Effects of elevated atmospheric CO_2 concentration on leaf dark respiration of *Xanthium strumarium* in light and in darkness

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Leaf dark respiration (*R*) is an important component of plant carbon balance, but the effects of rising atmospheric CO_2 on leaf *R* during illumination are largely unknown. We studied the effects of elevated CO_2 on leaf *R* in light (R_L) and in darkness (R_D) in *Xanthium strumarium* at different developmental stages. Leaf R_L was estimated by using the Kok method, whereas leaf R_D was measured as the rate of CO_2 efflux at zero light. Leaf R_L and R_D were significantly higher at elevated than at ambient CO_2 throughout the growing period. Elevated CO_2 increased the ratio of leaf R_L to net photosynthesis at saturated light (A_{max}) when plants were young and also after flowering, but the ratio of leaf R_D to A_{max} was unaffected by CO_2 levels. Leaf R_N was significantly higher at the beginning but significantly lower at the end of the growing period in elevated CO_2-grown plants. The ratio of leaf R_L to R_D was used to estimate the effect of light on leaf *R* during the day. We found that light inhibited leaf *R* at both CO_2 concentrations but to a lesser degree for elevated (17–24%) than for ambient (29–35%) CO_2-grown plants, presumably because elevated CO_2-grown plants had a higher demand for energy and carbon skeletons than ambient CO_2-grown plants in light. Our results suggest that using the CO_2 efflux rate, determined by shading leaves during the day, as a measure for leaf *R* is likely to underestimate carbon loss from elevated CO_2-grown plants.

Photosynthesis and mitochondrial respiration (also referred to as dark respiration, as opposed to photorespiration) are metabolic pathways that produce ATP and reductants to meet energy demands for plant growth and maintenance. Although the light reaction in photosynthesis provides ATP and reductants for biosynthesis in a leaf cell during illumination, mitochondrial respiration in light is necessary for biosynthetic reactions in the cytosol, such as sucrose synthesis (1, 2). Respiratory activity in light can even be considered part of the photosynthetic process, because it is needed to regulate the state of stromal redox during photosynthesis (3) and to maintain the cytosolic ATP pool (1). Mitochondrial respiration might also be a source for biosynthetic precursors, such as acetyl-CoA or acetate for chloroplastic fatty acid synthesis in light (1). The required magnitude of mitochondrial respiration in light is therefore determined by the potential need for this process to provide energy and carbon skeletons in the light (2).

Mitochondrial respiratory activity during illumination varies between 25 and 100% of the respiratory activity in darkness (1). The lower rate of nonphotorespiratory mitochondrial CO_2 release during illumination has been interpreted as evidence for partial inhibition of leaf respiration by light (4–6). The magnitude of light inhibition of respiration seems to depend on the photosynthetic capacity (1), but the mechanism of light regulation of mitochondrial respiration is not clearly understood (2, 3). Although there has been much study of, albeit little agreement on, the effects of elevated CO_2 on plant respiration (7–9), the effects of elevated CO_2 on mitochondrial respiration in light have been little studied and hence are largely unknown (5, 10). The commonly used method for estimating daytime leaf respiration as affected by CO_2 concentration is measurement of the rate of CO_2 efflux by shading leaves during the day (8, 11). However, light inhibition of mitochondrial respiration found in a variety of species (6, 12, 13) calls into question the validity of this method, because it assumes leaf respiration continues at the same rate in the light as in darkness. Leaf dark respiration is an important component in plant carbon balance and can return as much as 40–50% of photosynthetically fixed carbon to the atmosphere (14, 15). It is therefore essential that we understand whether light has a differential effect on dark respiration of ambient and elevated grown CO_2 plants to more accurately estimate the extent of respiratory carbon loss in terrestrial ecosystems as atmospheric CO_2 rises.

