range of different nutrients, especially if a host is a poor source of a particular nutrient (Struthers et al. 1986; Lion, personal observation). The effect of supplementary nursery nutrition on survival and growth in the field has been examined by Radomiljak (1998). Supplementary nursery nutrition did not significantly influence the survival of *S. album* parasitising *Alternanthera nana* (Radomiljak 1998), and the author therefore concluded that supplementary nursery nutrition is not required for S. *album* parasitising an efficient pot host (like *A. nana*).

The large variation in the growth response between individual sandal plants during the present study may have been due to insufficient haustorial connections. However, the presence of *A. dentata* led to a strong correlation between the number of haustoria with each of root number, root area and leaf area (see RESULTS). This observation indicates that functional haustorial connections had been established, but further research should include detailed examinations of haustorial connections for all tested individuals.

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