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# **Integrated nitrogen, carbon, and water relations of a xylem-tapping mistletoe following nitrogen fertilization of the host**

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**Abstract** Xylem-tapping mistletoes transpire large volumes of water ( $E$ ) while conducting photosynthesis ( $A$ ) at low rates, thus maintaining low instantaneous water-use efficiency ( $A/E$ ). These gas-exchange characteristics have been interpreted as a means of facilitating assimilation of nitrogen dissolved at low concentration in host xylem water; however, low  $A/E$  also results in substantial heterotrophic carbon gain. In this study, host trees (*Juniperus osteosperma*) were fertilized and gas exchange of mistletoe (*Phoradendron juniperinum*) and host were monitored to determine whether mistletoe  $A/E$  would approach that of the host if mistletoes were supplied with abundant nitrogen. Fertilization significantly increased foliar N concentrations ( $N$ ), net assimilation rates, and  $A/E$  in both mistletoe and host. However, at any given N concentration, mistletoes maintained lower  $A$  and lower  $A/E$  than their hosts. On the other hand, when instantaneous water-use efficiency and  $A/N$  were calculated to include heterotrophic assimilation of carbon dissolved in the xylem sap of the host, both water-use efficiency and  $A/N$  converged on host values. A simple model of *Phoradendron* carbon and nitrogen budgets was constructed to analyze the relative benefits of nitrogen- and carbon-parasitism. The model assumes constant  $E$  and includes feedbacks of tissue nitrogen concentration on photosyn-

thesis. These results, combined with our earlier observation that net assimilation rates of mistletoes and their hosts are approximately matched (Marshall et al. 1994), support part of the nitrogen-parasitism hypothesis: that high rates of transpiration benefit the mistletoe primarily through nitrogen gain. However, the low ratio of  $A/E$  is interpreted not as a means of acquiring nitrogen, but as an inevitable consequence of an imbalance in C and N assimilation.

**Key words** Parasitic higher plant · Nitrogen parasitism  
Water-use efficiency · Heterotrophy  
*Phoradendron juniperinum*

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## Introduction

Xylem-tapping mistletoes are obligate parasites that rely completely on their hosts for water but are capable of positive net photosynthesis ( $A$ ) (Hellmuth 1971; Hollinger 1983; Schulze et al. 1984; Ehleringer et al. 1985; Davidson et al. 1989; Goldstein et al. 1989; Marshall and Ehleringer 1990; Johnson and Choinski 1993). However, photosynthetic rates of mistletoes are among the lowest measured on  $C_3$  plants (Stewart and Press 1990). In contrast, mistletoes typically transpire ( $E$ ) at high rates (Glatzel 1983; Hollinger 1983; Schulze et al. 1984; Ehleringer et al. 1985; Davidson et al. 1989; Goldstein et al. 1989), up to 9 times higher than those of their hosts (Ullman et al. 1985; Küppers et al. 1992). Schulze et al. (1984) noted that one benefit of the high transpiration rates of mistletoes would be enhanced assimilation of nitrogen from the host xylem sap, which might be necessary to meet the mistletoe's requirements for growth. Because xylem-tapping mistletoes have no direct access to nitrogen which may be present in the host phloem, they are entirely dependent on the dilute nitrogen that they parasitize from host xylem sap.

The "nitrogen-parasitism hypothesis" has been supported by comparisons of mistletoes growing on nitrogen-fixing and non-fixing hosts, which differ in xylem

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nitrogen supply; generally, mistletoes with high nitrogen status were most similar to their hosts in instantaneous water-use efficiency ( $A/E$ ) the ratio of net photosynthesis to transpiration (Schulze and Ehleringer 1984; Ehleringer et al. 1985). Givnish (1986), however, pointed out that the water-use efficiency pattern was as much due to variation in the gas-exchange characteristics of the host as to that of the mistletoe.

Mistletoes acquire more than nitrogen from their hosts (Raven 1983). Recent analyses of the stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of mistletoes provide evidence of a second benefit, parasitic carbon gain, as a result of high transpiration rates. Marshall and Ehleringer (1990) noted discrepancies in the theoretically expected relationship between  $\delta^{13}\text{C}$  and  $A/E$  in the mistletoes that were similar to discrepancies observed in *Striga*, a  $\text{C}_3$  root parasite, on *Sorghum*, its  $\text{C}_4$  host (Press et al. 1987). They inferred that mistletoes must obtain a substantial carbon subsidy from passive uptake and assimilation of carbon compounds dissolved in the xylem sap of the host. This inference is supported by studies of Schulze et al. (1991) who showed that  $\text{C}_3$  mistletoes growing on CAM hosts also maintain  $\delta^{13}\text{C}$  intermediate between that of the host and that of mistletoe photosynthate. Both mistletoe studies estimated heterotrophy ( $H$ ), i.e., the proportion of carbon in the mistletoe derived from host photosynthate (Press et al. 1987), as approximately 60%. Marshall et al. (1994) have recently estimated proportional heterotrophy in several taxa of Australian mistletoes as between 5 and 21%, similar to the earlier estimate of Pate et al. (1991) of 24% for another Australian host-mistletoe pair.

Distinguishing the transpirational carbon benefit from the nitrogen benefit becomes particularly difficult in mistletoe since carbon and nitrogen assimilation are partially coupled. We might expect that mistletoes with higher foliar nitrogen concentrations would tend to have higher photosynthetic rates (as reviewed in Field and Mooney 1986). On the other hand, these mistletoes have no other available storage organs and a higher N concentration ( $N$ ) could reflect storage for later reproductive allocation (Ehleringer et al. 1986). Moreover, the low water-use efficiency of mistletoes leads one to ask whether  $A/N$  might not also be relatively low.

In this paper we present a test of the nitrogen-parasitism hypothesis (Schulze et al. 1984), in which we measured gas-exchange responses of mistletoes and their hosts to experimental nitrogen fertilization. Specifically, we evaluate photosynthetic  $A/N$ ,  $A/E$ ,  $\delta^{13}\text{C}$ , and proportional heterotrophy ( $H$ ) of hosts and mistletoes, comparing  $A/N$  and  $A/E$  between mistletoes and their hosts. As a means of analyzing the complex interactions that control C and N accumulation by the plant, we developed a model that incorporates the experimental results of our effort to test the nitrogen-parasitism hypothesis.

