Gas Exchange and Water Relations of the Root Hemi-parasite *Santalum album* L. in Association with Legume and Non-legume Hosts

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This paper examines foliar nitrogen (N) levels, photosynthesis, transpiration, water use efficiency and tissue water relations of the xylem-tapping root hemi-parasite *Santalum album* in pot culture with various N_2-fixing woody hosts, a non-fixing host (a eucalypt), or in the absence of a host. Foliar N concentrations of *Santalum* were significantly greater than corresponding hosts and higher when on N_2-fixing hosts than on the eucalypt, or without a host. Strong positive relationships were evident in *Santalum* between foliar N concentration, rates of net photosynthesis and instantaneous water use efficiencies. Photosynthesis rate and water use efficiency of *Santalum* were generally lower than in corresponding hosts, but transpiration rates were not noticeably different between associations. $\delta^{13}C$ values of total shoot dry matter of *Santalum* were poorly correlated with instantaneous water use efficiency as measured by gas exchange, but associations involving the three legumes showed less negative $\delta^{13}C$ values and better water use efficiencies for hosts, than corresponding parasites. Interpretation of such differences was difficult in view of an earlier demonstration of substantial heterotrophic gain of C from certain hosts. Diurnal profiles of gas exchange and leaf water potential of hosts and parasites indicated closely coordinated diurnal stomatal responses of the parasite water relations to its host, thus resulting in transpiration rates of the parasite generating leaf water potential gradients favouring continuous abstraction of water and nutrients from a host. Tissue water relations of *Santalum* generally resembled those of water-stress tolerant species. Host-specific effects on relative water content and osmotic adjustment were slight and rated unimportant in regulation of water flow to the parasite, or in protecting it from temporary water stress in a host.   © 1999 Annals of Botany Company

Key words: *Santalum album*, Indian sandalwood, root hemi-parasite, parasite:host relations, leaf nitrogen, photosynthesis, water use efficiency, water relations.

INTRODUCTION

A number of species of the root hemi-parasite *Santalum* (Santalaceae) produce highly valued aromatic heartwood known as sandalwood, of which the major aromatic constituents, α- and β-santalol, are widely used in the perfumery and cosmetics industries (Srinivasan *et al.*, 1992). The associated potential for commercial culture of sandalwood is now well recognized, particularly in the case of Indian sandalwood (*S. album* L.), for which research is now being conducted widely in the tropics (see Hamilton and Conrad, 1990; McKinnell, 1993; Gjerum, Fox and Ehrhart, 1995) including northern Western Australia (Radomiljac *et al.*, 1998*c*). Just as in the case of other root hemi-parasites, sandalwood is partly dependent on host species for water and nutrients, with leguminous hosts being generally better sources of nitrogen than other species (Tennakoon, Pate and Stewart, 1997*b*; Radomiljac *et al.*, 1998*b*). Proper selection of host species to give maximum benefit to *S. album* is accordingly proving to be critical for economically effective plantation culture (Rai, 1990; Srinivasan *et al.*, 1992; Havel and McKinnell, 1993; Fox *et al.*, 1996; Radomiljac *et al.*, 1998*c*).

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The recent demonstration of targeted haustorial initiation on nitrogen fixing hosts, and evidence of greatest benefit in terms of nitrogen from these as opposed to non-fixing species in native habitats [see study on *S. acuminatum* (R. Br.) A. DC. by Tennakoon, Pate and Stewart (1997*a*)] has raised the issue of the precise physiological basis for such benefit, whether simply in terms of providing nitrogen *per se* or follow-on advantages from such acquisition in terms of improved photosynthesis and water usage. Some of the features involved, such as growth and xylem solute composition when parasitizing different hosts, have already been studied for pot cultured *S. album* (Radomiljac, McComb and McGrath, 1998*a*; Radomiljac *et al.*, 1998*b*). In the present study we further evaluate host-specific responses of *S. album* on beneficial (N_2-fixing) and non-beneficial hosts or no hosts, specifically examining the impact of host type on net assimilation and transpiration rates and water use efficiencies of host and parasite.

MATERIALS AND METHODS

Pot culture

Santalum and woody hosts were grown as single plant pairings in 25 l pots in a nursery near Kununurra (15° 46′ S, 128° 44′ E), Western Australia as recently described by Radomiljac *et al.* (1998*a*). The woody host species selected

were the N_2-fixing legumes *Sesbania formosa* (F. Muell.) N. Burb. (Papilionaceae), *Acacia trachycarpa* E. Pritzel (Mimosaceae), *Acacia ampliceps* Masln. (Mimosaceae) and the non N_2-fixing *Eucalyptus camaldulensis* Dehnh. (Myrtaceae). Seedlings of host species were inoculated with appropriate *Bradyrhizobium*. Pots containing a single *Santalum* seedling were established as controls lacking a woody host. *Santalum* and host seedlings were 180 and 90 d old, respectively, at the time of transfer to 25 l pots. Controlled release fertilizer, Scotts® Osmocote Plus 8-9M, (N 16·0%, P 3·5%, K 10·0%, S 2·4%, Mg 1·2%, B 0·02%, Cu 0·05%, Fe 0·4%, Mn 0·06%, Mo 0·02%, Zn 0·015%) was applied to the potting media surface at a rate of 10 g per pot at the time of transplanting the parasite and hosts. Potting media consisted of coarse graded river sand:peat:perlite at 3:2:2. The experiment consisted of eight replicates, with each replicate containing five fully randomized pots of each parasite:host association. The associations were grown on for a further 300 d in the nursery in full sun, with overhead watering twice daily to near field capacity.

