Host-species-dependent physiological characteristics of hemiparasite *Santalum album* in association with N$_2$-fixing and non-N$_2$-fixing hosts native to southern China

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Understanding the interactions between the hemiparasite *Santalum album* L. and its hosts has theoretical and practical significance in sandalwood plantations. In a pot study, we tested the effects of two non-N$_2$-fixing (*Bischofia polycarpa* (Levl.) Airy Shaw and *Dracontomelon duperryanum* Pierre) and two N$_2$-fixing hosts (*Acacia confusa* Merr. and *Dalbergia odorifera* T. Chen) on the growth characteristics and nitrogen (N) nutrition of *S. album*. Biomass production of shoot, root and haustoria, N and total amino acid were significantly greater in *S. album* grown with the two N$_2$-fixing hosts. Foliage and root $\delta^{15}$N values of *S. album* were significantly lower when grown with N$_2$-fixing than with non-N$_2$-fixing hosts. Significantly higher photosynthetic rates and ABA (abscisic acid) concentrations were seen in *S. album* grown with *D. odorifera*. Similarity in the proportional amounts of amino acid of root xylem sap between *S. album* and its host *D. odorifera* was also evident, suggesting major access to nitrogenous solutes from *D. odorifera* to *S. album*. Irrespective of host species, *S. album* clearly appeared to optimize xylem sap extraction from its hosts by higher transpiration and lower water-use efficiency than its host. The growth of two non-N$_2$-fixing hosts parasitized by *S. album* was significantly greater than the equivalent values for unparasitized treatments, and lower growth and photosynthesis were observed for parasitized *A. confusa*, and significant decreases in root N, photosynthesis and transpiration for parasitized *D. odorifera* compared with unparasitized treatments. Furthermore, foliage ABA concentrations were significantly higher in all hosts parasitized by *S. album* than in their unparasitized counterparts. Our study is probably the first to report on host dependence and preference in the hemiparasite *S. album*, and the generated results may have important implications for understanding of the physiological interactions between host species and parasitic plants, and for successfully mixing plantations of *S. album* with *D. odorifera*.

**Keywords:** abscisic acid, amino acids, $\delta^{15}$N, haustorium, net leaf photosynthesis rate, transpiration.

Introduction

Among parasitic angiosperms, the sandalwood species (*Santalum* spp.), such as the most precious trees (*S. album*, *S. acuminatum* and *S. spicatum*), parasitizes the roots of host plants (Tennakoon et al. 1997a, 1997b, Radomiljac et al. 1999c). *Santalum* species are hemiparasites as they have functional chloroplasts to perform photosynthesis while partially relying on their host plants to take up water and nutrients through the haustorium, a specialized absorbing structure of parasitic plants, especially for nitrogenous compounds (Pate 2001, Bell and Adams 2011). Hence, host preference is an important aspect from which the parasite could gain measurable benefit (Jiang et al. 2008, Irving and Cameron 2009, Bell and Adams 2011).
Indian sandalwood (S. album L.) has been over-exploited for its aromatic heartwood and root, which have cosmetic, religious and medicinal significance (Kim et al. 2005, Ochi et al. 2005, Dhanya et al. 2010). Over the last two decades, several large-scale plantations of S. album have been established to meet future market requirements in Australia, China, India and Indonesia (Dhanya et al. 2010). Experiments on both pot and field plantations have shown that the growth performance of S. album is greatly enhanced by its successful attachment to suitable hosts, particularly to N$_2$-fixing species (e.g., Acacia, Casuarina and Sesbania) (Radomiljac et al. 1999a, Li 2003). Nevertheless, these hosts have a relatively low market value and the timely screening of suitable high-value host trees for S. album plantations is needed.

Dalbergia odorifera T. Chen, one of most precious rose-woods in the world with diverse medicinal and commercial values (Yu et al. 2007), has been successfully planted with S. album in the Jianfengling Arboretum of Hainan Island, China (18°42′N, 108°49′E), since 1989. Recently, we have reported an appropriated detailed understanding of N transfer between parasitized D. odorifera and S. album (Lu et al. 2013). This investigation has shown substantial N transfer from D. odorifera to S. album, while N$_2$-fixation of D. odorifera could enhance the N transfer. Nevertheless, a further understanding of the physiological interactions between S. album and D. odorifera is needed. As far as is known, no studies have investigated the direct or indirect effect of a host on S. album parasitism, which may provide useful management strategies for D. odorifera/ S. album mixed plantations, and potentially provide significantly increased value to the existing sandalwood plantation industry with another high-value woody species.

