

ORIGINAL PAPER

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Effects of low and elevated CO₂ on C₃ and C₄ annuals**I. Growth and biomass allocation**

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Abstract In order to study C₃ and C₄ plant growth in atmospheric CO₂ levels ranging from past through predicted future levels, *Abutilon theophrasti* (C₃) and *Amaranthus retroflexus* (C₄) were grown from seed in growth chambers controlled at CO₂ partial pressures of 15 Pa (below Pleistocene minimum), 27 Pa (pre-industrial), 35 Pa (current) and 70 Pa (predicted future). After 35 days of growth, CO₂ had no effect on the relative growth rate, total biomass or partitioning of biomass in the C₄ species. However, the C₃ species had greater biomass accumulation with increasing CO₂ partial pressure. C₃ plants grown in 15 Pa CO₂ for 35 days had only 8% of the total biomass of plants grown in 35 Pa CO₂. In 15 Pa CO₂, C₃ plants had lower relative growth rates and lower specific leaf mass than plants grown in higher CO₂ partial pressures, and aborted reproduction. C₃ plants grown in 70 Pa CO₂ had greater root mass and root-to-shoot ratios than plants grown in lower CO₂ partial pressures. These findings support other studies that show C₃ plant growth is more responsive to CO₂ partial pressure than C₄ plant growth. Differences in growth responses to CO₂ levels of the Pleistocene through the future suggest that competitive interactions of C₃ and C₄ annuals have changed through geologic time. This study also provided evidence that C₃ annuals may be operating near a minimum CO₂ partial pressure for growth and reproduction at 15 Pa CO₂.

Key words *Abutilon theophrasti* · *Amaranthus retroflexus* · Growth · Low CO₂ · Reproduction

Introduction

Atmospheric CO₂ has fluctuated gradually through geologic time and has only recently increased rapidly due to fossil fuel consumption and deforestation of tropical regions. CO₂ partial pressure has risen from 27 Pa to 35 Pa

since the onset of the industrial revolution 120 years ago, and is expected to rise to 70 Pa CO₂ before the end of the next century (Keeling et al. 1989). However, analyses of air trapped within the Vostoc ice core indicate that CO₂ partial pressure was as low as 18 Pa during the Last Glacial Maximum of the Pleistocene Epoch (20,000 years ago (Barnola et al. 1987)). It has been stated that 15 Pa CO₂ is the minimum CO₂ level necessary for photosynthesis in C₃ plants (Lovelock and Whitfield 1982), suggesting that C₃ plants were operating near a critical partial pressure of CO₂ during the Last Glacial Maximum.

Studies comparing the growth responses of C₃ and C₄ species to CO₂ partial pressures below current levels are relatively rare (Polley et al. 1992). C₃ species grown in low CO₂ partial pressure generally have reduced productivity and photosynthetic rates because of limitations in the availability of CO₂ at the chloroplasts and increased photorespiration (Farquhar and Sharkey 1982; Pearcy et al. 1987). In contrast, C₄ species grown in low CO₂ partial pressure generally show no reduction in photosynthetic rates or productivity because CO₂ is concentrated in chloroplasts near rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase), reducing competitive inhibition by oxygen and thereby reducing photorespiration (Pearcy et al. 1987). C₄ species have been predicted to have a competitive advantage over C₃ species under low CO₂ conditions as a result of relatively higher rates of photosynthesis and productivity (Polley et al. 1992).

The responses of C₃ and C₄ species to past levels of CO₂ are of particular interest because the composition of plant communities has been shown to be sensitive to CO₂ (Wray and Strain 1987; Bazzaz et al. 1989). Ehleringer et al. (1991) have suggested that low atmospheric CO₂ during the Miocene (26–7 million years before present) was a major selective force favoring the evolution and proliferation of C₄ species. This idea has been supported by pollen records indicating the establishment of open grasslands during the Miocene (Thomas and Spicer 1987), and by carbon-isotope evidence from northern Pakistan showing a shift from C₃ to C₄ vegetation during the Miocene (Quade et al. 1989). Furthermore, it has

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been suggested that reductions in CO₂ partial pressure may have supported the further expansion of C₄ species between 15,000 and 20,000 years ago (Ehleringer et al. 1991).

