

High Temperature-induced Sink Limitation Alters Growth and Photosynthetic Acclimation to Elevated CO₂ in Bean (*Phaseolus vulgaris* L.)

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ABSTRACT. Average global surface temperatures are predicted to rise due to increasing atmospheric CO₂ and other greenhouse gases. Attempts to predict plant response to CO₂ must take into account possible temperature effects on phenology and reproductive sink capacity for carbohydrates. In this study, we investigated the effects of atmospheric CO₂ partial pressure [35 Pa ambient CO₂ (aCO₂) vs. 70 Pa elevated CO₂ (eCO₂)] and temperature (26/15 vs. 35/21 °C day/night) on short- and long-term net CO₂ assimilation (A_n) and growth of red kidney bean (*Phaseolus vulgaris*). During early vegetative development [14–31 days after planting (DAP)], A_n and relative growth rate (RGR) at eCO₂ were significantly greater at the supra-optimum (35/21 °C) than at the optimum (26/15 °C) temperature. At 24 DAP, the CO₂ stimulation of A_n by eCO₂ was 49% and 89% at optimum and supra-optimum temperature, respectively, and growth enhancement was 48% and 72% relative to plants grown at aCO₂. This high temperature-induced growth enhancement was accompanied by an up-regulation of A_n of eCO₂-grown plants. In contrast, during later reproductive stages (31–68 DAP) the eCO₂ stimulation of A_n was significantly less at the supra-optimum than at optimum temperature. This was associated with reduced seed set, greater leaf carbohydrate accumulation, and down-regulation of A_n at the higher temperature. At final harvest (68 DAP), the eCO₂ stimulation of total dry weight was 31% and 14% at optimum and supra-optimum temperature respectively, and eCO₂ stimulation of seed dry weight was 39% and –18% at optimum and supra-optimum temperature, respectively. These data indicate substantial shifts in the response to eCO₂ during different phenological stages, and suggest that impaired reproductive development at high temperature could reduce the potential for CO₂ stimulation of photosynthesis and productivity in bean and possibly other heat-sensitive species.

One of the outcomes of the sustained emission of carbon dioxide and other radiatively active “greenhouse” gases into the atmosphere is an increase in mean global surface temperatures of ≈2–5 °C (Intergovernmental Panel on Climate Change, 2001; Stokstad, 2004). An increase in the frequency of extreme weather events such as periods of high temperature is also possible (Rosenzweig and Hillel, 1998). Such unfavorable environmental factors could limit crop productivity in a future CO₂-enriched world. A better understanding of high-temperature stress effects on the response of plants to elevated CO₂ (eCO₂) will be important to predict the environmental and economic impacts of a continued increase in greenhouse gases.

In general, the eCO₂ stimulation of net CO₂ assimilation (A_n) in C₃ plants tends to increase as temperatures rise in the non-stress range of 15–30 °C (Long, 1991; Sage et al., 1995; Wilks et al., 1995). However, when temperatures are high enough to impair reproductive development, the eCO₂ stimulation of A_n may not translate into higher crop yields due to reduced seed or fruit set. Also, a reduced reproductive sink capacity could exacerbate

downward photosynthetic acclimation to eCO₂ (Crawford and Wolfe, 1999).

Downward photosynthetic acclimation, defined here as a diminished stimulatory effect of eCO₂ on A_n over time, is frequently observed when either genotypic or environmental factors limit the plant’s capacity for utilization of the products of photosynthesis (Arp, 1991; Nowak et al., 2004; Wolfe et al., 1998). The source–sink imbalance results in an accumulation of hexose sugars in the leaf, and a suppression of genes encoding for the key photosynthetic enzyme, Rubisco (Moore et al., 1999). Other mechanisms of downward acclimation at eCO₂ have been reported, such as reduced light penetration or thylakoid membrane damage resulting from starch granule accumulation in the chloroplasts (Cave et al., 1981; Yelle et al., 1989). The importance of source–sink carbon balance on the sustainability of photosynthetic capacity has been documented in studies where this balance has been manipulated for example genetically (Peet et al., 1986; Sonnewald et al., 1994) or by defruiting (Clough et al., 1981). Indicators of downward acclimation include: a reduction in Rubisco activity and/or quantity; a reduction in leaf nitrogen (N) concentration; and a lower A_n of eCO₂-grown plants compared to plants grown at ambient CO₂ (aCO₂) when both are measured at a common aCO₂ level (Sage, 1994).