Growth performance, nutrient elements, organic solutes, gas exchange, haustorial anatomy and hormone variation have been studied between S. album and its respective hosts, including N$_2$-fixing hosts Acacia amplexica, Acacia trachycarpa, Sesbania formosa, and non-N$_2$-fixing hosts Eucalyptus camaldulensis, Kuhnia rosamarinifolia and Tithonia diversifolia (Radomiljac et al. 1999a, 1999b, 1999c, Tennakoon and Cameron 2006, Zhang et al. 2012, Yang et al. 2014). Differences in nutrient concentration, amino acid concentration and composition of xylem saps, photosynthesis and transpiration rates, δ$^{15}$N and δ$^{13}$C values, when S. album is associated with different host species in these studies, have been used alongside parasite growth to explain why some hosts are markedly superior to others in terms of overall benefit to the parasite. Generally, legumes, rather than non-legumes, are superior hosts due to their N$_2$-fixation capacity to provide the parasite with abundant N. However, some contrary evidence demonstrated that host N is not always a reliable predictor of parasite performance (Atsatt and Strong 1970, Kelly 1990, Marvier 1996, Jiang et al. 2008).

On the other hand, parasitic plants have deleterious direct and indirect effects on their host in terms of growth, photosynthesis and other physiological characteristics (Watling and Press 2001, Cameron et al. 2005, 2008, Li et al. 2012, Fisher et al. 2013). These effects could substantially influence the growth and survival of host and/or parasitic plants. Thus, studies on how interactions between hosts and parasites are important in selecting host plants for S. album plantation have been carried out.

We therefore investigated the host dependence and preference in the growth and performance of the parasitic S. album. Four different host species (two N$_2$-fixing legumes Acacia confusa Merr. and D. odorifera, and two non-N$_2$-fixing plants Bischofia polycarpa (Levl.) Airy Shaw and Dracontomelon duperreranum Pierre) have been reported as superior host candidates of S. album native to southern China (Li 2003). We aimed to address the following questions: (i) if S. album could successfully parasitize host plants, what physiological alterations did the host have after parasitization? (ii) Did the growth of S. album depend on its host species characteristics, e.g., non-N$_2$-fixing vs N$_2$-fixing host? If yes, among these four host species which one could be the best candidate to provide a brand-new mixed plantation pattern of host and S. album? Answers to these questions could improve our understanding of the physiological interactions between S. album and different host species in order to identify better mixed patterns for S. album field plantations in China.

Materials and methods

Plant material

Two N$_2$-fixing legumes of A. confusa and D. odorifera and two non-N$_2$-fixing plants of B. polycarpa and D. duperreranum were selected as hosts for S. album. Seeds of these five woody species were surface-sterilized with 3% sodium hypochlorite for 5 min and rinsed with distilled water. Seeds of S. album were also soaked in 0.1% gibberellic acid (GA) for 12 h and rinsed with distilled water to promote germination (Radomiljac et al. 1999a). The rinsed seeds were germinated on sterilized sand at 28–30 °C for ~2–3 weeks until germination. Two emerging seedlings with 1 cm radicles, either in a single species or with S. album as a pair, were moved into one 15 × 11 × 14 cm plastic pot with 250 g potting mix (vermiculite : perlite = 2 : 1, v/v, pH 6.8 ± 0.2) in April 2009. Seedlings were fertilized with 2.5 ml of Hoagland solution once weekly and watered twice weekly to field capacity. Seedlings of N$_2$-fixing hosts were inoculated with Bradyrhizobium elkanii DG, an effective strain isolated from Pterocarpus macarocarpus Kurz. nodules (Lu et al. 2012). Meanwhile, seedlings of non-N$_2$-fixing hosts were also inoculated with the same amounts of autoclaved B. elkanii inoculants.

