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Oxygen and carbon isotope composition of parasitic plants and their hosts in southwestern Australia

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Abstract We measured leaf dry matter $\delta^{18}O$ and $\delta^{13}C$ in parasitic plants and their hosts growing in southwestern Australia. Parasite/host pairs included two mistletoe species, three species of holoparasites, and five species of root hemiparasites. Among these parasite functional types, significant variation was observed in parasite/host isotopic differences for both $\delta^{18}O$ ($P<0.0001$, $n=65$) and $\delta^{13}C$ ($P<0.0001$, $n=64$). Mistletoes were depleted in both ^{18}O and ^{13}C compared to their hosts; parasite/host differences were −4.0‰ for $\delta^{18}O$ ($P<0.0001$) and −1.9‰ for $\delta^{13}C$ ($P<0.0001$). The lower $\delta^{18}O$ in mistletoe leaf dry matter compared to their hosts is consistent with the frequently observed high transpiration rates of these parasites. Root hemiparasites were also depleted in ^{18}O and ^{13}C compared to their hosts, but not to the same extent as mistletoes; parasite/host differences were −1.0‰ for $\delta^{18}O$ ($P=0.04$) and −1.2‰ for $\delta^{13}C$ ($P=0.0006$). In contrast to mistletoes and root hemiparasites, holoparasites were enriched in both ^{18}O and ^{13}C compared to their hosts; parasite/host differences were +3.0‰ for $\delta^{18}O$ ($P<0.0001$) and +1.5‰ for $\delta^{13}C$ ($P=0.02$). The enrichment in ^{18}O for holoparasite dry matter did not result from more enriched tissue water; holoparasite tissue water $\delta^{18}O$ was less than host leaf water $\delta^{18}O$ by a difference of −3.8‰ when sampled at midday ($P=0.0003$). Enrichment of holoparasites in ^{13}C compared to their hosts is consistent with a generally observed pattern of enrichment in heterotrophic plant tissues. Results provide insights into the ecology of parasitic plants in southwestern Australia; additionally, they provide a context for the formulation of specific hypotheses aimed at elucidating mechanisms underlying isotopic variations among plants.

Keywords Holoparasite · Mistletoe · Resource acquisition · Root hemiparasite

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Introduction

The evolution of a parasitic habit has been widespread in flowering plants, with examples found in 18 families and encompassing over 3,000 species (Kuijt 1969). The common feature uniting almost all parasitic angiosperms is the presence of a haustorium, an organ that attaches the parasite to its host and allows for extraction of water and solutes from the host's vascular system. Parasitic plants are often classified as hemiparasitic or holoparasitic, depending on the extent of their inability to produce their own reduced carbon for growth and respiration. Hemiparasites can be further divided into facultative or obligate parasites, depending on whether or not they are capable of completing their lifecycle in the absence of a host. In some cases, a distinction between holoparasitism and hemiparasitism is not easily made (Stewart and Press 1990). Parasitic plants may be further categorized as stem or root parasites depending on their position of haustorial attachment to the host. In the present study, we analyzed two species of mistletoes, which are obligate stem hemiparasites; three species of holoparasites, one root-feeding and two stem-feeding; and five species of facultative root hemiparasites, two annual and three perennial. The range in parasitic habits represented a diversity of strategies in parasite resource acquisition from the hosts, or from hosts and the surrounding environment.

Analyses of stable isotope ratios have already played an important role in providing information about the carbon, water, and nutrient relations of parasitic plants and their hosts (Ehleringer et al. 1985; Press et al. 1987; Marshall and Ehleringer 1990; Schulze et al. 1991; Richter et al. 1995; Ducharme and Ehleringer 1996; Tennakoon and

Pate 1996b; Bannister and Strong 2001; Pate 2001). Analyses to date have primarily focused on stable isotopes of carbon for estimating water use efficiency and heterotrophic carbon gain, and nitrogen for identifying sources of nitrogen used by parasites. In the present study, we extended the use of stable isotopes in analyzing parasite/host interactions by measuring oxygen isotope ratios ($\delta^{18}O$), in order to obtain time-integrated estimates of variation in transpiration rates (Barbour and Farquhar 2000; Barbour et al. 2000; Cernusak et al. 2003a). Such analyses are simplified when plants are exposed to the same evaporative conditions, source water $\delta^{18}O$ ($\delta^{18}O_s$), and atmospheric vapor $\delta^{18}O$ ($\delta^{18}O_v$), as is likely the case for parasitic plants growing along side their hosts. Leaf water $\delta^{18}O$ at the evaporative sites in leaves ($\delta^{18}O_e$) has been modeled as (Craig and Gordon 1965; Dongmann et al. 1974; Farquhar and Lloyd 1993)

$$\delta^{18}O_e = \delta^{18}O_s + \varepsilon^* + \varepsilon_k + \left(\delta^{18}O_v - \delta^{18}O_s - \varepsilon_k\right)\frac{e_a}{e_i}, \quad (1)$$

where ε^* is the equilibrium fractionation between liquid and vapor, ε_k is the kinetic fractionation during diffusion through the stomata and leaf boundary layer, and e_a/e_i is the ratio of ambient to intercellular vapor pressures. The ε_k can be calculated as $\varepsilon_k(‰)=(32r_s+21r_b)/(r_s+r_b)$, where r_s and r_b are the stomatal and boundary layer resistances to water vapor diffusion (Farquhar et al. 1989), and the coefficients 32‰ and 21‰ are fractionation factors for water vapor diffusion through stomata and boundary layer, respectively (Cappa et al. 2003). The $\delta^{18}O$ of leaf water at the evaporative sites in leaves has in turn been related to the average $\delta^{18}O$ of leaf mesophyll water ($\delta^{18}O_L$) by (Farquhar and Lloyd 1993)

$$\delta^{18}O_L = \delta^{18}O_s + \frac{(\delta^{18}O_e - \delta^{18}O_s)(1 - e^{-\wp})}{\wp}, \quad (2)$$

where $\wp$ is a Péclet number, defined as $EL/(CD)$, where E is transpiration rate (mol H_2O m^{-2} s^{-1}), L is a scaled effective path length (m), C is the molar concentration of water (mol m^{-3}), and D is the diffusivity of $H_2{}^{18}O$ in water (m^2 s^{-1}).

Equations 1 and 2 predict that at a given air temperature and humidity, an increase in stomatal conductance (and therefore transpiration rate) will cause a decrease in $\delta^{18}O_L$ as a result of increasing e_a/e_i (caused by evaporative cooling of the leaf), decreasing ε_k, and increasing $\wp$. The $\delta^{18}O_L$ can then be related to the $\delta^{18}O$ of plant cellulose ($\delta^{18}O_c$) as follows (Barbour and Farquhar 2000):

$$\delta^{18}O_c = \delta^{18}O_s + \left(\delta^{18}O_L - \delta^{18}O_s\right)(1 - p_{ex}p_x) + \varepsilon_{wc}, \quad (3)$$

where p_{ex} is the proportion of oxygen atoms exchanging with medium water during cellulose synthesis, p_x is the proportion of unenriched water in the developing cell (coming from xylem rather than leaf mesophyll), and ε_{wc} is the equilibrium fractionation between carbonyl oxygen and medium water, estimated as 27‰ (Sternberg and DeNiro 1983; Sternberg et al. 1986). We have presented Eqs. 1, 2, 3 in terms of little delta values (δ). In the present study, we relied upon the assumption that the parasite and host had the same source water, so that comparison of δ values implies a direct comparison of enrichment above source water ($\Delta^{18}O$).

The model presented in Eq. 3 can be extended to total leaf dry matter $\delta^{18}O$ by adding the term ε_{cp}, which describes the difference between dry matter $\delta^{18}O$ ($\delta^{18}O_p$) and cellulose $\delta^{18}O$ (Barbour and Farquhar 2000):

$$\delta^{18}O_p = \delta^{18}O_s + \left(\delta^{18}O_L - \delta^{18}O_s\right)(1 - p_{ex}p_x) + \varepsilon_{wc} + \varepsilon_{cp}\ . \quad (4)$$

Although the mechanisms contributing to ε_{cp} are not well understood, $\delta^{18}O_p$ and $\delta^{18}O_c$ have been strongly correlated when measured on the same samples in both leaves and wood (Borella et al. 1999; Barbour and Farquhar 2000; Barbour et al. 2000, 2001). Moreover, $\delta^{18}O_p$ was found to be more sensitive to variation in stomatal conductance than $\delta^{18}O_c$ by a factor of two (Barbour et al. 2000). Additionally, $\delta^{18}O_p$ was found to be an excellent integrator of spatial (Gan et al. 2002) and temporal (Cernusak et al. 2002) variation in $\delta^{18}O_L$. Accordingly, recent studies have begun taking advantage of the simplicity of analyzing $\delta^{18}O_p$ without the additional step of cellulose extraction (Saurer et al. 2000, 2001, 2002; Scheidegger et al. 2000). This is the approach that we took in the present study of parasite/host interactions. However, we analyzed $\delta^{18}O_c$ in approximately half the samples that we collected in order to determine whether similar patterns in parasite/host differences existed for both $\delta^{18}O_p$ and $\delta^{18}O_c$.

