

SEXES SHOW CONTRASTING PATTERNS OF LEAF AND CROWN CARBON GAIN IN A DIOECIOUS RAINFOREST SHRUB¹

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The sexes of dioecious species may differ in a range of vegetative and reproductive traits as well as in physiological traits. In *Siparuna grandiflora*, a Neotropical dioecious shrub, we examined differences in leaf-level photosynthesis of different classes of leaf age and, using simulation models, explored whether differences in leaf-level carbon gain led to sex differences in whole-plant daily carbon gain. Male plants had higher photosynthetic capacity at the leaf level. As leaves of both sexes aged their photosynthetic capacity and specific leaf area declined as expected. Simulations of daily carbon gain using the architecturally explicit model Y-Plant and a non-architectural model incorporating a wide range of realistic light environments revealed that the difference in leaf-level photosynthetic capacity did not translate into greater crown-level carbon gain for males. Rather, differences in patterns of allocation to leaf area allow females to achieve higher crown-level carbon gain. The results demonstrate that sex differences at the leaf level do not necessarily predict patterns at the whole-plant level.

Key words: crown architecture; daily carbon gain; dioecy; leaf age; photosynthesis; sexual dimorphism; *Siparuna*.

Male and female plants of dioecious species may differ in many leaf and whole-plant traits, including life history, reproductive allocation, phenology, and growth (see references in Delph, 1999, and Nicotra, 1999a, b). Yet, few studies have attempted to link physiological processes at the leaf level to patterns at the whole-plant level (Dawson and Geber, 1999; Retuerto et al., 2000). In the dioecious tropical understory shrub *Siparuna grandiflora* (Siparunaceae), allocation to reproduction in females exceeds that of males. Only females suffer significant indirect costs of reproduction in terms of lost growth and decreased likelihood of reproduction in the subsequent season (Nicotra, 1999a). Despite these differences in reproductive allocation and indirect costs, reproductively mature females and males have equivalent growth rates on average, and before reproduction, female plants actually have greater rates of leaf production and stem length growth (Nicotra, 1999b). These findings present a paradox: How do reproductively mature female plants maintain equivalent growth rates in similar understory environments, while incurring higher reproductive cost? In this study of *Siparuna grandiflora*, we explored whether there are differences between the sexes in photosynthetic carbon gain traits at the leaf level. We determined whether such differences in photosynthesis might be influenced by reproductive state. We further examined whether differences in leaf-level carbon gain lead to sex differences in whole-plant daily carbon gain.

To gain better insight into the potential adaptive value of

sex-based differentiation in leaf-level traits, it is essential to ascertain the impact of those leaf-level traits at the whole-plant level. Although studies of physiological differentiation in other dioecious species have explored differences in leaf-level carbon gain and water use, few general patterns emerge. Sexual differentiation in nonreproductive traits may arise in dioecious species as innate traits that are adaptations to different optimal reproductive functions in the sexes. Alternatively, these functional differences may be apparent only at particular times in a plant's life and as a direct effect of reproductive allocation. In some species males have higher photosynthetic rates (per unit leaf area) than females, but these differences do not necessarily result in growth differences (Dawson and Bliss, 1989a, b; Marshall et al., 1993; Gehring and Monson, 1994; Laporte and Delph, 1996).

Discrepancies between leaf-level traits (such as photosynthetic capacity) and whole-plant traits (such as growth rate) are not uncommon, and they compromise our ability to assess the importance of variation in leaf-level physiology to whole-plant performance. Discrepancies arise, in part, because leaf measurements are often made at saturating light levels on leaves of a single age at a single point in time, whereas the whole plant is composed of leaves of many ages as well as stems, roots, and reproductive structures, all in a complex spatial array (Chazdon et al., 1988; Chazdon and Montgomery, 2001). The effects of leaf age on carbon gain in particular are critical to relationships between leaf-level photosynthetic characteristics and whole-plant function (Field, 1983; Field and Mooney, 1983; Dawson and Bliss, 1993). The photosynthetic capacity of a leaf increases until near full leaf expansion then declines as the leaf ages and as microenvironmental conditions change (Field and Mooney, 1983). Age-dependent changes in carbon gain are associated with structural, biochemical, and microenvironmental changes (Dawson and Bliss, 1993). Species-level traits, such as leaf longevity and average photosynthetic capacity, may also influence the pattern of decline of

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photosynthetic capacity (Chabot and Hicks, 1982). In addition to specifics of the age-related decline in carbon gain capacity, age distribution and the total number and spatial arrangement of the leaves are likely to have a major impact on realized total plant carbon gain and growth rates (e.g., Chazdon, 1985; Dawson and Bliss, 1993). Sex differences in the pattern of decline in photosynthetic rates with age, the spatial distribution of leaves, and patterns of longevity and phenology in dioecious species have not been investigated before.

The ability of female *S. grandiflora* plants to achieve greater pre-reproductive growth and equivalent post-reproductive growth could depend on each of the mentioned factors and upon interactions among them. Here we characterize the effect of leaf age on photosynthetic light response at the leaf-level to determine whether there are sex-based differences in light response within and among leaf age classes. To assess the direct impact of reproduction on carbon gain, we compare carbon gain in reproductive plants with those found in equivalent microenvironments in which reproduction is suppressed. Finally, we use two simulation approaches to assess differences in whole-plant carbon gain. To assess whether sex-based differences in patterns of leaf display or architecture influence whole-plant carbon gain, we applied the architecturally explicit simulation model, Y-Plant (Percy and Yang, 1996). To compare patterns of whole-plant carbon gain in a larger sample of plants and over a wide range of light environments, we simulated carbon gain using a non-architectural model. Both models incorporated effects of leaf age and were based on measured values of leaf area on real plants. In combination, the approaches enable us to determine whether leaf-level traits lead to differences in carbon gain between the sexes at the whole-plant level.