There is considerable variability among species in their sensitivity to high temperature and the CO₂ × temperature interactive effects (Morrison and Lawlor, 1999). For *Phaseolus* L., even moderately high temperatures (e.g., day >32 °C and/or night temperature >27 °C) can negatively affect pollen viability, pod set, and yield (Gross and Kigel, 1994; Konsens et al., 1991; Monteroso and Wien, 1990; Rainey and Griffiths, 2005). Prasad et al.

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(2003) reported that eCO_2 did not offset the negative effects of high temperature stress on pollen viability and pod set of beans, but they did not examine the effects of stress on CO_2 response in relation to growth stage, source–sink balance, or photosynthetic acclimation.

Understanding how external factors such as temperature control internal processes such as whole plant source–sink balance and photosynthetic acclimation will be important for predicting the effects of rising atmospheric CO_2 on growth and yield of heat-sensitive crops. In this study, the combined effects of air temperature [optimum (26 °C) or supra-optimum (35 °C)] and air CO_2 partial pressure (pCO_2) [ambient (35 Pa) and twice ambient (70 Pa)] on leaf gas exchange and other indicators of photosynthetic acclimation, as well as growth and dry weight allocation, were measured throughout development to examine how temperature effects on reproductive development would alter A_n and yield responses to eCO_2 .

Materials and Methods

PLANT MATERIAL AND GROWTH CONDITIONS. Red kidney bean (cv. Redcloud) seeds were sown in 10-L plastic pots (0.24 m diameter, 0.23 m deep) containing a mixture of 2 peat : 2 vermiculite : 1 perlite (by volume). Following germination (7 DAP), seedlings were thinned to one per pot and randomly assigned to each of four, temperature-controlled, walk-in growth chambers constructed within two adjacent, sunlit glasshouses (two chambers in each glasshouse) at Cornell Univ., Ithaca, N.Y. (lat. 42°27'N, long. 76°29'W, elev. 500 m). The growth chambers (previously described by Jifon and Wolfe, 2002), were constructed of aluminum frames 2.4 m long, 2.4 m wide, and 1.8 m tall and covered with transparent polyethylene. Natural sunlight was supplemented with 1000-W multi-vapor metal halide lamps (model MVR1000/U; GE Lighting Systems, Cleveland) located above each chamber. Maximum photosynthetic photon flux (PPF) within each chamber was $\approx 1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at a height of 1 m and the photoperiod was 14 h. One glasshouse was maintained at a supra-optimum day/night temperature of 35/21 °C and the other at 26/15 °C, which is close to optimum for maximum growth of beans (Rainey and Griffiths, 2005). Growth chamber air temperatures were controlled to within 3 °C of the target points using the glasshouse temperature control system supplemented by thermostat-controlled air-conditioning units (Kenmore model 79056, constant fan; Sears Corp., Hoffman Estates, Ill.) installed in each chamber. Relative humidity (RH) varied between 60% and 95%. A two-by-two factorial combination of air temperature (35/21 °C or 26/15 °C, day/night) and pCO_2 [ambient (35 Pa) and twice ambient (70 Pa)] was maintained throughout the experimental duration. The CO_2 treatments (35 or 70 Pa) were randomly assigned to each of the two growth chambers in each glasshouse. Plants were watered at least once per day using an automatic drip irrigation system, and fertilized three times per week with a complete water-soluble fertilizer (20N–8.7P–16.6K, Peters 20–20–20; The Scotts Co., Marysville, Ohio). Mutual shading among plants was minimized by placing pots at least 30 cm apart, and as plants were harvested during the course of the experiment, the spacing was increased to accommodate the increased canopy sizes.