Leaf gas exchange and leaf water potentials of *Santalum* and hosts

Net rates of leaf photosynthesis (P_s) and transpiration (E_t) of foliage of host and parasite were assessed on three replicate pots of each association over a 2 d period in high summer (February 1997). In all associations *Santalum* was well attached to its associated host (Radomiljac, 1998). Measurements were conducted using a Parkinson broad-leaf chamber (PLC-B) coupled to a portable flow through ADC infra red gas analyser (Model LCA-3 ADC Instruments, Cambridge, UK). Measurements of P_s and E_t were made on 6·25 cm² enclosed leaf surfaces between 0800 and 1045 h, using the fifth fully expanded leaf on the uppermost main shoot of the host or parasite plant. Instantaneous water efficiencies (WUE) were then assessed as P_s/E_t. Leaf gas exchange data were analysed using ANOVA and Tukey's pairwise *t*-test. All analyses were performed using Systat® statistical software (Systat, 1992).

Using information provided by the curvette in terms of photosynthetic photon flux density (PPFD), relative humidity and leaf temperature, it was possible to target measurements to unshaded conditions of stable PPFD value, ambient humidities between 43–59% and temperatures within the range 29 to 38 °C. Under such conditions vapour pressure deficit (VPD) values lay within the range 1·69–3·71 (kPa) and CO_2 concentrations and PPFD readings in the cuvette within 329–365 μmol mol^{-1} and 1670–2290 μmol m^{-2} s^{-1}, respectively.

In a further study also undertaken in summer (1997) diurnal courses of P_s and E_t and WUE of host and parasite leaf material from the same replicated pots of each host:parasite association were examined at 2 h intervals between 0400 and 1600 h on a single day of measurements. Parallel measurements of leaf water potential (ψ) were undertaken on sample leafy shoots harvested from the same replicate pot material over the same day, using a standard pressure chamber technique (Scholander *et al.*, 1965; Turner, 1988).

Nitrogen and chlorophyll contents of foliage

At the end of the studies of leaf P_s and E_t, leaves of hosts and parasite used in the measurements were harvested, oven dried for 48 h at 80 °C, milled and subjected to Kjeldahl analysis for total N content (McKenzie and Wallace, 1954). Foliar N concentration data were analysed using ANOVA and Tukey's pairwise *t*-test.

Mean total chlorophyll concentrations of intact leaves of *Santalum* were determined using a Minolta® SPAD-502 Chlorophyll Meter (Minolta Co., Ltd. Japan) on three replicate leaves of a similar age to those used in the gas exchange studies from each of the five replicate pots of each association, including *Santalum* grown without a host. Calibration of the meter was effected using a selected series of leaves ranging in colour from the lightest yellow (most N deficient) to the deepest green found in the pot cultures. Chlorophyll ($a+b$) was extracted in 90% acetone from three discs of 1·2 cm diameter (combined weight $\cong$ 50 mg) from the above range of leaves and measured spectophotometrically as detailed by Lichtenthaler and Wellburn (1983). A regression equation was then fitted to convert readings from the SPAD-502 to chlorophyll ($a+b$) content per unit leaf area (Systat, 1992).

Carbon isotope discrimination ($\delta^{13}C$) of shoot dry matter of *Santalum* and hosts

A selective harvest of hosts and parasite from three pots from each *Santalum*:parasite association was made at the end of the experiment in late February (1997), using our previously published protocol (see Radomiljac *et al.*, 1998*a*) in which one plant of the three *Santalum* plants selected was approximately equal to, the second, one standard deviation (s.d.) unit greater, and the third, one s.d. less than the current mean current stem diameter (2 cm above ground level) of all plants in a treatment. Shoots of the selected plants were cut at soil level and sub samples of their whole milled dry matter combusted in an Isoprep 13 apparatus (VG Isogas, Cheshire, UK) and the resulting emissions analysed for the $^{13}C/^{12}C$ using a SIRA9 mass spectrometer (VG Isogas, Cheshire, UK) as described by Tennakoon *et al.*, (1997*a*).

Tissue water relations of *Santalum*

Terminal shoot segments of *S. album*, each including six–eight pairs of leaves were harvested in March 1997 from *Santalum* and immediately cut under water before transfer for 15 h in a dark humid chamber to restore full leaf turgidity. Each branch tip was then recut and the four youngest leaf pairs on a shoot retained. This material was quickly wrapped in plastic film, its ψ measured using a pressure bomb and quickly weighed before being left to dry for 4–6 h on the laboratory bench. Further measurements of ψ and mass were then undertaken nine–12 times during this drying phase (Hinckley *et al.*, 1980; Turner, 1988). The shoot was finally dried for 48 h at 80 °C to assess its dry weight (DW). Pressure volume curves, total water content, dry weight:turgid weight ratio (DW:TW), turgid weight:dry weight ratio (TW:DW), relative water content

(RWC), osmotic pressure at full turgor (π_{100}), osmotic pressure at zero tugor (π_0) and relative water content at zero turgor (RWC_0) of all samples were then assessed using standard techniques described by Melkonian, Wolfe and Stephonkus (1982), Turner (1988) and Stoneman (1992).