MATERIALS AND METHODS

Study site and species description—The study was conducted at the Organization for Tropical Studies' La Selva Biological Station in a Costa Rican lowland wet tropical forest (for detailed site description, see McDade et al., 1994). *Siparuna grandiflora* is a common understory shrub in secondary forests of the Sarapiquí region of Costa Rica. The shrub flowers at just over 1 m in stem length and can reach over 5 m in height. Leaves are opposite and decussate on the main stem. Plants generally consist of between one and three main stems with few other branches. Leaves are held nearly horizontal, drooping toward the tips, and reach sizes exceeding 250 cm². At La Selva, flowering occurs primarily between January and May. The red flowers (2–4 mm diameter) are borne cauliflorously; fruits (10–25 mm) mature between June and December (Antonio, 1983). There are no records of *S. grandiflora* or any other *Siparuna* switching sex, nor did any individual change sex during the course of this study (A. Nicotra, personal observation; Antonio, 1983; Feil, 1992).

Two groups of study plants were used. Leaf age differences and simulations of whole-plant carbon gain were investigated on plants grown from cuttings in field plots. Results of growth analyses for these plants were published in Nicotra (1999b). The plants used to determine whether reproductive state influenced carbon gain were naturally occurring plants measured in situ. The impact of reproduction on growth in these plants was previously published (Nicotra, 1999a).

Effects of leaf age on carbon gain—The plants used in the study of leaf age differences were grown from cuttings in 10-m² field plots established in two small existing light gaps in the species' natural secondary rainforest habitat. The cuttings were planted into the plots in May 1994, using a structured random approach, so that the sexes and stock genotypes were distributed evenly throughout plots (for details of cutting sources, selection and propagation see Nicotra, 1999b). Growth was measured on these plants every 3 mo

between June 1994 and 1996. We could therefore determine the age of all leaves on the plant within a 3-mo range. Average leaf lifespan for *S. grandiflora* plants of both sexes in the field is 14 mo (Nicotra, 1999b). In the plots, few leaves were more than 12 mo old, although there were many leaves in the 9- to 12-month cohort, suggesting that leaf longevity in the plots was similar to that of naturally occurring plants. In June 1996, the cuttings were just over 2 yr old and were reproducing for the first time; males had reached the end of the flowering season and females were maturing fruits.

The width of each leaf on 23 female and 38 male plants was measured between June and July 1996. We used a regression model to estimate leaf area per cohort from leaf width measurements. To derive the regression model, the perimeter of 47 leaves from five age cohorts was traced in June 1996. Leaf width was measured and area determined using a leaf area meter (LI-COR, Lincoln, Nebraska, USA). A regression revealed a tight fit between width and leaf area ($r^2 = 0.91$). The sexes did not differ in slope of the regression line.

For gas-exchange measurements, one leaf from each of four leaf-age cohorts (<2, 2–5, 6–8, and 9–12 mo) was selected from each of four reproductively mature plants per sex. The most recently initiated fully flattened leaf on the plant was chosen as the youngest leaf (<2 mo); this youngest leaf had not yet achieved full area. There were insufficient leaves in the >12-mo cohort to enable measurement.

Gas exchange was measured using a LI-COR 6200 portable photosynthesis system. Saturating light levels were obtained using a 150-W halogen bulb. Light was reflected off a heat-absorbing mirror to minimize temperature increases in the chamber. Light level was controlled using a dimmer and measured using a photodiode mounted on the leaf within the chamber. The photodiode was connected to a Campbell 21× data-logger and calibrated to the photosynthetically active radiation (PAR) sensor mounted on the photosynthesis system. For all measurements, CO₂ concentrations were between 345 and 365 ppm, relative humidity ranged from 60 to 85%, and leaf temperatures did not exceed 35°C. Measurements were not taken after 1300 or when cuvette temperature exceeded 35°C. Prior to measurement, each leaf was illuminated at a 1200 μmol-m⁻²·s⁻¹ for 30 min so that photosynthesis was fully induced. Light level was then raised to 1400 μmol-m⁻²·s⁻¹ and lowered incrementally to 0 μmol-m⁻²·s⁻¹. There was no indication of photo-inhibition at these light levels. The leaf was allowed to equilibrate to each new light level between measurements. Before dark respiration was measured, the leaf was covered with black fabric and left in the dark for at least 10 min or until CO₂ levels in the closed chamber were consistently rising. Measurements were more numerous at lower light levels on all plants (~5 at light levels below 300 μmol), but exact matching of light levels for each measurement was not achieved among plants.

Specific leaf area (SLA) and chlorophyll concentration were determined for each leaf using leaf disks collected 7 to 10 d after gas exchange measurements. Chlorophyll was extracted following the method of Moran and Porath (1980). Chlorophyll concentration was determined according to the equations of Inskeep and Bloom (1985). Nitrogen content was determined for each leaf following Kjeldahl tissue digestion. Samples were analyzed using the salicylate/nitroprusside method and a Technicon Auto-Analyzer (Anderson and Ingram, 1993).

Effect of reproductive state on carbon gain—Naturally occurring reproductively mature plants were used to study the impact of reproductive activity on photosynthetic capacity. The treatments used in the gas exchange measurements were controls (reproductive, no manipulation) and suppressed (all flower buds removed from plants as soon as a flower had matured and plant could be sexed, early to mid-January 1995). Suppression effectively prevented the plants from allocating resources to flowering and, for females, also prevented allocation of resources to fruiting.