Three types of dark respiration were studied in our experiment: leaf respiration in light estimated by using the Kok method during the day (R_L), leaf respiration in darkness measured as rate of CO_2 release by shading leaves during the day (R_D), and leaf dark respiration at the end of the dark period (R_N). Our primary objective was to study the effects of elevated CO_2 on leaf R_L at different developmental stages of *Xanthium strumarium*, especially before and after flowering. We hypothesized that leaf R_L would be higher at elevated CO_2 than at ambient CO_2, because plants grown at elevated CO_2 produce more biomass (16, 17), and higher biomass production requires a higher demand for ATP, reductants, and biosynthetic precursors. Our secondary objective was to investigate whether leaf R_N had similar responses to CO_2 enrichment as leaf R_L or R_D. We also examined the relationship between photosynthetic rate at saturated photosynthetically active radiation (PAR) [net photosynthesis at saturated light (A_{max})] and respiration, two biological processes that link organic carbon in the biosphere with inorganic carbon in the atmosphere. Our study will therefore help to elucidate the mechanisms of higher atmospheric CO_2 effects on plant respiration and to construct a more accurate carbon budget of plants under elevated CO_2.

Materials and Methods

Growth Conditions. We grew *X. strumarium* L. (common cocklebur), a developmentally determinate cosmopolitan species, in environmentally controlled conditions. *X. strumarium* is a qualitative short-day plant that flowers only when days are shorter than 15.7 h and nights are longer than 8.3 h (18). Seeds of *X. strumarium* were obtained from a single seed source in Lubbock, TX. Plants were germinated and grown in 8.4-liter pots filled

Abbreviations: A_{max}, net photosynthesis at saturated light; PAR, photosynthetically active radiation; *R*, dark respiration; R_D, daytime *R* measured by shading leaves; R_L, daytime *R* in light; R_N, nighttime *R*.

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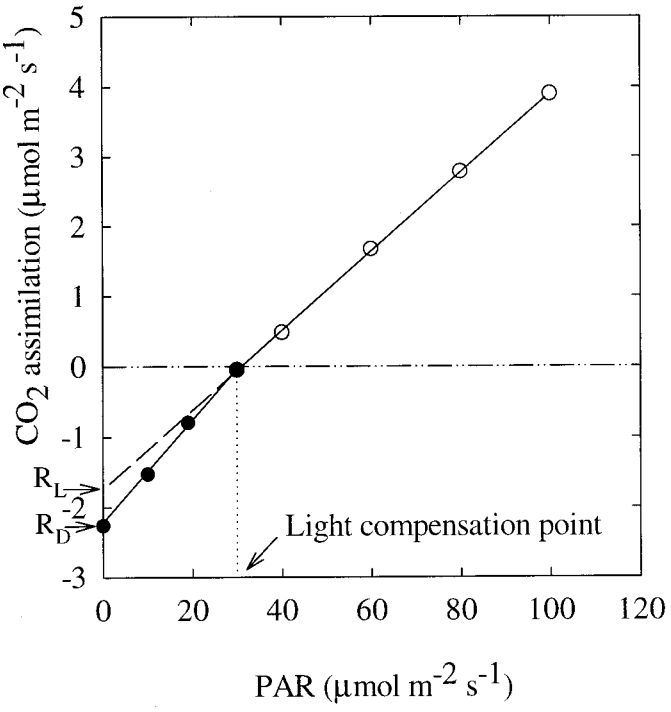

Fig. 1. Representative photosynthetic light-response curve of *X. strumarium* at low PAR. The CO_2 efflux rate at PAR = 0 was considered to be daytime leaf mitochondrial respiration in darkness (R_D). The part of light-response curve before the abrupt change in slope was extended to *y* axis, and the intercept was considered to be daytime leaf mitochondrial respiration in light (R_L) according to Kok (19). Change in slope of the curves occurs near the light compensation point.