Experimental design

In April 2010, parasitic associations were established between 1-year-old S. album (20 ± 1.2 cm height and
3.3 ± 0.3 mm diameters) seedlings and their four 1-year-old host species. One parasitized pair was then transplanted to a 40 × 30 × 35 cm pot containing 5 kg of the above-mentioned potting mix. A 20-cm distance was fixed between these two seedlings in each pot to reduce distance effects. In addition, two individuals of each species were also transplanted into one pot for determination of intraspecific competition. As a result, with three replicates or pots for each paired association plants, a total of 72 pots were grown in a greenhouse in a random arrangement under 33/25 °C day/night and 65/75% day/night relative humidity, at the Research Institute of Tropical Forestry in Guangzhou (23°11′N, 113°23′E), China. Plants were watered three times weekly to near field capacity and fertilized with 50 ml of Hoagland nutrition monthly.

Measurements of leaf photosynthesis and transpiration
Using a LI-6400 portable photosynthesis system with a standard 6-cm² leaf chamber (LI-COR, Inc., Lincoln, NE, USA), net rates of leaf photosynthesis (Pn, µmol CO₂ m⁻² s⁻¹) and transpiration (E, mmol H₂O m⁻² s⁻¹) were measured 6 months after transplantation between 09:30 and 10:30 am on two consecutive sunny days in October 2010. Photosynthetic irradiance was provided by an integrated red-blue light-emitting diode source (Model 6400-02B; LI-COR, Inc.) at 1000 µmol m⁻² s⁻¹. During measurements on these 2 days, the CO₂ concentration inside the greenhouse was 387.8 ± 4.7 ppm. Leaf temperature was 30 °C. To ensure a similar leaf age and development, the third, fourth and fifth fully expanded phyllodes of A. confusa and leaves of S. album, and the second, third and fourth fully expanded leaves of B. polycarpa, D. duperreryanum and D. odorifera were sampled. Water-use efficiency (WUE, µmol CO₂ mmol H₂O⁻¹) as Pn/E was then calculated.

Plant harvesting and sampling
Six months after transplantation, the shoot and root of S. album and its host plants were separately harvested on 25 October 2010, oven dried at 70 °C for 72 h and then ground with a ball mill (Retsch GmbH & Co. KG, Haan 1, Germany) for further analyses. Root xylem sap was collected by an aspirator (A-1000S; EYELA, Tokyo, Japan, Figure 1) from freshly excavated root branches. To collect root sap, root segments were tightly inserted into a plastic pipette tip and a mild vacuum (−0.04 to −0.07 MPa) was then applied to the apparatus via a rubber hose that was connected to an aspirator (Pate et al. 1994). Approximately 300 µl xylem sap for each treatment was then bulked and frozen for further analyses.

Numbers of haustoria of S. album on each host plant were counted and harvested from those forming typical appressed contacts with root xylem and those directly parasitizing root nodules on N₂-fixing hosts.

Analyses of N and ¹⁵N natural abundance
Total N in plant foliage and roots was determined by the Kjeldahl digestion method (Knowles and Blackburn 1993). δ¹⁵N values of leaves and roots were analyzed using an isotope ratio mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at the State Key Laboratory of Tree Genetics and Breeding in Beijing, China.

Abscisic acid analysis
The second, third and fourth fully expanded leaves of both S. album and hosts were selected to measure their abscisic acid (ABA) concentrations. Briefly, 0.5 g deep frozen tissue, collected from a small strip in the middle portion of these leaves, but avoiding their mid-rib, was ground with 2 ml of 80% methanol containing butylated hydroxy toluene (0.001%, w/v). The extract was mechanically shaken (120 rpm) for 1 h at 4 °C and then subjected to centrifugation at 13,000g for 20 min. The supernatants were assayed for ABA using an enzyme-linked immunosorbent assay with the monoclonal antibody (AFRC MAC 252; Sigma, Poole, Dorset, UK) (Asch et al. 2001).

Analyses and identifications of amino acids
The collected root xylem sap (300 µl) was left at room temperature, and then diluted 10 times with 3% trichloroacetic acid solution and centrifuged at 10,000 rpm for 15 min. A total

![Figure 1. Conceptual diagram of xylem sap collection. AS, aspirator; EP, Eppendorf tube; PG, pressure gauge; PT, pipette tip; RH, rubber hose; S, rubber stopper; SF, suction flask.](image-url)
of 20 µl collected supernatant was injected into an L-8800 High Speed Amino Acid Analyser (HITACHI High-Technologies, Tokyo, Japan). Separation was primarily achieved on a HITACHI column 855-350, and the reaction temperature was 134 °C. The analyses of amino acids were followed with the instrumental manual using the ninhydrin reaction methods. The identifications of amino acids were compared with the standard amino acid solutions, type AN and type B, which were purchased from the Wako Pure Chemical Industries, Osaka, Japan.