Studies comparing the responses of C₃ and C₄ species to elevated CO₂ are of interest because rising CO₂ levels are expected to have major impacts on plant communities (Bazzaz 1990). Numerous comparative studies have shown that elevated CO₂ increases the productivity of C₃ species more than C₄ species (Patterson and Flint 1980; Carter and Peterson 1983; Patterson et al. 1984). Wray and Strain (1987) demonstrated that *Aster pilosus* (C₃) had greater leaf area and biomass at 65 Pa CO₂ compared with 35 Pa CO₂, whereas *Andropogon virginicus* (C₄) had similar leaf area and biomass at 35 Pa and 65 Pa CO₂. Furthermore, *Festuca elatior* (C₃) and *Glycine max* (C₃) performed better against *Sorghum halapense* (C₄) in elevated CO₂ compared with current CO₂ conditions (Carter and Peterson 1983; Patterson et al. 1984).

Many studies comparing plant responses to elevated CO₂ have involved the C₃ species, *Abutilon theophrasti*, and the C₄ species, *Amaranthus retroflexus*. Zangerl and Bazzaz (1984) demonstrated that in a co-occurring assemblage of C₃ and C₄ annuals, including *Ab. theophrasti* and *Am. retroflexus*, the proportion of total biomass contributed by C₃ species increased relative to C₄ species in elevated CO₂. However, when grown individually at 28° C the final biomass of *Am. retroflexus* was stimulated under elevated CO₂, whereas the final biomass of *Ab. theophrasti* was not stimulated (Coleman and Bazzaz 1992). When grown in competition, elevated CO₂ stimulated the production of biomass to a greater extent in *Am. retroflexus* than in *Ab. theophrasti* (Bazzaz et al. 1989). These counter-intuitive responses of C₄ plants responding strongly to CO₂ should be considered further.

The objective of this study was to compare the effects of low, current and elevated CO₂ on the growth and biomass allocation of *Ab. theophrasti* (C₃) and *Am. retroflexus* (C₄). Plants were grown under CO₂ partial pressures of 15 Pa (below Pleistocene minimum), 27 Pa (pre-industrial), 35 Pa (current) and 70 Pa (predicted future). Plant growth was assessed by measurements of biomass, growth rate and leaf area.

Materials and methods

Growth conditions

Am. retroflexus and *Ab. theophrasti* are annual weeds found in agricultural fields and disturbed areas (Garbutt et al. 1990). Seeds of both species were planted in large 3.5 l plastic pots to reduce possible complications of root restriction (Thomas and Strain 1991) in a 3:3:1 (v/v) medium of gravel, "Turface" and sterilized topsoil, respectively. Following emergence, seedlings were thinned to one individual per pot. Pots were watered to saturation with half-strength Hoagland's solution (Downs and Hellmers 1978) each morning and with de-ionized water each afternoon.

Seeds were germinated and grown in one of four CO₂-controlled growth chambers at the Duke University Phytotron. Chambers were maintained at CO₂ partial pressures of 15 Pa, 27 Pa, 35

Pa and 70 Pa. CO₂ partial pressure within each chamber was automatically monitored and controlled by continuous CO₂ injection equipment and infrared gas analyzers (Hellmers and Giles 1979). The 15 Pa and 27 Pa CO₂ chambers were scrubbed of CO₂ when necessary by passing chamber air over a hydrated lime/vermiculite mixture. Within each chamber, *Am. retroflexus* and *Ab. theophrasti* were placed on opposite sides of the chamber to avoid interspecific shading. Light/dark periods were 14 h/10 h, with corresponding air temperatures of 28° C/22° C. The photosynthetic photon flux density (PPFD) during the light period was maintained at 1000±50 μmol photons m⁻² s⁻¹ using sodium vapor and metal halide lamps. Relative humidity was approximately 70% during the light period and 100% during the dark period. Because of careful daily monitoring of air temperature, light, and humidity, it was assumed that chamber effects were minimal and only CO₂ partial pressure varied between chambers.

One drawback of studying the responses of plants to past CO₂ partial pressures is that present genotypes may not be representative of genotypes existing in the past. C₃ plants which existed during the Pleistocene may have had heritable traits which made them more tolerant of low CO₂ conditions. Furthermore, the relatively rapid rate of change in atmospheric CO₂ level which has occurred during this century would require rapid and sensitive selection. Therefore, conclusions concerning past responses of plants to low atmospheric CO₂ are speculative.

