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CO_2 AND TEMPERATURE EFFECTS ON LEAF AREA PRODUCTION IN TWO ANNUAL PLANT SPECIES[1]

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Abstract. We studied leaf area production in two annual plant species, *Abutilon theophrasti* and *Amaranthus retroflexus,* under three day/night temperature regimes (18°/14°, 28°/22°, and 38°/31°C) and two concentrations of carbon dioxide (400 and 700 μL/L). The production of whole-plant leaf area during the first 30 d of growth was analyzed in terms of the leaf initiation rate, leaf expansion, individual leaf area, and, in *Amaranthus,* production of branch leaves. Temperature and CO_2 influenced leaf area production through effects on the rate of development, determined by the production of nodes on the main stem (the plastochron index), and through shifts in the relationship between whole-plant leaf area and the number of main stem nodes. In *Abutilon,* leaf initiation rate was highest at 38°, but area of individual leaves was greatest at 28°. Total leaf area was greatly reduced at 18° due to slow leaf initiation rates. Elevated CO_2 concentration increased leaf initiation rate at 28°, resulting in an increase in whole-plant leaf area. In *Amaranthus,* leaf initiation rate increased with temperature, and was increased by elevated CO_2 at 28°. Individual leaf area was greatest at 28°, and was increased by elevated CO_2 at 28° but decreased at 38°. Branch leaf area displayed a similar response to CO_2, but was greater at 38°. Overall, whole-plant leaf area was slightly increased at 38° relative to 28°, and elevated CO_2 levels resulted in increased leaf area at 28° but decreased leaf area at 38°. The effects on leaf area closely parallel rates of biomass accumulation in the same experiment, suggesting that responses of developmental processes to elevated CO_2 and interacting factors may play an important role in mediating effects on plant growth.

Key words: Abutilon theophrasti; Amaranthus retroflexus; *annual plants; CO_2 enrichment; leaf area development; plastochron index; temperature.*

INTRODUCTION

The prospect of significant changes in global climate, due to increasing concentrations of CO_2 accompanied by a predicted increase in temperature, has focused renewed attention on the environmental factors that influence plant growth and the potential for changes in climate to alter patterns of plant productivity (NAS 1988). Both CO_2 and temperature are known to influence plant growth in numerous ways. Increasing CO_2 concentrations often increase the productivity of individual plants (reviews in Strain and Cure 1985, Kimball 1986, Bazzaz 1990), apparently due to effects on both leaf area production and carbon assimilation rates. Temperature has numerous effects on plant growth, due to its pervasive role in the regulation of biochemical reaction rates, morphogenetic processes, and matter and energy exchange with the environment (Long and Woodward 1988). However, the effects on growth of increasing temperature and increasing CO_2 may operate through different pathways and have contrasting results. Coleman and Bazzaz (1992) demonstrated that CO_2 and temperature interact strongly to influence resource acquisition and growth in two annual plant species. Their results suggest that the effects of the various treatments on growth were primarily due to changes in leaf area production and loss and to a lesser degree to effects on photosynthesis, nitrogen use efficiency, and water use efficiency. These results are consistent with other studies showing that variation in growth is more strongly correlated with patterns of allocation and leaf area development than with either photosynthetic rate or whole plant net assimilation rates (e.g., Potter and Jones 1977, Lechowicz 1984, Mooney and Chiariello 1984, Poorter and Remkes 1990). Thus, a mechanistic understanding of factors influencing individual and community level productivity must include studies of the developmental mechanisms underlying the production of leaf area, comparable to the extensive research on the biochemical basis of gas exchange characteristics.

The development of leaf surface area can be broken into two components: (1) the rate of overall development, as indicated by the rate of leaf initiation on the main stem, and (2) the rate of leaf area production relative to the developmental stage of the plant (cf. Jones and Hesketh 1980). The latter component is the

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result of the rate and duration of individual leaf expansion, and hence of individual leaf area, and of the rate of production of lateral branches and branch leaves. The rate of leaf initiation by individual meristems (plastochron rate) increases with increasing temperature in most species; studies of soybean (*Glycine max*), sunflower (*Helianthus annus*), and cucumber (*Cucumis sativa*) have reported a fairly consistent increase in initiation rate of 0.015–0.03 leaves (lvs)·d^{-1}·$°C^{-1}$ in the range of 15°–30° (Milthorpe 1959, Hofstra et al. 1977, Rawson and Hindmarsh 1982, Snyder and Bunce 1983). Temperature also has marked effects on leaf size, and the rate and duration of leaf expansion. Individual leaf areas are frequently greatest at intermediate temperatures, which is primarily due to increased rates of leaf expansion (Milthorpe 1959, Hesketh and Baker 1969). The rate and duration of expansion usually decrease at higher temperatures (Snyder and Bunce 1983, Rawson and Dunstone 1986).