RESULTS

Leaf nitrogen and chlorophyll contents

As expected due to the higher xylem N concentrations in N_2-fixing than non-fixing hosts (Radomiljac *et al.*, 1998*b*), foliar N concentrations in the parasite were significantly higher when associating with the three legumes than when on *E. camaldulensis* (Fig. 1). The N concentration of foliage of *Santalum* grown on its own was slightly greater than on *E. camaldulensis*, reinforcing earlier conclusions suggesting minimal benefit, if not a negative response, in terms of competition for N when the parasite associates with the latter host (Radomiljac *et al.*, 1998*b*). Foliage of *Santalum* consistently had higher N concentrations than corresponding host foliage, this effect being most noticeable in partnerships with *A. trachycarpa*. A positive relationship ($r^2 = 0.56$, $P = 0.248$) was evident between foliar N concentration of parasite and associated host.

A significant positive relationship was evident between leaf chlorophyll, measured with the SPAD-502 chlorophyll metre, and the foliar N concentration of parasite ($r^2 = 0.739$, $P = 0.000$) and chlorophyll ($a+b$) concentration of parasite foliage ($r^2 = 0.824$, $P = 0.005$) (data not shown). Chlorophyll ($a+b$) concentrations in foliage of *Santalum*
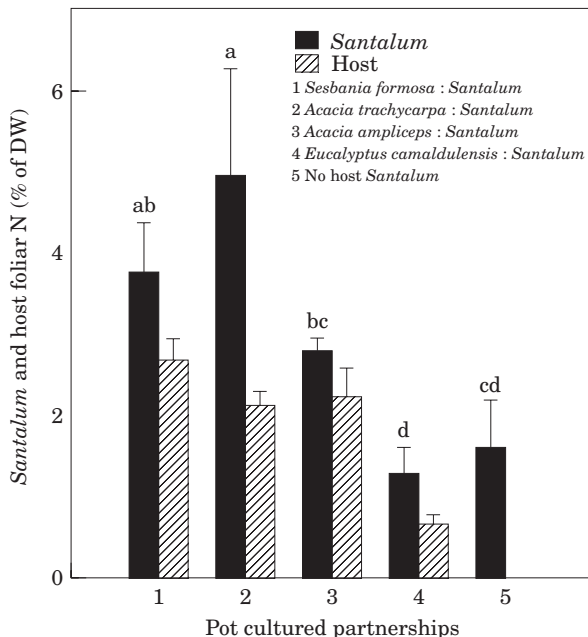

FIG. 1. Mean foliar nitrogen concentration of the root hemi-parasite *Santalum album* and host plant when grown in association with four different host species (1–4) or without a host (5). *Santalum* foliar N concentration means followed by the same letter are not significantly different from one another using Tukey's pairwise *t*-test ($P > 0.05$). Mean host foliar N concentrations are significantly different from one another ($F = 78.1$, $P < 0.000$), pairwise comparison not shown. Bars = s.e. $n = 6$.
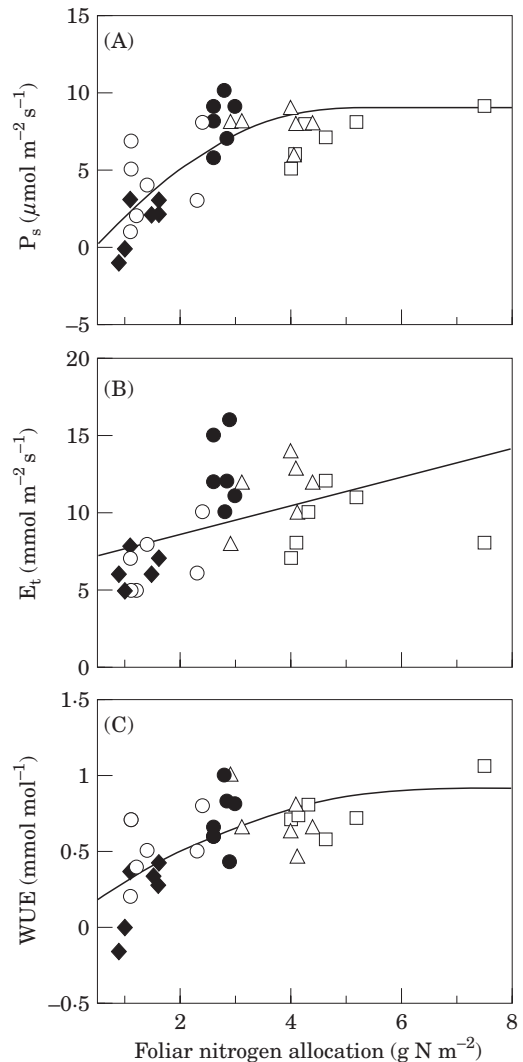

FIG. 2. Relationship between *Santalum album* foliar nitrogen and (A) leaf photosynthesis ($y = -1.87 + 4.224x - 0.414x^2$ over increasing part of response from 0 to 5.2, $r^2 = 0.637$, $P = 0.000$), (B) leaf transpiration ($y = 6.779 + 0.905x$; $r^2 = 0.201$, $P = 0.013$) and (C) instantaneous water use efficiency ($y = 0.06 + 0.254x - 0.019x^2$ over increasing part of response from 0 to 6.5, $r^2 = 0.479$, $P = 0.000$). Associated hosts were *Sesbania formosa* (△), *Acacia trachycarpa* (□), *A. ampliceps* (●) and *Eucalyptus camaldulensis* (◆) and *Santalum* without a host (○).

were strongly related to N concentrations, the relationship fitting the equation:

foliar chlorophyll concentration (g m^{-2}) =

$-0.348 + 0.914 \times$ foliar N concentration (% of DW)

Relationship between %N, P_s, E_t and WUE of parasite foliage

Mean P_s values for the parasite (Fig. 2 A) increase as foliar N increases up to about 4%, with essentially no further increase up to 8% N. The corresponding rise in E_t (Fig. 2 B) with rising foliar N was less pronounced, resulting in a significant, almost three-fold, increase in WUE (P_s/E_t, Fig.