The carbon isotope ratio of C_3 plants ($\delta^{13}C_p$) is correlated with the ratio of intercellular to ambient carbon dioxide concentrations (c_i/c_a) in photosynthesizing leaves (Farquhar et al. 1982):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a)\frac{c_i}{c_a}, \quad (5)$$

where $\delta^{13}C_a$ is the carbon isotope ratio of atmospheric CO_2 (~8‰), a is the fractionation caused by gaseous diffusion (4.4‰), and b is the effective fractionation caused by carboxylating enzymes (~27‰). In turn, the ratio c_i/c_a can be related to the instantaneous water use efficiency of a plant (A/E) as

$$\frac{A}{E} = \frac{c_a\left(1 - \frac{c_i}{c_a}\right)}{1.6v}, \quad (6)$$

where A is the photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$), E is the transpiration rate (mmol H_2O m^{-2} s^{-1}), and v is the vapor pressure difference between the leaf and air (mmol H_2O mol^{-1}). The ambient carbon dioxide concentration, c_a, is expressed as μmol mol^{-1}. Equations 5 and 6 indicate that for plants experiencing the same v, $\delta^{13}C_p$ of the leaves can be used to make integrated comparisons of water use efficiency. For parasitic plants growing along side their hosts, the assumption of a common v is reasonable; however, we note that small differences in leaf temperature caused by different transpiration rates will cause variation in v between plants.

We used $\delta^{18}O_p$ and $\delta^{13}C_p$ in leaves of parasitic plants and their hosts growing in southwestern Australia to make inferences about contrasting strategies of resource acquisition, both within and among different functional types of parasites. Additionally, results indicated some situations in which variation in $\delta^{18}O_p$ and $\delta^{13}C_p$ could not be accounted for using Eqs. 1, 2, 3, 4, 5, 6. These discrepancies were used to highlight areas in which further research into the mechanisms causing isotopic variations would be helpful.

Materials and methods

The locations of sampling sites, names of species sampled at each site and their authorities, and the functional type of the parasite are given in Table 1. Of the root hemiparasite species, three were perennial (*E. odoratus*, *E. sparteus*, and *O. phyllanthi*), and two were annual (*B. trixago*, and *P. viscosa*). William Bay National Park,

Table 1 Locations of sampling sites and pairs of species sampled at each site

Site	Location	Parasite species	Host species	Type of parasite
William Bay National Park	35°01′25″S, 117° 14′12″E	*Cassytha* sp.	*Acacia littorea* Maslin	Holoparasite
			Leucopogon capitellatus DC.	
			Spyridium globulosum (Labill.)Benth.	
		Cuscuta campestris Yunck.	*Cakile maritima* Scop.	Holoparasite
			Euphorbia paralias L.	
			Muehlenbeckia adpressa (Labill.) Meisn.	
			Pecarisonium sp.	
			Senecio elegans L.	
			Unknown host	
		Exocarpos odoratus (Miq.)A.DC.	*Spyridium globulosum* (Labill.)Benth.	Root hemiparasite
		Olax phyllanthi (Labill.) R.Br.	*Acacia littorea* Maslin	Root hemiparasite
			Spyridium globulosum (Labill.)Benth.	
Ocean Beach	35°02′12″S, 117°19′53″E	*Cassytha* sp.	*Exocarpos odoratus* (Miq.)A.DC.	Holoparasite
		Exocarpos odoratus (Miq.)A.DC.	*Bossiaea linophylla* R.Br.	Root hemiparasite
			Hibbertia sp.	
			Spyridium globulosum (Labill.)Benth.	
		Exocarpos sparteus R.Br.	*Pelargonium australe* Willd.	Root hemiparasite
			Spyridium globulosum (Labill.)Benth.	
		Olax phyllanthi (Labill.) R.Br.	*Hibbertia* sp.	Root hemiparasite
			Leucopogon parviflorus (Andrews) Lindl.	
			Spyridium globulosum (Labill.)Benth.	
Lights Beach	35°01′20″S, 117°16′31″E	*Bartsia trixago* L.	*Conocephalum* sp.	Root hemiparasite
			Senecio lautus Willd.	
		Exocarpos sparteus R.Br.	*Scaevola nitida* R.Br.	Root hemiparasite
Pate Property	34°57′55″S, 117°17′19″E	*Olax phyllanthi* (Labill.) R.Br.	*Scaevola nitida* R.Br.	Root hemiparasite
		Orobanche minor Sm.	*Ribes sanguineum*	Holoparasite
		Parentucellia viscosa (L.) Caruel	*Anthoxanthum odoratum* L.	Root hemiparasite
			Hypochaeris radicata L.	
			Ricinocarpus sp.	
			Senecio sp.	
Tunney	32°33′19″S, 119° 17′55″E	*Amyema miquelii* (Miq.) Tiegh.	*Eucalyptus wandoo* Blakely	Mistletoe
Williams	32°59′49″S, 116° 51′01″E	*Amyema preissii* (Miq.) Tiegh.	*Acacia acuminata* Benth.	Mistletoe

Ocean Beach, and Lights Beach are coastal heath sites in close proximity to the township of Denmark, Western Australia. The Pate Property is located on Mount Shadforth near Denmark and is an open woodland dominated by a mixture of marri and karri, *Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson and *Eucalyptus diversicolor* F.Muell., respectively. The Tunney site is an open woodland dominated by *Eucalyptus wandoo* Blakely and *Eucalyptus marginata* Sm., and the Williams site is native shrubland bordering farmland. The root hemiparasites were chosen such that the individuals sampled were growing in close proximity to only a single putative host. Several of the parasitic plant species have been studied previously in these same habitats with regard to various aspects of their biology and strategies of resource acquisition and use (Pate et al. 1990a, 1990b, 1990c, 1991; Tennakoon and Pate 1996a; Pate and Bell 2000; Pate 2001). Sampling took place between 7 and 13 December 2001, and again between 19 and 25 February 2002.

In the December sampling, a representative sample of 10–20 leaves was collected from both parasite and host and oven dried at 80°C. In the case of the annual root hemiparasites and their hosts, the whole aboveground biomass was sampled. The whole biomass was sampled for the *Cuscuta* and *Cassytha* holoparasites, and the whole aboveground biomass for the *Orobanche* holoparasite. For the mistletoes, host leaves were collected from the same branch that the mistletoe was parasitizing. In the February sampling, leaves of parasites and their hosts were immediately placed into gas-tight glass vials and frozen at −20°C. Leaf water sampling took place between 1100 and 1600 hours local time. Samples remained frozen until tissue water was extracted by cryogenic vacuum distillation. The dry matter was retained for carbon and oxygen isotope analyses. Dry matter samples were ground to a fine powder and analyzed for carbon isotope ratio in an Isochrom mass spectrometer (Micromass, Manchester, UK) following combustion in a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy). Oxygen isotope analyses of both tissue water and dry matter took place in an Isochrom mass spectrometer following pyrolysis in a Carlo Erba elemental analyzer (Farquhar et al.1997). Isotope ratios have been expressed in delta (δ) notation using PeeDee Belemnite as the standard for carbon and Vienna Standard Mean Ocean Water as the standard for oxygen. Analytical precision was ±0.1‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{18}O$. The nitrogen concentration of leaf dry matter was measured for the mistletoes and their hosts in a Carlo Erba elemental analyzer (CE Instruments, Milan, Italy).

We extracted cellulose for isotopic analysis from the leaf samples collected in the December sampling, except the annual root hemiparasites and their hosts. Cellulose extractions were performed as described by Barbour and Farquhar (2000), based on the modified technique of Loader et al. (1997). The term ε_{cp}, describing the difference in $\delta^{18}O$ between total dry matter and cellulose, was calculated as $\varepsilon_{cp}=\delta^{18}O_p-\delta^{18}O_c$.