Growth measurements

Plants of each species in each CO₂ treatment ($n=5-6$ per sampling period) were harvested 7, 14, 21, 28 and 35 days after planting. Leaf area was measured with a LI-3100 leaf area meter (LI-COR Inc., Lincoln, Neb.). Plant material was separated into roots, stems, leaves and reproductive structures (buds, flowers) and oven dried (70° C) for 48 h. Root-to-shoot ratio (RSR) was calculated as the ratio of root biomass to shoot biomass. Specific leaf mass (SLM) was calculated as the ratio of leaf biomass to leaf area, and leaf area ratio (LAR) was calculated as the ratio of leaf area to total plant biomass (Kvet et al. 1971). Instantaneous relative growth rate (RGR) (change in biomass per unit biomass per unit time) and net assimilation rate (NAR) (change in biomass per unit leaf area per unit time) were estimated using the regression method of Hunt and Parsons (1974) which allowed 95% confidence intervals to be determined. Instantaneous RGR can be defined by the following equation:

$$\text{RGR}=\text{NAR}\times\text{LAR}$$

Statistical analyses

Data from all harvests were tested for normality and log_e transformed when necessary. Two-way analyses of variance (ANOVA) were used to test for main effects of species, CO₂ and their interactions. Multiple comparisons of means were made using the Scheffe post hoc test. Treatment effects were considered significant if $P<0.05$.

Results

Biomass production

Total biomass of *Ab. theophrasti* (C₃) significantly increased as CO₂ partial pressure increased (Fig. 1). At 35 days, plants grown in 15 Pa CO₂ had only 8% of the total biomass of plants grown in 35 Pa CO₂. Total biomass was 24% lower in 27 Pa CO₂ and 22% higher in 70 Pa CO₂ compared with 35 Pa CO₂ at 35 days.

Partial pressure of CO₂ had significant effects on the biomass of all organs of *Ab. theophrasti*. Final leaf bio-

mass of *Ab. theophrasti* grown in 35 Pa CO₂ was approximately 8 times greater than plants grown in 15 Pa CO₂ (Table 1). However, there were no differences between leaf biomass of plants grown in 27 Pa, 35 Pa and 70 Pa CO₂. Final stem biomass was 30 times greater for plants grown in 35 Pa CO₂ compared with 15 Pa CO₂ and was similar between the 35 Pa and 70 Pa CO₂ treatments (Ta-

ble 1). Final root biomass of *Ab. theophrasti* in 35 Pa CO₂ was 20 times greater than in 15 Pa CO₂, and plants in 70 Pa CO₂ had 54% greater root biomass than plants in 35 Pa CO₂ (Table 1). Final reproductive biomass of *Ab. theophrasti* was greatly reduced in 15 Pa CO₂ compared with 35 Pa CO₂ (Table 1). Reproductive structures which developed in 15 Pa CO₂ consisted only of small flower buds which aborted before anthesis, but those which developed in higher CO₂ appeared as normal flowers and buds.

Total biomass produced by *Am. retroflexus* (C₄) was unaffected by CO₂ partial pressure throughout the duration of the experiment (Fig. 1). Final biomass of leaves, stems, roots and reproductive structures of *Am. retroflexus* were similar between all CO₂ treatments (Table 1).

Biomass partitioning

As *Ab. theophrasti* aged, the proportion of leaf biomass decreased as stem biomass increased in 27 Pa, 35 Pa and 70 Pa CO₂ (Fig. 2). In 15 Pa CO₂, the biomass partitioning between leaves, stems and roots remained relatively constant throughout the 35 day growth period (Fig. 2). In all treatments, the proportion of root biomass increased between 7 days and 14 days and changed slightly thereafter. At 35 days, *Ab. theophrasti* grown in 15 Pa CO₂ had the lowest RSR due to very low root biomass, and plants grown in 70 Pa CO₂ had the highest RSR (Table 1).

As *Am. retroflexus* aged, the proportion of leaf biomass decreased as stem and reproductive biomass increased (Fig. 3). In all treatments, the proportion of root biomass reached a maximum at 21 days and declined thereafter. *Am. retroflexus* had similar RSR in all CO₂ treatments at 35 days (Table 1). CO₂ partial pressure affected the phenology of *Am. retroflexus* by delaying the onset of reproduction by 4 days in 70 Pa CO₂ compared with other CO₂ treatments (data not shown).

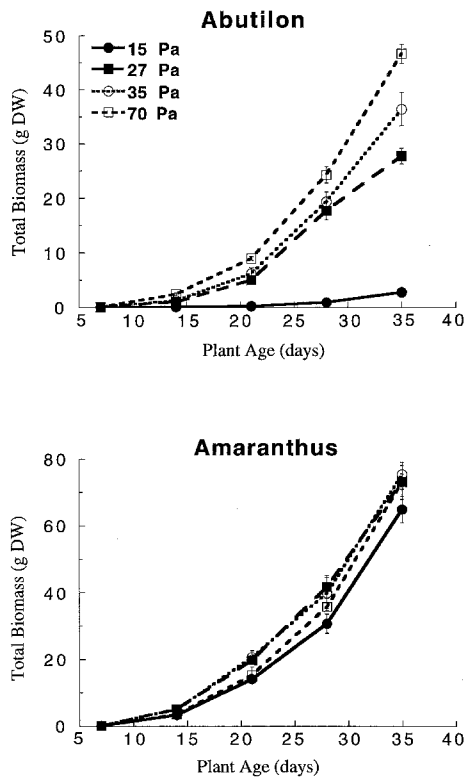


Fig. 1 Total biomass as a function of plant age and CO₂ partial pressure for *Abutilon theophrasti* and *Amaranthus retroflexus*. Symbols represent means and error bars represent ± 1 SE (note difference in scale between graphs)