TABLE 1. *Mean ($\pm$ s.e.) leaf gas exchange and growth data of the root hemiparasite* Santalum album *when grown in pot culture as single plant pairings with* Sesbania formosa, Acacia trachycarpa, A. ampliceps *and* Eucalyptus camaldulensis *hosts or without a host*

	Parasite: S. album	Host S. formosa	Parasite: S. album	Host A. trachycarpa	Parasite: S. album	Host A. ampliceps	Parasite: S. album	Host E. camaldulensis	S. album grown without a host
Leaf photosynthesis (μmol m^{-2} s^{-1})	7·83$\pm$0·98[a]	19·16$\pm$6·46[A]	6·83$\pm$1·17[a]	9·50$\pm$2·43[B]	8·83$\pm$1·17[a]	14·33$\pm$6·35[AB]	1·50$\pm$1·64[b]	7·67$\pm$5·24[B]	3·83$\pm$2·48[b]
Leaf transpiration (mmol m^{-2} s^{-1})	11·50$\pm$2·16[ab]	11·67$\pm$2·07[A]	9·33$\pm$1·97[bc]	11·00$\pm$2·10[A]	12·67$\pm$2·34[a]	11·17$\pm$3·31[A]	6·50$\pm$1·05[c]	9·33$\pm$2·66[A]	6·83$\pm$1·94[c]
Instantaneous water use efficiency (mmol mol^{-1})*	0·71$\pm$0·18[a]	1·68$\pm$0·71[A]	0·74$\pm$0·10[a]	0·86$\pm$0·13[B]	0·73$\pm$0·20[a]	1·26$\pm$0·56[AB]	0·21$\pm$0·24[b]	0·78$\pm$0·41[B]	0·52$\pm$0·22[ab]
N use efficiency in P_s (μmol s^{-1} mg^{-1} N)‡	0·021	0·072	0·014	0·045	0·032	0·062	0·012	0·115	0·024
Leaf N conc (%)	3·8$\pm$0·6	2·7$\pm$0·3	4·9$\pm$1·3	2·1$\pm$0·2	2·8$\pm$0·2	2·2$\pm$0·3	1·3$\pm$0·3	0·7$\pm$0·1	1·6$\pm$0·6
Total conc of N from xylem sap amino acid solutes (μg N ml^{-1})‡	77	—	78	—	41	—	14	—	10
Shoot DW (g)§	182$\pm$47[a]	—	57$\pm$30[b]	—	123$\pm$58[a]	—	17$\pm$9[b]	—	36$\pm$11[b]
Leaf area (cm^2)§	7473$\pm$2255[a]	773$\pm$520[B]	2203$\pm$1612[b]	985$\pm$1202[b]	5296$\pm$2691[a]	5448$\pm$1740[A]	489$\pm$173[b]	3779$\pm$1123[A]	940$\pm$340[b]
Specific leaf area (cm^2 g^{-1})§	109$\pm$4·1	122	99·4$\pm$3·1	65·0	90·3$\pm$11·5	44·9	120$\pm$36·6	46·86	102$\pm$11·3
Air temp. (°C)	33·3	33·2	34·2	34·2	33·7	34·5	36·8	35·7	36·8
Relative hum. (%)	49·2	48·8	48·0	47·0	46·7	46·8	47·2	47·0	45·8
VPD (KPa)	2·5	2·6	2·7	2·8	2·8	3·0	3·2	3·0	3·3

Means followed by the same superscript are not significantly different using Tukey's pairwise *t*-test ($P > 0.05$). Lower case letters are for *S. album* data. Upper case letters are for host data.
* Instantaneous water use efficiency (mmol mol^{-1}) = leaf photosynthesis (μmol m^{-2} s^{-1})/leaf transpiration (mmol m^{-2} s^{-1}).
† N use efficiency = leaf photosynthesis (μmol m^{-2} s^{-1})/foliar nitrogen concentration (mg g^{-1}).
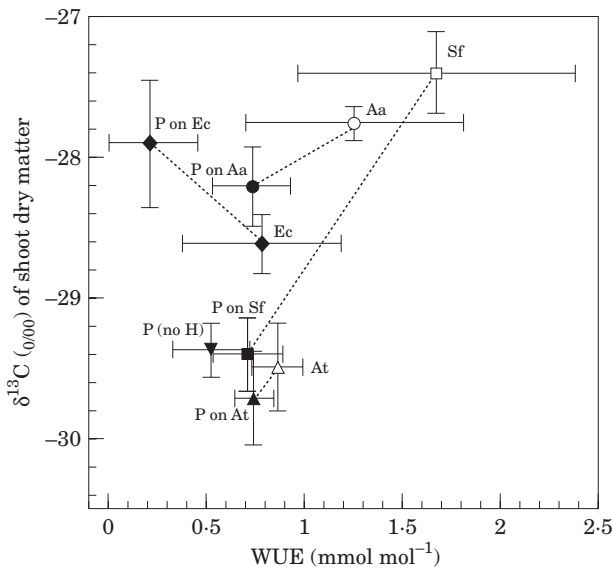‡ Data from Radomiljac *et al.* (1998*a*); § data from Radomiljac *et al.* (1998*b*).