**Statistical analysis**

Data (means ± SE, n = 3) were subjected to one-way analysis of variance and the significant differences between treatments were compared using the least significant difference at the P = 0.05 level. The impact of *S. album* on hosts was assessed by comparing the parasitized and unparasitized hosts using Student’s t-test. All analyses were performed with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

**Results**

**Growth performances**

In general, significantly greater diameter, height, shoot and root biomass of *S. album* were observed in seedlings associated with the two N₂-fixing hosts *A. confusa* and *D. odorifera* than in seedlings with the two non-N₂-fixing hosts *B. polycarpa* and *D. duperreranum* (Table 1).

Height, diameter and total biomass of both non-N₂-fixing hosts parasitized by *S. album* were significantly greater than the equivalent values for the unparasitized treatment (Student’s t-test; P < 0.05) (see Table S1 available as Supplementary Data at Tree Physiology Online). In contrast, lower growth indices were observed for parasitized *A. confusa* but not for *D. odorifera* compared with the unparasitized treatment (see Table S1 available as Supplementary Data at Tree Physiology Online).

**Haustorial number and biomass production**

After 1 year of growth with host plants, haustoria had been successfully formed between roots of *S. album* and its host (data not shown). Bell-shaped *S. album* haustoria attached to the roots of both 1.5-year-old N₂-fixing *A. confusa* and *D. odorifera* (Figure 2a and b) and non-N₂-fixing *B. polycarpa* and *D. duperreranum* (Figure 2e and f). Meanwhile, *S. album* haustoria also penetrated into 1.5-year-old N₂-fixing *A. confusa* and *D. odorifera* nodules (Figure 2c and d). All haustoria were classified into three size categories: <0.2 cm, 0.2–0.5 cm and >0.5 cm. The majority of haustoria were <0.2 cm in most species, and there were significantly more haustoria of this size formed on *B. polycarpa* than on other host species (Table 2). Numbers of 0.2–0.5 cm haustoria were significantly higher in *D. odorifera* than in other host species (Table 2). In addition, *D. odorifera* had significantly greater biomass production in seedlings with the two non-N₂-fixing hosts compared with the two N₂-fixing hosts (Table 1).
of attached haustoria than other host species (Table 2). Moreover, the biomass of per haustorium was generally significantly higher in N<sub>2</sub>-fixing hosts than in non-fixing hosts, except when comparing A. confusa with D. duperreranum (Table 2).

Numbers of haustoria (r<sup>2</sup> = 0.29, P = 0.07, n = 12) were not correlated with S. album biomass production, while biomass production of all haustoria (r<sup>2</sup> = 0.44, P = 0.02, n = 12) and per haustorium (r<sup>2</sup> = 0.79, P = 0.00, n = 12) in the hosts significantly correlated with biomass production in S. album (see Figure S1a and b available as Supplementary Data at Tree Physiology Online). In addition, comparatively low numbers of haustoria penetrated nodules in the two N<sub>2</sub>-fixing hosts A. confusa and D. odorifera (Figure 2c and d; Table 2).

**Nitrogen concentrations**

Concentrations of both foliage and root N and total amounts of plant (all aboveground and belowground tissues) N were significantly higher in S. album when associated with N<sub>2</sub>-fixing hosts than with non-fixing hosts (Figure 3). Foliage N concentrations were significantly higher in S. album than in their non-N<sub>2</sub>-fixing hosts, whereas root N concentrations were lower in S. album than in their N<sub>2</sub>-fixing hosts. In addition, S. album seedlings associated with D. duperreranum showed considerably lower N concentrations in both foliage and roots than in seedlings associated with other hosts. Amounts of plant total N were significantly higher in N<sub>2</sub>-fixing than in non-N<sub>2</sub>-fixing plants, while they were without significant differences in plant total N between N<sub>2</sub>-fixing or between non-N<sub>2</sub>-fixing species (Figure 3c).