Ten plants of each sex were selected from each treatment ($n = 40$ plants). Gas exchange was measured in June 1995 when females were maturing fruit and males had ceased flowering. One healthy, fully expanded leaf (approximately 3 mo post-initiation) was measured on each plant using a LI-COR 6200 portable photosynthesis system as described earlier except that light

TABLE 1. Parameters obtained for light response curves for each combination of sex and leaf age. Data from four leaves were used to fit each curve. For details of curve-fitting methodology see text.

Leaf age (mo)	Sex	Pmax (μmol·m ⁻² ·s ⁻¹)	Quantum yield	Respiration (μmol·m ⁻² ·s ⁻¹)	Curvature term	r ²
<2	Female	6.694	0.049	-0.636	0.799	0.96
	Male	7.663	0.045	-0.367	0.066	0.85
2-5	Female	5.592	0.061	-0.448	0.195	0.94
	Male	8.630	0.065	-0.440	-0.047	0.90
6-8	Female	6.039	0.043	-0.351	0.631	0.86
	Male	7.315	0.044	-0.365	0.577	0.87
9-12	Female	3.723	0.049	-0.407	0.209	0.93
	Male	4.554	0.052	-0.366	0.236	0.76

levels were adjusted using screen filters. Leaf nitrogen content was analyzed as described earlier.

Leaf display and daily whole-plant carbon gain—Y-Plant simulation—The Y-Plant simulation model was used to assess differences in plant architecture between the sexes and to determine whether such differences influenced whole-plant light interception capacity (Pearcy and Yang, 1996). Y-Plant uses geometrical measures to reconstruct the projected canopy of each plant. Inputs include all elevation angles, azimuths and lengths of stem, branch, and leaf such that a three-dimensional reconstruction can be made of each plant. Absorbed light is calculated throughout the day for all leaf surfaces using a hemispherical canopy photograph, coupled with physiological data, and used to estimate a daily photosynthetic rate for the whole plant. We developed models for three representative plants of each sex (six plants total) at a single representative understory microsite. Y-Plant simulations gave projected leaf area and displayed leaf area of the crown as a function of measured azimuth and solar elevation angle for individual plants. Projection efficiency (E_p) is calculated as the projected area of all leaves, divided by the total leaf area, thus accounting for deviations in leaf angle from horizontal but not for self-shading. Display efficiency (E_d) includes the effect of leaf overlap, as well as angle. Thus, the difference between E_p and E_d provides an index of leaf overlap or self-shading within the crown (Pearcy and Yang, 1996).

Y-Plant also estimates a daily assimilation rate taking into account patterns of self-shading (A_{day} , measured as moles of CO₂ per square meter per day). At each sampling step, the simulation model computes the whole-plant instantaneous assimilation rate (in diffuse and direct light). We specified a 30-min time step, which provided good resolution of whole-plant photosynthetic measurements, while minimizing computation times. The instantaneous photosynthetic rate is based on the calculation of light absorption of the cross-sectional area of the crown at each time step. This measure incorporates the changing leaf area exposed to the sun during the day and accounts for shifts in self-shading as the sun moves across the sky. These instantaneous rates are integrated to yield total daily whole-plant assimilation rate. We estimated daily carbon gain by multiplying the whole-plant rate by the total leaf area calculated by the model. This estimate represents a simplification since the effective leaf area is less than the total leaf area.

Non-architectural simulations—Geometric measurement of plants for fitting of Y-Plant models is extremely labor intensive, taking approximately 1 d per plant. To simulate whole-plant carbon gain on a larger sample of plants, we developed a simple non-architectural model that incorporated measured leaf area data and light response curves fit for each leaf age cohort by sex combination. Simulations assume a single set of photosynthetic rates for each leaf cohort and sex category and do not incorporate any effects of light acclimation. The non-architectural model assumed that all foliage was horizontal and that there was no within-crown shading. Simulations of whole-plant daily carbon gain were made under eight different light regimes using light data from representative habitats. Instantaneous light readings (PAR, measured as micromoles per square meter per second) were collected every 5 s and averaged to 30 s from 0600 to 1700 on a single day in November 1990. Thirty-second averages allowed most sunflecks to be detected. Readings were re-

corded along a transect through a large gap (projected area 300 m²) and into the understory using photodiodes (calibrated to a quantum sensor) connected to a Campbell 21× datalogger. Data from eight sensors ranging in daily PFD from 0.68 to 9.13 mol·m⁻²·d⁻¹ were used in simulations. Naturally occurring individuals of *S. grandiflora* individuals have daily PFD levels ranging from 0.13 to 9.86 mol·m⁻²·d⁻¹, with a mean of 3.78 mol·m⁻²·d⁻¹; the sexes do not differ in average light level (A. B. Nicotra, unpublished data; Nicotra, 1998). At each time step, instantaneous photosynthetic rate for each leaf age cohort was multiplied by the measured leaf area for that cohort and by 30 s to give a total carbon gain per time increment per leaf area. Totals for each plant were summed over the day to calculate daily carbon gain. Because the data set contained only 21 females with leaves in at least two cohorts, 21 males were selected at random from the total of 38 male plants for which we had leaf area data. All simulations were done using Microsoft Excel.