FIG. 3. Relationship between instantaneous water use efficiency and $\delta^{13}C$ values of *Santalum album* partnered singly with various associated hosts. Host codings: *Sesbania formosa* (□ Sf), *Acacia trachycarpa* (△ At), *A. ampliceps* (○ Aa), *Eucalyptus camaldulensis* (◇ Ec). *Santalum* parasitizing *S. formosa* (■ P on Sf), parasitizing *A. trachycarpa* (▲ P on At), parasitizing *A. ampliceps* (● P on Aa), parasitizing *E. camaldulensis* (◆ P on Ec) and in absence of a host (▼ P no H). Dotted lines connect values for the parasite with that of the relevant host, enabling one to visualize how $\delta^{13}C$ and water use efficiency values compare for each partnership.

2C) over the non-saturating part of the range of foliar N values.

Commenting on the effects of host on the above relationships, P_s of *Santalum* was significantly higher when on the three legumes than on the eucalypt or no host, and the same applied to E_t and WUE in respect of *A. ampliceps* and *S. formosa vs. E. camaldulensis* or no host.

Interesting relationships emerge from comparisons of P_s, E_t and WUE of parasite and host of each partnership and ranking respective performances in terms of apparent N use efficiency in photosynthesis i.e. mean P_s per unit foliar N (Table 1). Host P_s values are always appreciably greater than for corresponding parasites, the effect being most noticeable in respect of the *Santalum: S. formosa* and *Santalum: E. camaldulensis* associations. Differences between host and parasite in respect of E_t are slight and insignificant and the same applies to WUE, except for the over two-fold greater WUE of host than parasite in the case of *Sesbania* and the eucalypt. With foliar N concentrations always greater in parasite than host (Fig. 1A) but with the opposite true for P_s ratings, values for photosynthetic-N use efficiency (Table 1) are less in the parasite than the host in all cases.

$\delta^{13}C$ values for shoot dry matter and instantaneous WUE of host and parasite

In keeping with the well-watered unstressed conditions experienced by the species in pot culture, $\delta^{13}C$ values of young shoot dry matter (Fig. 3) varied between host species and *Santalum: host* associations within a relatively narrow range of just over 2‰ ($-27\cdot4$ to $-29\cdot7$‰). The $\delta^{13}C$ values for host plants were not consistently less negative than the partner parasite, but data for the three beneficial legume: parasite partnerships bore evidence of better WUE of hosts being associated with less negative $\delta^{13}C$ values for host than partner parasite. The reverse of this trend applied to the essentially non-beneficial relationship between *Santalum* and the eucalypt.

Matched diurnal profiles of P_s, E_t, WUE and leaf ψ for different host: parasite pairings and parasite without a host

This analysis, following changes in all four quantities from dawn to dusk on a single day, produced the data sets depicted in Fig. 4. Note that the two hosts *S. formosa* and *A. ampliceps* were highly beneficial to *Santalum* with shoot dry weights of *Santalum* being 182 and 123 g per plant and corresponding leaf areas 7473 and 5296 cm² per plant, respectively (see Table 1). These contrast with the much poorer responses by the parasite, namely 57 g per plant and 2203 cm² leaf area per plant for *A. trachycarpa*, 36 g and 940 cm² per plant for the unpartnered *Santalum* and only 17 g and 489 cm² per plant for parasitism on *E. camaldulensis*. Changes in PAR, VPD and air temperature for the day of study are shown in Fig. 4F.

There was a general tendency for P_s values of host and parasite to achieve peak values in early morning (Fig. 4), coinciding with sharply increasing PAR but still low temperature and VPD values. As shown earlier for the mean data of Fig. 2, P_s values throughout the day were higher in *Santalum* on leguminous than on the non-legume host or no host, and corresponding data for hosts segregated *S. formosa* and *A. ampliceps* as exhibiting higher P_s than other hosts. With the exception of *A. trachycarpa* peak values for P_s of all hosts exceeded those of the partner parasite by a considerable margin.

As predicted from the higher temperatures and VPD values later in the day, diurnal courses of E_t showed maxima later in the day than for corresponding P_s. Differences between host and parasite tended to be smaller than for P_s. In some cases host E_t slightly exceeded that of the parasite for a period of the day, in others the reverse situation applied.

Values for WUE also showed relatively small differences between parasite and associated hosts, but there was some evidence of better WUE of the host than the parasite between mid-morning to just after noon. This was not the case for the *Santalum: A. trachycarpa* relationship.