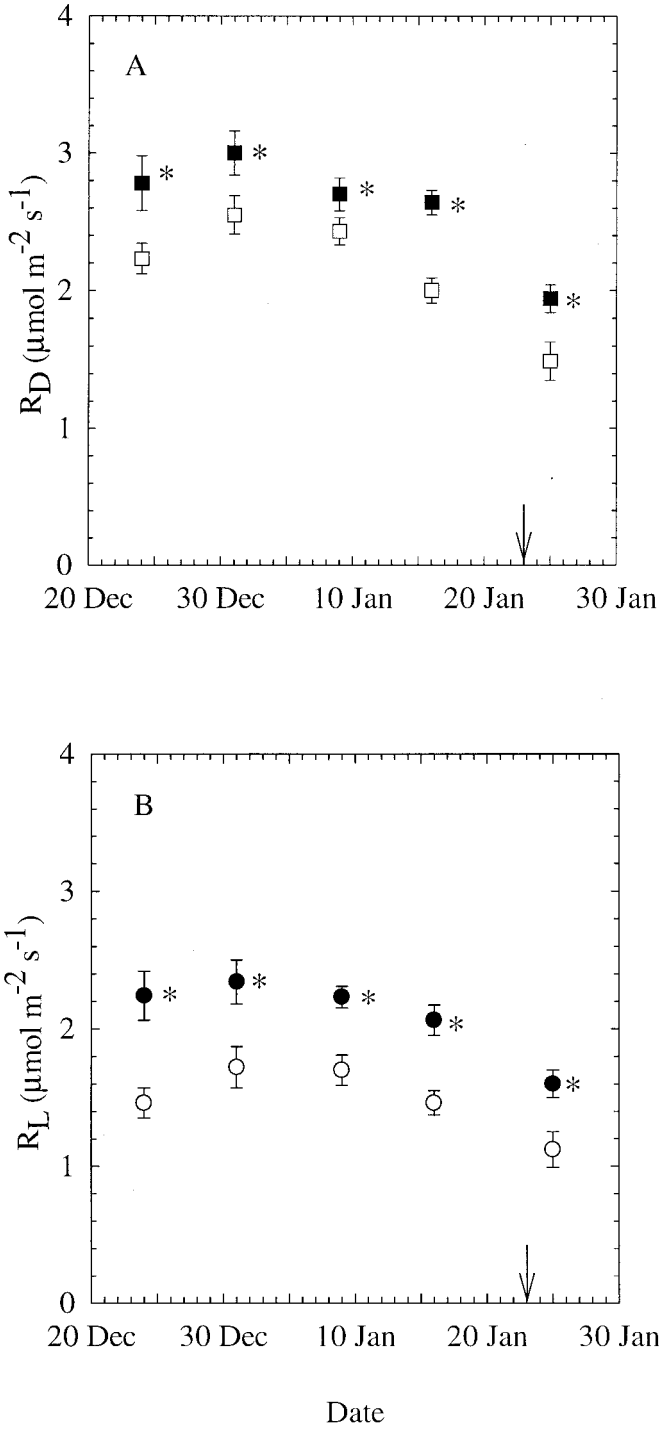

Fig. 2. Leaf R_D (*A*) and leaf R_L (*B*) of *X. strumarium* grown at ambient (open symbols) or elevated (closed symbols) CO_2. Leaf R_D and R_L were measured at growth CO_2 concentration on five different dates. Because there was no effect of planting date on R_D or R_L, all measurements from plants of different ages were averaged for each CO_2 treatment. Arrow indicates date of flowering for all plants. Mean ± 1 SE; $n = 18$ for December 24, 1999, and $n = 24$ for all other dates. *, $P < 0.05$.

with sand in four 1.4-m^2 growth chambers (Conviron, Controlled Environments, Winnipeg, MB, Canada) at the Lamont–Doherty Earth Observatory. To examine the possible effects of developmental stage on plant respiratory responses to elevated CO_2, six cohorts were planted at 5-day intervals starting November 23, 1999. After germination, seedlings were thinned to one for each pot. Carbon dioxide concentrations were maintained at 730 μmol mol^{-1} in two chambers (elevated CO_2 treatment) and at 365 μmol mol^{-1} in the other two chambers (ambient CO_2 treatment). The elevated CO_2 treatment was created by adding pure CO_2 to a mixing fan within the chambers. Air temperature was maintained at 28/20°C (day/night) and relative humidity at 50%. PAR was approximately 300–400 μmol m^{-2} s^{-1} at leaf surface for the photoperiod from 09:00 to 03:00 h during the entire growing period. Flowering was induced by changing the photoperiod from 18 h to 12 h on January 11, 2000, when the youngest plants were approximately 3 weeks old. Photoperiod was changed back to 18 h 2 days later, and the 18-h photoperiod was maintained for the rest of the experiment. All of the plants started flowering on January 23, 2000, regardless of planting dates. All pots were watered to saturation daily with distilled water throughout the experiment. Soil nutrients were supplemented by adding Osmocote Plus (15–11-13, 90269, Scotts–Sierra Horticultural Products, Marysville, OH). The experimental design was a complete factorial with six replicates per treatment for a total of 72 plants (two CO_2 levels × six planting dates × six replicates).