Analysis—Curves were fit to the photosynthetic light response data following the equation of Marshall and Briscoe (1980):

$$A = (\phi I + A_{max} - \sqrt{(\phi I + A_{max})^2 - 4\phi\theta I A_{max}}) / 2\theta - R$$

where A is the photosynthetic rate, ϕ is the quantum yield, I is the irradiance level, A_{max} is maximum photosynthetic capacity, θ is a curvature term, and R is the dark respiration rate. Curves were fitted using MacCurveFit (Raner, 1992). Light response curves fit for each leaf had r^2 of 0.98 or better. ANOVA was conducted on photosynthetic capacity, respiration rate, quantum yield, and the light compensation point obtained from the curve fit to measured light response for each leaf. ANOVA models included terms for sex and leaf age and the interaction between these terms. Leaf nitrogen, SLA, and chlorophyll data were analyzed using ANOVA models with terms for sex and leaf age and the interaction between them. All variables except chlorophyll content met assumptions of homogeneity of variance and normality. Chlorophyll content was transformed to achieve normality. Data on reproductive states were analyzed using two-way ANOVA. Scheffé post-hoc tests were used to test for differences among means.

Curves fitted to aggregated data for each sex by leaf age combination were used in simulations of daily carbon gain (r^2 ranged from 0.72 to 0.96, Table 1). Data from the non-architectural model were analyzed using repeated measures ANOVA, including fixed terms for sex and light with plant as a random effect. Analyzed data were log transformed to meet assumptions of normality. All analyses were done using Datadesk software (Data Description, Ithaca, New York, USA) and GenStat (2000).

RESULTS

Leaf age and light response—Males had higher saturated area-based leaf-level photosynthetic rates than females at every leaf age (Fig. 1). By analyzing parameters estimated from the full light response curves, we were able to test for differences in leaf-level photosynthetic capacity, dark respiration rates, quantum yield of the light response, and the light compensation point for all leaf age classes (Table 2, Fig. 2). Males had significantly greater photosynthetic capacity than females

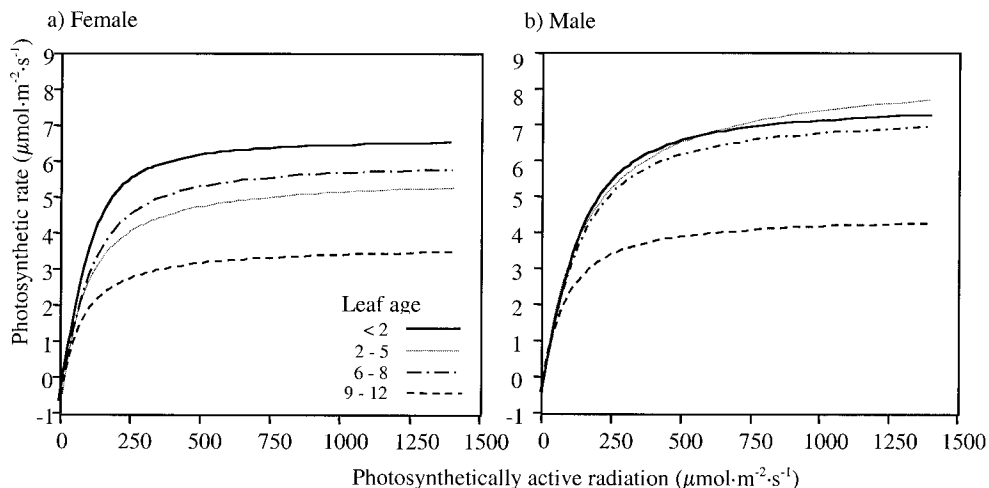


Fig. 1. Photosynthetic light response curves for *Siparuna grandiflora* leaves of four ages on male and female plants. Each curve was fit to the data from four leaves.

(mean [\pm SE] of 6.83 [\pm 0.54] and 5.59 [\pm 0.39] $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively), and the oldest leaf age cohort had significantly lower photosynthetic capacity than the three younger cohorts (Fig. 2a). Males had slightly greater quantum yield than females (mean [\pm SE] of 0.054 [\pm 0.003] and 0.048 [\pm 0.002], respectively) yielding a marginally significant sex effect for quantum yield. Quantum yield did not vary with leaf age. Sex and leaf age did not significantly affect dark respiration rate or light compensation point.

Leaf age and nitrogen, chlorophyll, and SLA at the leaf level—The youngest leaf cohort had lower nitrogen content and significantly greater SLA than older cohorts, which were fairly uniform for both traits (Table 2, Fig. 2b, c). Because they had greater photosynthetic rates at lower leaf nitrogen content on an area basis, the youngest leaves also had greater photosynthetic nitrogen use efficiency (PNUE, measured as micromoles per gram N per second, Table 2, Fig. 2d). As would be expected given their leaf nitrogen content, the youngest leaves had significantly lower chlorophyll content than older cohorts (Table 2, Fig. 2e). The youngest leaves also had

higher chlorophyll a/b ratios than all other leaf age cohorts, and significantly more so than the 6–8- or 9–12-mo-old leaves (Table 2, Fig. 2f).

Males had slightly (but not significantly) more chlorophyll than females (mean [\pm SE] of 609.9 [\pm 35.3] and 545.7 [\pm 35.8] $\mu\text{mol}/\text{m}^2$, respectively, $P = 0.08$, Table 2), but there were no significant sex differences for chlorophyll a/b ratio, leaf nitrogen content, or SLA. Because males achieved greater photosynthetic capacity at equivalent nitrogen levels to females, males had greater PNUE on average; but this difference was not statistically significant ($P = 0.17$).

Reproductive state and carbon gain—In both sexes, reproduction was associated with decreased mass-based, leaf-level photosynthetic capacity relative to plants in which reproduction was suppressed ($F_{1,36} = 6.47$, $P = 0.02$ for effect of reproductive state, Fig. 3). Males had marginally higher photosynthetic capacity on a mass basis than females regardless of treatment ($F_{1,36} = 4.02$, $P = 0.05$ for sex effect). The pattern, though similar for area-based measurements was not significant because of variance in SLA. There was no significant

TABLE 2. Results of ANOVA examining effects of sex and leaf age on (A) gas exchange parameters obtained from curves fit to light response curves for individual leaves; (B) leaf nitrogen content, specific leaf area (SLA), and photosynthetic nitrogen use efficiency (PNUE); and (C) leaf chlorophyll content and chlorophyll a/b ratios.