## Materials and methods

### Study site

The study was conducted in southern Utah on Coral Pink Sand Dunes State Reserve (37°3'N, 112°45'W, elevation 1855 m), east of Zion National Park. The site, described previously (Dawson et al. 1990b; Flanagan et al. 1992), is dominated by a pinyon-juniper woodland growing on stabilized sand dunes over sandstone. The most common species was *Juniperus osteosperma*, the Utah juniper. Associated woody vegetation included *Pinus edulis*, *Artemisia tridentata*, and *Chrysothamnus nauseosus*. The United States National Weather Service Station at Kanab, Utah, which is 20 km to the south, received an average 319 mm precipitation annually. The plot is near the center of a contiguous zone of mistletoe infection covering several hundred square kilometers. The site was fenced in spring 1988 to exclude grazing. Trees were tagged and mapped, diameters were measured, and mistletoe age structure, sex ratio and infection status were determined (Dawson et al. 1990a, b). Trees were chosen for inclusion in this study by first eliminating all trees without mistletoe infections, then randomly selecting from among those remaining. After a tree was selected, all trees within 5 m were eliminated from further consideration. This procedure ensured a 2.5 m buffer around each treated tree, which helped to minimize effects of fertilization on adjacent trees. This method was used to select 42 individuals in an area of approximately 0.56 ha.

### Nitrogen fertilization

Urea was placed under the crowns of selected trees in November 1988; April 1989, June 1989, November 1989, May 1990, and July 1990. Different trees were fertilized at rates equivalent to 0, 30, 60, 90, 120, and 150 kg N ha<sup>-1</sup>. Nitrogen addition rates were scaled to tree size based on estimates of leaf area, which were obtained from equations based on stem diameter (Miller et al. 1987). The scaled fertilization rate was calculated using the following equation:

$$N \text{ added tree}^{-1} = F \frac{LA}{LAI} \quad (1)$$

where  $F$  is the fertilization rate (0–150 kg N ha<sup>-1</sup>),  $LA$  is the leaf area of the individual tree (m<sup>2</sup>), and  $LAI$  is leaf area index (m<sup>2</sup>ha<sup>-1</sup>). Six applications at the highest rate resulted in N addition equivalent to 900 kg N ha<sup>-1</sup> over a 2-year period.

Granulated sugar added to the ground surface at a rate equivalent to 1000 kg ha<sup>-1</sup> constituted the seventh treatment. Sugar additions favor microbial immobilization of N, which reduces N availability for plants, an effect opposite that of the urea additions (Waring and Schlesinger 1985). This treatment has been demonstrated to create marked differences in several coniferous forest types (see Schimel et al. 1992 and references therein). Sugar additions were made on the same dates as the urea additions.

Six trees received each of the seven treatments. N additions increased foliar N levels beginning in June 1989 for the mistletoes and August 1989 for the junipers, which is 8 months after the first treatment. Because nitrogen concentrations did not rise until after stems had elongated in 1989, we have used only the data from 1990 in this paper. Treatment effects were variable among trees within a treatment (Fig. 1). Because of the variability within treatments, we have analyzed the data using regression analysis, with foliar N as independent variable, rather than using analysis of variance of treatment effects. The regression approach has the further advantage of making the results more general by emphasizing functional relationships among physiological variables rather than detailing site-specific treatment effects.

### Photosynthetic gas exchange

Photosynthetic gas-exchange was measured in August 1990 with a Licor-6200 portable photosynthesis system. Diurnal measurements

on three branches of one or two trees preceded each set of measurements of the experimental trees. Data from the diurnal courses were used to determine when during the day photosynthetic rates reached their maxima (not presented); maximum photosynthesis ( $A_{\max}$ ) was then measured in the treated trees, and their mistletoes, over the course of the next two days. The healthiest foliage that could be found in full sunlight was chosen; juniper foliage on mistletoe-infected branches was avoided. Measured foliage was then collected and returned to the lab, where it was oven-dried, weighed and ground. Leaf surface area was determined on a subsample of the foliage by cutting the foliage into the smallest units that would lay flat on the belt of a Licor 3000-leaf area meter. Specific leaf area did not vary among treatments or dates (data not presented), and in August 1990 averaged  $14.40$  ( $SE=0.13$ )  $\text{cm}^2 \text{g}^{-1}$  for junipers,  $12.10$  ( $0.11$ )  $\text{cm}^2 \text{g}^{-1}$  for mistletoes. Leaf samples were analyzed for Kjeldahl nitrogen (Chapin and Van Cleve 1989) and for carbon isotope ratio in the Stable Isotope Ratio Facility for Environmental Research at the University of Utah, Salt Lake City, Utah, United States, following the methods presented in Ehleringer and Osmond (1989).

### Statistical analyses

Treatment effects on foliar nitrogen concentration were initially evaluated by analysis of variance. The effect of nitrogen on  $A_{\max}$  was then evaluated by nonlinear regression fitting  $A_{\max}$  to foliar N concentrations using a rectangular hyperbola. Residuals were not evenly distributed, presumably because the proportion of foliar nitrogen not involved in photosynthesis was higher in mistletoes with lower N concentrations. Therefore, a third term, adjusting for nonphotosynthetic nitrogen, was included in the analysis. Functional relationships were compared between mistletoes and their hosts using analysis of covariance, with foliar nitrogen concentrations as the covariate.