Despite differences between partnerships in absolute values and diurnal amplitudes of values for leaf ψ of respective partner species, all associations exhibited relatively unstressed ψ values ($-0\cdot5$ MPa or less) at dawn with consistently slightly lower (more negative) values for parasite than host. The following morning ψ values of hosts and parasite became more negative but with values for the parasite more negative than those of the host. By late morning or shortly after, maximum differences in ψ between parasite and host were recorded, after which ψ values showed some recovery, more or less in parallel, until the end of measurements at 1600 h. The diurnal course of changes in
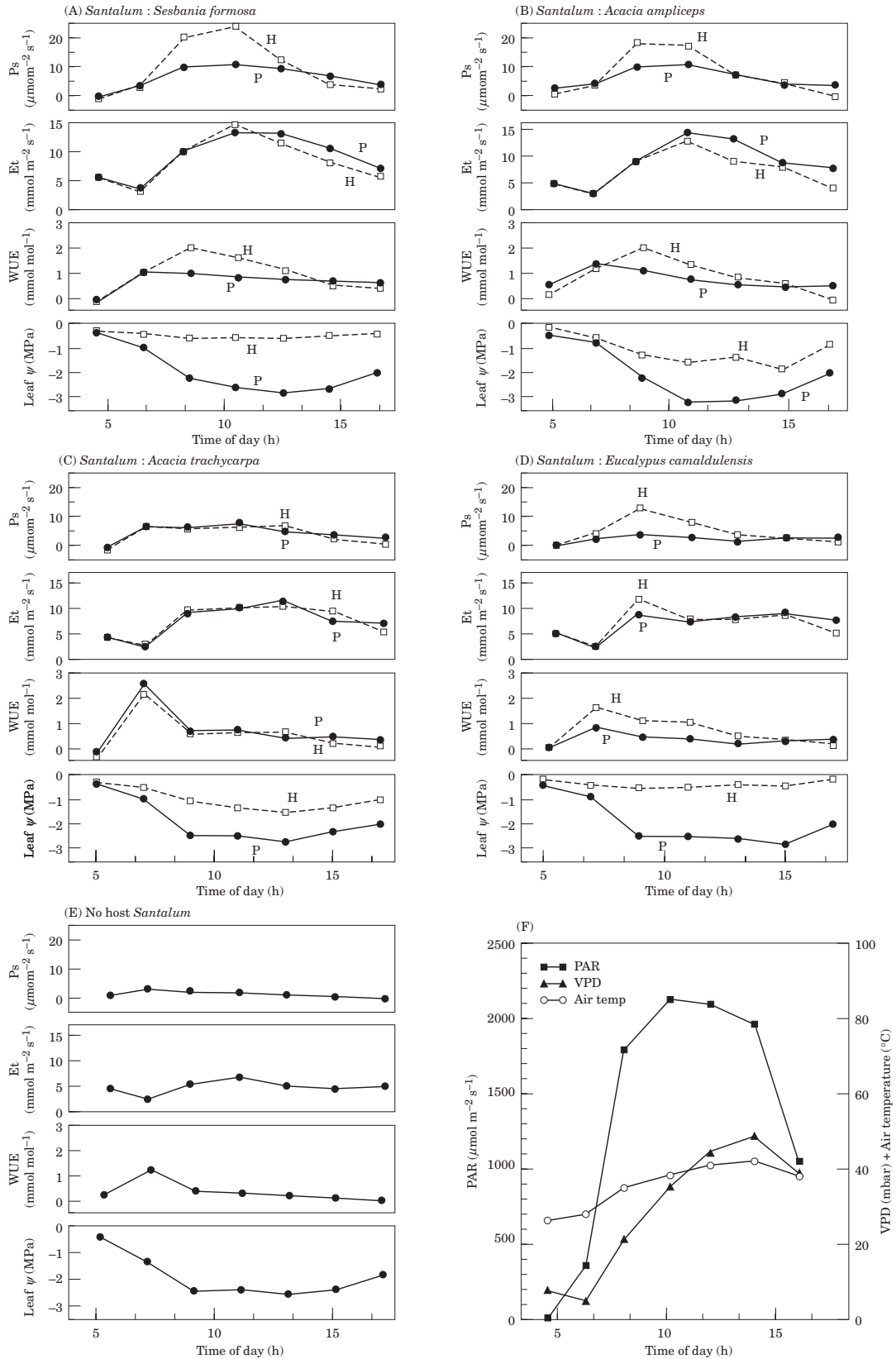
FIG. 4. For legend see facing page.

TABLE 2. *Tissue water relations of shoots of the root hemi-parasite* Santalum album *when grown in pot culture as single plant pairings with* Sesbania formosa, Acacia trachycarpa, A. ampliceps, Eucalyptus camaldulensis *hosts or without a host*

	S. album: *S. formosa*	*S. album:* *A. trachycarpa*	*S. album:* *A. ampliceps*	*S. album:* *E. camaldulensis*	Unattached *S. album*	*F*	*P*
Turgid mass/dry mass ratio	3.47 ± 0.18	3.74 ± 0.58	3.22 ± 0.04	3.97 ± 0.93	3.69 ± 0.17	0.968	0.466
Dry weight/turgid weight ratio	0.29 ± 0.01	0.28 ± 0.04	0.30 ± 0.01	0.26 ± 0.05	0.27 ± 0.01	1.216	0.363
Osmotic pressure at full tugor (MPa)	-2.70 ± 0.28	-2.56 ± 0.65	-2.70 ± 0.07	-2.78 ± 0.16	-2.73 ± 0.09	0.788	0.559
Osmotic pressure at zero turgor (MPa)	-3.10 ± 0.19	-3.13 ± 0.09	-3.09 ± 0.05	-3.20 ± 0.15	-3.13 ± 0.10	0.800	0.552
Relative water content at zero turgor (%)	75.67 ± 3.79	72.83 ± 5.53	75.67 ± 2.52	83.33 ± 3.22	78.33 ± 4.73	2.800	0.085

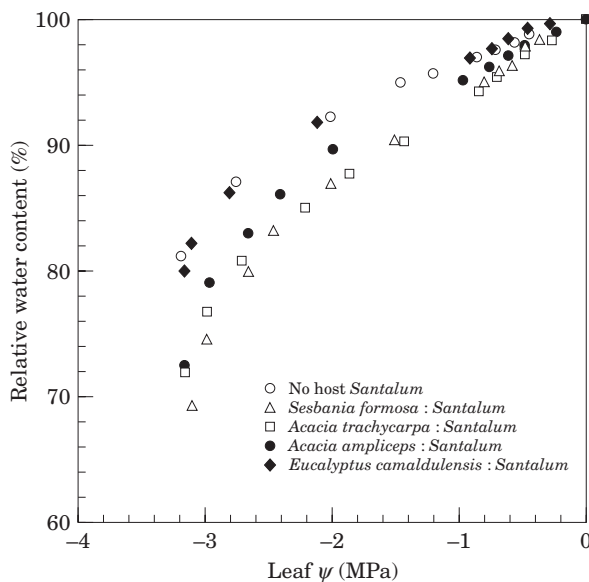Values are means $\pm$ s.e. Data are from three replicates.