Elevated CO_2 is often reported to increase whole plant leaf area. In contrast to temperature, however, elevated CO_2 generally has little or no effect on leaf initiation rates (e.g., Ford and Thorne 1967, Rogers et al. 1980, Jones et al. 1984), though some studies report small increases in rate or in the total number of main stem nodes (Rogers et al. 1984, Cure et al. 1987, Sionit et al. 1987, Sasek and Strain 1991). In a study of soybean seedlings, Leadley and Reynolds (1989) suggested that elevated CO_2 accelerated the time of appearance of the first leaves but that the rate of production of successive leaves was unchanged relative to plants growing at ambient CO_2. Increases in whole plant leaf area may also be due to increased branching or tillering (Rogers et al. 1984, Acock and Allen 1985, Sasek and Strain 1991) and/or to increase in individual leaf area (e.g., Ford and Thorne 1967, Jones et al. 1984). Elevated CO_2 has been observed to increase the rate of expansion in soybean (Rogers et al. 1984, Cure et al. 1989), and to increase the rate and decrease the duration of expansion in *Populus* (Gaudillére and Mousseau 1989). However, in a more detailed study, Leadley and Reynolds (1989) observed no effect of elevated CO_2 on the rate and duration of leaflet expansion or on final leaflet area of the first six leaflets of soybean. These conflicting results suggest that effects of CO_2 on leaf development may be sensitive to other environmental factors or the developmental stage of the plant.

In this study of the annual plant species *Abutilon theophrasti* Medic. and *Amaranthus retroflexus* L. we examined the effects of temperature and CO_2 concentration on the rate of leaf initiation, and on the production of leaf area as a result of individual leaf expansion and the production of branch leaves (*Amaranthus* only). We show that elevated CO_2 and temperature can both have significant effects on leaf area in the early phases of growth but that there is a strong interaction between the two factors as well as marked differences between the two species.

Materials and Methods

Species and growth conditions

Abutilon theophrasti and *Amaranthus retroflexus* are co-occurring annuals of disturbed environments in the American Midwest; photosynthesis is by the C_3 pathway in the former and the C_4 pathway in the latter. Both are monopodial, bearing simple leaves in a spiral on an erect primary stem. *Abutilon* produces few branches and bears flowers in the leaf axils, while *Amaranthus* produces numerous lateral branches from various nodes, and the terminal meristem switches from vegetative to reproductive growth at flowering. Individuals of both species were grown in growth chambers under three day/night temperature treatments (18°/14°, 28°/22°, and 38°/31°C) and two CO_2 levels (400 and 700 μL/L). Temperatures were adjusted gradually between day and night values over a 2-h period, and the average daily temperatures for the three treatments were 34°, 24.8°, and 16°C. The plants used for these measurements were part of a larger experiment, described by Coleman and Bazzaz (1992), which provides additional information on growth conditions.

Data collection

For 10 plants of each species in each treatment, main stem plastochron index was determined at roughly 3-d intervals from day 13 to 31. The plants in the 18°C treatment grew more slowly so measurements continued until day 73. In five plants in each treatment, more detailed measurements were made of the length of all main stem leaves, node position of all lateral branches, and number of leaves on each branch. This time interval was chosen in order to focus on leaf area production during the vegetative growth phase of the plants. After day 31, in the 28° and 38° treatments, the rate of leaf production slowed markedly, coincident with the onset of reproduction (data not shown), and up to this point five or fewer leaves had died on each plant, representing <15% of leaf area. Total plant nitrogen content peaked at roughly the same time and leaf nitrogen concentrations were relatively high (Coleman and Bazzaz 1992).

The plastochron index (PI, Erickson and Michelini 1957) was used to quantify leaf initiation and to provide a developmental time scale for analysis of leaf area production and branch initiation. PI provides a continuous measure of main stem node number based on the relative lengths of the two most recently expanding leaves on a stem, according to the following equation:

$$\mathrm{PI} = n + \frac{\ln(L_n/L_{\mathrm{ref}})}{\ln(L_n/L_{n+1})}.$$

L_{ref} equals an arbitrary reference length that the leaves attain during the exponential phase of expansion (15 mm in this experiment); n is the node number of the newest leaf that is longer than the reference length; L_n

is the length of leaf n, and L_{n+1} is the length of the next youngest leaf after leaf n. The leaf plastochron index for each leaf (LPI) is defined as the plastochron index of the shoot minus the node number of the leaf, and is used as a measure of the developmental age of a leaf.

For plants in the 28° and 38°C treatments, leaf area production was estimated from leaf lengths, using regressions derived from an independent harvest (Coleman and Bazzaz 1992, day 30). The following equations, forced through the origin, were used for main stem leaves:

$$\textit{Abutilon}: \quad A = -0.3811 \cdot L + 0.9771 \cdot L^2 \quad (N = 253, \quad r^2 = 0.994)$$

$$\textit{Amaranthus}: \quad A = -1.059 \cdot L + 0.819 \cdot L^2 - 0.033 \cdot L^3 \quad (N = 366, \quad r^2 = 0.998),$$

where A is individual leaf area (in square centimetres) and L is leaf length (in centimetres). In addition, for *Amaranthus* the regression of total branch leaf area on number of branch leaves was determined from data of Coleman and Bazzaz (1992), days 20 and 30. Branch leaf area was square-root transformed in order to provide a linear regression, and the equation was forced through the origin:

$$\text{SQRT(branch leaf area)} = 0.197 \cdot \text{(branch leaf number)} \quad (N = 21, \quad r^2 = 0.974).$$

From these equations whole-plant leaf area was estimated for *Abutilon* on days 22, 24, 28, and 31 and for *Amaranthus* on days 24 and 31. The leaf area estimates obtained by this method for plants at 28° and 38° on day 31 were not significantly different from the areas measured in the destructive harvest on day 30 by Coleman and Bazzaz (1992, $P > .5$ for both species).