We used Eqs. 1, 2, 3, 4 to estimate proportional differences in transpiration rates between mistletoes and their hosts from measurements of $\delta^{18}O_p$ and $\delta^{18}O_c$. The average November to February midday temperature and humidity for a weather station near to the mistletoe collection sites (Narrogin, Western Australia) were used to parameterize the model; values were 44% and 22°C, respectively. The $\delta^{18}O_v$ was assumed to be in equilibrium with $\delta^{18}O_s$ at the midday air temperature. The difference between leaf temperature and air temperature and the transpiration rate were calculated as described by Barbour et al. (2000). The photosynthesis-weighted average stomatal conductance of the hosts was assumed to be 50 mmol m^{-2} s^{-1}. Boundary layer conductance for mistletoes and hosts was assumed to be 2.5 mol m^{-2} s^{-1}. The scaled effective path length in Eq. 2 was assumed to be 25 mm for both mistletoes and hosts (Flanagan et al. 1993). After a leaf water enrichment had been calculated for the hosts, stomatal conductance of the mistletoe was increased in the model until the modeled $\delta^{18}O_L$ difference between host and mistletoe was equal to that inferred from measurements of $\delta^{18}O_p$ or $\delta^{18}O_c$, calculated as $\delta^{18}O_{L(h)} - \delta^{18}O_{L(m)} = (\delta^{18}O_{p(h)}-\delta^{18}O_{p(m)})/(1-p_{ex}p_x)$, where subscripts (h) and (m) refer to host and mistletoe, respectively; the $\delta^{18}O_p$ was replaced by $\delta^{18}O_c$ for measurements of cellulose, rather than dry matter. The $p_{ex}p_x$ was assumed to be 0.4 (Barbour and Farquhar 2000), such that a 1.67‰ difference in $\delta^{18}O_L$ between host and mistletoe was inferred from a 1‰ difference in $\delta^{18}O_p$ or $\delta^{18}O_c$. Proportional enhancements in mistletoe transpiration rates were calculated by dividing the mistletoe transpiration rate by the host transpiration rate and subtracting one. Thus, a proportional enhancement in transpiration rate of zero indicates equivalent transpiration rates between mistletoe and host, whereas a proportional enhancement of 2, for example, indicates that the mistletoe transpiration rate was three times that of the host.

Parasite/host variation in isotopic parameters was investigated with analyses of variance. The parasite/host difference in $\delta^{13}C$ or $\delta^{18}O$ was taken as the dependent variable. Independent variables were parasite functional type and parasite species nested within parasite functional type. After the analysis of variance model was estimated, Tukey's method for pairwise comparisons was used to determine which parasite functional types or species within functional types differed from one another. Additionally, hypothesis tests were conducted to determine whether the parasite/host isotopic difference for each parasite functional type was significantly different from zero. Sampling date (December or February) was initially considered as a factor in the models, but was not significant in analyses of parasite/host differences in either $\delta^{18}O_p$ or $\delta^{13}C_p$, and thus was not considered further. Although parasite/host isotopic differences did not change with sampling date, there did appear to be a shift in $\delta^{18}O_p$ values for all species between December and February. Analysis of variance was used to test whether this difference was significant, using sampling date and species as independent variables. Variation in foliar nitrogen concentrations between mistletoes and their hosts and between mistletoe species was assessed using analysis of variance. Statistical analyses were conducted in SYSTAT 9.0 (SPSS, Chicago, Ill., USA).

Results

Mistletoe $\delta^{18}O$

There were strongly contrasting patterns in parasite/host isotopic differences for $\delta^{18}O_p$ among the different parasite functional types ($P<0.0001$, $n=65$); data are shown in Fig. 1. Parasite species within functional types was not a significant term in this analysis ($P=0.47$). Mistletoe $\delta^{18}O_p$ values were less than those of their hosts by a mean parasite/host difference of −4.0‰ ($P<0.0001$); this value differed significantly from corresponding values for both holoparasites ($P<0.0001$) and root hemiparasites ($P<0.0001$). The transpiration modeling exercise based on $\delta^{18}O_p$ measurements suggested proportional enhancements in transpiration rates for *A. preissii* and *A. miquelii* compared to their hosts of 2.8 and 3.9, respectively. Note that this analysis assumed that ε_{cp} was invariant between mistletoe and host.

Parasite/host differences in $\delta^{18}O$ of leaf cellulose also varied significantly among parasite functional types ($P=0.0002$, $n=34$). In this case, parasite species within functional types was a significant term in this analysis ($P=0.02$). Mistletoe $\delta^{18}O_c$ values were less than those of their hosts by a mean parasite/host difference of −3.6‰ ($P<0.0001$). There was a significant difference in parasite/host $\delta^{18}O_c$ differences between the two mistletoe species ($P=0.05$); values are given in Table 2. The transpiration modeling exercise based on $\delta^{18}O_c$ measurements suggested proportional enhancements in transpiration rates for

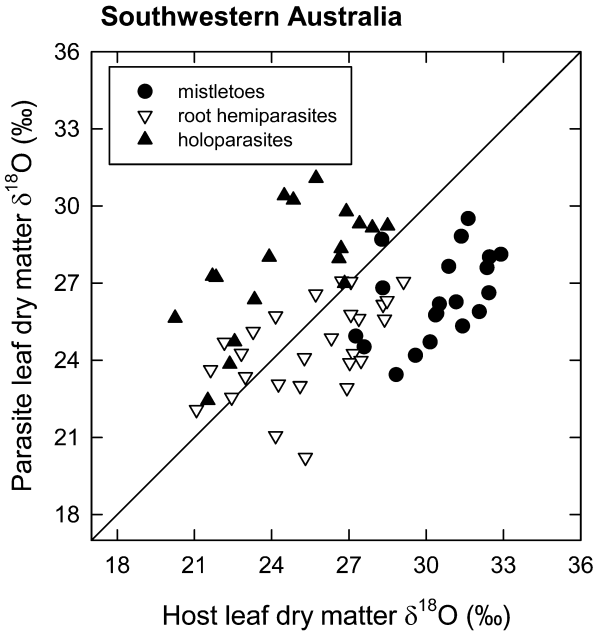

Fig. 1 Leaf dry matter $\delta^{18}O$ values of parasitic plants growing in southwestern Australia plotted against the leaf dry matter $\delta^{18}O$ values of their hosts. Data points below the *one-to-one line* indicate a lower $\delta^{18}O$ in the parasite than in the host, whereas those above the line indicate a higher $\delta^{18}O$

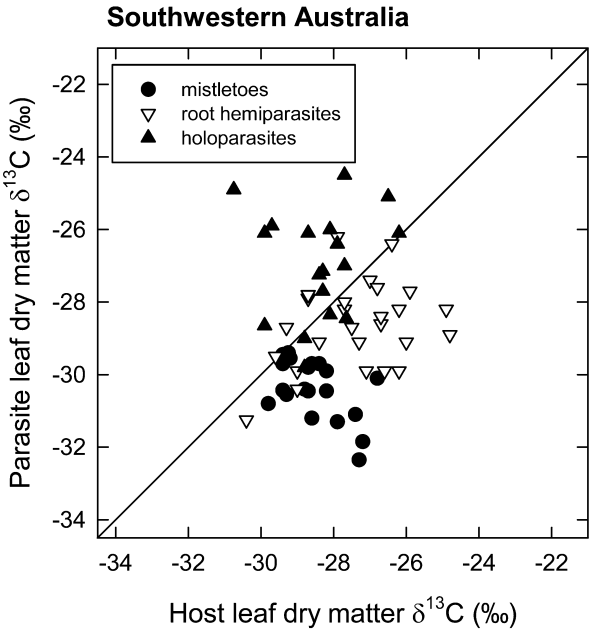

Fig. 2 Leaf dry matter $\delta^{13}C$ values of parasitic plants growing in southwestern Australia plotted against the leaf dry matter $\delta^{13}C$ values of their hosts. Data points below the *one-to-one line* indicate a more negative $\delta^{13}C$ in the parasite than in the host, whereas those above the line indicate a less negative $\delta^{13}C$

A. preissii and *A. miquelii* compared to their hosts of 5.0 and 1.1, respectively.

There was significant variation among parasite functional types in parasite/host differences in leaf water $\delta^{18}O$ (P=0.01, n=24), whereas variation among parasite species within functional types was not significant (P=0.64). However, in contrast to the results for dry matter and cellulose, the mean parasite/host difference in $\delta^{18}O_L$ for mistletoes was not significantly different from zero (P =0.96). Mean $\delta^{18}O_L$values for the mistletoes and their hosts are given in Table 2.

Mistletoe $\delta^{13}C$

The contrasting isotopic pattern of the parasite functional types in relation to their hosts was similarly reflected in the $\delta^{13}C$ of leaf dry matter (P<0.0001, n=64); data are shown in Fig. 2. For parasite/host $\delta^{13}C_p$ differences, there was significant variation among parasite species within parasite functional types (P=0.003). Mistletoe $\delta^{13}C_p$ values were more negative than those of their hosts, showing an average difference of −1.9‰ (P<0.0001). This mean difference was significantly different from that of holoparasites (P<0.0001), but not root hemiparasites (P=0.21). Additionally, the parasite/host difference in $\delta^{13}C_p$ was significantly different between the two mistletoe species (P=0.03); mean values for the two species are given in Table 3.