### Model calculations

The carbon-budget model runs on a 1-day timestep and assumes 12 h of light-saturated photosynthesis. Heterotrophic carbon gain is calculated as:

$$C_H = E \times c_x / k \quad (2)$$

where  $C_H$  = heterotrophic carbon gain ( $\text{mol g}^{-1} \text{day}^{-1}$ ),  $E$  is transpiration rate ( $\text{mol g}^{-1} \text{day}^{-1}$ ),  $c_x$  is the carbon concentration of xylem sap, and  $k$  is 55.56, the number of moles of water per liter. Total daily carbon gain is calculated by summing net photosynthesis and  $C_H$  and then subtracting respiration, which is assumed to consume 40% of daily carbon gain. Respiration includes both construction and maintenance costs. The remaining carbon is converted to biomass by dividing by 0.43, the proportion of carbon in mistletoe tissue (J. D. Marshall, unpublished work). Plant biomass is incremented daily. The proportion of heterotrophic carbon gain ( $H_{\text{mod}}$ ) is calculated in the model as:

$$H_{\text{mod}} = C_H / (A + C_H) \quad (3)$$

The proportion of heterotrophic carbon gain can also be estimated from carbon isotope ratio and photosynthetic gas exchange data (Press et al. 1987) as:

$$H = (\delta_{\text{mg}} - \delta_{\text{m}}) / (\delta_{\text{mg}} - \delta_{\text{h}}) \quad (4)$$

where  $\delta_{\text{m}}$  is the measured carbon isotope ratio of mistletoe tissue and  $\delta_{\text{h}}$  is the measured carbon isotope ratio of host tissue. The carbon isotope ratio of mistletoe photosynthate ( $\delta_{\text{mg}}$ ) is predicted from Farquhar et al. (1982):

$$\delta_{\text{mg}} = \delta_{\text{air}} - a - (b-a) \times (p_i / p_a) \quad (5)$$

where  $\delta_{\text{air}}$  is  $-8\%$ ,  $a$  is  $4.4\%$ ,  $b$  is  $27\%$ , and  $p_i$  and  $p_a$  are the intercellular  $\text{CO}_2$  partial pressure and ambient  $\text{CO}_2$  partial pressure, respectively.

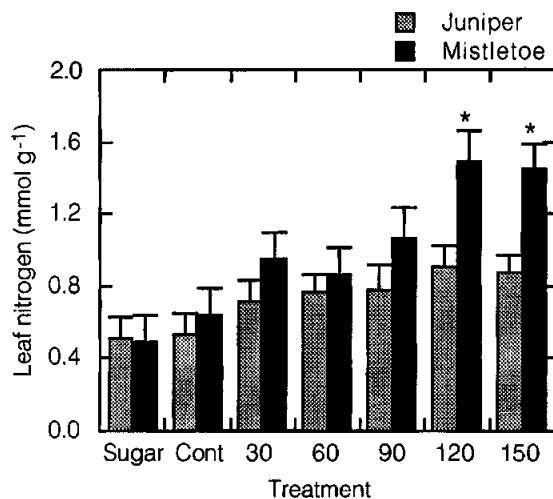
**Table 1** Constants in model of mistletoe gas exchange and growth

Constant	Value	Source
$E$	$6 \text{ mmol m}^{-2} \text{ s}^{-1}$	calculated from Ehleringer et al. (1986)
Xylem C	$46 \text{ mmol l}^{-1}$	Gregg (1991)
Xylem N	$0.76 \text{ mmol l}^{-1}$	Gregg (1991)
Biomass/C	$25.7 \text{ g mol C}^{-1}$	J.D. Marshall, unpublished data
Daylength	12 h	
Respiratory losses	$0.4 \text{ mol mol}^{-1}$	
LAR	$0.4 \text{ g g}^{-1}$	

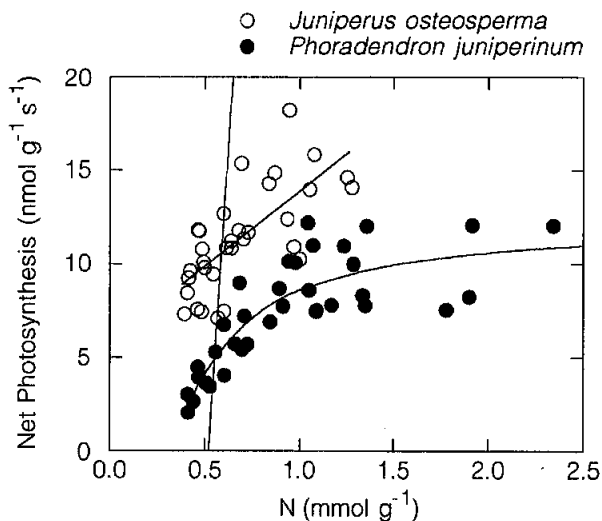
Nitrogen assimilation rate is estimated by multiplying the nitrogen concentration of xylem sap by the daily transpiration rate. Tissue nitrogen concentrations are updated daily and photosynthetic rates are estimated as a function of the updated nitrogen concentration using the functional relationship described in the Results section below. Our estimates of the constants in the model are presented in Table 1. To simulate responses to different host xylem composition, the model was run following a quadrupling of the xylem nitrogen concentration, from  $0.76 \text{ mmol l}^{-1}$  to  $3.04 \text{ mmol l}^{-1}$ . A third run assumed no assimilation of xylem carbon. Each of these sets of conditions was modeled starting from high ( $2.5 \text{ mmol N g}^{-1}$ ) and low ( $0.25 \text{ mmol N g}^{-1}$ ) foliar N concentrations. The computer model, written both in Microsoft Basic for the Macintosh and in Stella II format, is available upon request.