Gas-Exchange and Leaf Nitrogen Measurements. Leaf photosynthetic rate (*A*) was measured by using a LI-6400 Portable Photosynthesis System (Li-Cor, Lincoln, NE) on the youngest mature leaves. Leaf A was first measured at saturating PAR of 1,500 μmol m^{-2} s^{-1} (A_{max}) and then at lower levels of PAR (100, 80, 60, 40, 30, 20, 10, 0, 0, 0 μmol m^{-2} s^{-1}) at growth CO_2. Leaves were allowed to equilibrate for at least 5 minutes at each light level before any reading was recorded. Leaf temperature was

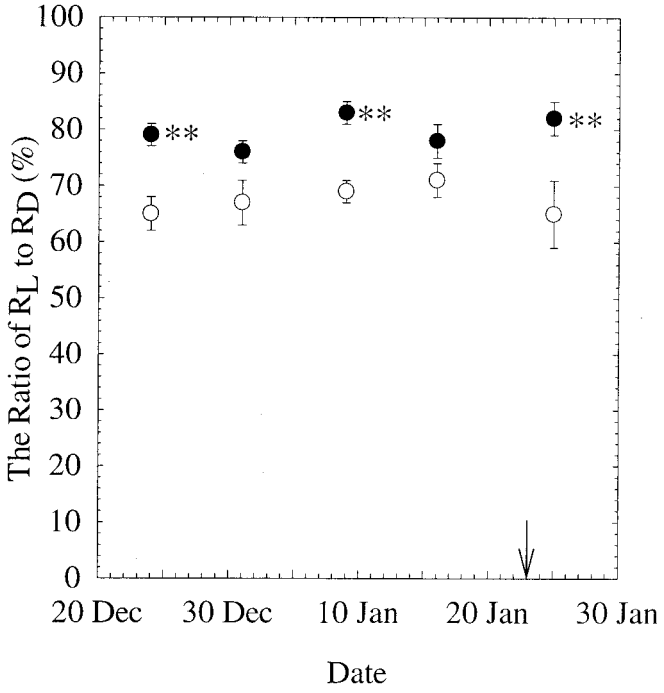

Fig. 3. The ratio of leaf R_L to leaf R_D of *X. strumarium* grown at ambient (open symbols) or elevated (closed symbols) CO_2 for five different sampling dates. The ratio of R_L/R_D ranged from 65–71% for ambient and 76–83% for elevated CO_2-grown plants. *P* values for CO_2 treatment on December 31, 1999 and January 5, 2000 were 0.056 and 0.121, respectively. Mean ± 1 SE; $n = 18$ for December 24, 1999, and $n = 24$ for the other measuring dates. **, $P < 0.01$.

maintained at 27.3 ± 0.05°C (mean ± SE) and relative humidity at ≈50% inside the cuvette.

The Kok (19) and Laisk methods (20) are the most commonly used methods for estimating leaf respiration in light. We chose the Kok method, because the Laisk method is not appropriate for studying the effect of different CO_2 concentrations on leaf R_L, as intercellular CO_2 concentration would have to be changed during the course of the measurement (6). Photosynthetic rates at low light levels were plotted against the eight PAR levels (Fig. 1). There was an obvious change in the slope of the line near the light compensation point (the Kok effect). The upper part of the light curve, before the obvious change in slope, was extended to the axis of *A*, and the intercept was considered to be leaf R_L under growth conditions (19). Leaf R_D was obtained by averaging the three CO_2 efflux rates at zero PAR for each plant, which was equal to the intercept of the lower part of the curve at the axis of *A* (Fig. 1). Leaf R_N was measured at the end of the daily dark period, i.e., from 07:00 to 09:00 h, by using the same Photosynthesis System. After stable CO_2 flow was achieved, three readings were recorded at a 30-s interval, and the average was taken as leaf R_N. Leaf temperature was 20.0 ± 0.05°C during the measurement of leaf R_N.

After the last set of gas-exchange measurements, all plants were harvested. Leaf samples were collected for leaf nitrogen assay. Leaf N concentration was determined in dried and ground material by using an NCS autoanalyzer (Carlo Erba NCS 2500, Milan, Italy).