Table 1 Effects of different CO₂ partial pressures on biomass production (as grams dry weight) and root-to-shoot ratio for *Abutilon theophrasti* and *Amaranthus retroflexus* after 35 days of growth. Values are means \pm standard errors for five to six plants per species per CO₂ treatment. Different superscript letters within a row indicate significant differences at $P < 0.05$

	(Experimental CO ₂ conditions)			
	15 Pa	27 Pa	35 Pa	70 Pa
Leaf biomass (g DW)				
<i>Abutilon</i>	2.06 \pm 0.27 ^a	4.4 \pm 0.73 ^b	17.3 \pm 1.3 ^b	19.3 \pm 1.4 ^b
<i>Amaranthus</i>	17.1 \pm 1.4 ^a	19.9 \pm 0.63 ^a	19.9 \pm 0.77 ^a	18.0 \pm 1.2 ^a
Stem biomass (g DW)				
<i>Abutilon</i>	0.37 \pm 0.06 ^a	8.0 \pm 0.5 ^b	11.3 \pm 0.9 ^{bc}	15.3 \pm 1.3 ^c
<i>Amaranthus</i>	25.9 \pm 1.2 ^a	30.1 \pm 0.9 ^a	31.5 \pm 1.5 ^a	32.5 \pm 2.4 ^a
Root biomass (g DW)				
<i>Abutilon</i>	0.38 \pm 0.06 ^a	5.2 \pm 0.3 ^b	7.6 \pm 1.0 ^b	11.7 \pm 1.0 ^c
<i>Amaranthus</i>	8.7 \pm 1.0 ^a	9.6 \pm 0.6 ^a	10.1 \pm 0.4 ^a	11.1 \pm 0.7 ^a
Reproductive biomass (g DW)				
<i>Abutilon</i>	0.01 \pm 0.003 ^a	0.15 \pm 0.02 ^b	0.30 \pm 0.05 ^c	0.32 \pm 0.05 ^c
<i>Amaranthus</i>	13.1 \pm 0.7 ^a	13.7 \pm 1.0 ^a	13.9 \pm 1.6 ^a	11.5 \pm 1.6 ^a
Root-to-shoot ratio				
<i>Abutilon</i>	0.17 \pm 0.049 ^a	0.23 \pm 0.0030 ^b	0.26 \pm 0.015 ^b	0.34 \pm 0.022 ^c
<i>Amaranthus</i>	0.16 \pm 0.012 ^a	0.15 \pm 0.0065 ^a	0.16 \pm 0.0044 ^a	0.18 \pm 0.014 ^a

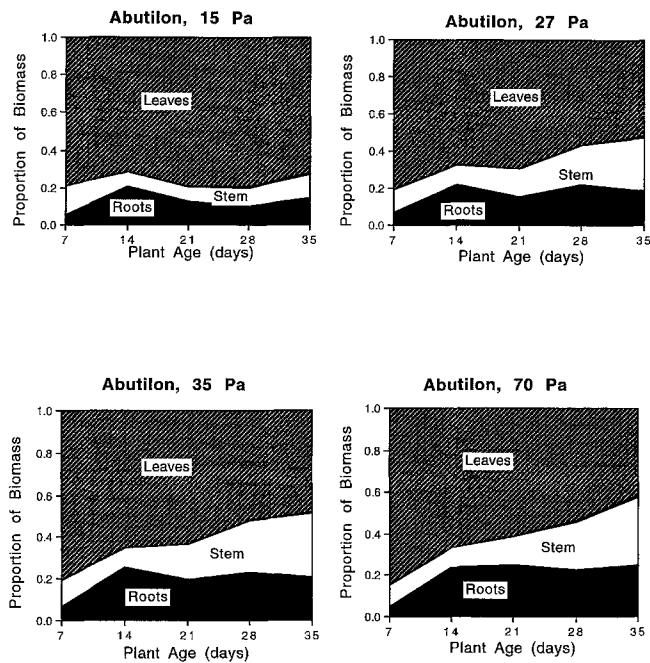


Fig. 2 Partitioning of biomass between leaves, stems and roots as a function of plant age and CO₂ partial pressure for *Abutilon theophrasti* (note proportion of reproductive biomass is too small to be seen on this scale)