## Results

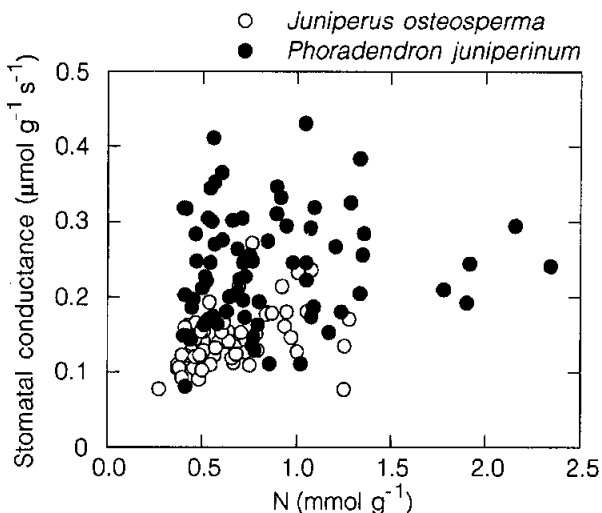
Nitrogen fertilization of host trees increased foliar nitrogen concentrations of the mistletoes for the two highest nitrogen application rates (one-way ANOVA:  $F=6.07$ ,  $P=0.0004$ ). Fertilization had no significant effect on foliar N of the junipers ( $F=1.75$ ,  $25 \text{ df}$ ,  $P=0.15$ , Fig. 1). Sugar additions, which were expected to immobilize nitrogen, had no significant effect on either species (Fig. 1). Regression analysis yielded evidence of a strong effect of foliar N on net photosynthesis of both species (Fig. 2). Because the relationship was not linear for the mistle-



**Fig. 1** Foliar Kjeldahl nitrogen concentrations of mistletoes and their hosts, summarized by nitrogen treatment, in July and August, 1990. Asterisks denote treatments in which significant differences ( $\alpha=0.05$ ) were detected by analysis of variance



**Fig. 2** Photosynthetic rates versus foliar nitrogen concentrations. Fitted curve of mistletoe data is presented as Eq. 5 in text. Regression equation for juniper data is:  $A$  ( $\text{nmol g}^{-1} \text{s}^{-1}$ ) =  $6.07 + 7.57 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=32$ ,  $r^2=0.48$ ,  $P<0.0001$ ). The nearly vertical line is the regression equation of Field and Mooney (1986) describing the relationship in a broad range of plant species



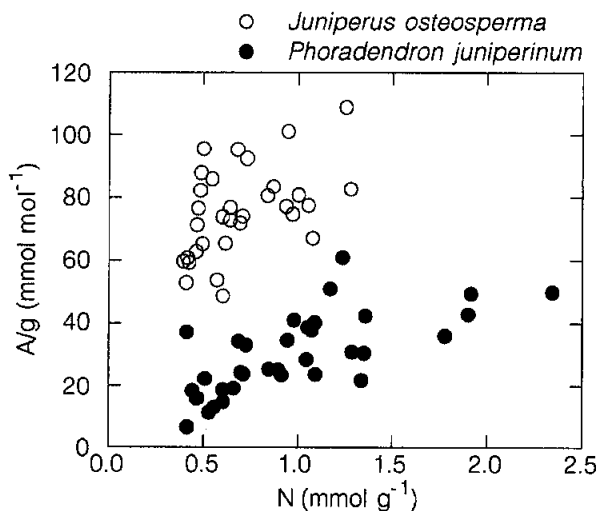
**Fig. 3** Stomatal conductance versus foliar nitrogen concentration. Regression lines not significant for either mistletoes ( $n=36$ ,  $r^2=0.003$ ,  $P=0.77$ ) or junipers ( $n=34$ ,  $r^2=0.002$ ,  $P=0.78$ )

toes, we used a curve-fitting routine (JMP 2.0, SAS Institute, Cary, N. Carolina, USA) to fit the data to a rectangular hyperbola. The distribution of the residuals was improved when we added a term to account for that part of foliar nitrogen apparently not involved in photosynthesis ( $0.35 \text{ mmol g}^{-1}$ ). The curve fitting procedure yielded the following equation:

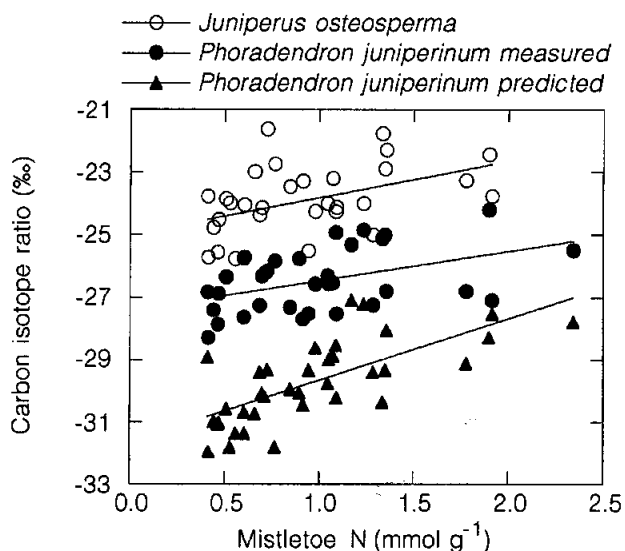
$$A_{\max} = \frac{12.45 \times (N - 0.35)}{0.29 + (N - 0.35)} \quad (6)$$

where  $A_{\max}$  is in  $\text{nmol g}^{-1} \text{s}^{-1}$ ,  $N$  is in  $\text{mmol g}^{-1}$ .

In contrast, nitrogen fertilization had no detectable effect on stomatal conductance of mistletoes ( $F=0.09$ , 35



**Fig. 4** Intrinsic water-use efficiency ( $A/g$ ) versus foliar nitrogen concentrations. Regression equations are as follows: mistletoe  $A/g$  ( $\text{mmol mol}^{-1}$ ) =  $11.43 + 18.70 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=35$ ,  $r^2=0.48$ ,  $P<0.0001$ ); Juniper  $A/g$  ( $\text{mmol mol}^{-1}$ ) =  $56.98 + 26.67 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=32$ ,  $r^2=0.23$ ,  $P=0.006$ )