Foliar N concentrations varied significantly between the two mistletoe/host species pairs (P<0.0001, n=40), but not between mistletoes and their hosts (P=0.14). The mean N concentration for leaf dry matter of *Amyema preissii* was 23.6±8.0 mg g^{-1} (mean±1SD); that for its host *Acacia acuminata* was 27.5±3.0 mg g^{-1}. The mean N concentration for leaf dry matter of *Amyema miquelii* was 8.4 ±2.2 mg g^{-1}; that for its host *Eucalyptus wandoo* was 8.7 ±1.7 mg g^{-1}. The regression line relating mistletoe N concentration to host N concentration was $[N]_{mistletoe}$=0.83 $[N]_{host}$+1.0 mg g^{-1} (R^2 =0.70, P<0.0001, n=20). Mistletoe/host differences in $\delta^{13}C_p$ became less negative with increasing host leaf N concentration according to the following equation: $\delta^{13}C_p$(difference)=0.11$[N]_{host}$−3.8 (R^2 =0.51, P=0.0002, n=20).

There was significant variation among parasite functional types in parasite/host differences in $\delta^{13}C_c$ (P=0.02, n=31). Mistletoe $\delta^{13}C_c$ values were significantly more negative than those of their hosts (P=0.0002). The mean parasite/host $\delta^{13}C_c$ difference for mistletoes was −2.2‰. Interestingly, when cellulose was analyzed instead of leaf dry matter, variation in parasite/host $\delta^{13}C$ differences was no longer observed among species within parasite functional types (P=0.82). Thus, the two mistletoes species had very similar parasite/host differences in $\delta^{13}C_c$, in contrast to the results for $\delta^{13}C_p$ (Table 3).

Root hemiparasite $\delta^{18}O$

Root hemiparasites had $\delta^{18}O_p$ values that were significantly lower than those of their hosts (P=0.04). The mean parasite/host $\delta^{18}O_p$ difference for root hemiparasites was −1.0‰. The difference in $\delta^{18}O_c$ between root hemiparasites and their hosts was similar to that in $\delta^{18}O_p$, with a

Table 2 Oxygen isotope composition of parasitic plants and their hosts. Values are given with standard deviations where more than one pair of individuals was sampled. The column headed *n* gives the number of parasite/host pairs sampled for leaf dry matter $\delta^{18}O$, leaf cellulose $\delta^{18}O$, and leaf water $\delta^{18}O$, in that order

Parasite species	Host species	*n*	Leaf dry matter $\delta^{18}O$ (‰)			Leaf cellulose $\delta^{18}O$ (‰)			Leaf water $\delta^{18}O$ (‰)		
			Parasite	Host	Difference	Parasite	Host	Difference	Parasite	Host	Difference
Mistletoes											
Amyema miquelii	*Eucalyptus wandoo*	10, 6, 4	27.0±1.4	31.6±0.8	−4.6	33.1±2.6	34.6±2.1	−1.5	8.9±0.5	9.4±0.6	−0.5
Amyema preissii	*Acacia acuminata*	10, 6, 4	25.9±1.8	29.4±1.6	−3.5	34.5±2.2	40.1±1.4	−5.6	17.1±1.5	16.7±4.7	0.4
Holoparasites											
Cassytha sp.	*Acacia littorea*	3, 1, 2	29.6±2.0	24.0±2.1	5.6	31.4	30.8	0.6	13.4±2.3	12.8±1.6	0.6
	Exocarpus odoratus	1, 1, 0	24.7	22.6	2.1	29.6	29.2	0.4			
	Leucopogon capitellatus	4, 1, 3	28.2±1.0	26.2±1.6	2.0	32.5	31.2	1.3	9.5±1.7	14.1±1.3	−4.6
	Spyridium globulosum	2, 1, 1	29.5±0.4	27.7±1.1	1.8	31.5	29.9	1.6	8.8	14.9	−6.1
Cuscuta campestris	*Cakile maritima*	1, 1, 0	22.4	21.5	0.9	28.3	29.1	−0.8			
	Euphorbia paralias	1, 0, 1	29.2	27.9	1.3				5.3	13.1	−7.8
	Muehlenbeckia adpressa	2, 1, 1	25.9±2.9	24.5±3.0	1.4	29.0	28.6	0.4	4.9	10.5	−5.6
	Pecarisonium sp.	1, 1, 0	26.4	23.3	3.1	30.0	27.1	2.9			
	Senecio elegans	1, 0, 1	30.2	24.8	5.4				7.1	9.8	−2.7
	Unknown host	1, 1, 0	25.6	20.3	5.3	29.2	28.4	0.8			
Orobanche minor	*Ribes sanguineum*	1, 1, 0	27.2	21.8	5.4	28.8	25.4	3.4			
Root hemiparasites											
Bartsia trixago	*Conocephalum* sp.	1, 0, 0	23.9	27.0	−3.1						
	Senecio lautus	1, 0, 0	24.3	22.8	1.5						
Exocarpus odoratus	*Bossiaea lynophylla*	1, 1, 0	22.6	22.5	0.1	29.2	30.8	−1.6			
	Hibbertia sp.	1, 0, 1	27.1	27.1	0.0				13.3	14.5	−1.2
	Spyridium globulosum	3, 2, 1	24.7±1.7	27.2±1.1	−2.5	28.4±0.7	31.7±0.6	−3.3	11.6	13.9	−2.3
Exocarpus sparteus	*Pelargonium australe*	2, 1, 1	26.2±0.6	24.9±1.1	1.3	32.2	-	-	12.1	9.3	2.8
	Scaevola nitida	1, 1, 0	24.7	22.2	2.5	30.9	27.2	3.7			
	Spyridium globulosum	2, 1, 1	26.4±0.9	28.1±1.4	−1.7	29.2	30.5	−1.3	11.0	12.2	−1.2
Olax phyllanthi	*Acacia littorea*	1, 1, 0	23.4	23.0	0.4	30.9	31.6	−0.7			
	Hibbertia sp.	1, 1, 0	23.1	24.3	−1.2	29.1	31.6	−2.5			
	Leucopogon parviflorus	2, 1, 1	22.5±2.0	25.8±2.3	−3.3	29.3	29.2	0.1	12.3	13.5	−1.2
	Spyridium globulosum	5, 3, 2	25.7±1.0	27.6±0.7	−1.9	30.6±2.4	32.3±0.8	−1.7	13.7±0.7	12.9±0.4	0.8
	Scaevola nitida	1, 1, 0	23.6	21.6	2.0	31.2	28.3	2.9			
Parentucellia viscosa	*Anthoxanthum odoratum*	2, 0, 0	21.6±2.0	25.2±0.1	−3.6						
	Hypochaeris radicata	1, 0, 0	25.1	23.3	1.8						
	Ricinocarpus sp.	1, 0, 0	24.1	25.3	−1.2						
	Senecio sp.	1, 0, 0	22.1	21.1	1.0						

Table 3 Carbon isotope composition of parasitic plants and their hosts. Values are given with standard deviations where more than one pair of individuals was sampled. The column headed *n* gives the number of parasite/host pairs sampled for leaf dry matter $\delta^{13}C$ and leaf cellulose $\delta^{13}C$, in that order