Statistical Analysis. Data were analyzed by using a two-way analysis of variance with CO_2 treatment and planting date as the main effects and chamber as a nested effect within CO_2 treatment by using SPSS (Ver. 10.0.2, SPSS, Chicago). Measurements from different plants sowed at the same time in each chamber were averaged before being analyzed, because planting date did not have a significant effect on these measurements. Comparisons among means for CO_2 levels and planting dates were made by least significant difference for *a priori* comparisons. Treatment effects were considered to be significant if $P < 0.05$.

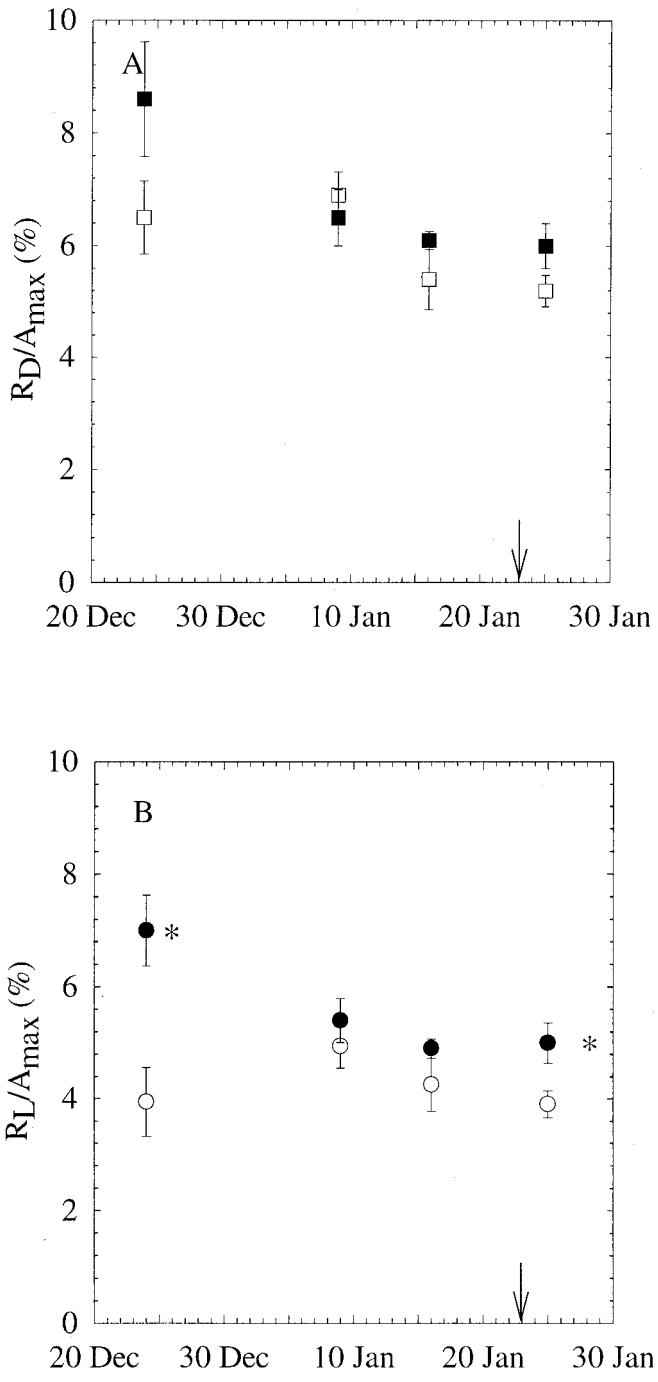

Fig. 4. Percentages of leaf R_D (*A*) and leaf R_L (*B*) to maximum net photosynthetic rate (A_{max}) of *X. strumarium* grown at ambient (365 μmol mol^{-1}, open symbols) or elevated (730 μmol mol^{-1}, closed symbols) CO_2 for four different sampling days. Arrow indicates starting date of flowering. $n = 8–20$; *, $P < 0.05$.