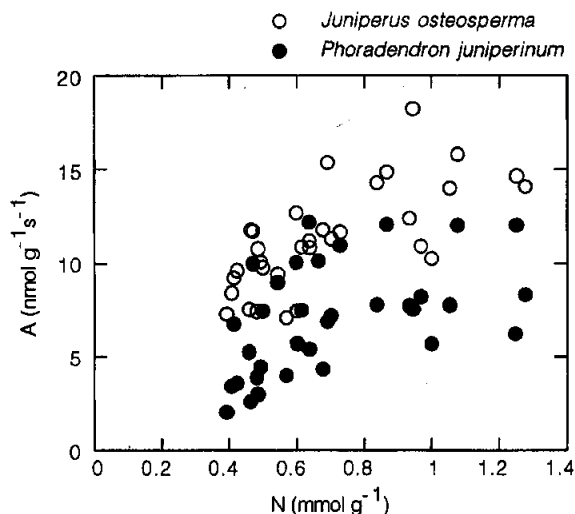
**Fig. 5** Carbon isotope ratio versus foliar Kjeldahl nitrogen concentration. Mistletoe  $\delta^{13}\text{C}$  predicted from gas exchange ( $\text{‰}$ ) =  $-32.22 + 2.09 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=36$ ,  $r^2=0.50$ ,  $P<0.0001$ ). Measured mistletoe  $\delta^{13}\text{C}$  ( $\text{‰}$ ) =  $-27.41 + 0.94 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=33$ ,  $r^2=0.20$ ,  $P=0.010$ ). Juniper  $\delta^{13}\text{C}$  ( $\text{‰}$ ) =  $-24.99 + 1.16 \times N$  ( $\text{mmol g}^{-1}$ ), ( $n=31$ ,  $r^2=0.20$ ,  $P=0.011$ )

$df$ ,  $P=0.77$ ) or junipers ( $F=0.08$ , 33  $df$ ,  $P=0.78$ , Fig. 3). An increase in photosynthetic rates without a change in stomatal conductance increases intrinsic water-use efficiency, expressed here as  $A/g$ . In fact, mistletoe  $A/g$  was tripled over the range of  $N$  concentrations induced by this experiment (Fig. 4). The increase in  $A/g$  was also manifested in carbon isotope ratios of the mistletoes;  $\delta^{13}\text{C}$  increased by about  $2\text{‰}$  as foliar  $N$  concentrations rose (Fig. 5).

One might have predicted a decrease in mistletoe heterotrophy given that increased  $A/g$  would increase auto-

**Table 2** Comparison of gas exchange and isotope parameters between mistletoes and junipers. All comparisons, except for nitrogen, were made using one-way ANOVA, with foliar nitrogen as a covariate. The nitrogen comparison was made with an  $F$ -test calculated from a simple one-way ANOVA

Variable	Mistletoe Mean (SE)	Juniper Mean (SE)	Significance level	
			Intercept	Slope
$N$ ( $\text{mmol g}^{-1}$ )	0.97 (0.06)	0.71 (0.07)	0.006	
$g$ ( $\mu\text{mol g}^{-1} \text{s}^{-1}$ )	0.259 (0.010)	0.151 (0.010)	<0.0005	0.49
$A$ ( $\text{nmol g}^{-1} \text{s}^{-1}$ )	7.8 (0.6)	16.9 (0.6)	<0.0001	<0.0001
$A/g$ ( $\mu\text{mol mol}^{-1}$ )	29.8 (2.2)	75.2 (2.3)	<0.0001	0.37
$\delta^{13}\text{C}$ (‰)	-26.4 (0.2)	-23.9 (0.2)	0.18	<0.0001

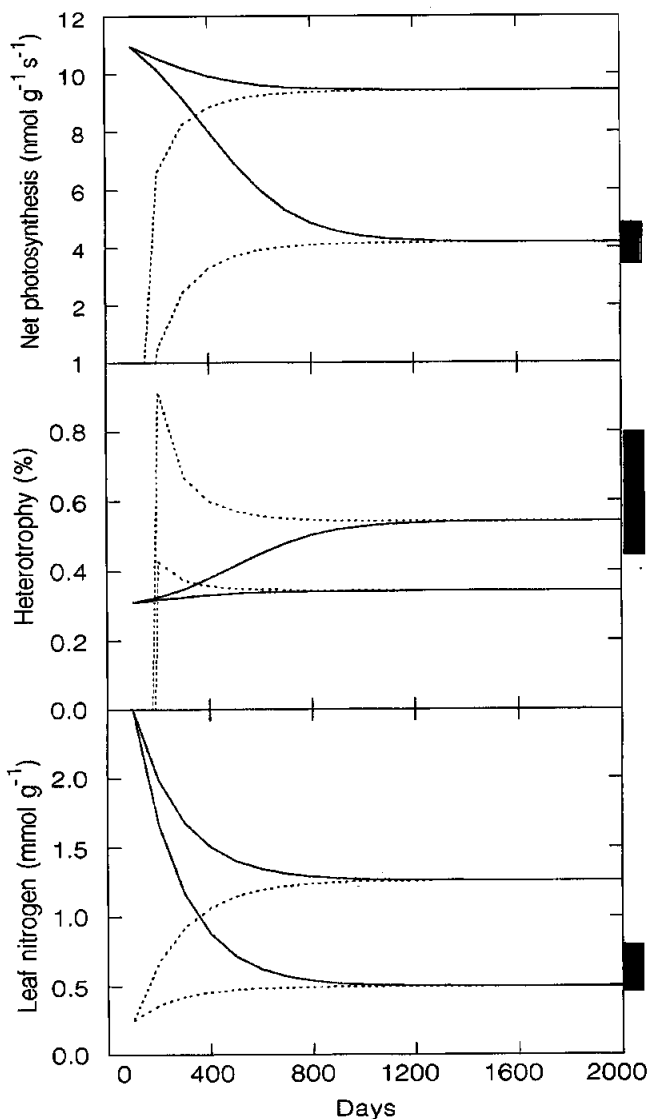


**Fig. 6** Net photosynthetic rates ( $\text{nmol g}^{-1} \text{s}^{-1}$ ) of mistletoes compared to those of their individual hosts presented as a function of the foliar nitrogen concentrations ( $\text{mmol g}^{-1}$ ) of the junipers