Parasite species	Host species	*n*	Leaf dry matter $\delta^{13}C$ (‰)			Leaf cellulose $\delta^{13}C$ (‰)		
			Parasite	Host	Difference	Parasite	Host	Difference
Mistletoes								
Amyema miquelii	*Eucalyptus wandoo*	10, 6	−31.0±0.7	−28.0±0.8	−3.0	−27.8±0.6	−25.5±0.9	−2.3
Amyema preissii	*Acacia acuminata*	10, 6	−29.8±0.4	−29.0±0.5	−0.8	−27.0±0.7	−25.0±0.4	−2.0
Holoparasites								
Cassytha sp.	*Acacia littorea*	3, 1	−26.7±2.1	−29.8±1.0	3.1	−27.5	−25.2	−2.3
	Exocarpus odoratus	1, 1	−26.1	−26.2	0.1	−24.1	−23.5	−0.6
	Leucopogon capitellatus	4, 1	−27.5±1.2	−28.5±0.9	1.0	−24.3	−26.9	2.6
	Spyridium globulosum	2, 1	−28.1±2.4	−28.4±0.6	0.3	−25.1	−25.5	0.4
Cuscuta campestris	*Cakile maritima*	1, 1	−27.2	−28.3	1.1	−26.0	−27.1	1.1
	Euphorbia paralias	1, 0	−25.1	−26.5	1.4			
	Muehlenbeckia adpressa	2, 1	−26.1±2.3	−28.0±0.4	1.9	−27.3	−25.6	−1.7
	Pecarisonium sp.	1, 1	−26.0	−28.1	2.1	−25.2	−25.3	0.1
	Senecio elegans	1, 0	−26.1	−28.7	2.6			
	Unknown host	1, 0	−28.7	−29.9	1.2			
Orobanche minor	*Ribes sanguineum*	1, 1	−27.0	−27.7	0.7	−25.9	−24.9	−1.0
Root hemiparasites								
Bartsia trixago	*Conocephalum* sp.	1, 0	−28.7	−29.3	0.6			
	Senecio lautus	1, 0	−29.9	−29.0	−0.9			
Exocarpus odoratus	*Bossiaea lynophylla*	1, 1	−26.2	−27.9	1.7	−23.5	−25.5	2.0
	Hibbertia sp.	1, 0	−28.7	−27.5	−1.2			
	Spyridium globulosum	3, 2	−28.9±1.2	−27.1±0.3	−1.8	−25.0±1.6	−25.1±0.2	0.1
Exocarpus sparteus	*Pelargonium australe*	2, 1	−28.3±0.1	−25.8±1.3	−2.5	−24.2	−25.5	1.3
	Scaevola nitida	1, 1	−29.1	−26.0	−3.1	−27.0	-	-
	Spyridium globulosum	2, 1	−29.4±0.7	−25.5±1.0	−3.9	−27.8	−24.1	−3.7
Olax phyllanthi	*Acacia littorea*	1, 1	−27.8	−28.7	0.9	−25.7	−24.9	−0.8
	Hibbertia sp.	1, 1	−28.0	−27.7	−0.3	−25.8	−24.3	−1.5
	Leucopogon parviflorus	2, 1	−28.7±0.6	−28.1±0.5	−0.6	−26.5	−25.1	−1.4
	Spyridium globulosum	5, 3	−28.2±1.3	−26.4±0.3	−1.8	−23.8±0.3	−25.7±1.4	1.9
	Scaevola nitida	1, 1	−27.4	−27.0	−0.4	−24.4	-	-
Parentucellia viscosa	*Anthoxanthum odoratum*	1, 0	−30.4	−29.0	−1.4			
	Hypochaeris radicata	1, 0	−27.9	−28.7	0.8			
	Ricinocarpus sp.	1, 0	−29.5	−29.6	0.1			
	Senecio sp.	1, 0	−31.3	−30.4	−0.9			

mean value of −0.9‰; however, this difference was not statistically significant (P=0.31). We did not observe a significant difference in leaf water $\delta^{18}O$ between root hemiparasites and their hosts (P=0.82); the mean difference was −0.2‰. There was no significant variation among parasite species within the root hemiparasite functional type for any of the $\delta^{18}O$ analyses. Values for individual species are detailed in Table 2.

Root hemiparasite $\delta^{13}C$

Root hemiparasites had $\delta^{13}C_p$ values more negative than those of their hosts (P=0.0006); the mean parasite/host $\delta^{13}C_p$ difference was −1.2‰. There were no significant differences among individual root hemiparasite species in their $\delta^{13}C_p$ values relative to their hosts. However, sample sizes were rather small for all but a couple of species (Table 3). In contrast to the results for $\delta^{13}C_p$, values of $\delta^{13}C_c$ for perennial root hemiparasites did not differ between parasite and host (P=0.94); the mean parasite/host $\delta^{13}C_c$ difference for root hemiparasites was 0.2‰.

Holoparasite $\delta^{18}O$

Variation in $\delta^{18}O_p$ between holoparasites and their hosts was opposite in sign to that observed for mistletoes and root hemiparasites (Fig. 1). The mean parasite/host difference was positive, having a value +3.0‰ (P<0.0001); this value was significantly different from that observed for mistletoes (P<0.0001) and root hemi-

parasites (P<0.0001). Holoparasite $\delta^{18}O_c$ was also higher than that of hosts, showing a mean difference of +1.2‰, which was moderately significant (P=0.06). In contrast, however, holoparasite tissue water was significantly less enriched in ^{18}O than host leaf water; the parasite/host $\delta^{18}O_L$ difference was −3.8‰ (P=0.0003); mean $\delta^{18}O_L$ values were 9.1±3.3‰ and 12.9±2.0‰ for parasite and host, respectively. This was an interesting result because the sign of the difference in $\delta^{18}O_L$ between holoparasites and their hosts was opposite to that of the differences in $\delta^{18}O_p$ and $\delta^{18}O_c$. There were no significant differences among parasite species within the holoparasite functional type for any of the $\delta^{18}O$ analyses.

Holoparasite $\delta^{13}C$

Parasite/host variation in $\delta^{13}C_p$ for holoparasites also differed in sign compared to that observed for mistletoes and root hemiparasites (Fig. 2). Holoparasites were enriched in ^{13}C compared to their hosts, showing an average parasite/host $\delta^{13}C_p$ difference of +1.5‰ (P=0.02). This mean value differed from that for both mistletoes (P<0.0001), and root hemiparasites (P=0.0005). However, a similar difference in $\delta^{13}C$ was not observed in cellulose; holoparasite $\delta^{13}C_c$ was not different from that of the hosts (P=0.59). There were no significant differences among parasite species within the holoparasite functional type for either of the $\delta^{13}C$ analyses.

All species $\delta^{18}O$ and $\delta^{13}C$

The oxygen isotope ratio of leaf dry matter increased between December and February for all species sampled on both dates except *E. wandoo* (Fig. 3A). The mean $\delta^{18}O_p$ for all plants sampled in December was 25.4‰, whereas the mean value for February was 28.0‰. Sampling date (December or February) was a significant term in the analysis of variance in individual $\delta^{18}O_p$ values (P<0.0001, n=130). In contrast, $\delta^{13}C_p$ did not change consistently across species between December and February (Fig. 3B), with mean values of −28.5‰ and −28.3‰ for the two dates respectively, which were statistically indistinguishable (P=0.67, n=129).

Leaf cellulose was enriched in ^{18}O compared to leaf dry matter for all of the samples in which both analyses were performed. The mean value for ε_{cp}, the difference between $\delta^{18}O_p$ and $\delta^{18}O_c$, across all samples was −6.2±2.9‰ (mean±1 SD). Values of ε_{cp} for individual species varied from −11.2‰ for *A. acuminata* to −2.8‰ for *E. wandoo* (Table 4). There was a significant, positive correlation between $\delta^{18}O_p$ and $\delta^{18}O_c$ (r=0.63, P<0.0001, n=67). Additionally, parasite/host differences in $\delta^{18}O_p$ were significantly correlated with parasite/host differences in $\delta^{18}O_c$ (r=0.69, P<0.0001, n=33).

Leaf cellulose $\delta^{13}C$ was less negative than leaf dry matter $\delta^{13}C$ for all samples in which both analyses were performed, except two. The mean difference between $\delta^{13}C_p$ and $\delta^{13}C_c$ was −2.6±1.1‰, with the two being

Table 4 Values for ε_{cp}, the difference between total leaf dry matter $\delta^{18}O$ and leaf cellulose $\delta^{18}O$, for species sampled in the present study and others available in the literature. Values reported from the present study are limited to species in which three or more individuals were sampled, and are given as the mean ±1 SD. The overall mean is −6.8‰

Species	ε_{cp} (‰)	Reference
Acacia acuminata	−11.2±2.2	This study
Amyema miquelii	−6.3±2.5	This study
Amyema preissii	−9.8±1.6	This study
Exocarpus odoratus	−5.6±1.7	This study
Exocarpus sparteus	−5.3±1.7	This study
Eucalyptus wandoo	−2.8±2.1	This study
Gossypium hirsutum	−7.5	Barbour and Farquhar 2000
Olax phyllanthi	−6.4±1.6	This study
Picea abies	−6.4	Jäggi et al. 2003
Spyridium globulosum	−4.4±1.0	This study
Triticum aestivum	−9.1	Barbour et al. 2000