FIG. 5. Representative pressure-volume curves (relationship between relative water content and leaf water potential) for detached shoots of *Santalum album* previously growing in association with *Sesbania formosa* ($\triangle$), *Acacia trachycarpa* ($\square$), *A. ampliceps* ($\bullet$), *Eucalyptus camaldulensis* ($\blacklozenge$) and without a host ($\circ$).

ψ of parasite without a host essentially followed those shown when a host was present.

Tissue water relations of Santalum *on different hosts or without a host*

As shown in Table 2, values for the various measures of tissue water relations of *Santalum* showed only slight differences when on different hosts or in the absence of a host. These differences proved to be insignificant implying relatively little effect of a host on directing osmotic adjustment or other attributes of the parasite. However, as shown in Fig. 5, relative water content (RWC) of leaves

varied in a highly predictable fashion in relation to leaf ψ, with some evidence of differences in *Santalum* responses in this respect (e.g. see plots for *Santalum* on *A. trachycarpa vs. Santalum* on *E. camaldulensis*). In general terms, RWC showed a change of 20–30 % as ψ of the parasite changed over approx. 3 MPa.

DISCUSSION

When grown in association with N_2-fixing hosts, *Santalum* shoot growth (Table 1) was markedly greater than when grown with *E. camaldulensis* or without a host. This difference has been directly attributed to the substantial benefit in terms of nitrogen gain accruing from the intake of xylem solutes from a well nodulated legume host (Radomiljac *et al.*, 1998*b*). Following on from this effect would be increased foliar N concentration, leaf chlorophyll contents and leaf photosynthesis and eventually large increases in leaf area. Following in turn from improved leaf photosynthesis on N_2-fixing hosts one finds increase in the instantaneous water use efficiency of the parasite, presumably reflecting an adaptive response to the greater N benefit derived per unit of transpirational loss from the xylem stream of a legume, as opposed to a non-legume host or no host. These findings are fully consistent with data for xylem-tapping mistletoes (Schulze and Ehleringer, 1984; Marshall, Dawson and Ehleringer, 1994), but contrast with the findings of Seel, Cooper and Press (1993) who found no consistent relationship between host foliar N and water use efficiency in the herbaceous root hemi-parasite *Rhinanthus minor* L. on various hosts.

It is now widely recorded that parasitic angiosperms have generally lower water use efficiency values than their associated hosts (Schulze, Turner and Glatzel, 1984; Press, Tuohy and Stewart, 1987; Shah, Smirnoff and Stewart, 1987; Press, Graves and Stewart, 1988; Davidson, True and Pate, 1989), but notable exceptions to this rule have been

FIG. 4. Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of (A) the *Santalum album* (P—$\bullet$) and *Sesbania formosa* (H—$\square$) association, (B) the *S. album* and *Acacia ampliceps* association, (C) the *S. album* and *Acacia trachycarpa* association, (D) the *S. album* and *Eucalyptus camaldulensis* association and (E) *S. album* grown without a host. Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) (F).

reported for *S. acuminatum* by Tennakoon *et al.* (1997*a*) and *O. phyllanthi* by Pate *et al.* (1990). In the present study values for water use efficiency of *Santalum* attached to *A. trachycarpa* and *A. ampliceps* were 0·74 and 0·73 mmol mol^{-1}, and those of corresponding hosts 0·86 and 1·23 mmol mol^{-1}, respectively. In comparable studies on *S. acuminatum* attached to *A. rostellifera* Benth. Tennakoon *et al.* (1997*a*) recorded values for the parasite in the range 1·34 to 3·72 mmol mol^{-1}, again not noticeably different from the host (2·40 to 4·98 mmol mol^{-1}). In light of these findings $\delta^{13}C$ values for shoot dry matter of *Santalum* would be expected to become less negative (more water use efficient) as a result of parasitizing N_2-fixing hosts. This proved to be the case for all associations except the poorly performing *Santalum* on eucalypt. However, one must be cautious when interpreting the significance of the $\delta^{13}C$ values. Firstly, whole shoot material rather than discrete phenologically equivalent foliar samples were compared for each host and parasite. The values thus represent long-term integrated measures of isotope discrimination, which may have been compromised by differing phenologies of growth. Secondly, the actual differences in $\delta^{13}C$ values were relatively small in certain cases (e.g. less than 1‰ for all but one of the partnerships) and compositional differences in dry matter in respect, say, of relative amounts of lignin might have had a relatively large impact in determining the differences concerned. Thirdly, and perhaps most importantly, *S. album* effects large heterotrophic gains of C from certain hosts (Radomiljac 1998) and such acquisition would be expected to change $\delta^{13}C$ values of the parasite significantly towards those of the donor host. Use of $\delta^{13}C$ values to assess instantaneous WUE of the parasite would clearly be unwarranted in such circumstances.

Arguing more generally, Hogberg, Johannisson and Hallgren (1993) and Hogberg *et al.* (1995) suggested that higher levels of foliar N in any species should tend to promote higher rates of leaf photosynthesis and, by lowering p_i/p_a, generate leaf dry matter with less negative $\delta^{13}C$ values than one would expect of low N foliage. It is important to note that $\delta^{13}C$ values are not simply a reflection of p_i/p_a but could also include variation in the resistance to CO_2 diffusion within leaves. Unfortunately, differences in $\delta^{13}C$ values recorded in the present study between hosts and parasite were relatively slight, and probably inconclusive in view of the well-watered pot conditions used. Studies of $\delta^{13}C$ signals of foliage of host and parasite under stressed field conditions are clearly called for.