GAS EXCHANGE MEASUREMENTS AND EVALUATION OF PHOTOSYNTHETIC ACCLIMATION. Light-saturated ($PPF = 1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) net CO_2 assimilation rates (A_n) of the youngest fully expanded leaves were measured with a portable photosynthesis system

(LI-6200; LI-COR, Lincoln, Nebr.). Assimilation rates were measured at least once before anthesis (14–24 DAP) and after anthesis (31–54 DAP) to coincide with periods of predictable changes in whole-plant source/sink balance associated with vegetative and reproductive growth. During each measurement period, A_n was measured first at the growth pCO_2 and then repeated at the reciprocal CO_2 concentration by lowering or raising growth chamber pCO_2 . Plants were equilibrated for about 60 min in the new CO_2 environment prior to measurements. All measurements were made at the growth temperature. Water vapor pressure deficit during measurements varied from 1.5 to 2.8 kPa. A photosynthetic acclimation index (AI) (Stirling et al., 1997) for CO_2 -enriched plants was calculated from A_n measurements conducted at a common pCO_2 (35 Pa, based on the reciprocal measurements) as follows:

$$AI = A_{n35E} / A_{n35A}$$

where A_{n35E} is the net assimilation rate of plants grown at eCO_2 and measured at 35 Pa, and A_{n35A} is the net assimilation rate of plants grown and measured at 35 Pa. All A_{n35} measurements were made on the same leaves as A_n measurements. A comparison of assimilation rates measured at the same pCO_2 allowed us to make inferences about CO_2 and temperature effects on photosynthetic capacity; however, this approach does not allow for the comprehensive analysis of a complete A_n vs. intercellular pCO_2 (A-C) curve (Sage, 1994). By definition, the AI of plants grown and measured at ambient pCO_2 (aCO_2) is 1.0, whereas values <1.0 or >1.0 for eCO_2 -grown plants are indicative of downward or upward photosynthetic acclimation, respectively.

LEAF AND WHOLE-PLANT MEASUREMENTS. Following the A_n measurements, plants were returned to their growth environments, and in the mid-afternoon (1500–1600 HR), leaf disks (2.5 cm² each) were sampled (avoiding major veins) from the same leaflet used in gas exchange measurements. Two disks were used to determine specific leaf area [SLA (leaf area per unit leaf dry weight)]. Leaf total nonstructural carbohydrate [TNC (glucose, sucrose, and starch)] concentrations were assayed as glucose equivalents following the enzymatic methods described by Hendrix (1993). Groups of four to five plants from each treatment were harvested at least twice during the experiment (before and after anthesis), and the components—leaves, stems, roots, and pods—were dried to constant weight. Total leaf area per plant was calculated from SLA and leaf weight data. Leaf area ratio [LAR (total leaf area/total plant dry mass)] and harvest index [HI (the ratio of seed yield to total biomass yield per plant)] were also computed.

STATISTICAL ANALYSIS. Three complete repetitions (serving as replications or “blocks”) of the experiment were conducted during the summer months of 1995–98. After each repetition, CO_2 treatments were randomly re-assigned among the growth chambers in each glasshouse. Analysis of variance (ANOVA) using the general linear model procedures of SAS (SAS, Cary, N.C.) was conducted as a split-split plot design, with growth temperature as main plot, CO_2 as sub-plot, measurement date as sub-sub plot, and three replications (blocks). Even though measurements were made at approximately the same growth stage in each experiment, some physiological variables differed between experiments because of slight differences in plant size and possibly differences in absolute air vapor pressure between measurement dates. However, the response patterns were similar among measurement periods and experiments. In some cases, only data from one repetition of the study are presented.