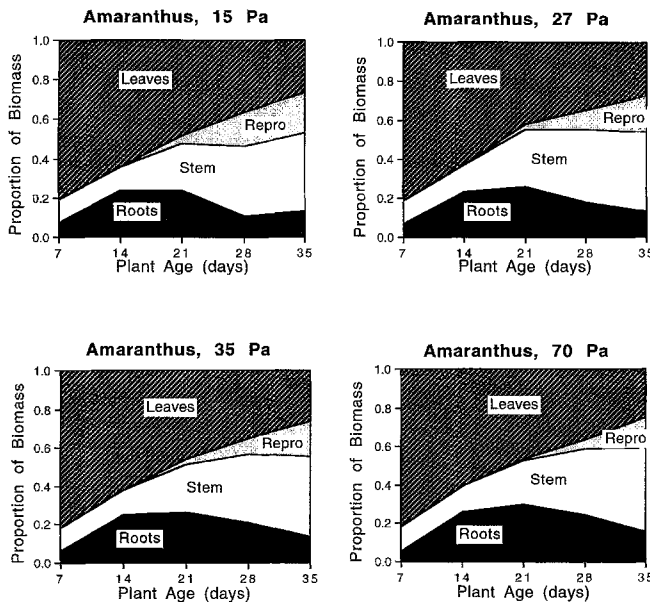


Fig. 3 Partitioning of biomass between leaves, reproductive structures, stems and roots as a function of plant age and CO₂ partial pressure for *Amaranthus retroflexus*

Growth rates

Partial pressure of CO₂ affected RGR of *Ab. theophrasti* before 21 days and had no effect thereafter (Fig. 4). At 7 days, plants grown in 70 Pa CO₂ had higher RGR and plants grown in 15 Pa CO₂ had lower RGR than plants grown in 27 Pa and 35 Pa CO₂. RGR of plants grown in

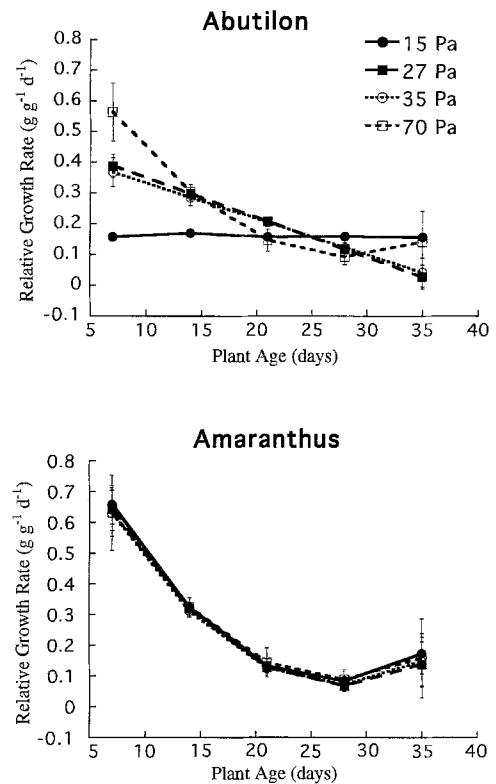


Fig. 4 Relative growth rate (RGR) as a function of plant age and CO₂ partial pressure of *Abutilon theophrasti* and *Amaranthus retroflexus*. RGR values and 95% confidence intervals (error bars) were estimated from the regression method of Hunt and Parsons (1974)

15 Pa CO₂ remained relatively constant throughout the growth period, whereas plants grown in 70 Pa CO₂ had decreasing RGR.

Partial pressure of CO₂ had the greatest effect on NAR of *Ab. theophrasti* before 28 days (Table 2). Prior to 28 days, *Ab. theophrasti* generally had the lowest NAR in 15 Pa CO₂ and the highest NAR in 70 Pa CO₂. NAR of plants grown in 15 Pa CO₂ remained relatively constant as the plants aged, whereas plants grown in 70 Pa CO₂ exhibited decreasing NAR. *Ab. theophrasti* had the highest LAR in 15 Pa CO₂ and the lowest LAR in 70 Pa CO₂ (Table 2).

RGR (Fig. 4) and NAR (Table 2) of *Am. retroflexus* were unaffected by CO₂ partial pressure. In all treatments, RGR and NAR declined between 7 days and 28 days and rose between 28 days and 35 days. *Am. retroflexus* showed no differences in LAR between CO₂ treatments (Table 2).