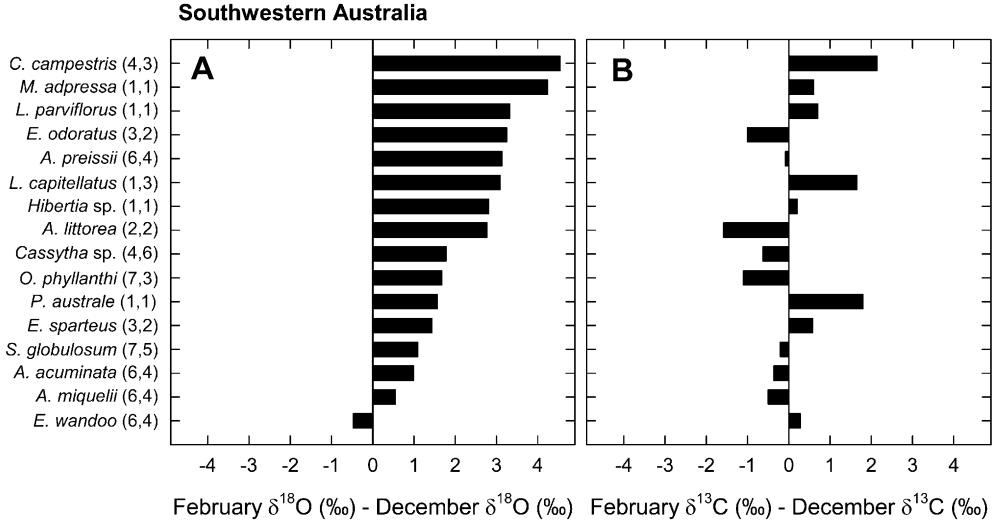

Fig. 3 The mean difference between leaf dry matter $\delta^{18}O$ values sampled in February, 2002 and those sampled in December, 2001 for all species sampled on both dates (**A**), and the corresponding differences in leaf dry matter $\delta^{13}C$ values (**B**). Values in parentheses are the number of individuals sampled in December, followed by the number of individuals sampled in February

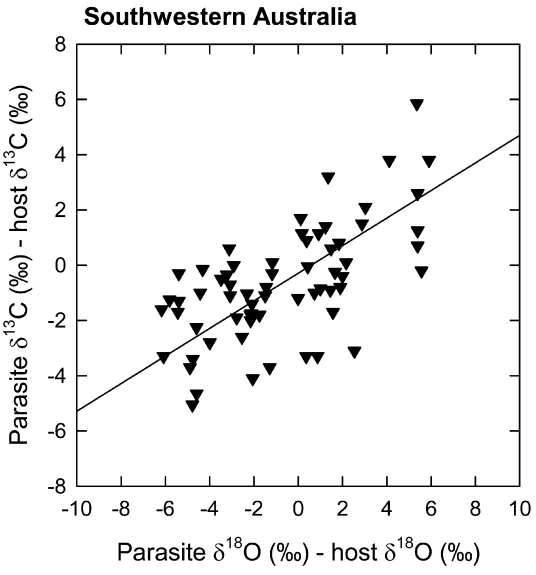

Fig. 4 Parasite/host differences in leaf dry matter $\delta^{13}C$ plotted against the corresponding parasite/host difference in leaf dry matter $\delta^{18}O$. The two parameters are significantly, positively correlated (r=0.63, P<0.0001, n=64). The line drawn on the graph has a slope of 0.5 and a y -intercept of 0.3. These coefficients were calculated using principle components analysis, such that neither parameter was explicitly dependent on the other

significantly, positively correlated (r=0.70, P<0.0001, n=64). Parasite/host differences in $\delta^{13}C_p$ were also significantly correlated with parasite/host differences in $\delta^{13}C_c$ (r=0.55, P=0.002, n=30).

There was a general relationship across all parasite/host pairs between the $\delta^{13}C_p$ parasite/host difference and the $\delta^{18}O_p$ parasite/host difference (Fig. 4). Parasites with higher $\delta^{13}C_p$ values than their hosts also tended to have higher $\delta^{18}O_p$ values than their hosts. The holoparasites were a good example of such a pattern. Conversely, parasites such as the mistletoes, which had lower $\delta^{13}C_p$ values than their hosts, also tended to have lower $\delta^{18}O_p$ values than their hosts.

Discussion

We observed consistent differences in $\delta^{18}O_p$ and $\delta^{13}C_p$ between parasitic plants and their hosts within the different parasite functional types. Results for leaf dry matter isotopic analyses generally agreed with those for leaf cellulose, although there were some exceptions. Such variation in isotopic composition suggests contrasting strategies of resource acquisition and use among the various parasitic plants in relation to each other and to their hosts. For example, lower $\delta^{18}O_p$ and $\delta^{13}C_p$ in mistletoes and root hemiparasites than their hosts suggests a relatively more liberal use of water and a lower water use efficiency by both functional types in the seasonally dry, Mediterranean-type environment of southwestern Australia. However, the three-fold larger parasite/host difference in $\delta^{18}O_p$ for mistletoes compared to root hemiparasites highlights the greater extent to which mistletoes rely on such a strategy to acquire carbon and nutrients compared to the root hemiparasites. Additionally, some of the results serve to highlight areas in which the mechanisms leading to isotopic variation among plants have yet to be unraveled, such as the higher $\delta^{18}O_p$ and $\delta^{13}C_p$ in holoparasites compared to their hosts. In such cases, the observations presented here may provide a basis for designing experimental systems aimed at elucidating these mechanisms. In the following discussion, we expand upon the implications of our results for understanding the ecology of parasitic plants and the mechanisms underlying their isotopic variations.

Mistletoe $\delta^{18}O$

The mean differences in $\delta^{18}O_p$ of −3.5‰ for the mistletoe *A. preissii* growing on *A. acuminata*, and −4.6‰ for the mistletoe *A. miquelii* growing on *E. wandoo*, suggest substantially higher transpiration rates in these two mistletoe species compared to their hosts in southwestern Australia. Transpiration rates of mistletoes growing in Australia were previously observed to be consistently higher than those of their hosts (Hellmuth 1971; Ullmann et al. 1985; Davidson et al. 1989; Davidson and Pate 1992; Marshall et al. 1994b; Tennakoon and Pate 1996a); estimates of the proportional enhancement in mistletoe transpiration rates compared to those of their hosts range from 0.4 to 7.9. Similar observations have been reported for mistletoes in other regions as well (Glatzel 1983; Schulze et al. 1984; Ehleringer et al. 1986; Marshall et al. 1994a). Based on $\delta^{18}O_p$ measurements, we calculated time-integrated, proportional enhancements in transpiration of 2.8 and 3.9 for *A. preissii* and *A. miquelii* compared to their respective hosts, whereas $\delta^{18}O_c$ measurements indicated proportional enhancements of 5.0 and 1.1, respectively. Such values are well within the range observed for Australian mistletoes. Nonetheless, the difference in estimates based on $\delta^{18}O_p$ as compared to $\delta^{18}O_c$ highlights the need for a mechanistic understanding of processes controlling ε_{cp}, the difference in $\delta^{18}O$ between dry matter and cellulose.

Because mistletoes are known to assimilate organic solutes from the host's transpiration stream (Raven 1983; Marshall and Ehleringer 1990; Schulze et al. 1991), it is reasonable to ask whether this process might have contributed to the difference that we observed in $\delta^{18}O_p$ and $\delta^{18}O_c$ between mistletoes and their hosts. The effect of mistletoe heterotrophy on $\delta^{18}O_c$ can be included in Eq. 3 as follows:

$$\begin{aligned}\delta_{c(m)} =& \delta_s + (1-\rho)\left(\delta_{L(m)} - \delta_s\right)\left(1 - p_{ex(m)}p_{x(m)}\right) \\ &+ \rho\left(\delta_{ox} - \varepsilon_{wc} - \delta_s\right)\left(1 - p_{ex(ox)}p_{x(m)}\right) \\ &+ \varepsilon_{wc}.\end{aligned} \tag{7}$$

The δ terms in Eq. 7 refer to $\delta^{18}O$. The subscript *m* refers to the mistletoe, and the subscript *ox* refers to xylem-borne

organic solutes procured from the host's transpiration stream. The ρ is the proportion of photosynthate acquired from the host's xylem sap, and (1−ρ) is the proportion supplied by the mistletoe's own photosynthesis. We assume similar oxygen concentrations in the two pools. In the one case that we are aware of in which the $\delta^{18}O$ of xylem sap dry matter was measured, it was found to be enriched relative to source water by 34.9‰ in *Lupinus angustifolius* (Cernusak et al. 2002). In this case, the term $(\delta_{ox}-\varepsilon_{wc}-\delta_s)$ would have a value of 7.9‰. The effect of this term on the $\delta^{18}O$ of mistletoe foliage depends on the terms ρ and $(1-p_{ex(ox)}p_{x(m)})$. The term ρ has been estimated from measurements of gas exchange and $\delta^{13}C$ values for some mistletoe species (Marshall and Ehleringer 1990; Marshall et al. 1994b). The term $p_{ex(ox)}$ is not known, but detailed investigation of the chemical composition of xylem-borne solutes and likely pathways for their incorporation into organic material could provide a range of expected values. However, until specific data become available, it is difficult to predict whether heterotrophy should increase, decrease, or not change the $\delta^{18}O$ of mistletoe leaves.