Data analysis

The rate of leaf initiation was determined as the slope of the linear regression of plastochron index on time (plastochron rate, PR). During the vegetative growth phase, up to day 30, leaf initiation rate was nearly constant within each plant, as indicated by r^2s of 0.969 to 1 in all 112 regressions calculated. The regression slopes for each species, as well as the other variables described below, were analyzed with the MGLH module of SYSTAT (Wilkinson 1987), using a two-way ANOVA model with temperature and CO_2 level as main effects; differences between individual treatments were analyzed using post-hoc linear contrasts. Homogeneity of variances was tested with the F_{max} statistic (Sokal and Rohlf 1981), normal probability plots were examined to test for satisfactory normality of residuals, and all tests were performed at the 95% confidence level. For plastochron rate the two species were analyzed separately, due to significant heteroscedasticity, and the plastochron rates for *Abutilon* were log transformed.

Effects on individual leaf size were analyzed using the maximum length measured during the experiment for those leaves that grew to maturity in all treatments during the vegetative growth phase (nodes 1–6 in *Amaranthus* and nodes 1–8 in *Abutilon*). Treatment effects on leaf length were analyzed with two-way ANOVA on untransformed data. For *Amaranthus* one outlier was removed from leaf 5 and one from leaf 6 due to significant heterogeneity of variances. The rate and duration of leaf area expansion were calculated for the fifth main stem leaf, based on repeated measurements of area plotted against leaf plastochron index as an index of leaf age; data from all plants in each treatment were combined in order to provide adequate resolution of the expansion curve. A modified form of the monomolecular equation (Constable and Rawson 1980) was used to describe leaf area (A) expansion in time (LPI):

$$A = A_{max} \cdot [1 - e^{(-C \cdot \text{LPI}^p)}].$$

C and p are rate constants that were fitted using the NONLIN module of SYSTAT (Wilkinson 1987). From these parameters the duration of leaf expansion was defined as the time (in plastochrons) required to grow from 5 to 95% of maximum leaf area (Dennet et al. 1978, Leadley and Reynolds 1989), and the mean rate of leaf expansion was calculated as the maximum area divided by the duration. These results were then converted to chronological time based on the plastochron rates for each treatment. Because these parameters were analyzed using pooled data from all plants, no statistical comparisons of the results were possible and they are presented as preliminary observations.

The rates of branch and branch leaf production in *Amaranthus* were analyzed based on data from days 25 and 31 for the 28° and 38°C treatments and days 53 and 61 for the 18° treatments. For this interval the rate of lateral branch production, the overall rate of leaf production on branches and the mean rate of leaf production per branch were calculated for each plant, using time scales based on both days and plastochrons, and the results were analyzed using two-way ANOVA on untransformed data with two outliers removed.

Whole-plant leaf area on day 31 was analyzed in terms of treatment effects on plastochron index and on the relationship between PI and leaf area. No results were available for *Amaranthus* at 18° because the plants were too small to determine plastochron index, and for *Abutilon* the 18° treatment had to be analyzed separately from the higher temperatures due to extremely slow growth. Regressions of whole-plant leaf area, and main stem and branch leaf area for *Amaranthus,* on plastochron index were calculated for each plant individually. Using the regression equations, predicted leaf areas were calculated for each plant at the mean PI for all treatments (13.64 and 3.17 for *Abutilon* at 28° or 38° and at 18°, respectively; 20.85 for *Amaranthus* at 28° and 38°). The regression slopes and predicted leaf areas were compared by ANOVA. Differ-

ences in leaf area among treatments are interpreted in terms of two components: effects of main stem development, reflected in the mean PI on day 31, and changes in the allometry of leaf area production relative to main stem development as indicated by the predicted leaf area at a common PI. All analyses were performed on square-root transformed data, but results (Tables 3 and 4) are presented on back-transformed values. One outlying individual was removed from the 38°, ambient CO_2 treatment for each species prior to analysis; in *Abutilon* this outlier was due to early abscission of a large number of leaves, while in *Amaranthus* the outlier had extremely low production of branch leaf area.

Results and Discussion

Leaf initiation rate

The rate of leaf initiation ranged from ≈0.26 lvs/d at 18°C to 0.76 lvs/d at 38° for *Abutilon,* and from ≈0.4 lvs/d at 18° to 1.06 lvs/d at 38° for *Amaranthus* (Fig. 1). For both species there was a very strong effect of temperature on plastochron rate (Table 1). Between 18° and 28° the plastochron rate increased at 0.040 and 0.065 lvs·d^{-1}°C^{-1} in *Abutilon* and *Amaranthus,* respectively, but rates increased less between 28° and 38°. The sensitivity of plastochron rate to temperature was considerably higher than that observed in other studies, as cited above. In *Abutilon* there was a small but significant increase in plastochron rate at elevated CO_2. In *Amaranthus* there was no main effect of CO_2, but there was a strong temperature × CO_2 interaction. The interaction was due to a significantly increased plastochron rate at elevated CO_2 in the 28° treatment (0.901 and 1.039 lvs/d at 400 and 700 μL/L, respectively), while there was no effect of CO_2 at the lower or higher temperature levels (Fig. 1). Effects of CO_2 on leaf initiation rates are dependent on other environmental conditions and species, which may help to explain the conflicting results reported in previous studies.