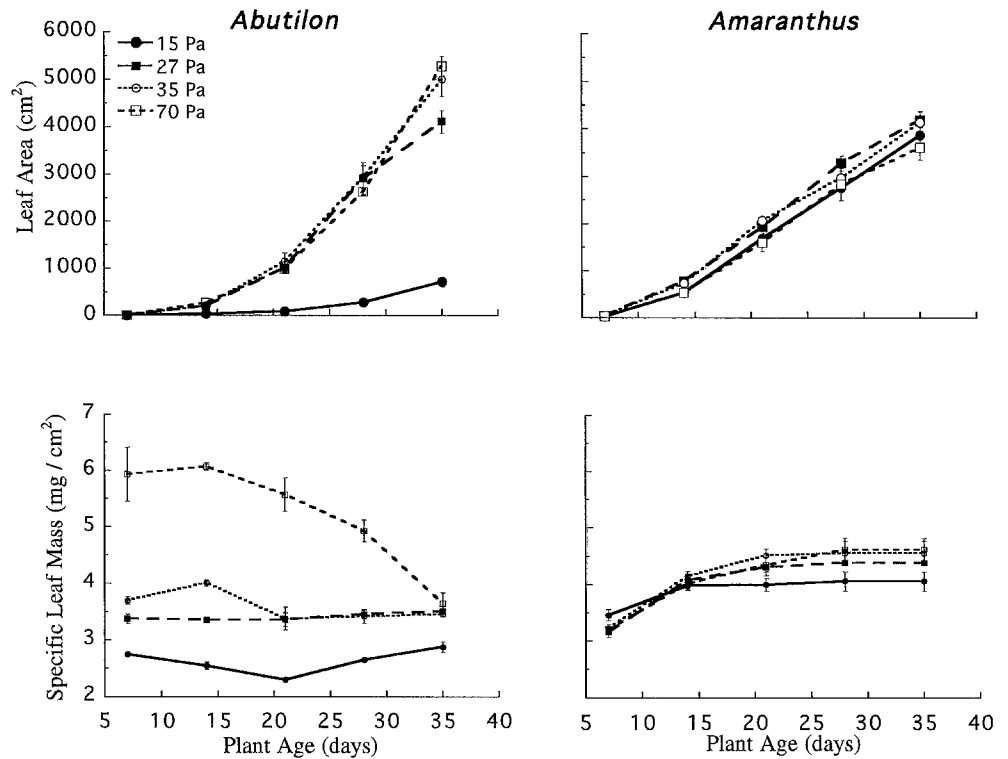
Leaf measurements

Ab. theophrasti grown in 15 Pa CO₂ had much lower leaf area compared with plants grown in higher CO₂ partial pressure throughout the 35 day growth period (Fig. 5). At 35 days, *Ab. theophrasti* grown in 27 Pa CO₂ had significantly lower leaf area than plants grown in 35 Pa

Table 2 Effects of different CO₂ partial pressures on net assimilation rate (NAR) and leaf area ratio (LAR) for *Abutilon theophrasti* and *Amaranthus retroflexus* at different ages. NAR values were generated from the method of Hunt and Parsons (1974) and LAR values are means of five to six plants. Different superscript letters within a column for the same species and measurement indicate significant differences at $P < 0.05$

	CO ₂ (Pa)	Plant age (days)				
		7	14	21	28	35
NAR (g dm⁻² d⁻¹)						
<i>Ab. theophrasti</i>	15	0.06 ^a	0.05 ^a	0.05 ^a	0.05 ^a	0.06 ^a
	27	0.17 ^b	0.14 ^b	0.11 ^b	0.07 ^a	0.02 ^a
	35	0.18 ^b	0.15 ^b	0.12 ^b	0.08 ^a	0.03 ^a
	70	0.39 ^c	0.27 ^c	0.14 ^b	0.08 ^a	0.13 ^b
<i>Am. retroflexus</i>	15	0.28 ^a	0.20 ^a	0.11 ^a	0.09 ^a	0.29 ^a
	27	0.25 ^a	0.21 ^a	0.12 ^a	0.09 ^a	0.24 ^a
	35	0.25 ^a	0.21 ^a	0.12 ^a	0.09 ^a	0.27 ^a
	70	0.25 ^a	0.22 ^a	0.13 ^a	0.11 ^a	0.32 ^a
LAR (dm² g⁻¹)						
<i>Ab. theophrasti</i>	15	2.88 ^a	2.82 ^a	3.46 ^a	3.01 ^a	2.52 ^a
	27	2.39 ^b	2.00 ^b	2.08 ^b	1.64 ^b	1.48 ^b
	35	2.18 ^b	1.63 ^b	1.89 ^b	1.53 ^b	1.38 ^b
	70	1.47 ^c	1.10 ^c	1.12 ^c	1.10 ^c	1.14 ^c
<i>Am. retroflexus</i>	15	2.34 ^a	1.61 ^a	1.21 ^a	0.91 ^a	0.60 ^a
	27	2.58 ^a	1.55 ^a	0.98 ^a	0.80 ^a	0.58 ^a
	35	2.53 ^a	1.50 ^a	1.03 ^a	0.78 ^a	0.55 ^a
	70	2.54 ^a	1.52 ^a	1.08 ^a	0.80 ^a	0.50 ^a