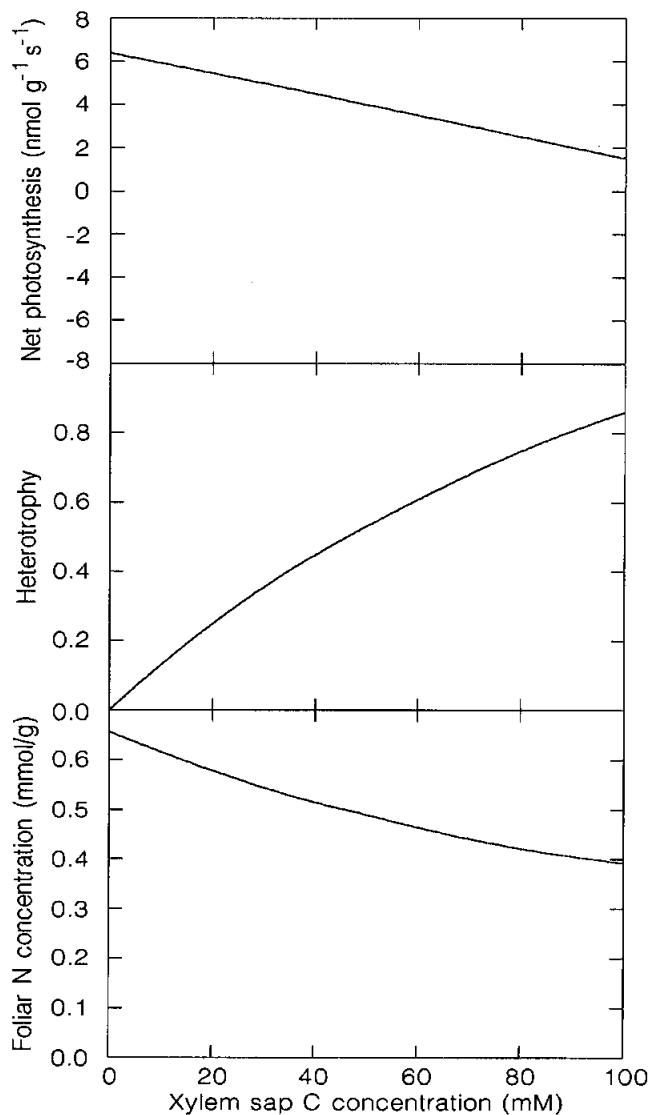
trophic carbon gain if the concentration of solutes in host xylem were held constant. The data are equivocal. Analysis of covariance found differences near the detection limit in the slopes of regression lines relating  $\delta^{13}\text{C}$  to  $N$  for mistletoe photosynthate, mistletoe tissues and mistletoe hosts ( $F=2.90$ , 2  $df$ ,  $n=100$ ,  $P=0.060$ , Fig. 5). The mistletoe carbon isotope ratios lay midway between the observed host carbon isotope ratios and those predicted for the mistletoe based on  $p_i/p_a$  values, increasing somewhat less rapidly than the predicted values. Heterotrophy calculated from the least-squares regressions presented in Fig. 5 decreased from 64 to 47% as mistletoe foliar  $N$  increased from 0.4 to 2.0  $\text{mmol N g}^{-1}$ . Heterotrophy calculated from measurements of individual mistletoes did not vary with foliar  $N$  ( $F=2.14$ , 27  $df$ ,  $P=0.16$ ); mean heterotrophy was 58 (SE=17)%.

How did the gas exchange of N-fertilized mistletoes compare to that of their hosts? Mistletoe stomatal conductance was consistently higher than that of junipers (Fig. 2, Table 2), and photosynthetic rates were consistently lower (Fig. 3, Table 2). Thus  $A/g$  was consistently lower among mistletoes than their hosts (Fig. 4, Table 2). Carbon isotope ratios tended to be more negative in mistletoes than in hosts at any given nitrogen concentration (Fig. 5, Table 2).

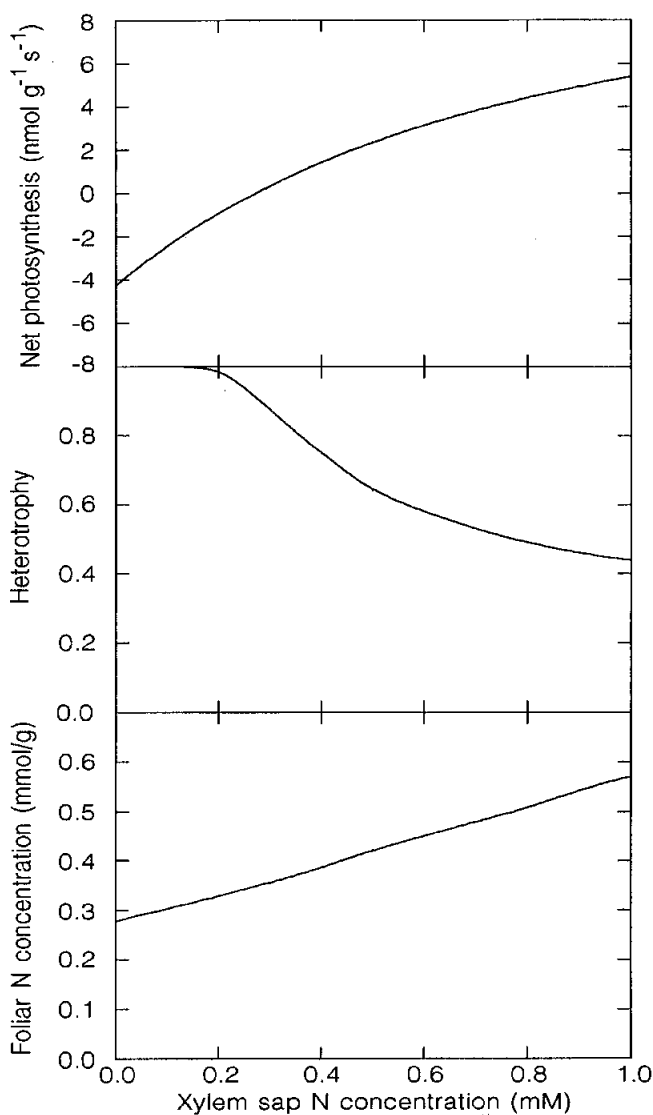
Mistletoes reached photosynthetic rates similar to those of junipers, but only if high-nitrogen mistletoes were compared to low-nitrogen hosts (Fig. 2). As a con-