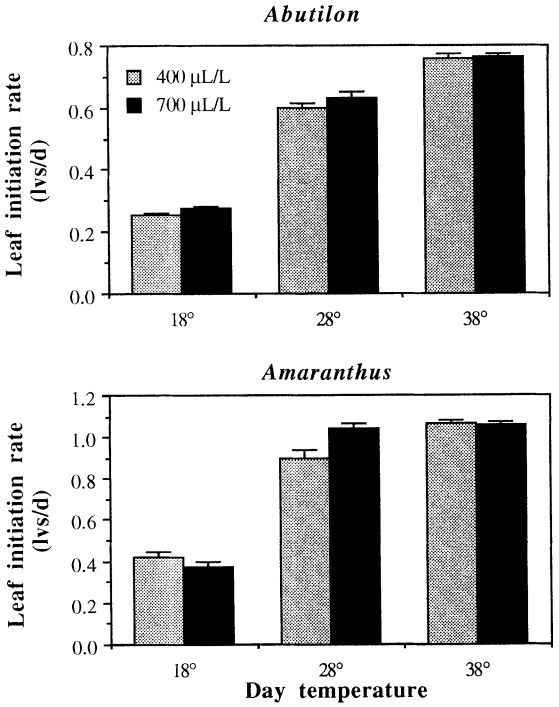

FIG. 1. Rate of leaf initiation (=plastochron rate) in response to temperature and CO_2 concentration for *Abutilon theophrasti* and *Amaranthus retroflexus.* Error bars show 1 SE of the mean.

Individual leaf area and leaf expansion

Maximum individual leaf lengths displayed a nonlinear response to temperature, with the greatest area at 28° for all nodes and both species (Fig. 2). Two-way ANOVA analyzed separately for each node and species showed highly significant effects of temperature at all nodes but no main effects of CO_2 at any node (ANOVA results not shown). There was a significant interaction between CO_2 and temperature at nodes 1, 4, 5, and 6 in *Amaranthus* and nodes 1 and 3 in *Abutilon.* In almost all cases elevated CO_2 caused a significant increase in leaf area at 28°, but a significant decrease at 38° for some nodes. Treatment effects on leaf area at node 5 were apparently due to effects on the rate of leaf expansion (Table 2). Neither temperature nor CO_2 appeared to have any effect on the duration of expansion, when measured on a developmental time scale. Leaf expansion rates were highest at 28° in both species, measured in days or in plastochrons, and in *Amaranthus* were apparently increased by high CO_2 at 28°, but decreased at 38°. The nonlinear effect of temperature

TABLE 1. Analysis-of-variance tables for the effect of temperature and CO_2 on plastochron rate (rate of leaf initiation). See patterns of response in Fig. 1. Data for *Abutilon* are $\log_e$ transformed; data for *Amaranthus* are untransformed.

Species	Source	df	MS	*F* ratio	*P*
Abutilon	Temp	2		1279.868	.000
	CO_2	1		7.216	.010
	Temp × CO_2	2		1.176	.316
	Error	54	.005		
Amaranthus	Temp	2		338.838	.000
	CO_2	1		0.996	.323
	Temp × CO_2	2		6.704	.003
	Error	48	0.007		