Fig. 5 Leaf area and specific leaf mass as a function of plant age and CO₂ partial pressure for *Abutilon theophrasti* and *Amaranthus retroflexus*. Symbols represent means and error bars represent ± 1 SE



CO₂. *Ab. theophrasti* showed no significant differences in leaf area between the 27 Pa, 35 Pa and 70 Pa CO₂ treatments before 35 days. Specific leaf mass of *Ab. theophrasti* grown in 15 Pa CO₂ was always lower than SLM of plants grown in higher CO₂ (Fig. 5). The SLM of plants grown in 70 Pa CO₂ declined with time and was significantly higher than all other CO₂ treatments before 35 days.

Leaf area and SLM (Fig. 5) of *Am. retroflexus* were unaffected by CO₂ partial pressure throughout the 35 day growth period.

Discussion

Ab. theophrasti grown in 15 Pa CO₂ had extremely low total biomass compared with plants grown in the current CO₂ level. Other studies have also demonstrated that C₃ species have reduced biomass when grown in the low CO₂ levels of the Pleistocene. Soybean (*Glycine max*) had 61% less biomass when grown in 16 Pa CO₂ compared with 35 Pa CO₂ (Allen et al. 1991), and biomass of oats (*Avena sativa*) and wild mustard (*Brassica kaber*) increased linearly between 16 Pa and 35 Pa CO₂ (Polley

et al. 1993). This evidence suggests that low levels of atmospheric CO₂ during the Pleistocene reduced the potential productivity of C₃ species.

Past studies have shown that biomass of C₃ species increases when CO₂ is raised from pre-industrial levels to current levels. For instance, mixed cultures of cowpea (*Vigna unguiculata*), okra (*Abelmoschus esculentus*) and radish (*Raphanus sativus*) had 8% greater biomass in 35 Pa CO₂ compared with 27 Pa CO₂ (Overdieck et al. 1988). Thomas and Strain (1991) showed that cotton (*Gossypium hirsutum*) had 34% more biomass when grown in 35 Pa CO₂ compared with 27 Pa CO₂. In this study, the biomass of *Ab. theophrasti* increased by 24% between 27 Pa and 35 Pa CO₂. Therefore, the productivity of C₃ species has most likely increased over the past 120 years due to anthropogenic CO₂ emissions.

Ab. theophrasti grown in 15 Pa CO₂ had a higher proportion of leaf biomass relative to root biomass than plants grown in 35 Pa CO₂. Similarly, Allen et al. (1991) found that soybean grown in 16 Pa CO₂ had a high proportion of leaf biomass relative to root biomass. Although Allen et al. (1991) did not measure photosynthesis, they suggested that partitioning more carbon to leaves may result in an enhancement of the photosynthetic apparatus under low CO₂ conditions. This enhancement may be minimal if nitrogen and other nutrients are unavailable for sufficient production of rubisco and other photosynthetic components (Tissue et al. 1993). In this study, *Ab. theophrasti* grown in 15 Pa CO₂ had extremely low root biomass which may have limited nutrient availability to leaves. Tissue et al. (1994) found leaf N content (g m⁻²) greatly reduced in *Ab. theophrasti* grown in 15 Pa CO₂. In addition, although the relative investment in rubisco versus light reaction components increased by 26% in 15 Pa CO₂ compared with 35 Pa CO₂, rubisco capacity still limited photosynthesis in 15 Pa CO₂ (Tissue et al. 1994). Greater allocation of carbon to leaves rather than roots still resulted in low rates of carbon assimilation, which limited biomass accumulation of *Ab. theophrasti* grown in 15 Pa CO₂. In contrast, *Ab. theophrasti* grown in 70 Pa CO₂ had higher root-to-shoot ratio (RSR) than plants grown in lower CO₂ treatments. C₃ plants grown in elevated CO₂ often show increased RSR due to the partitioning of additional photosynthate to below-ground tissues (Acock et al. 1983; Norby et al. 1984). Plants which produce greater root biomass in response to elevated CO₂ have the potential for exploring more soil volume, thereby increasing the uptake of soil moisture and nutrients.