Root N concentrations of D. odorifera were significantly lower in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in parasitized than in unparasitized treatments, but N concentrations in foliage and root were significantly higher in paras.
Foliage and root $\delta^{15}$N values

Foliage and root $\delta^{15}$N values were significantly higher in non-$N_2$-fixing hosts *B. polycarpa* and *D. duperreranum* (4.56–4.62 and 3.21–3.99‰) than in $N_2$-fixing hosts *A. confusa* and *D. odorifera* (0.31–0.43 and 1.18–2.01‰) (Figure 4). Foliage and root $\delta^{15}$N values of *S. album* were significantly lower when associated with $N_2$-fixing hosts (−0.23 to 1.22‰ and 1.29–1.64‰) than with non-$N_2$-fixing hosts (2.33–3.62 and 2.40–2.93‰) (Figure 4).

Amino acid profiles in xylem sap

Total concentrations of amino acids in the xylem sap were generally higher in the $N_2$-fixing hosts than in the non-$N_2$-fixing hosts (Figure 5). This was also true for *S. album* when it was associated with an $N_2$-fixing host.

The principal amino acid in the host root xylem varied between species (Figure 5). Aspartic acid dominated in *A. confusa*, hydroxylysine in *B. polycarpa* and phosphoserine
in other plant species, i.e., the \( \text{N}_2 \)-fixing \textit{D. odorifera} and non-\( \text{N}_2 \)-fixing \textit{D. duperreranum}. Phosphoserine was the most common amino acid in the xylem sap of \textit{S. album} when associated with any host. Other major amino acids in the xylem sap were similar between \textit{S. album} grown with \( \text{N}_2 \)-fixing hosts and its hosts. However, the amino acid composition of root xylem sap in \textit{S. album} differed from that of their associated non-\( \text{N}_2 \)-fixing hosts, \textit{B. polycarpa} and \textit{D. duperreranum}. For instance, both parasitized non-\( \text{N}_2 \)-fixing hosts contained aspartic acid and glutamic acid, whereas glutamine or asparagine was absent in \textit{S. album} associated with \textit{B. polycarpa} or \textit{D. duperreranum}.

Concentrations and amounts of ABA

\textit{Acacia confusa} had significantly higher foliar ABA concentration than other hosts, whether they were in the parasitized or unparasitized treatments (Figure 6a, see Table S2 available as Supplementary Data at \textit{Tree Physiology} Online). Foliage ABA was highest in \textit{S. album} grown with \textit{D. odorifera} (0.62 ng g\(^{-1}\) DW), followed by with \textit{A. confusa} (0.52 ng g\(^{-1}\) DW), then with \textit{D. duperreranum} (0.43 ng g\(^{-1}\) DW) and least with \textit{B. polycarpa} (0.26 ng g\(^{-1}\) DW) (Figure 6a). The ABA concentration of \textit{S. album} associated with either non-\( \text{N}_2 \)-fixing or \( \text{N}_2 \)-fixing hosts was significantly higher than its corresponding hosts \((P < 0.05)\), except \textit{A. confusa} (Figure 6a). In addition, foliage ABA was significantly higher in all hosts when parasitized by \textit{S. album} than their unparasitized counterparts \((P < 0.05)\) (see Table S2 available as Supplementary Data at \textit{Tree Physiology} Online).

Amounts of foliage ABA were significantly higher in \( \text{N}_2 \)-fixing hosts \textit{A. confusa} and \textit{D. odorifera} (10.48 and 11.00 ng per plant) than in non-\( \text{N}_2 \)-fixing hosts \textit{B. polycarpa} and \textit{D. duperreranum} (7.57 and 7.27 ng per plant) (Figure 6b). \textit{Santalum album} L. grown with \( \text{N}_2 \)-fixing hosts had significantly higher total foliage ABA (13.38 and 20.75 ng per plant) than with non-\( \text{N}_2 \)-fixing hosts (1.13 and 1.29 ng per plant) (Figure 6b). Interestingly, foliage ABA concentrations were higher but foliage ABA amounts were lower than their hosts when \textit{S. album} grew with the two non-\( \text{N}_2 \)-fixing hosts.