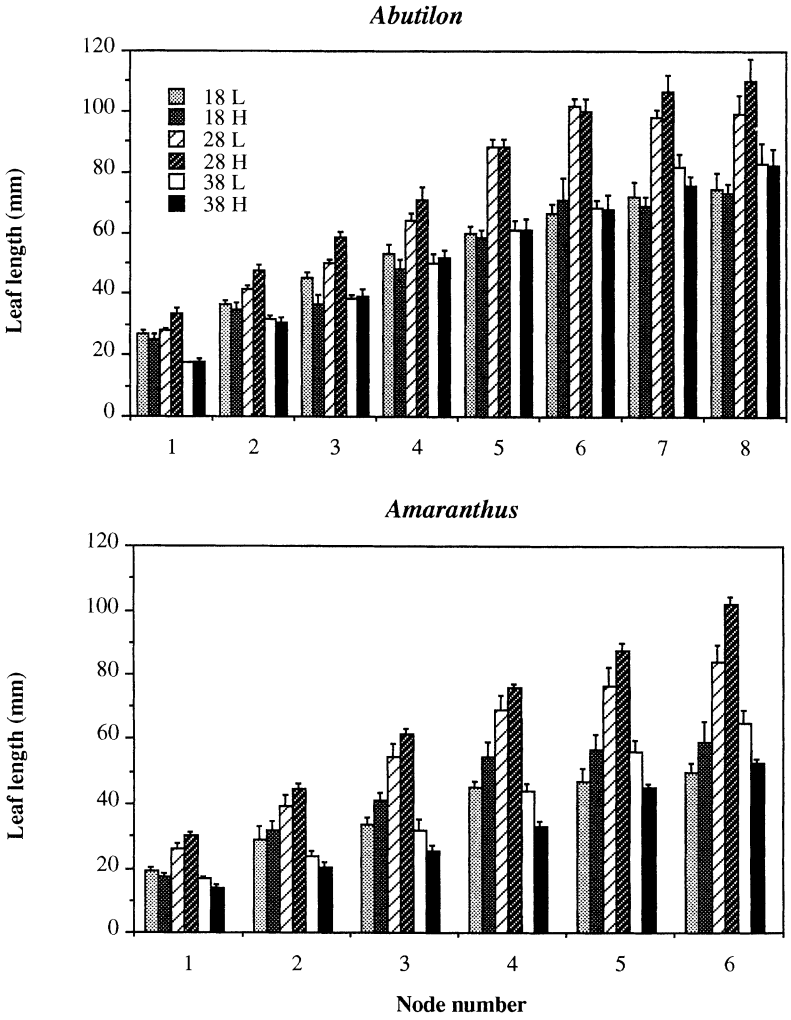

FIG. 2. Fully expanded leaf length (mean and 1 SE) by node for *Abutilon theophrasti* and *Amaranthus retroflexus*. (L and H stand for 400 and 700 μL/L CO_2, respectively.)

TABLE 2. Leaf area and the rate and duration of leaf expansion for leaf 5 as a function of daytime temperature (°C) and CO_2 concentration (μL/L).*

Species	Treatment		Leaf area (cm^2)	Duration (P)	Rate (cm^2/P)	Duration (d)	Rate (cm^2/d)
	Temp	CO_2					
Abutilon	18°	400	30.887	4.93	5.64	19.34	1.44
	18°	700	31.524	5.41	5.74	19.67	1.58
	28°	400	73.559	5.78	11.46	9.68	6.84
	28°	700	68.038	6.34	9.65	10.02	6.11
	38°	400	28.760	6.57	3.94	8.70	2.98
	38°	700	34.806	6.34	4.94	8.32	3.77
Amaranthus	18°	400	10.219	7.12	1.29	16.78	0.55
	18°	700	14.922	6.16	2.62	16.64	0.97
	28°	400	25.675	8.41	2.75	9.33	2.48
	28°	700	31.846	8.91	3.22	8.58	3.34
	38°	400	14.623	7.83	1.68	7.34	1.79
	38°	700	9.397	8.61	0.98	8.16	1.04

* Rate and duration determined from parameters of nonlinear model of leaf expansion, as explained in text. Rate and duration of expansion are expressed in time units of plastochrons (P, the interval between successive leaves) and days, which are related by plastochron rate (Fig. 1). Data from all leaves from each treatment were necessary to estimate model, precluding statistical comparisons of results.

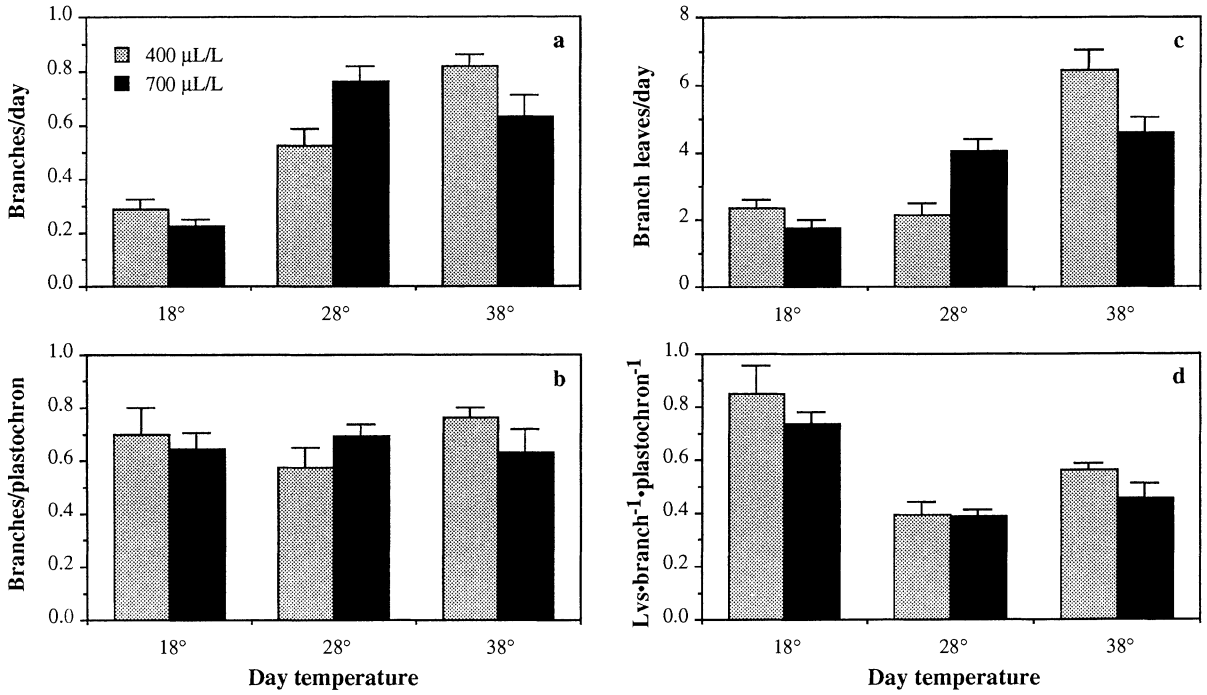

FIG. 3. *Amaranthus retroflexus*: rate of branch production on (a) chronological and (b) developmental time scales; (c) rate of branch leaf production, and (d) rate of leaf production per branch relative to main stem development. Data for days 25–31 for the 28° and 38°C treatments and days 53–61 for the 18° treatments. Error bars show 1 SE of the mean.

on leaf expansion rate and leaf area is similar to patterns observed in sugar beet, sunflower, and soybean (Terry 1968, Hofstra et al. 1977, Rawson and Dunstone 1986). Effects of CO_2 concentration on leaf expansion differ in different studies, and as with plastochron rate the patterns observed here demonstrate that CO_2 effects are dependent on associated environmental factors and on species.