Results

Daytime Leaf R_D and R_L. Leaf R_D and R_L were significantly higher in elevated CO_2 compared with ambient CO_2-grown plants throughout the experiment ($P < 0.05$; Fig. 2). The difference in

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Table 1. Effects of CO_2 concentration and planting date on leaf dark respiration at night (R_N; μmol $m^{-2}\cdot s^{-1}$) of *X. strumarium*

	Dec 31 99		Jan 9 00		Jan 31 00	
	365 ppm	730 ppm	365 ppm	730 ppm	365 ppm	730 ppm
Effect of CO_2						
	1.68 ± 0.32[b]	2.21 ± 0.47[a]	1.99 ± 0.47	1.66 ± 0.51	1.40 ± 0.32[a]	1.00 ± 0.39[b]
Effect of planting date						
0	1.65 ± 0.35	2.14 ± 0.20	1.92 ± 0.56	1.89 ± 0.39	1.58 ± 0.24	0.69 ± 0.28
5	1.59 ± 0.10	1.74 ± 0.49	1.93 ± 0.21	1.35 ± 0.22	1.43 ± 0.18	1.23 ± 0.24
10	1.68 ± 0.16	2.19 ± 0.36	1.59 ± 0.33	1.72 ± 0.22	1.23 ± 0.30	0.85 ± 0.20
15	1.81 ± 0.16	2.78 ± 0.53	2.10 ± 0.60	2.13 ± 0.39	1.42 ± 0.18	1.08 ± 0.23
20			1.93 ± 0.47	1.62 ± 0.53	1.36 ± 0.33	0.80 ± 0.26
25			2.49 ± 0.25	1.26 ± 0.37	1.36 ± 0.22	1.36 ± 0.29

Leaf temperature during respiration measurements was 20.0 ± 0.05°C. Different letters ([a] and [b]) indicate statistical significance at $P \leq 0.05$ within the same day. $n = 24$ for CO_2 treatments and $n = 4–6$ for planting dates. Mean ± 1 SE.

daytime leaf R between plants grown at ambient CO_2 and elevated CO_2 concentration ranged from 11 to 32% for leaf R_D and 31 to 53% for leaf R_L. There was a gradual decrease of both leaf R_D and R_L as plants grew in size, although there was no significant effect of planting date on any particular measuring date. After flowering initiation, leaf R_D and R_L dropped ≈30% from their preflowering levels in ambient as well as in elevated CO_2-grown plants.

Elevated CO_2-grown plants had significantly higher leaf R_L/R_D ratio than ambient CO_2-grown plants on three measuring dates ($P < 0.01$). The ratio of leaf R_L to R_D, which reflects the magnitude of inhibition of daytime leaf dark respiration by light, remained remarkably constant during the experiment (Fig. 3). The ratio of R_L/R_D was marginally higher ($P = 0.056$ and $P = 0.121$) at elevated than at ambient CO_2 on the other two measuring days. Across the experiment, leaf R_L was 65–71% of leaf R_D at ambient and 76–83% at elevated CO_2. Therefore, the inhibition by light on daytime leaf dark respiration was 29–35% for ambient and 17–24% for elevated CO_2-grown plants.

Leaf R_D and R_L were 5.2–8.6% and 3.9–7.0% of net maximum photosynthesis, respectively. There was no significant CO_2 effect on R_D/A_{max} on any measuring dates (Fig. 4*A*). The ratio of R_L/A_{max} was significantly higher at elevated CO_2 early in the experiment and after plants started flowering, but there was no significant difference between CO_2 treatments in the middle of the growing period (Fig. 4*B*).

Nighttime Leaf Respiration. Carbon dioxide concentration had no consistent effect on leaf R_N, which was measured at the end of the daily dark period on 3 days during the experiment. Although leaf R_N was 32% higher at elevated than at ambient CO_2 before flowering initiation, it was 29% lower when flowers were in full bloom ($P < 0.05$, Table 1 *Upper*). No difference in leaf R_N was observed between plants grown at different CO_2 levels in the middle of the growing period. For ambient CO_2-grown plants, percentage of leaf R_N to leaf R_D increased from 66 to 94% from the beginning to the end of the experiment. For elevated CO_2-grown plants, the percentage decreased from 74 to 52% for the same period, because leaf R_N at ambient CO_2 remained little changed, whereas leaf R_N at elevated CO_2 showed a 55% decrease during that period. As observed in daytime leaf R_D or R_L, we found no significant effect of planting date on leaf R_N (Table 1 *Lower*).