	Sex (df = 1)		Leaf age (df = 3)		Sex \times Leaf age (df = 3)		Error	
	MS	P	MS	P	MS	P	df	MS
A) Gas exchange								
Pmax ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	12.286	0.034	14.404	0.004	1.716	0.558	24	2.431
Respiration rate ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	0.009	0.532	0.036	0.211	0.025	0.369	24	0.022
Quantum yield ^a	0.027	0.060	0.006	0.467	0.007	0.391	23	0.007
Light compensation point	14.629	0.280	12.779	0.381	6.968	0.632	24	11.951
B) Leaf nitrogen and SLA								
Leaf nitrogen (g/cm ²)	0.056	0.424	0.627	≤ 0.001	0.032	0.770	24	0.085
Specific leaf area (cm ² /g)	0.001	0.708	0.047	≤ 0.001	0.002	0.664	24	0.005
PNUE ($\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{s}^{-1}$)	1.189	0.174	7.622	≤ 0.001	0.427	0.559	24	0.606
C) Chlorophyll content								
Chlorophyll ($\mu\text{mol}/\text{m}^2$)	32 881.0	0.084	116 075	≤ 0.001	5078.9	0.685	24	10 138
Chlorophyll a/b ratio	2.049	0.252	8.645	0.004	0.973	0.588	24	1.488

^a One extreme outlier was excluded so that data could be transformed to normality.

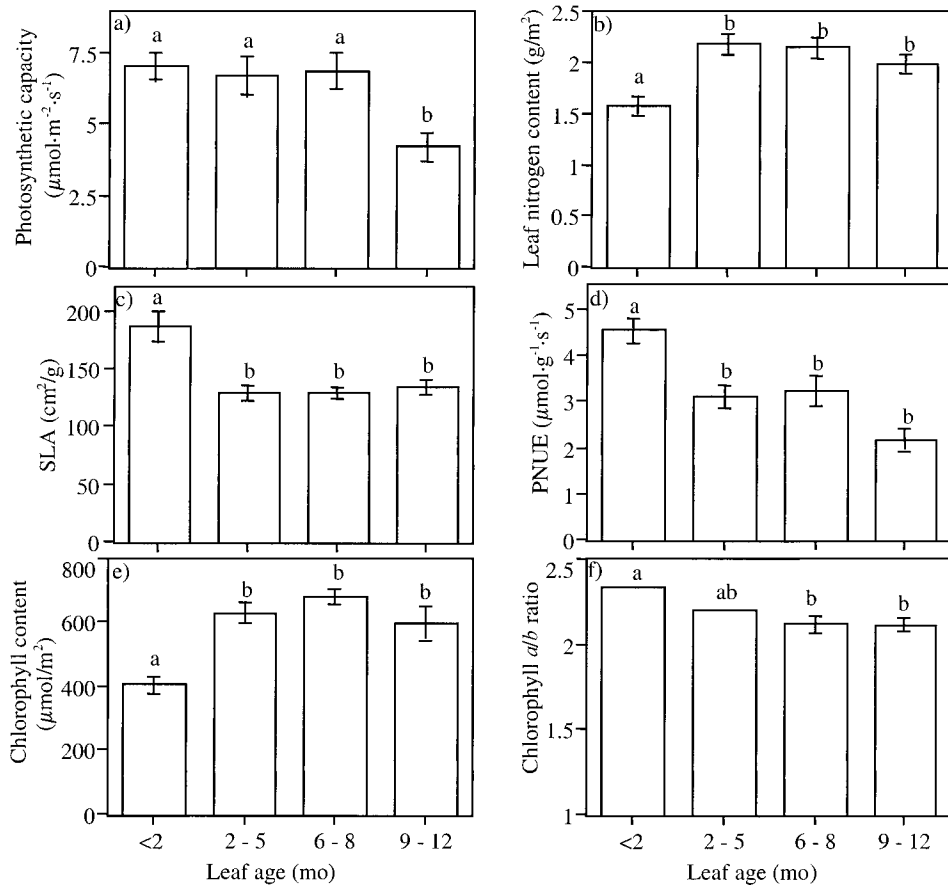


Fig. 2. Photosynthetic capacity, specific leaf area (SLA), nitrogen, photosynthetic nitrogen use efficiency (PNUE), and chlorophyll content of leaves of four different ages averaged across sex (means ± 1 SE). Different letters indicate statistically significant differences among means for leaf age according to Scheffé post-hoc tests. Where error bars are not apparent, they were too small for resolution.

interaction between sex and reproductive state. There were no differences in nitrogen content between sexes or reproductive states.

Whole-plant leaf area—Females had significantly (1.7 times; ANOVA, $F_{1,40} = 6.93, P = 0.01$) more total leaf area than males. The sexes did not differ in proportional allocation of leaf area to different leaf age cohorts (Fig. 4, analysis not shown). There is no difference between the sexes in leaf size.

The greater leaf area on female plants is a result of greater leaf production rate (measured on these same plants; Nicotra, 1999b).