Branch leaf production

In *Amaranthus,* temperature and temperature × CO_2 interaction had significant effects on the rate of branch production, measured in days. The rate of branch production was significantly greater under elevated CO_2 at 28°C, but there were no significant differences between CO_2 treatments at the lower or higher temperatures (Fig. 3a). The pattern among treatments, however, closely mirrored that of plastochron rate, so there were no significant differences among treatments when measured in plastochrons (Fig. 3b). In all treatments, branches were initiated at roughly two-thirds the rate of leaf initiation on the main stem. The mean rate of branch leaf initiation, on all branches combined, ranged from 1.77 to 6.47 lvs/d (with a high of >14 lvs/d for one plant in the 38°, low CO_2 treatment). As above, temperature and temperature × CO_2 interaction had significant effects on branch leaf production, which was increased by elevated CO_2 at 28° but decreased at 38° (Fig. 3c). The rate of leaf initiation was slower on branches than on the main stem, as indicated by initiation rates of 0.39 to 0.85 lvs·branch^{-1}·plastochron^{-1}, but was highest at 18°, followed by 38° and 28°, the reverse of the pattern for most other variables (Fig. 3d).

The high rates of branch leaf initiation are similar to those in other annual plants growing at low densities, such as *Linum usitatissimum* and *Ambrosia trifida* (Bazzaz and Harper 1977, Abul-Fatih and Bazzaz 1980). The increased relative rate of branch leaf production at low temperature is similar to results for sugar beet (Terry 1968). The relatively constant rates of branch production on a plastochron basis (Fig. 3b) suggest that temperature and CO_2 levels did not influence the general developmental patterns in these plants. This interpretation is supported by an analysis of covariance of the number of branches present on day 31, using PI as a covariate, in which there were no significant effects of temperature, CO_2, or their interaction (data not shown). Based on these data, it appears that the CO_2 effects on branch number may be due to effects on the overall rate of development and not to changes in the pattern of branch production. In the vine *Lonicera japonica,* on the other hand, branch production increased under elevated CO_2 while main stem node number actually decreased, suggesting a shift in the relationship between these processes (Sasek and Strain 1991); in future studies it would be useful to distinguish between these two mechanisms, which may underlie changes in degree of branching.

TABLE 3. Leaf area parameters for *Abutilon* on day 31 as a function of daytime temperature (°C) and CO_2 (μL/L). The slope of leaf area (LA) on plastochron index (PI†) and the predicted values of leaf area are based on regressions calculated separately for each plant.‡

Treatment		Plastochron index	Observed total leaf area	Predicted total leaf area at PI = 13.64	Slope of LA on PI for PI > 4	Predicted total leaf area at PI = 3.17
Temp	CO_2					
18°	400	3.22a	14.2a	···	···	13.54a
18°	700	3.12a	12.4a	···	···	11.99a
28°	400	12.48b	451.1b	603.1a	2.171a	12.97a
28°	700	13.78b	697.8c	682.1a	2.442a	15.53a
38°	400	13.75b	477.0b	421.5b	2.205a	7.28b
38°	700	14.00b	417.7b	359.9b	1.661b	8.31b
ANOVA						
Temp		***	***	***	*	***
CO_2		NS	NS	NS	NS	NS
Temp × CO_2		NS	*	NS ($P = .075$)	*	NS

† See *Materials and methods: Data collection* for definition of PI.

‡ All calculations were performed on square-root transformed data; results presented here were retransformed to actual area. Predicted leaf areas are presented for PI = 13.64, the mean of all plants in the 28° and 38°C treatments, and PI = 3.17, the mean of the plants in the 18° treatments. One outlier was removed from the 38°, 400 μL/L treatment prior to analysis. (Areas in square centimetres, slopes calculated using square-root transformed values as shown in Fig. 4; ANOVA significance values: * $P < .05$; ** $P < .01$; *** $P < .001$; treatment means followed by different letters are significantly different at the $P < .05$ level, as determined by pairwise orthogonal contrasts.)

Whole-plant leaf area

In both species, elevated CO_2 caused an increase in total leaf area at 28° but a decline at 38°C, creating significant temperature × CO_2 interaction effects (Tables 3 and 4). In *Abutilon* this interaction was due to increased leaf area under elevated CO_2 at 28° (Table 3). This effect on leaf area was apparently due to increases in both PI and in leaf area relative to PI (Fig. 4), though neither of these component increases was statistically significant (Table 3). Predicted leaf areas at similar PI were significantly higher at 28° than at 38°, indicating that temperature caused a shift in the allometry between leaf area and main stem development (Table 3, Fig. 4). The decrease in leaf area relative to PI at 38° was compensated by the higher leaf initiation rates, such that total leaf area of the 28°, ambient CO_2, and the 38° treatments were similar (Table 3). There was no effect of CO_2 on leaf area at 38°. Total leaf area was also greatly decreased at 18° relative to the higher temperatures. This was entirely due to the decrease in leaf initiation rate; the 28° and 38° plants had similar or decreased leaf areas at equivalent PI (Table 3).