The consistent difference that we observed in $\delta^{18}O_p$ of mistletoes compared to their hosts, whereby mistletoes were less enriched in ^{18}O, contrasts with previous results comparing dry matter δD of Australian mistletoes to that of their hosts (Ziegler 1994). For dry matter δD, mean values of −51‰ for mistletoes and −73‰ for hosts were reported. Thus, in the case of deuterium, mistletoes were consistently *more* enriched than their hosts. We assume that the difference in $\delta^{18}O_p$ between mistletoes and hosts results from a differential enrichment between the two in leaf water isotopes (e.g. Flanagan et al. 1993); because $\delta^{18}O$ and δD in leaf water are strongly correlated, one might expect that the $\delta^{18}O$ and δD patterns in dry matter would be similarly correlated. Presumably then, the difference in the direction of mistletoe/host variation in δD as opposed to $\delta^{18}O$ of leaf dry matter results from variation between the mistletoe and host in post-photosynthetic, biochemical fractionation of δD. To a first approximation, the higher δD for mistletoe dry matter may imply a proportionally larger heterotrophic metabolism than in the host (Yakir 1992).

In the leaf water $\delta^{18}O$ samples that we examined, we did not observe a statistically significant difference between mistletoes and hosts. However the sample size was rather small (n=8) and therefore may not have been sufficient to capture variation between the two. Whereas the $\delta^{18}O_p$ and $\delta^{18}O_c$ values represent integrated measures of the leaf water $\delta^{18}O$, a single measurement of the leaf water itself represents only a snapshot in time in the dynamic leaf water system. Therefore, one would expect the power to detect differences between two populations to be greater for analyses of $\delta^{18}O_p$ or $\delta^{18}O_c$ than for $\delta^{18}O_L$. For example, in a sampling of 30 *Eucalyptus globulus* Labill. leaves over the course of a diurnal cycle, the coefficient of variation for $\delta^{18}O_p$ was 0.03, whereas that for $\delta^{18}O_L$ was 0.24 (L. Cernusak, unpublished data). For samples collected at the same time within that diurnal cycle, the average coefficient of variation for $\delta^{18}O_p$ was 0.01, whereas that for $\delta^{18}O_L$ was 0.13. Similar differences in variability between $\delta^{18}O_p$ and $\delta^{18}O_L$ were observed for cotton plants grown at constant humidity (Gan et al. 2002). The situation is analogous to that encountered when one analyzes variation among populations in instantaneous measurements of c_i/c_a, as compared to measurements of $\delta^{13}C_p$ (e.g. Cernusak and Marshall 2001).

Mistletoe $\delta^{13}C$

We found a significant variation in the parasite/host $\delta^{13}C_p$ differences between *A. miquelii* growing on a non-nitrogen fixing *Eucalyptus* host and *A. preissii* growing on a potentially nitrogen fixing *Acacia* host; parasite/host $\delta^{13}C_p$ differences were −3.0‰ and −0.8‰, respectively. This result is consistent with results for two *Phoradendron* mistletoe species growing on a non-nitrogen fixing host and an *Acacia* host, where the parasite/host $\delta^{13}C_p$ differences were −3.0‰ and −0.2‰, respectively (Schulze and Ehleringer 1984), and with results from three continents showing a general pattern of smaller parasite/host $\delta^{13}C_p$ differences on nitrogen fixing hosts than on non-nitrogen fixing hosts (Ehleringer et al. 1985). We observed a significant relationship between mistletoe/host $\delta^{13}C_p$ differences and host leaf N concentrations, consistent with previous reports (Ehleringer et al. 1985; Schulze et al. 1991; Bannister and Strong 2001).

The observations of $\delta^{18}O_p$ and $\delta^{18}O_c$ and foliar N concentration in the present study can be used to formulate hypotheses aimed at explaining the variation in mistletoe/host $\delta^{13}C_p$ differences. Parasite/host differences in $\delta^{13}C_p$ between *A. preissii* and *A. miquelii* could suggest a proportionally lower c_i/c_a ratio in *A. preisii* relative to its host compared to that in*A. miquelii* relative to its host. The observations of $\delta^{18}O_p$ suggest that the parasite/host differences in transpiration rate were similar for the two mistletoe species, whereas observations of $\delta^{18}O_c$ suggest a larger parasite/host difference in transpiration for *A. preisii* than *A. miquelii*. These observations indicate that a lower parasite/host difference in c_i/c_a in *A. preissii* compared to *A. miquelii* would have to result from a proportionally higher photosynthetic rate in *A. preissii* relative to its host compared to that in *A. miquelii* relative to its host. However, we found that foliar N concentrations were similar between *A. preissii* and its *Acacia* host, as was the case for *A. miquelii* and its *Eucalyptus* host. Therefore, the inference of a higher photosynthetic rate in *A. preissii* relative to its host compared to *A. miquelii* relative to its host based on measurements of foliar N concentration alone is not justified.

An alternative explanation is that *A. preissii* assimilated a larger amount of dissolved organic carbon from its host's transpiration stream than *A. miquelii*, thereby taking on a higher degree of partial heterotrophy. In a previous study of mistletoes and their hosts in southwestern Australia (Pate et al. 1991), the *Eucalyptus* host of *A. miquelii* was reported to have a xylem sap total amino acid concentra-

tion of 0.83 mmol l^{-1}, whereas the *Acacia* host of *A. preissii* had a xylem sap total amino acid concentration of 2.4 mmol l^{-1}, approximately three-fold higher. In the few cases where the $\delta^{13}C$ of xylem sap dry matter has been measured, it was consistently less negative than that of leaf dry matter from the same plant (Richter et al. 1995; Yoneyama et al. 1998; Cernusak et al. 2002), with an average difference of 1.0‰ for four *Acacia* species (Richter et al. 1995). Thus, we can hypothesize that the assimilation of a larger amount of amino acids and other compounds relatively enriched in ^{13}C and dissolved in the host's transpiration stream caused the $\delta^{13}C_p$ of *A. preissii* to resemble its host more closely than that of *A. miquelii* did its host.

Root hemiparasite $\delta^{18}O$ and $\delta^{13}C$

We found that leaf dry matter of root hemiparasites was less enriched in ^{18}O and ^{13}C than that of their hosts; average parasite/host differences were −1.0‰ for $\delta^{18}O_p$, and −1.2‰ for $\delta^{13}C_p$. Results were the same for both annual and perennial root hemiparasites. This suggests a general trend of higher transpiration rates and lower water use efficiency for root hemiparasites when compared to their hosts in southwestern Australia, although notably not to the same extent as the mistletoe species that we examined. Previous assessments of the water use efficiency of root hemiparasites in relation to their hosts have resulted in both lower estimates (Press et al. 1988, 1993; Ducharme and Ehleringer 1996; Lechowski 1996; Pate and Bell 2000), and similar or higher estimates (Pate et al. 1990a; Tennakoon et al. 1997; Loveys et al. 2001). Pate et al. (1990a) previously observed no difference in $\delta^{13}C_p$ between *O. phyllanthi* and its hosts in the coastal heath near Denmark, Western Australia. In contrast, we observed a difference of −1.0‰ for $\delta^{13}C_p$, and a corresponding difference of −1.5‰ for $\delta^{18}O_p$ for *O. phyllanthi* in the same location. The contrasting results might relate to changes in the successional status of the vegetation community, or to temporal variations in resource availability. It is worth noting that root hemiparasites can also gain carbon by assimilating xylem-borne solutes from their host's transpiration stream (Press et al. 1987, 1988; Ducharme and Ehleringer 1996; Tennakoon and Pate 1996b), and that such heterotrophy will cause variation in leaf dry matter $\delta^{13}C$ unrelated to parasite c_i/c_a. However, the likely net effect is to cause parasite $\delta^{13}C$ to be less negative than it otherwise would be, thereby causing differences in water use efficiency between parasite and host to be underestimated, if anything.

Parasite/host differences in $\delta^{18}O_c$ appeared to be similar to those in $\delta^{18}O_p$. In contrast, $\delta^{13}C_c$ did not show the same parasite/host difference that $\delta^{13}C_p$ did. It is possible that leaf cellulose $\delta^{13}C$ contains a signature that is temporally separated from that contained in total leaf dry matter, or that post-photosynthetic fractionations differ between the parasites and their hosts. Further research would be helpful in clarifying the contrasting results between $\delta^{13}C_p$ and $\delta^{13}C_c$ for root hemiparasites compared to their hosts.