\( P_n \), \( T \), and WUE for different host–parasite pairings

There were no clear trends between the photosynthesis rates \((P_n)\) of \textit{S. album} and its hosts (Figure 7). Values of \( P_n \) were significantly higher in parasitized \textit{A. confusa} and \textit{B. polycarpa},...
but significantly lower in parasitized *D. duperreranum* and *D. odorifera*, than their corresponding parasite *S. album*. Values of $P_{n}$ in *S. album* were enhanced by N$_{2}$-fixing hosts (5.96 and 8.23 µmol CO$_{2}$ m$^{-2}$ s$^{-1}$ in *A. confusa* and *D. odorifera*) but decreased by non-N$_{2}$-fixing hosts (3.69 and 4.49 µmol CO$_{2}$ m$^{-2}$ s$^{-1}$ in *B. polycarpa* and *D. duperreranum*) (Figure 7). Values of $P_{n}$ were significantly higher in non-N$_{2}$-fixing hosts *B. polycarpa* and *D. duperreranum*, but significantly lower in N$_{2}$-fixing hosts *A. confusa* and *D. odorifera*, under parasitized than unparasitized conditions ($P < 0.05$, see Table S2 available as Supplementary Data at *Tree Physiology* Online).

In all associations, transpiration rates ($T_{r}$) were significantly higher in *S. album* than in its associated hosts (Figure 7). Rates of $T_{r}$ were similar between the parasitized and unparasitized conditions.
non-N₂-fixing hosts, but were significantly higher or lower in parasitized N₂-fixing A. confusa or D. odorifera than in their unparasitized counterparts (see Table S2 available as Supplementary Data at Tree Physiology Online).

Values of WUE were significantly higher in any associated host plant than in S. album (Figure 7). The WUE values D. duperreranum were significantly higher in parasitized A. confusa and D. duperreranum, but similar in parasitized B. polycarpa and D. odorifera, than in their corresponding unparasitized counterparts (see Table S2 available as Supplementary Data at Tree Physiology Online).

Discussion

This study investigated a number of interrelated physiological factors involved in the complex interactions between S. album and its four potential plant hosts native to China, growing in pairs in pots under greenhouse conditions, with a view towards developing a reliable D. odorifera/S. album mixture plantation in southern China.

Growth responses on S. album

The two N₂-fixing species (A. confusa and D. odorifera) used in this study were better hosts than the two non-N₂-fixing hosts (B. polycarpa and D. duperreranum) in promoting S. album growth (Table 1). This is in accordance with previous studies, which concluded that xylem-tapping root hemiparasites grow better when associated with N₂-fixing hosts, apparently as a result of higher N concentrations in the xylem of legumes compared with non-legumes (Radomiljac et al. 1999b, 1999c, Press and Phoenix 2005, Bell and Adams 2011). The data for the two N₂-fixing plants in the present study clearly showed that D. odorifera was better at promoting the growth and photosynthesis of S. album than A. confusa, but was considerably poorer than the latter species when considering foliar N. Nevertheless, by the end of the 6-month study period, the growth of A. confusa was severely constrained under parasitism. Thus, our results indicate that S. album possibly parasitizes various angiosperm hosts, but some are more heavily infected than others.

An interesting finding from this study was that the growth of S. album in the absence of a host was greater than that of plants grown with non-N₂-fixing host B. polycarpa and D. duperreranum (data not shown), supporting earlier studies suggesting a minimal benefit, if not disadvantage, in terms of competition for nutrients when the parasite associates with an inferior host (Radomiljac et al. 1999a). Our studies also found that the most suitable host quality index for S. album were the biomass production per haustoria and the total haustorial biomass production instead of the number of haustoria attached to the host’s roots. This is in accordance with previous studies that not all species attacked by parasites act as suitable hosts because some attached haustoria are not able to penetrate their vascular tissues (Cameron et al. 2006, Cameron and Seel 2007, Suetsugu et al. 2012).

Nitrogenous solute flux from host to S. album

Since the xylem sap of host and parasite are linked by a haustorium, it has been suggested that organic N is acquired by the passive, non-selective flux of xylem sap from the host to the parasite (Pate et al. 1994, Radomiljac et al. 1998, Hibberd and Jeschke 2001, Pageau et al. 2003). Our results revealed substantial and consistent differences between the amino acid composition of the root xylem sap in the host species and associated S. album (Figure 5). The concentrations of amino compounds in the root xylem sap of the two N₂-fixing hosts and associated S. album were higher than the two non-N₂-fixing hosts and their associated S. album (Figure 5). Therefore, our results provide evidence that a direct bulk transfer of major nitrogenous solute did indeed take place from the xylem of N₂-fixing hosts to S. album. This finding is consistent with the ‘nitrogen parasitism hypothesis’ that the primary physiological function of importing organic N compounds from the host xylem sap is to supply N to the parasite (Schulze et al. 1984, Marshall et al. 1994). However, a total of 13 or 19 amino acids with two distinctive organic acids (piperolic acid and djenkolic acid) were detected (Tennakoon et al. 1997b, Radomiljac et al. 1998), while a total of 26 amino acids were found in this study (see Figure 5). We assume these differences are mainly from the utilization of different plants species and tissues (stem sap vs root sap). Meanwhile, a new generation of amino acids analyzer was also employed in this study.