In *Amaranthus* on day 31 elevated CO_2 increased leaf area at 28° and decreased it at 38°C. At 28° the CO_2 effect was due to both the increase in plastochron rate, and consequently in PI, and the shift in leaf area relative to PI. In contrast, at 38° there was no significant difference in PI, but leaf area relative to PI was sig-

TABLE 4. Leaf area parameters of *Amaranthus* on day 31 as a function of daytime temperature (°C) and CO_2 (μL/L). See legend to Table 3 for explanation. Predicted leaf areas are presented for PI = 20.85, the mean of all plants in the 28° and 38°C treatments. One outlier was removed from the 38°, 400 treatment previous to analysis. (Units and ANOVA results presented as in Table 3.)

Treatment		Plastochron index†	Total leaf area			Main stem leaf area			Branch leaf area		
			Observed	Predicted at PI = 20.85	Slope relative to PI	Observed	Predicted at PI = 20.85	Slope relative to PI	Observed	Predicted at PI = 20.85	Slope relative to PI
Temp	CO_2										
28°	400	17.74a	338.2a	460.4a	1.014a	288.6a	401.1a	0.956a	42.8a	59.0a	0.346a
28°	700	21.18b	652.6b	532.5b	1.036a	413.8b	416.2a	0.814a	120.8b	113.4b	0.678ab
38°	400	23.24c	767.2c	498.3ab	1.435b	250.3ac	217.2b	0.388b	448.5c	267.5c	1.643c
38°	700	21.35bc	439.5a	345.6c	0.858a	210.9c	209.2b	0.458b	144.4b	136.1b	0.845b
ANOVA											
Temp		**	*	**	NS	***	***	***	***	***	***
CO_2		NS	NS	*	*	NS	NS	NS	*	NS	NS
Temp × CO_2		**	***	***	*	***	NS	NS	***	***	***

† See *Materials and methods: Data collection* for definition of PI.

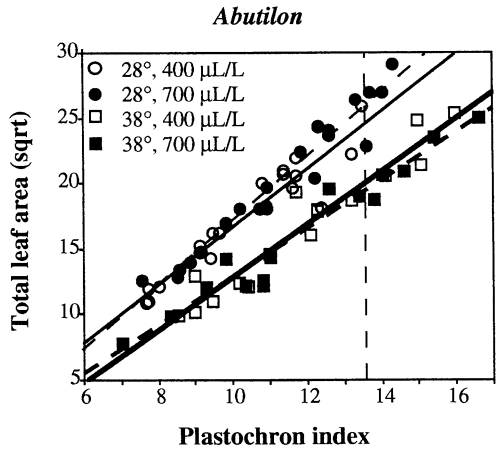

FIG. 4. Relationship between whole-plant leaf area (in square centimeters, square-root transformed) and main stem plastochron index (a continuous measure of node number) in *Abutilon theophrasti*. Regression lines shown here are calculated using all points within each treatment from day 23 to 31; statistical analyses and results in Table 3 were based on individual regressions for each plant. Vertical dashed line indicates mean plastochron index of 28° and 38°C treatments on day 31. (Regression lines: thin lines—28°, thick lines—38°; solid lines—ambient CO_2, dashed lines—elevated CO_2.)

nificantly reduced (Table 4, Fig. 5a). These patterns were due to contrasting effects of temperature and CO_2 on main stem and branch leaf area. Main stem leaf area was significantly increased relative to PI at 28°, and there was no effect of CO_2 (Fig. 5c). Branch leaf area, however, was decreased relative to PI at 28°, and there was a strong temperature × CO_2 interaction. Elevated CO_2 increased predicted branch leaf area at 28° but decreased it at 38° (Table 4, Fig. 5b). Differences in main stem leaf area on day 31 were due primarily to differences in PI, while differences in branch leaf area were due to effects on PI and on the allometry of branch leaf area relative to PI. The rate of development of main stem and branch leaf area relative to PI were negatively correlated, resulting in fairly similar rates of total leaf area development across treatments (Table 4).

These effects on whole-plant leaf area are consistent with the response of biomass accumulation, determined in plants grown simultaneously (Coleman and Bazzaz 1992). At 28°C both *Abutilon* and *Amaranthus* grew more rapidly under elevated CO_2; in *Abutilon* both leaf area and photosynthetic rate increased under elevated CO_2 but in *Amaranthus* CO_2 did not influence photosynthetic rate. At 38° in *Abutilon* neither leaf area nor biomass responded to elevated CO_2 during the first 30 d of growth. In *Amaranthus* at 38° leaf area declined significantly under elevated CO_2 (Table 4), but we do not know whether the biomass of these individuals declined in parallel. This reduction in leaf area was not readily apparent in the replicate individuals harvested at the same time by Coleman and Bazzaz (1992: Fig. 2).