Leaf display and daily whole-plant carbon gain—Leaf angles calculated using whole-plant averages by Y-Plant for three plants per sex were 41° and 37° for females and males, re-

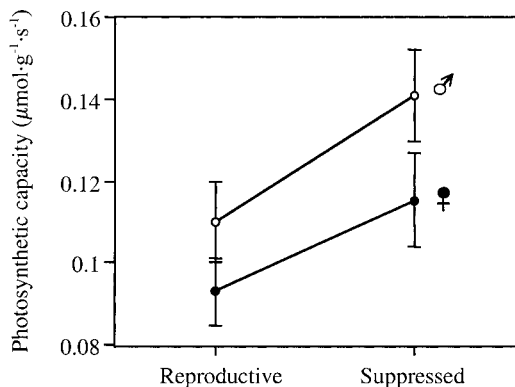


Fig. 3. Photosynthetic capacity of individual leaves on a mass basis for male and female plants as a function of reproductive state (means ± 1 SE).

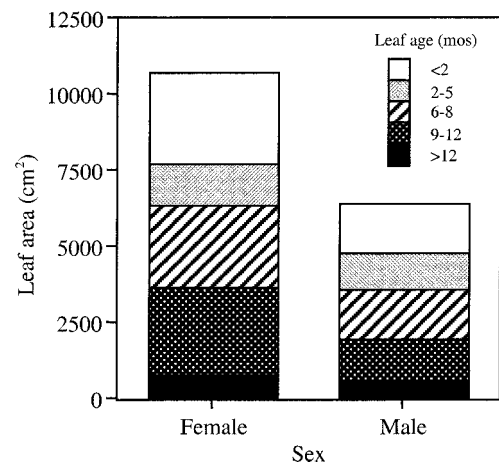


Fig. 4. Total plant leaf area, divided into leaf-age cohorts for each sex.

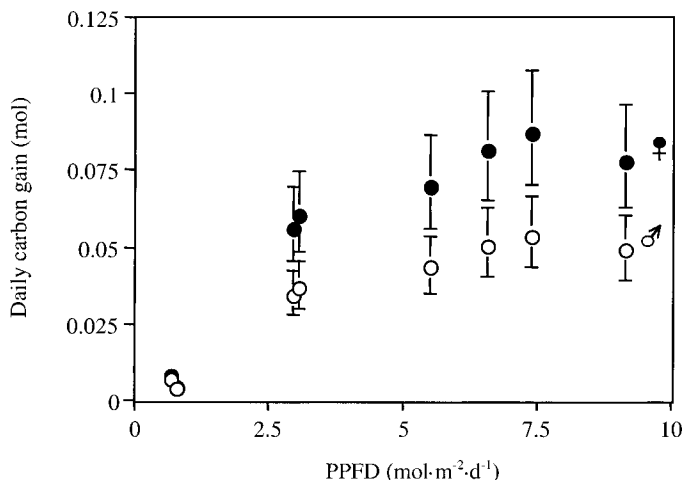


Fig. 5. Results of simulation of whole-plant daily carbon gain by males and females. Asterisks indicate significant differences between the sexes (means \pm 1 SE).

spectively, and did not differ significantly between the sexes (ANOVA, $F_{1,4} = 0.16$, $P = 0.71$). Nor did the efficiency with which the leaves were projected (E_p) or displayed (E_d) differ between the sexes (ANOVA E_p , $F_{1,4} = 0.002$, $P = 0.96$; ANOVA E_d , $F_{1,4} = 2.65$, $P = 0.25$). Females had equivalent mean E_d (0.55) and slightly lower mean E_p (0.37 vs. 0.44) than males.

Y-Plant was also used to estimate whole-plant assimilation rates for three plants of each sex at a typical understory light level (similar to the lowest light levels used in non-architectural model below) based on 30-min averages of light absorption, including effects of leaf overlap. Rates did not differ between the sexes (0.0315 and 0.0319 mol CO₂·m⁻²·d⁻¹; female and male, respectively, ANOVA, $F_{1,4} = 0.01$, $P = 0.94$). When the estimated rate was multiplied by actual leaf area to determine whole-plant carbon gain, females assimilated 0.0217 mol CO₂/d, whereas males assimilated 0.0130 mol CO₂/d. These estimates were quite variable within sex and whole-plant carbon gain did not differ significantly with sex (ANOVA, $F_{1,4} = 2.05$, $P = 0.23$).

The non-architectural model provides a robust opportunity to assess daily carbon gain under a wide range of light levels at a very small time scale (eight environments at 30-s intervals vs. one light environment at 30-min intervals for Y-Plant). Given that the sexes did not differ in leaf display patterns, the results are likely to be representative of a more explicit architectural model. The non-architectural model demonstrated significant sex-based differences in whole-plant diurnal carbon gain at light levels above 2 mol·m⁻²·d⁻¹ (Fig. 5, Table 3). There were no differences between the sexes in whole-plant rate of carbon gain (measured as moles per square centimeter per day, analysis not shown). Both male and female plants had higher diurnal carbon gain at higher light levels. Males, despite having a higher photosynthetic capacity at the leaf level, did not achieve greater whole-plant daily carbon gain. Rather, females achieved greater daily carbon gain at all but the lowest light levels. The greater whole-plant carbon gain of females was due to higher whole-plant leaf area.

DISCUSSION

Males of *S. grandiflora* had greater photosynthetic capacity and nitrogen content than females regardless of leaf age or

TABLE 3. Results of repeated measures ANOVA examining effects of sex and leaf age on daily carbon gain (log) in eight different light environments.