All *Ab. theophrasti* plants showed the first visible signs of flower bud production between 21 days and 22 days, indicating that CO₂ partial pressure did not affect the onset of reproduction. However, all flower buds produced in 15 Pa CO₂ aborted approximately 2 days after they appeared. A possible explanation for this result is that limitations in the availability of carbon prevented reproduction from proceeding beyond the bud stage. Low CO₂ partial pressure during the Pleistocene may

have disrupted the sexual reproduction of some C₃ species.

In *Ab. theophrasti*, differences in final biomass between CO₂ treatments can be attributed to the effect of CO₂ partial pressure on RGR during early growth. *Ab. theophrasti* in 15 Pa CO₂ had approximately half the RGR of plants in 35 Pa CO₂ during the first 14 days of growth, resulting in lower biomass accumulation throughout the growth period. Likewise, Allen et al. (1991) demonstrated that soybean (*Glycine max*) grown in 16 Pa CO₂ had lower RGR during early growth than soybean grown in 33 Pa CO₂. In contrast, *Ab. theophrasti* in 70 Pa CO₂ had higher RGR during the first 14 days of growth than plants in 33 Pa CO₂, resulting in greater biomass accumulation throughout the growth period. These differences in RGR can be explained by changes in NAR and LAR between CO₂ treatments. Low RGR in 15 Pa CO₂ was due to low NAR, despite high values of LAR; high RGR in 70 Pa CO₂ was due to high NAR, despite low values of LAR. Therefore, leaf area was highest relative to plant size in 15 Pa CO₂ compared to all other CO₂ treatments, but the efficiency of leaves in producing new growth was lowest in 15 Pa CO₂. Past studies have also demonstrated that NAR decreases and LAR increases with decreasing CO₂ partial pressure (Norby and O'Neill 1991; Bowler and Press 1993).

Ab. theophrasti grown in 70 Pa CO₂ had higher SLM throughout most of the growth period compared with plants grown in lower CO₂ partial pressure. C₃ species grown in elevated CO₂ commonly show increased SLM because of greater amounts of stored starch in leaf tissue (Allen et al. 1988; Vu et al. 1989), as has been shown in *Ab. theophrasti* (Tissue et al. 1994), or because of more cell layers within that tissue (Thomas and Harvey 1983). In *Ab. theophrasti* grown in 70 Pa CO₂, SLM decreased with time, in part due to diminishing sink demand. On the other hand, SLM of *Ab. theophrasti* grown in 15 Pa CO₂ remained low throughout the growth period because of limitations in the availability of carbon (Tissue et al. 1994).

CO₂ partial pressures between 15 Pa and 70 Pa had no effect on production or partitioning of biomass in the C₄ species, *Am. retroflexus*. This result suggests that C₄ species may have been more competitive against C₃ species during periods of low atmospheric CO₂ such as the Pleistocene. The only effect of CO₂ partial pressure on *Am. retroflexus* was a delay in the onset of reproduction in 70 Pa CO₂, as has been shown in *Sorghum halepense* (C₄) (Carter and Peterson 1983) and *Setaria faberii* (C₄) (Garbutt et al. 1990) under elevated CO₂ conditions. In the future, the timing of plant-pollinator interactions may be offset for species which exhibit delayed reproduction in response to elevated CO₂ (Bazzaz et al. 1985). Bazzaz et al. (1989) reported that between 15 days and 40 days of growth, *Am. retroflexus* accumulated greater biomass with increasing CO₂. This result contrasts with our study, probably because we grew *Am. retroflexus* with constant PPFD levels of 1000 μmol photons m⁻² s⁻¹, whereas

Bazzaz et al. (1989) grew *Am. retroflexus* under natural light conditions where PPFD reached a maximum of 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. High irradiance has been shown to increase the response of C_4 species to elevated CO_2 (Sionit and Patterson 1984).

In conclusion, CO_2 partial pressures increasing from 15 Pa through 70 Pa increased the growth of the C_3 species, *Ab. theophrasti*, and had little effect on the growth of the C_4 species, *Am. retroflexus*. These findings support other studies that show C_3 plant growth is more responsive to CO_2 partial pressure than C_4 plant growth. Differences in growth responses to CO_2 levels of the Pleistocene through the future suggest that competitive interactions of C_3 and C_4 annuals have changed through geologic time. Furthermore, *Ab. theophrasti* had very low biomass and aborted reproduction in 15 Pa CO_2 , indicating that some C_3 annuals may be operating near a minimum CO_2 partial pressure for growth and reproduction at 15 Pa CO_2 .

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