The mechanistic approach to leaf area production used in this study complements allocational studies often utilized by ecologists, but at this time the two are difficult to link. In growth analysis the role of leaf area is expressed in the leaf area ratio term, which is the product of leaf mass ratio and specific leaf area (Hunt 1990). This approach provides a powerful tool for

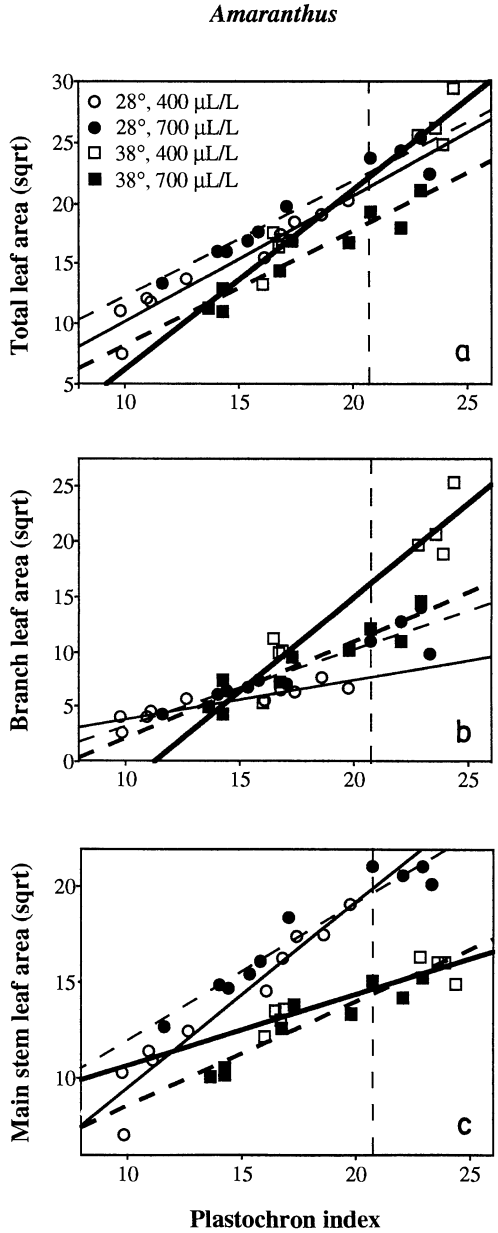

FIG. 5. Relationship of (a) whole-plant leaf area, (b) branch leaf area, and (c) main stem leaf area to main stem plastochron index in *Amaranthus retroflexus* (all data are in square centimeters, square-root transformed). All points within each treatment from day 25 and 31 were used to calculate regression lines shown here; statistical analyses and results in Table 4 were based on individual regressions for each plant. Vertical dashed line indicates mean plastochron index of 28° and 38° treatments on day 31. (Regression lines as in Fig. 4.)

quantitative interpretation of growth rate, but it remains very difficult to provide mechanistic explanations for patterns of allocation (Mooney and Chiariello 1984). In contrast, the developmental approach used here focuses directly on the processes responsible for the production of leaf area, but does not place these processes in the context of whole-plant allocation patterns. Elucidation of the relationship between individual developmental processes and whole-plant growth has proven much more difficult than the comparable analysis of the mechanistic basis of carbon assimilation and water relations. Bridging this gap remains one of the outstanding challenges in plant physiological ecology.

Conclusions

In *Abutilon* and *Amaranthus* temperature and CO_2 both influenced the development of whole-plant leaf area through effects on leaf initiation rate and on the relationship of leaf area production relative to main stem development. However, effects of CO_2 were all modified by growth temperature, particularly in *Amaranthus*. At 28° and 38°C elevated CO_2 displayed opposing effects on individual leaf area and leaf expansion rate, and in *Amaranthus* on branch production, branch leaf production, and whole-plant leaf area. Coleman and Bazzaz (1992) reported similar reversals of the effect of elevated CO_2 on relative growth rate and leaf area in both species and on total biomass in *Amaranthus*. Previous studies of these and other annual plant species from the same environment have shown that effects of CO_2 concentration on growth can also be influenced by light, nutrients, SO_2 exposure, and competition (Carlson and Bazzaz 1982, Zangerl and Bazzaz 1984, Bazzaz and Garbutt 1988, Bazzaz et al. 1989).

The interactions between CO_2 and temperature observed in this experiment emphasize that plant responses to CO_2 are not easily predictable, and depend critically on levels of other environmental factors and on the species. The sensitivity of CO_2 effects to other factors may explain some of the conflicting results observed in different studies, and emphasizes the importance of studying multiple factors simultaneously, as well as the responses of different species. Under different conditions, environmental influences on plant growth may result from effects on different aspects of the plants. In this study, the primary influences on growth apparently operated through effects on leaf area production and to a lesser extent on photosynthesis and water relations. Leaf area production reflects the interaction of several developmental processes, such as leaf initiation and expansion and branch development, all of which can be influenced by temperature and CO_2, as well as other factors. The responses of these factors observed here were largely consistent with results from other studies, e.g., the increase in plastochron rate and the nonlinear response of leaf expansion with increasing temperature, but their interactions resulted in highly variable growth responses in these two species. Consequently, long-term impacts of changing climate will depend critically on the changes in multiple environmental factors and may affect different species very differently. Predicting these changes will require a detailed understanding of physiological mechanisms, and how these mechanisms operate at various levels of organization.

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