Our analysis did not detect variation in parasite/host isotopic differences among the different species of root hemiparasites. However sampling frequencies were rather low for most of the species. Thus, an improved experimental design in the future may well show meaningful differences among different species of root hemiparasites in their isotopic behavior in relation to their hosts.

Holoparasite $\delta^{18}O$

The dry matter and cellulose of holoparasites were enriched in ^{18}O compared to their hosts, showing parasite/host $\delta^{18}O_p$ and $\delta^{18}O_c$ differences of +3.0‰ and +1.2‰, respectively. This was not simply a reflection of more enriched tissue water, as the tissue water in the holoparasites was less enriched than host leaf water by −3.8‰ when sampled at midday. Transpirational water loss is generally very low for holoparasites (Raven 1983); it has been assessed as less than 2% of the host's transpiration rate in *Orobanche* (Hibberd et al. 1999), and as roughly the same as the amount of water used for tissue growth in *Cuscuta* (Jeschke et al. 1994a, 1994b). If one assumes little or no evaporational enrichment after uptake, the holoparasite tissue water $\delta^{18}O$ values are consistent with host phloem water being the main water source. Within the host, the phloem water $\delta^{18}O$ is expected to be intermediate between that of leaf water and that of xylem water (Cernusak et al. 2002, 2003b). A symplastic connection between host sieve elements and haustorial cells in *Cuscuta* was recently demonstrated (Haupt et al. 2001), which would allow direct uptake of host phloem water together with solutes.

Equation 3 can be used to construct hypotheses aimed at explaining the enrichment in ^{18}O of holoparasite dry matter and cellulose relative to their hosts. In the case of holoparasites, the terms $\delta^{18}O_L$ and $\delta^{18}O_s$ in Eq. 3 would refer to values for the host, and thus would not differ between the two partners. Therefore, we can hypothesize that the difference between parasite and host lies in the term $p_{ex}p_x$. If the parasite uses exclusively phloem water, this may lead to a lower p_x value in the parasite, because developing cells in the host would be expected to contain a mixture of phloem water and xylem water (Lalonde et al. 2003). Alternatively, p_{ex} may be lower in the parasite than in the host. There is at least one report suggesting that p_{ex} may decrease if sink strength is high and carbohydrate supply is limiting (Barbour and Farquhar 2000). A final hypothesis is that p_x is effectively lower in the parasite, not, however, caused by a difference in use of phloem versus xylem water, but rather by evaporative enrichment of water within the holoparasite. Although evaporation rates are very low compared to the host, some evaporation must occur from the tissue of the holoparasite. The small enrichments in tissue water caused by this evaporation may build up over time because the residence time of

water in the holoparasite is likely to be long. In an analogous situation, water in twigs of deciduous trees was observed to increase in $\delta^{18}O$ by several per mil during the leafless winter months, before returning to the value of soil water when leaves developed (Förstel and Hützen 1983). Notably, the parasite/host trend for $\delta^{18}O$ in dry matter of holoparasites agrees with the trend for δD; holoparasites were consistently enriched in dry matter δD compared to their hosts (Ziegler 1994, 1996).

Holoparasite $\delta^{13}C$

We found that the holoparasites that we analyzed were consistently enriched in $\delta^{13}C_p$ relative to their hosts. In a previous comparison of holoparasite/host $\delta^{13}C_p$, it was concluded that the two were not significantly different (Ziegler 1994). We re-analyzed the data presented in Table 19.2 of that report and found that there was one anomalous pair in the parasite/host comparison that caused the two not to differ statistically; the pair in question lists a value of −24.8‰ for *Cuscuta hyalina* Heyne ex Roth growing on *Zea mays* L. with a value of −14.6‰. This datum is likely erroneous (H. Ziegler, personal communication), because it implies that *Cuscuta* assimilated most of its own carbon via the C_3 photosynthetic pathway. In contrast, *Cuscuta* is known to be a phloem-feeding, obligate holoparasite, and has been shown to assimilate less than 1% of its own carbon by its own photosynthesis (Jeschke et al. 1994a). Excluding this particular datum from the data set results in an average difference between holoparasites and hosts of +1.0‰ (P=0.007, n=27), similar to the average difference that we observed of +1.5‰. Such results are also consistent with observations of holoparasitic plants in South Africa (De la Harpe et al. 1981). We calculated an average enrichment of +1.4‰ (P=0.06, n=12), based on data presented in Table 3 of De la Harpe et al.

The less negative holoparasite $\delta^{13}C_p$ relative to host leaves is consistent with a general pattern of ^{13}C enrichment in heterotrophic plant tissues relative to the leaves supplying them with photosynthate. This pattern has been observed in wood (Craig 1953; Leavitt and Long 1982; Francey et al. 1985), roots (Gebauer and Schulze 1991; Ineson et al. 1996; Syvertsen et al. 1997), fruits (Yoneyama and Ohtani 1983; Cernusak et al. 2002), and emerging leaves (Terwilliger 1997; Terwilliger et al. 2001). In cases where $\delta^{13}C_c$ was analyzed, the pattern was generally the same as for $\delta^{13}C_p$ (Leavitt and Long 1982; Terwilliger et al. 2001). This contrasts with our results for holoparasitic plants, in which $\delta^{13}C_p$ was less negative than that of the leaves supplying them with photosynthate, but $\delta^{13}C_c$ was not. It should also be noted that not all holoparasitic plants show less negative $\delta^{13}C_p$ than their hosts; for example, the holoparasitic mistletoe *Tristerix aphyllus* consistently showed $\delta^{13}C_p$ values more negative than its hosts (Kraus et al. 1995).

The *Cuscuta* and *Cassytha* holoparasites that we sampled were assumed incapable of net photosynthesis, as has been demonstrated elsewhere by gas exchange measurements (De la Harpe et al. 1981; Jeschke et al. 1994a). However, members of both genera have been reported capable of low rates of $^{14}CO_2$ fixation (MacLeod 1961; De la Harpe et al. 1979). This photosynthetic capacity allows the plants to refix some respiratory CO_2 in the light. Thus, although there is not a net uptake of CO_2 from the atmosphere, there is a reduced rate of CO_2 efflux from the plants in the light compared to the CO_2 efflux rate in the dark (De la Harpe et al. 1981; Jeschke et al. 1994a). An analogous situation exists in woody tissues, where some respired CO_2 can be refixed in the light by photosynthetic bark (e.g. Cernusak and Marshall 2000). This process has been shown to result in the retention of carbon having a $\delta^{13}C$ value more negative than that of the dark-respired CO_2 (Cernusak et al. 2001). The same process would be expected during refixation in holoparasitic plants. However, it would appear that the refixation of CO_2 relatively depleted in ^{13}C is generally not sufficient to counter the process that causes the dry matter of holoparasites to be enriched in ^{13}C compared to their hosts.

All species $\delta^{18}O$ and $\delta^{13}C$

We observed an increase of 2.6‰ in $\delta^{18}O_p$ between the December sampling and the February sampling averaged across all species; in contrast there was no change in $\delta^{13}C_p$ (Fig. 3A, B). We previously observed a variation of 4‰ in $\delta^{18}O_p$ of *Lupinus angustifolius* L. leaves over the course of a diurnal cycle (Cernusak et al. 2002); the variation was attributed to the daily accumulation and export of carbohydrates in equilibrium with $\delta^{18}O_L$. Given the apparent plasticity of the $\delta^{18}O_p$ of leaves in response to variation in $\delta^{18}O_L$, a seasonal shift across most species is not surprising. The magnitude of seasonal variation that we observed in $\delta^{18}O_p$ in southwestern Australia agrees well with recent observations for *Picea abies* in Switzerland, where $\delta^{18}O_p$ of current-year leaves increased by 2.1‰ from spring to summer (Jäggi et al .2003).

We observed a large variation among species in ε_{cp}, the difference between $\delta^{18}O_p$ and $\delta^{18}O_c$ (Table 4). This may reflect differences among species in the timing and dynamics of leaf expansion. Other factors such as variation in leaf carbohydrate concentration or composition may also contribute. Until the processes underlying variation in ε_{cp} are better understood, it would be premature to conclude that $\delta^{18}O_p$ or $\delta^{18}O_c$ is likely to provide a more useful integrated record of $\delta^{18}O_L$.

Of the consistent differences that we observed in isotopic composition between parasitic plants and their hosts, some were easily interpretable in terms of mechanisms causing the variations. In other cases, data necessary for testing explanatory hypotheses were not available; these hypotheses await experimental testing in the future. Data describing the chemical and isotopic composition of solutes procured by parasites from the xylem and phloem

sap of their hosts would be especially helpful in enabling some of these hypotheses to be tested.

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