Results

LEAF GAS EXCHANGE RESPONSES. Net CO₂ assimilation rates varied with growth pCO₂, temperature, and measurement date [Fig. 1A ($P < 0.01$ for the measurement date \times CO₂ \times temperature interaction)]. During early vegetative development (14–24 DAP), the CO₂ stimulation of A_n was greater in leaves of plants grown at 35 °C (89%) than in plants grown at 26 °C (48%). In the late developmental stages (31–54 DAP), however, CO₂ stimulation of A_n declined significantly and more rapidly at 35 °C (12% at 54 DAP) than at 26 °C (26% at 54 DAP).

A significant measurement date \times CO₂ \times temperature interaction ($P < 0.01$) was also observed for A_{n35} [A_n measured at a common pCO₂ (35 Pa)]. During early development (14–24 DAP),

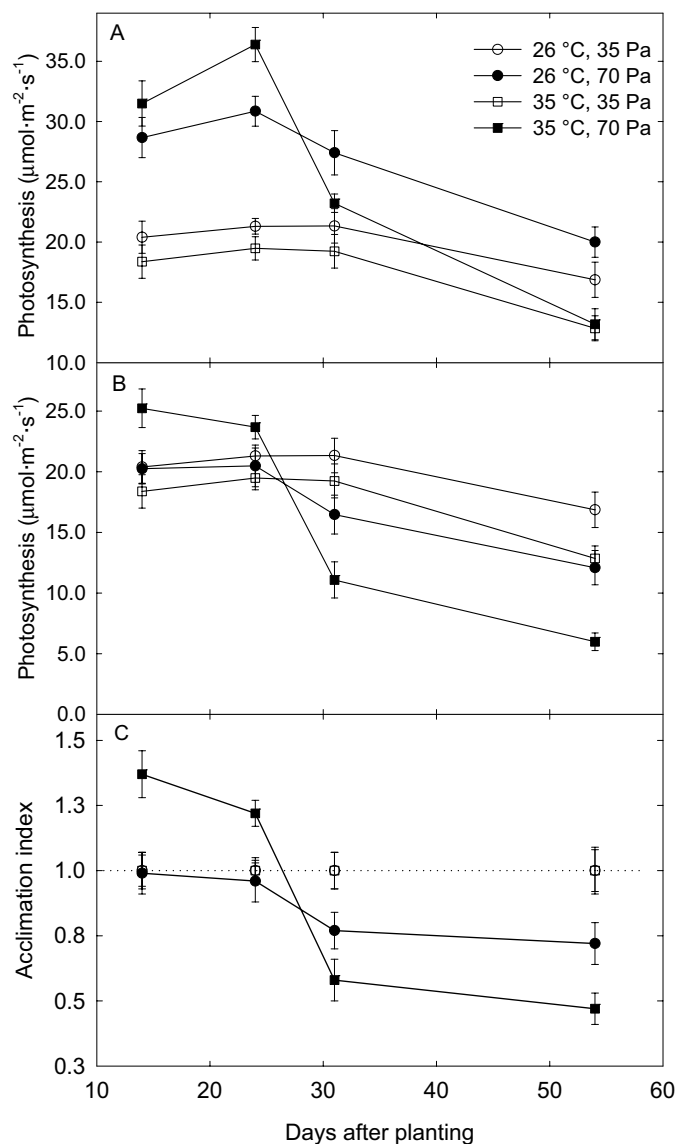


Fig. 1. Influence of growth temperature (26 or 35 °C) and CO₂ partial pressure [pCO₂ (35 or 70 Pa)] on leaf photosynthesis (A, B) and photosynthetic acclimation to elevated CO₂ (C) in beans. Photosynthetic rates were measured at the growth pCO₂ and temperature (A) or at a common pCO₂ (35 Pa) (B). The photosynthetic acclimation index (C) is the ratio A_{n35E}:A_{n35A}, where A_{n35E} is the net leaf CO₂ assimilation rate of plants grown at eCO₂ and measured at 35 Pa, and A_{n35A} is the net leaf CO₂ assimilation rate of plants grown and measured at 35 Pa. Each point is the mean \pm SE of four to eight measurements on leaves from four different plants.