**Fig. 7** Predicted photosynthetic rates, nitrogen concentrations, and proportional heterotrophy of mistletoes based on modelled mistletoe gas exchange. Initial nitrogen concentrations of 0.25 (dashed lines) or 2.5  $\text{mmol N g}^{-1}$  were assumed. The model was run with measured xylem  $N$  concentration ( $0.76 \text{ mmol l}^{-1}$ ), a four-fold increase in xylem  $N$ , or in the absence of xylem carbon assimilation. Simulations of the following variables are presented: *above* net assimilation rates, *below* nitrogen concentrations, and *centre* heterotrophy. Mean values,  $\pm$ SE, independently measured in the control treatment are presented for comparison as black bars on the right



**Fig. 8** Predicted asymptotic net photosynthetic rates, nitrogen concentrations, and proportional heterotrophy of mistletoes grown on hosts varying in xylem sap carbon concentrations



**Fig. 9** Predicted asymptotic net photosynthetic rates, nitrogen concentrations, and proportional heterotrophy of mistletoes grown on hosts varying in xylem sap nitrogen concentrations

sequence,  $A/N$  among mistletoes was less than half that of their hosts (Table 2). The photosynthetic rate of a mistletoe was compared to that of its individual host using N concentration of host foliage as the independent variable (Fig. 6); mistletoe photosynthetic rates reached those of their hosts only infrequently.

After incorporating these observations into the carbon-balance model described above we used the model to integrate the simultaneous transport, assimilation, and metabolism of carbon, nitrogen, and water. The model predicted relative growth rate,  $H$ ,  $A_{\max}$ , and foliar N as a function of  $E$  and the C and N concentrations of host xylem water. Simulation results are presented in Fig. 7. Independent of initial nitrogen concentration in the mistletoe, the model predicted an asymptotic approach to a specific foliar nitrogen concentration. Because photosynthetic rate is determined by nitrogen concentration in the

model, photosynthetic rates also approached an asymptote (Fig. 7a). Heterotrophy behaved similarly (Fig. 7c). When the model was run using measured xylem sap composition,  $0.76 \text{ mmol l}^{-1} \text{ N}$  and  $46 \text{ mmol l}^{-1}$  (Gregg 1991, Table 1), it yielded asymptotic values of net photosynthesis,  $N$ , and  $H$  similar to independent measurements (Fig. 7).

The influences of the carbon and nitrogen concentrations of xylem sap on asymptotic net photosynthetic rates, heterotrophy, and nitrogen concentrations are presented in Figs. 8 and 9, respectively. Low xylem nitrogen concentrations and high xylem C concentrations had similar effects, decreasing photosynthetic rates, increasing heterotrophy, and decreasing foliar nitrogen concentrations (Figs. 8 and 9).

## Discussion

This study revealed that mistletoes responded to nitrogen fertilization of their hosts with a threefold increase in photosynthetic rates (Fig. 2) while maintaining relatively constant stomatal conductance (Fig. 3). These gas-exchange responses resulted in clear increases in two measures of water-use efficiency: the ratio  $A/g$  (Fig. 5) and carbon isotope ratio (Fig. 6). These responses were among those predicted by earlier formulations of the nitrogen-parasitism hypothesis (Schulze et al. 1984; Ehleringer et al. 1985), which states that mistletoes maintain low water-use efficiency as a means of obtaining nitrogen from the host sufficient to meet their growth requirements. Through further analysis of our data we have refined this interpretation.

Photosynthetic rates of most plants are increased by improved nitrogen status (Field and Mooney 1986; Evans 1989; Sinclair and Horie 1989); in this the mistletoes are not unusual. Similarly, nitrogen-induced increases in  $A/E$  have previously been observed (von Caemmerer and Farquhar 1981; Toft et al. 1989; Heitholt et al. 1991). The increases in  $A$  and  $A/E$  of mistletoe and host were generally similar in direction and magnitude in this study. Therefore, to interpret these data in terms of parasitism it is necessary to concentrate on those characters that are unique to the mistletoe, specifically low  $A/N$ , low  $A/g$ , and heterotrophic carbon gain.

If nitrogen deficiencies have been an important selective force so that mistletoe gas exchange has been modified to enhance nitrogen assimilation, then it seems unlikely that  $A/N$  would have been sacrificed in the process.  $A/N$  varies remarkably little among  $C_3$  plants (Field and Mooney, 1986). However, as shown in Table 2 and Fig. 2,  $A/N$  of the mistletoes is much lower than that of their hosts. Where variation in  $A/N$  is observed it is often interpreted as the consequence of a tradeoff between  $A/N$  and  $A/E$ , presumably because the low internal  $CO_2$  concentration ( $c_i$ ) necessary for high  $A/E$  results in decreased availability of  $CO_2$  at the sites of carboxylation (Field et al. 1983; Lajtha and Whitford 1989; Reich et al. 1989). However, the low  $A/N$  in mistletoes is not caused by low  $c_i$ ; internal  $CO_2$  concentrations in mistletoe leaves are uniformly high ( $>280 \mu\text{mol mol}^{-1}$ ; see Marshall et al. 1993). Therefore, another explanation for low  $A/N$  must be sought.