Diurnal variations in transpiration of *Santalum* proved to be closely similar to those of the host it was parasitizing. This is in agreement with data reported for *S. acuminatum* by Tennakoon *et al.* (1997*a*) but contrasts with those reported for several herbaceous root hemi-parasites (Press *et al.*, 1987, 1998; Shah *et al.*, 1987). It is widely accepted that mistletoe transpiration rates are generally much greater than those of their hosts on the grounds that high transpiration would be required to take up sufficient xylem nitrogen from a host to produce the highly succulent, predominantly leafy shoot biomass typical of this class of parasite (Glatzel, 1983; Hollinger, 1983; Schulze *et al.*, 1984; Ehleringer *et al.*, 1985; Davidson *et al.*, 1989; Stewart

and Press, 1990). Schulze and Ehleringer (1984) extended this concept by suggesting that mistletoes should increase transpiration in adaptive response to situations where N levels in a host transpiration stream were low, but this supposition has been contested by Givnish (1986). In our study *S. album* transpiration was found to be significantly lower when attached to the low N yielding host *E. camaldulensis* than when attached to N_2-fixing hosts suggesting essentially no capacity to adapt to hosts with low N concentrations in their xylem stream. A similar conclusion has been drawn from studies with certain herbaceous Scrophulariacean root hemi-parasites by Press *et al.* (1993) and Seel, Cooper and Press (1993).

It seems more likely that the close similarities in transpiration of *Santalum* and host reflects principally an ability of the parasite to track closely the stomatal responses of its associated host and thereby maintain coordinated responses in leaf water potential continuously promoting uptake of xylem fluid from a host. This is in accord with the many demonstrations of similar responses in mistletoes (Pate, 1995) but contrasts with data for *Striga* showing limited capacity for control of transpiration as water stress develops in a host (Press *et al.*, 1987; Shah *et al.*, 1987).

Santalum associations examined in this study showed the midday leaf water potential to be some 2 MPa more negative than in the corresponding host, precisely as has been reported for a whole range of root parasites and mistletoes (Glatzel, 1983; Schulze *et al.*, 1984; Ullman *et al.*, 1985; Ehleringer *et al.*, 1986; Davidson *et al.*, 1989; Davidson and Pate, 1992; Veenendaal, Abebrese and Walsh, 1996). Although the well watered pot conditions of the study were unlikely to have stressed the parasite to the limits of its functioning, it was shown to continue transpiring at high rates (10–15 mmol m^{-2} s^{-1}) when leaf water potential indicated maximum daily water stress at or close to -3 MPa.

Relative water content of foliage of *Santalum* decreased slowly, but consistently, as more negative leaf water potential values were recorded for leaf xylem water potential. Bearing in mind that these values again relate to well watered pot conditions, relative water content values of 70–80 % for *Santalum* at a leaf water potential value of -3 MPa would still come close to the sort of values which one would associate with sclerophytic species such as eucalypts; e.g. *E. viminalis* Labill. (67 %), *E. melliodora* A. Cunn. ex Schauer (81 %), *E. microcarpa* (Maiden) Maiden (86 %), *E. behriana* F. Muell. (87 %), (Ladiges, 1975; Clayton-Greene, 1983; Myers and Neales, 1984) or the xerophytic species of *Acacia*, *A. aneura* F. Muell. ex Benth. (85 %) (Connor and Tunstall, 1968). Furthermore, water-stress tolerant species are regarded by Cowan (1981) as typically showing lesser proportional decreases in relative water content with decreasing ψ. Our study suggests this applies also to *S. album*, despite it being indigenous to high rainfall monsoonal tropics where exposure to drought may be limited due to habitats with a relatively cool and unstressed dry winter season.

Under conditions reported here *Santalum* exhibited little capacity for osmotic adjustment when associating with different hosts. Thus, when attached to *A. trachycarpa* an osmotic pressure at full turgor of $-2·56$ MPa was recorded

for *Santalum vs.* −2·78 MPa when attached to *E. camaldulensis*; host-specific osmotic adjustment of only 0·2 MPa. Similarly osmotic pressure at zero turgor values of −3·09 and −3·20 MPa were recorded for *Santalum* attached to *A. ampliceps* and *E. camaldulensis*, respectively, indicative of an adjustment of a mere 0·11 MPa. These findings suggest that the bulk tissue water relations of *S. album* are not appreciably compromised when associated with high or low quality host species.

Our study reports closely similar values for osmotic potential at full turgor and at turgor loss point, relative water content at turgor loss point and dry weight to turgid weight ratio for *Santalum* regardless of whether grown with a N_2 or non-N_2 fixing host or when grown without a host. In a similar vein, ratios of dry weight:turgid weight of *Santalum* ranged narrowly from 0·26 to 0·30 between treatments indicating similar leaf reserves of water for accommodating stress control irrespective of the type of host being parasitized. Findings are thus consistent with those recorded for the mistletoe *Amyena* (Davidson *et al.*, 1989; Davidson and Pate, 1992) where the relatively small differences in tissue water relations observed would carry little impact in establishing or maintaining the observed potential gradients between host and parasite. This reinforces our earlier conclusion that *Santalum* transpiration rate perpetuates a favourable water potential gradient from its host.

ACKNOWLEDGEMENTS

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