Source of variation	df	SS	MS	VR	F_{prob}
Between subjects (plant stratum)					
Sex	1	11.95	11.95	3.16	0.083
Error	40	151.52	3.79	711.95	
Within subjects					
Light	7	322.91	46.13	8670.31	<0.001
Sex \times Light	7	2.89	0.41	77.56	<0.001
Error	280	1.49	0.01		
Total	335	490.76			

reproductive state. Despite these differences, simulations of whole-plant carbon gain at a range of light levels indicate greater daily yield in females than males. The individual results each contribute to our understanding of the effects of leaf age, reproductive state, and light response on leaf-level carbon gain. Their synthesis explains the paradox presented in the introduction: how do field-grown females achieve average rates of stem growth and leaf production equivalent to males, despite greater reproductive allocation and measurable indirect costs of reproduction?

Sex-based differences in carbon gain traits, demonstrated in a number of dioecious species, are dependent on phenological state and measurement and growth conditions (see also Dawson and Bliss, 1989a, b; Marshall et al., 1993; Hogan et al., 1998; Jones et al., 1999; Retuerto et al., 2000). For example, in the arid-zone chenopod *Maireana pyramidata*, fruiting females have lower water-use efficiency than males, but non-fruiting females are not distinguishable from males (A. Leigh and A. B. Nicotra, unpublished data). Males of *Pistacia lentiscus* have higher photosynthetic capacity than females in the absence of stress, but equivalent rates and lower water-use efficiency under drought stress (Correia and Barradas, 2000). Notably, sex-based differences in carbon gain do not consistently lead to differences in growth rates (Dawson and Bliss, 1989a, b; Marshall et al., 1993; Gehring and Monson, 1994; Laporte and Delph, 1996; Retuerto et al., 2000). In some species, sex-based differences in ecophysiological traits do not even appear biologically significant (Liebig et al., 2001). Results to date therefore demonstrate that generalized patterns in physiological dimorphism will not be found between the sexes of dioecious species and that a broad understanding of the physiological differentiation in dioecious species requires investigations at multiple scales.

Leaf age—Photosynthetic capacity in *S. grandiflora* declined with leaf age in both sexes. The decline in photosynthetic capacity with leaf age most likely is influenced by decreasing SLA (Field, 1983; Reich et al., 1991; Reich and Walters, 1994) associated with an increase in leaf thickness and may be associated with increased investment in defense. Increased leaf thickness may increase relative stomatal limitations on photosynthesis by increasing resistance to diffusion of CO₂. The decline in photosynthetic capacity with age is generally associated with declining leaf nitrogen content and decreases in the activity of RuBP carboxylase (Field, 1983). In the present study, no decline in leaf nitrogen was detected. Rather, leaf nitrogen content was higher in leaves older than 2 mo and lower in the youngest leaf age class. Nor was the

decline in photosynthetic capacity with leaf age linear—there was a small difference in capacity among the three younger age classes, and the oldest age class had a marked decline. Long-lived leaves tend to exhibit a slower rate of photosynthetic decline with leaf age (Field, 1983). Clearly, in a species such as *S. grandiflora* that maintains a considerable proportion of leaf area in older leaves, decline in photosynthetic capacity with leaf age will have significant impact on whole-plant processes.

Reproductive state—In *S. grandiflora*, reproductive status also affected realized carbon gain. Reproductive plants of both sexes had lower photosynthetic capacity than plants in which reproduction was suppressed. Though some studies have demonstrated increases in carbon gain during reproduction, others, like the present study, demonstrate the reverse. Increases in carbon gain have been attributed to increased sink strength provided by developing fruits and flowers (Neales and Incoll, 1968; Herold, 1980; Reekie and Bazzaz, 1987; Saulnier and Reekie, 1995; Laporte and Delph, 1996; Giuliani et al., 1997; but see Hogan et al., 1998; Obeso et al., 1998). But, because reproduction also increases the demand for resources, such as nitrogen, at the whole-plant level, it may compete with photosynthetic processes. The tenets of resource allocation theory and evidence of demographic or somatic costs of reproduction in a variety of species (see references in Nicotra, 1999a; Rocheleau and Houle, 2001) indicate that resource limitation, not sink limitation, is more often the rule. Under conditions of light limitation, carbohydrates are not likely to accumulate. Under conditions of nitrogen limitation, assimilation is likely to be limited at the stage of carbon fixation. Consider the case of *Oenothera biennis* in which reproduction decreases photosynthetic rates at low nitrogen availability, but increases photosynthetic rates at high nitrogen availability (Saulnier and Reekie, 1995). The results of this study and of work on other woody species (Karlsson, 1994; Schechter et al., 1994; Obeso et al., 1998) suggest that among woody perennials, or perhaps any field-grown plant facing resource limitation, reproduction will be associated with a decrease in photosynthetic capacity.

Whether increased or decreased, the impact of reproduction on carbon gain might be expected to differ between the sexes in conjunction with different total reproductive allocation. In dioecious *Silene latifolia* and *Salix arctica*, photosynthetic capacity of females is greater during fruiting (Dawson and Bliss, 1989a; Laporte and Delph, 1996), but in the tree *Ilex aquifolium*, fruiting females have lower photosynthetic efficiency than non-fruiting females, and non-fruiting females have greater efficiency than males (see also Hogan et al., 1998; Obeso et al., 1998). The present study indicates that both sexes suffer an equivalent depression in photosynthetic capacity as a result of reproduction, though the sexes differ in the effects of reproduction on subsequent growth. Males show no decrease in growth over a 12-mo period as a result of reproduction, despite the decrease in photosynthetic capacity in reproductive individuals demonstrated here. Females, in contrast, show a decline in growth associated with reproduction in the previous season, as would be expected given the impact of reproduction on photosynthetic capacity (Nicotra, 1999b).