Leaf Respiration and Leaf Chemistry. At ambient CO_2, there was a significant positive correlation between leaf R_L and leaf N ($P = 0.04$), but only a marginally significant correlation between leaf R_D and leaf N ($P = 0.07$) (Fig. 5). The proportion of variation that can be attributed to the relationship between leaf R_L and leaf N, however, is small ($R^2 = 0.136$). At elevated CO_2 there was no correlation between leaf R_D or R_L and leaf N. Neither leaf R_L nor R_D was significantly correlated with leaf starch concentration (data not shown).

Discussion

Our results showed that leaf R_L was significantly higher in elevated CO_2 compared with ambient CO_2-grown *X. strumarium* plants, suggesting higher energy output in light in elevated CO_2-grown plants. There is abundant evidence showing that growth at elevated CO_2 will greatly increase biomass production (16, 17). Higher biomass production will likely require more energy and carbon skeleton output from chloroplasts and mitochondria. This higher requirement can be met by more efficient cell metabolism and/or a larger number of energy- and reductant-producing organelles, mainly mitochondria. Although there has been no cellular level study examining whether cells can metabolize more efficiently at a higher CO_2 concentration, studies have shown that the number of mitochondria increased dramatically at elevated CO_2 in 10 species representing 8 families (21, 22). These plants were grown in environments that included growth and open-top chambers and Free-Air-CO_2 Enrichment Facilities. Griffin *et al.* (22) also found a significant increase in the proportion of stroma thylakoid membranes to grana thylakoid membranes in leaves of the nine species studied. They hypothesized that plants adjusted cell ultrastructure for more ATP output from chloroplasts to meet the higher energy demand at elevated CO_2 during daytime. Our results support their hypothesis by showing a significantly higher leaf mitochondrial respiration during illumination at elevated CO_2. Their energy balance hypothesis is also supported by our findings showing that leaf R_N had no consistent response to CO_2 treatment during the experiment. It is possible that plants had different requirements for energy and carbon precursors and hence respiration at night.

Although to a different degree, daytime leaf R was significantly inhibited by light at both ambient and elevated CO_2 in *X. strumarium*. The magnitude of inhibition found in our study by using the Kok method, 29–35% for ambient and 17–24% for elevated CO_2-grown plants, was similar to the results found in seven *Poa* species by using the Laisk method (12). Villar *et al.* (6), however, found a much greater light inhibition of leaf R of 51 and 62% in two Californian chaparral shrubs also by using the Laisk method. The observed daytime inhibition of leaf respiration by light has been attributed to the accumulation of photosynthetic metabolites during illumination, such as ATP and NADPH, acting on respiratory enzymes as respiratory regulators (2, 23). Significantly higher daytime leaf R_L and mitochondria numbers at elevated CO_2 (21, 22), however, suggested that potential demand for ATP and reductants