The abundant nodulation of A. confusa and D. odorifera, a number of haustoria of S. album attached to both nodules and roots, and values of δ¹⁵N close to zero for both legumes and accompanying S. album provide evidence of a high dependence of host and parasite on fixed N. For most annual or woody hemiparasites, the proportion of nitrogenous compound transferred from the host ranged widely, from low (0.2–18%) via non-N₂-fixing hosts to high (56–70%) via N₂-fixing plants (Tennakoon et al. 1997b, Jiang et al. 2004, Cameron and Seel 2007); in such studies there is little relevance in distinguishing between N₂-fixation and N transfer. Bearing this in mind, the present study examines that D. odorifera with effective nodules do have enhanced N fluxes between host D. odorifera and S. album, suggesting that effective N₂-fixation could supply more nitrogenous substances in the xylem sap to the S. album haustoria (Lu et al. 2013). Hence, N₂-fixing legumes are assumed to be superior hosts by supplying N to parasites. In contrast, some studies have reported that N₂-fixing species do not enhance the growth of parasites compared with non-N₂-fixing hosts (Atsatt and Strong 1970, Kelly 1990, Marvier 1996, Matthes 1997). Recently, Jiang et al. (2008) suggested that N₂-fixation does not influence the quality of legumes as
hosts for the hemiparasite Rhinanthus minor, but rather the well-developed haustorium formed by the parasite, coupled with the lack of defensive mechanism of the host, and the presence of ample nitrogenous compounds in the xylem sap accessible to the parasite's haustorium, govern the host quality of legumes. Our results show that the comparison of δ¹⁵N signatures of the parasite and its potential hosts gives a good indication of the Nₚ-fixing plants likely to act as principal N sources to S. album.

**AB A concentrations in parasite and host**

Parasitic plants are known to accumulate high concentrations of ABA (Jiang et al. 2010), but its function in such unprecedented concentrations remains unclear. In any case, parasitism may significantly influence the associated host metabolism to favor nutrient acquisition by the parasite, such as elevated levels of ABA in host tissues (Taylor et al. 1996, Frost et al. 1997, Pageau et al. 2003). In common with other hemiparasites, such as Striga and Rhinanthus species, the foliar ABA concentration in S. album was found to be significantly higher than in its associated hosts (Figure 6). In addition, the foliar ABA concentration in all four hosts was significantly higher after attachment by S. album, as shown earlier for Striga and Rhinanthus by Taylor et al. (1996) and Frost et al. (1997). Taylor et al. (1996) suggested three possible sources for the extra ABA following host/parasite association: (i) a wounding response by haustorial attachment; (ii) a water deficiency of the host after parasitizing; (iii) ABA synthesis in the parasite and transport into the host. A recent study found another mechanism by which haustoria of S. album could synthesize phytohormones (e.g., ABA and GA), which was likely to be used for cell division and differentiation during haustorial development (Zhang et al. 2012).

In this study, a strong positive relationship was observed between S. album biomass and amounts of ABA ($r^2 = 0.83$, $P = 0.00$, $n = 12$, see Figure S1C available as Supplementary Data at Tree Physiology Online), suggesting that ABA content might be a reliable indicator of host quality. Zhang et al. (2012) verified that the ABA and GA contents of S. album were three times higher in attached haustoria than in non-attached haustoria, suggesting that endogenous hormones (e.g., indole-3-acetic acid, GA and ABA) might be involved in the haustorial formation of S. album and in water and nutrient transport for the host–parasite association (Zhang et al. 2012). Based on the haustorial research, our explanation for the high concentration and amounts of ABA in S. album grown with superior hosts is that it is possible that S. album may form more effective haustoria with suitable hosts (A. confusa and D. odorifera) than with inferior hosts (B. polycarpa and D. duperreranum) (Figure 6). Several studies have indicated that hormones (e.g., ABA, jasmonic acid) could be responsible for initiating the heartwood transformation (Davison and Young 1973, Shain and Hillis 1973, Taylor et al. 2002).