Simulated daily carbon gain—Our application of measured light response to simulations of whole-plant carbon gain provides insight into how leaf-level traits scale to whole-plant patterns. Males had higher leaf-level rates of carbon gain and

even had slightly (not significantly) higher whole-plant area based rates of daily carbon gain (per square centimeter per day). But, because of differences in leaf area, females had greater whole-plant carbon gain at all but the lowest light level. The difference in carbon gain at low light might reflect the impact of slightly higher quantum yield in males (see Table 1). In Nicotra (1999b), the sexes were erroneously reported to have equivalent whole-plant leaf area, rather than females having greater leaf area. Accordingly, males were presented as having larger leaves than females (fewer leaves, but equivalent area). The data in the present study correctly present females as having greater whole-plant leaf area on average.

All models of plant carbon gain contain simplifications. Our non-architectural model simplified aspects of leaf display, but incorporated a high degree of realism in terms of diurnal light variation. Notably, leaf display patterns (assessed using the Y-Plant model) in *S. grandiflora* suggested no sex differences in leaf angle or exposure efficiencies. As such, the results of the non-architectural model are likely to be indicative of real patterns of daily carbon gain. Although a model incorporating effects of leaf overlap and self-shading would provide a more accurate estimate of real daily carbon gain, we do not expect such a model would reveal a different pattern with regard to sexual differences.

The estimates of whole-plant carbon gain answer the lingering question about the biology of *S. grandiflora*: how do females achieve equivalent growth on average (over multiple seasons), despite having greater reproductive allocation and exhibiting short-term negative impacts of reproduction on growth? Females have the capacity to produce more leaves and maintain a greater leaf area, enabling them to achieve greater whole-plant carbon gain and on average, equivalent stem growth and leaf production.

Females might even be expected to achieve greater growth than males, on average, if it were not for the indirect costs of reproduction. Other factors not included in the models that could also offset the female advantage include overall respiratory costs or construction costs. Because the sexes did not differ in respiration rate at the leaf level, there is no reason to expect respiratory carbon loss at the leaf level to differ between the sexes. However, when females are provisioning fruits, respiratory costs are likely to differ markedly between the sexes (Gehring, 1993). The photosynthetic parameters used in the simulations were obtained on plants grown from cuttings and entering their first reproductive season. As such, effects of reproduction on photosynthetic capacity were not incorporated. The reduction in growth in females following reproduction could reflect longer duration of depressed carbon gain. However, as males reproduce more frequently than females (Nicotra, 1999a), males may express a reduced photosynthetic capacity more frequently than females. Although this study does not suggest a differential impact of reproduction on leaf-level photosynthetic capacity for the sexes, measurements of realized growth indicate that longer term measurements would reveal differences between the sexes during some phenological stages. Perhaps sustained decreases in photosynthetic capacity in females offset the advantage gained through greater leaf area, resulting in equivalent growth averaged over several seasons (Nicotra, 1999a, b).

Sexual dimorphism and carbon gain—What evolutionary advantage might these differences between the sexes confer (Kohorn, 1994)? Nicotra (1999a, b) has previously argued that

S. grandiflora males pay for reproduction primarily out of current photosynthetic yield, whereas females may supplement current photosynthesis with allocation of stored reserves to reproduction. In males, allocation to reproduction is linearly related to plant size (Nicotra, 1999a), suggesting that males do not modify proportional reproductive allocation in response to variation in resource availability during reproduction. The pressure to finance reproduction out of current assimilation, even though photosynthetic rate declines during reproduction, could give a selective advantage to higher photosynthetic rates in males. In females, on the other hand, allocation to reproduction is much less tightly correlated with plant size; thus, the ability to alter patterns of allocation of resources to fruits vs. leaves or storage is likely to be more advantageous than inherently high photosynthetic capacity (see also Delph, 1990; Delph and Meagher, 1995). Trade-offs between allocation to photosynthetic surfaces vs. to reproductive structures may explain why female *S. grandiflora* have greater allocation to leaves early in life (pre-reproductively) and why females generally initiate reproduction at a larger size than males.

Why don't males benefit from the traits under favorable selection in females or vice versa? One explanation would be a trade-off, for example, between the evolution of higher photosynthetic rates and the evolution of greater allocation to storage. Alternatively, if male fitness is not limited by stored reserves, but by pollinators or by the decrease in photosynthesis that accompanies reproduction, then traits that increase allocation to storage might be selectively neutral in males. If allocation to storage increases female fitness, whereas small increases in photosynthetic capacity do not, then an increase in photosynthetic capacity may be selectively neutral in females. In a sense, physiological differentiation between the sexes might be seen as an expression of alternative parts of the same reaction norm, mediated by differential resource availability and requirements (e.g., Schlichting and Pigliucci, 1998; Meagher, 1999).

Summary—Our assessment of leaf- and whole-plant level sexual differentiation in *S. grandiflora* provides a synthesis of the extent of differentiation between the sexes in a dioecious species. In *S. grandiflora*, sexual differentiation has been demonstrated in leaf-level physiology, in reproductive allocation, in delayed costs of reproductive allocation, and in patterns of growth and vegetative allocation (Nicotra, 1999a, b). The compounded impact of these differences underlies observed differences in life history patterns—sex ratio, size at first reproduction, reproductive frequency, and potentially, patterns of mortality (Nicotra, 1998). Previous studies have demonstrated differences at each of these levels, but few have scaled across these levels and assessed the importance of differences at each level to patterns at subsequent levels (Retuerto et al., 2000). Clearly, a study of differences at a single level would have provided a misleading impression of how sexual differentiation affects the ecology of the species. While it is not always possible to assess such a range of traits, the present study illustrates the importance of considering how patterns of differentiation at other levels may influence the impact of a given incidence of sexual differentiation on ecological patterns and evolutionary processes.

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