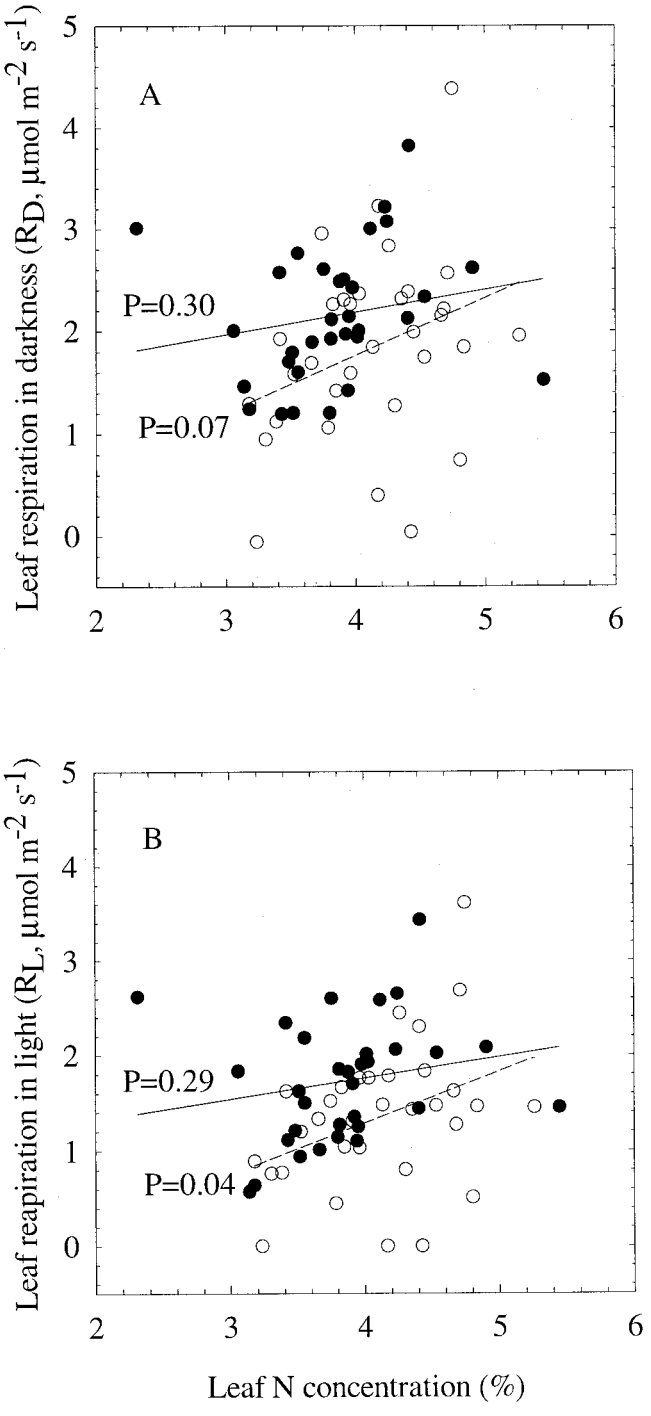

Fig. 5. Relationship between leaf R_D and leaf N concentration (*A*) and between leaf R_L and leaf N concentration (*B*) of *X. strumarium* grown at ambient (open symbols, dashed lines) or elevated (closed symbols, solid lines) CO_2. Also shown are the *P* values for the regressions between leaf respiration and leaf N concentration.

might be more important in determining the magnitude of light inhibition of leaf *R*.

It has been suggested that decreases in leaf *R* may be related to reduced leaf N content at elevated CO_2 (24). However, leaf N status did not explain the effect of CO_2 enrichment on leaf *R* in our study. Although leaf N was significantly lower at elevated CO_2, leaf *R* was significantly higher at elevated CO_2. It appears that higher leaf *R* in *X. strumarium* under CO_2 enrichment was because of increased photosynthate production, as suggested by Brooks and Farquhar (5), rather than reduced protein turnover (24).

Leaf *R* during the day is typically estimated as the CO_2 efflux from a leaf by either covering a clear cuvette with an opaque cloth (25) or turning off the light source and making the PAR zero (11). Leaf *R* measured in this manner is consequently used to assess the effects of elevated CO_2 on carbon loss on leaf, plant, and ecosystem levels (9). This is a valid approach if light affects leaf *R* of ambient and elevated CO_2-grown plants to the same extent. Our study, however, showed that this is not the case with *X. strumarium*. We found that the ratio of R_L/R_D at elevated CO_2 (76–83%) is significantly higher than at ambient CO_2 (65–71%), indicating less light inhibition of leaf *R* at elevated CO_2. When leaf R_D is used as an approximate measure of leaf R_L, which is a more accurate estimate of nonphotorespiratory carbon loss during the day, we are underestimating daytime carbon loss at elevated CO_2 by 11–12%. If light inhibits leaf *R* of ambient and elevated CO_2-grown plants of other species in a similar manner, this finding will have important implications for how daytime carbon loss should be incorporated into the construction of the global carbon budget.

In summary, we found that leaf R_D and R_L in *X. strumarium* were significantly greater at elevated CO_2 compared with ambient CO_2, but that they were not related to leaf N content. Light inhibited leaf *R* at both ambient and elevated CO_2, but the inhibition was greater for ambient than for elevated CO_2-grown plants, presumably because elevated CO_2-grown plants had a higher demand for energy and carbon skeletons. We demonstrated that using leaf *R* determined by shading leaves during the day as a measurement for respiratory carbon loss underestimates daytime leaf *R* of elevated CO_2-grown plants by 10–11%. If this differential effect of light on leaf *R* of ambient and elevated CO_2 exists in other species, the underestimate of carbon flux from ecosystems to the atmosphere at higher CO_2 should be taken into consideration in models of the global carbon budget.

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