Thus, we would intend to investigate whether such an effect is responsible for accelerating the heartwood formation of D. odorifera after parasitization by S. album.

**Gas exchange between S. album and host**

The results of this study showed that increased N derived from the xylem stream of the two N₂-fixing hosts resulted in an increase of N concentration in S. album tissue and high photosynthetic performance and eventually, improved biomass production (Table 1 and Figure 3). Improved rates of photosynthesis in S. album when grown in association with N₂-fixing A. confusa and D. odorifera provide circumstantial evidence that S. album has a lower dependence on such hosts for carbon compared with non-N₂-fixing B. polycarpa and D. duperreranum. However, S. album clearly appears to optimize root xylem sap extraction from its hosts in the same way as the obligate hemiparasite Striga hermonthica (Taylor et al. 1996) and facultative hemiparasite Rhinanthus minor (Jiang et al. 2003), by having higher transpiration rates and lower WUE values than in the host. However, notable exceptions include S. acuminatum (Tennakoon et al. 1997), Olax phyllanthis (Pate et al. 1990) and mistletoe (Press et al. 1993, Seel et al. 1993). Due to elevated parasite transpiration, xylem sap is drawn through the haustorium, providing vascular continuity between Striga and its host, and into the parasite via cohesion (Press and Graves 1995). But there is no vascular continuity between the haustorium of S. album and its host Tithonia diversifolia, instead the presence of massive interfascicular parenchyma was found at the host–parasite interface (Tennakoon and Cameron 2006).

**Host responses to parasitism**

It has long been known that parasitism can suppress the biomass and photosynthesis of the associated host plant (Hibberd et al. 1996, Watling and Press 2001, Cameron et al. 2005, 2008, Fisher et al. 2013). In Dalbergia, significant decreases in photosynthesis, transpiration and N concentration of root were noted compared with unparasitized plants (see Table S2 available as Supplementary Data at Tree Physiology Online), suggesting that S. album sequesters significant amounts of N from the host plant. These decreases were mirrored in Acacia, except for the N concentration and transpiration (see Table S2 available as Supplementary Data at Tree Physiology Online). In contrast, in the two non-N₂-fixing plants B. polycarpa and D. duperreranum, intraspecific competition had a worse influence on the growth of unparasitized plants than parasitism by S. album (see Table S1 available as Supplementary Data at Tree Physiology Online). From an evolutionary perspective, in order to acquire large amounts of xylem sap from hosts, parasites may have evolved by improving their attraction to potential hosts rather than by their absorbing ability. The hemiparasite S. album competed poorly with hosts B. polycarpa and D. duperreranum for available nutrients in pot studies, and thus both non-N₂-fixing hosts parasitized by S. album grew consistently better than their unparasitized treatments (see
Table S1 available as Supplementary Data at *Tree Physiology* Online. However, differences in defensive ability have been indicated to underpin these differences in host quality and the associated parasite-induced host damage (Cameron et al. 2006, Cameron and Seel 2007, Rümer et al. 2007). Because of the absence of obviously defensive structures, Fabaceae have always served as good hosts for *R. minor* (Rümer et al. 2007, Jiang et al. 2008). Nevertheless, poor host *Plantago lanceolata* exhibited strong reactions (e.g., releasing toxic secondary compounds and host cell disintegration) against the haustorial tissues (Rümer et al. 2007). Therefore, in investigations addressing *S. album*—host interactions, anatomical surveys on host defense may be important to get a complete picture of the complex interactions.

In conclusion, the results on the physiology of the root hemi-parasite *S. album* presented in this study collectively provide an insight into the complex interactions between the parasite and the host during the early growth stage of *S. album*. A range of questions remain concerning the heartwood development of both *S. album* and its suitable host *D. odorifera*. Further research in this area is needed to allow the development of a superior methodology for the concurrent plantation of these two valuable timber species.

**Supplementary data**

Supplementary data are available at *Tree Physiology* Online.

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**Conflict of interest**

None declared.

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