We propose that the low  $A/N$  of mistletoes might be a result of assimilation of heterotrophic carbon. It can be demonstrated that mistletoe  $A/N$  would be similar to that of other plants if it were corrected to account for assimilation of xylem carbon as well as photosynthetic carbon assimilation, as follows:

$$A/N_{\text{total}} = A_{\text{max}} \frac{(1 + H/(1 - H))}{N} \quad (6)$$

where  $A/N_{\text{total}}$  accounts for assimilation of xylem solutes in addition to photosynthesis and  $H$  is proportional heterotrophy (Eq. 3). When the data in Table 1 are corrected by Eq. 6, they yield a mean  $A/N_{\text{total}}$  of  $18.6 \mu\text{mol mol}^{-1} \text{N}$

$s^{-1}$ , 2.4 times the  $A/N$  calculated from net photosynthesis only and approximately equal to the  $A/N$  of the juniper. A similar correction may be applied to  $A/E$  of the mistletoe:

$$A/E_{\text{total}} = A/E_{\text{photosynthetic}} \times (1 + H/(1 - H)) \quad (7)$$

Equation 7 also yields  $A/E$  estimates similar to those of the juniper ( $71$  vs.  $75 \mu\text{mol mol}^{-1}$ ). Based on these analyses, we conclude that neither  $A/E$  nor  $A/N$  is particularly low when expressed in terms of summed autotrophic and heterotrophic carbon assimilation.

Similar fixation of xylem solutes probably occurs in the leaves of the host. However, if such fixation happens in the host it cannot be considered net assimilation on the whole-plant level; it is instead redistribution of fixed carbon with the plant. In contrast, mistletoe assimilation of xylem carbon from the host does contribute to the net carbon budget of the mistletoe plant. Therefore "heterotrophic" carbon fixation by the host leaves can be neglected when comparing mistletoes to hosts.

Another consideration in the interpretation of low mistletoe  $A/N$  is the presence of large quantities of nitrogen in metabolically inactive storage forms. Ehleringer et al. (1986) presented evidence of similarity in amino acid profiles between juniper and mistletoe leaves, except that arginine, which contains four N atoms per amino acid and is known to serve as a nitrogen storage compound, contained a considerably greater proportion of total N in the mistletoe than in the juniper leaves. We estimate from their data that arginine contained 31% of the nitrogen in mistletoes and only 13% in juniper leaves. We thus estimate that perhaps 18% of the nitrogen in mistletoe leaves is in storage forms; this would be sufficient to decrease  $A/N$ , but not to halve it.

The model integrating mistletoe gas exchange and nitrogen nutrition provides a means of analyzing the web of costs and benefits and to analyze the long-term consequences of low  $A/E$  and  $A/N$  in mistletoes. It accounts for the interrelated benefits of photosynthetic carbon dioxide assimilation, parasitic assimilation of carbon solutes from host xylem water, and assimilation of nitrogen-containing solutes from host xylem water. The model is able, with only a few assumptions, to make accurate predictions of photosynthetic rates, nitrogen concentrations and proportional heterotrophy from xylem sap composition and transpiration rates. The model also estimates relative growth rate as  $76\% \text{ year}^{-1}$  for a 200-day growing season, which, after correction upward by 20% to account for reproductive effort (Dawson et al. 1990b), agrees qualitatively with previous estimates of  $50\% \text{ year}^{-1}$  for mistletoes that are 5–10 years old (Schulze and Ehleringer 1984).

Perhaps most important, the model explains low photosynthetic  $A/E$  of mistletoes as a long-term consequence of nitrogen dilution resulting from assimilation of carbon from xylem sap. Whether the model run is started at high or low initial nitrogen concentrations ( $N$ ), the mistletoe converges on a value of  $N$ , net photosynthetic rate ( $A$ ),



and a proportional heterotrophy ( $H$ ) determined by xylem sap composition (Fig. 7). The point of convergence is determined by the balance of C and N assimilation. A mistletoe with high nitrogen status would have a high photosynthetic rate (Eq. 1). It would also import xylem carbon and small amounts of xylem nitrogen. The net effect, over time, would be a fall in the nitrogen concentration due to rapid carbon assimilation, both autotrophic and heterotrophic. As nitrogen concentration fell net photosynthetic rates would fall as well, reducing net carbon gain and thus slowing the decline in nitrogen concentration. This process would continue until an asymptote was approached. The reverse would occur, but ending at the same asymptote, in a mistletoe starting at low nitrogen concentration.

In the absence of xylem carbon assimilation a much higher nitrogen concentration would be maintained (0.66 vs. 0.50 mmol g<sup>-1</sup>, Figs. 7 and 8). Similarly, a higher photosynthetic rate would be maintained (6.4 vs 4.2 nmol g<sup>-1</sup> s<sup>-1</sup>, Figs. 7 and 8). Given that the model assumes constant transpiration, the increase in the photosynthetic rate translates directly to an increase in  $A/E$ . Increased  $A$  in the absence of xylem carbon assimilation would make up about 60% of the 150% difference in  $A/g$  between juniper and mistletoe (Fig. 4).

We revisit the question, do mistletoes maintain high  $E$  and low  $A/E$  to obtain sufficient nitrogen for growth? Marshall et al. (1994) have argued that absolute rates of carbon and nitrogen gain may be determined by the mistletoe's need to match the relative growth rate of the host to prevent either being overgrown by the host tissue or, at the other extreme, growing out of balance with the hydraulic bottleneck at the haustorium. Thus high  $E$  would indeed be necessary to meet the nitrogen requirements of growth if nitrogen concentration of xylem water were low or if the host branch grew rapidly.

Based on the results of these experiments, we conclude that high  $E$  and low  $A/E$  are maintained for quite different reasons. Low  $A/E$  is a necessary consequence of low nitrogen concentrations, which are in turn a consequence of having two routes of carbon assimilation. Although  $A/E$  of *Phoradendron juniperinum* is low relative to that of the host, the ratio of total carbon gain to transpiration is quite similar between mistletoe and host if one accounts for heterotrophic assimilation of xylem carbon. Similarly, although  $A/N$  is considerably lower in the mistletoe, the ratio of total carbon gain to nitrogen concentration becomes quite similar to that of the host if xylem carbon assimilation is accounted for. Under these circumstances it seems likely that the peculiarities of mistletoe gas exchange are beneficial for life as a parasite rather than a historical accident attributable to decreased selective pressure on autotrophic metabolism. The interpretation of high rates of transpiration as a means of acquiring sufficient nitrogen for growth (Schulze et al. 1984) is therefore supported. However, low photosynthetic water-use efficiency may simply be an unavoidable consequence of nitrogen dilution due to excess capacity for carbon assimilation.

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