A_{n35} values of CO₂-enriched plants were either similar to (at 26 °C) or significantly greater than (at 35 °C) A_{n35} of plants grown at 35 Pa CO₂ (Fig. 1B). At 31 and 54 DAP, however, A_{n35} of CO₂-enriched plants were significantly lower ($\approx 31\%$) than those of plants grown at 35 Pa CO₂, indicative of downward acclimation as defined here. The decline in A_{n35} of CO₂-enriched plants late in development was more severe in plants grown at 35 °C (-40%) compared to 26 °C-grown plants (-23%). Accordingly, acclimation indices (AI) were either greater ($+24\%$ at 35 °C) or not significantly different (-4% at 26 °C) from unity at 24 DAP. AI values were generally lower than unity at 54 DAP (-28% and -54% at 26 and 35 °C, respectively) (Fig. 1C).

Stomatal conductance [g_s (measured at growth conditions)] was significantly reduced at eCO₂ ($P < 0.01$) and high temperature [$P < 0.01$ (Table 1)]. g_s also declined on average 43% between 24 and 54 DAP ($P = 0.01$ for measurement date effect). The measurement date \times CO₂ \times temperature interaction was not significant ($P = 0.27$). C_i:C_a values varied from 0.60 to 0.85 and were generally lower at 54 DAP ($\approx 18\%$) compared to 24 DAP.

TISSUE N AND LEAF NONSTRUCTURAL CARBOHYDRATE CONTENT. Leaf N concentrations were reduced at eCO₂ (average -27% at 24 DAP and -30% at 54 DAP) compared to aCO₂-grown plants [$P < 0.01$ (Table 2)]. A significant temperature-induced reduction in leaf N was also observed at 54 DAP ($P = 0.01$ for the measurement date \times CO₂ \times temperature interaction). A strong positive linear relationship between leaf N and AI was observed at 54 DAP (Fig. 2A).

Total nonstructural carbohydrates (sum of leaf glucose, sucrose, and starch) concentrations were significantly higher in leaves of plants grown at eCO₂ compared to leaves grown at aCO₂ [Table 2 ($P < 0.01$ for the measurement date \times CO₂ \times temperature interaction)]. Individually, this increase was only significant for glucose and starch. Leaf glucose concentration of CO₂-enriched plants was increased by growth at high temperature but this effect was only significant at 54 DAP. At 24 DAP, leaf starch concentration of CO₂-enriched plants was reduced at 35 °C relative to 26 °C, but at 54 DAP, starch concentration was significantly increased at 35 °C (Table 2). Leaves with high TNC, and particularly high glucose concentration, had lower AI values than leaves with low TNC especially at 54 DAP (Fig. 2B).

LEAF PROPERTIES. At 24 DAP, total leaf area per plant (LA) was significantly higher in CO₂-enriched plants at both temperature regimes, whereas at 54 DAP, no CO₂ effects were observed (Table 3). The average LA of plants grown at 35 °C was reduced slightly at 54 DAP due to accelerated leaf senescence/abscission. Significant eCO₂-induced reductions in specific leaf area [SLA ($P = 0.02$)] and leaf area ratio [LAR ($P = 0.02$)] were also observed,

Table 1. Growth temperature (26 or 35 °C) and CO₂ partial pressure [pCO₂ (35 or 70 Pa)] effects on leaf stomatal conductance (g_s) and the ratio of leaf intercellular to ambient pCO₂ (C_i:C_a) of bean plants during vegetative [24 d after planting (DAP)] and reproductive (54 DAP) growth phases. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Duncan's multiple range test, $n = 4$ to 8).

Temp (°C)	pCO ₂ (Pa)	g_s (mol·m ⁻² ·s ⁻¹)		C _i :C _a	
		24 DAP	54 DAP	24 DAP	54 DAP
26	35	0.54 a	0.31 a	0.80 a	0.74 a
	70	0.35 b	0.15 b	0.82 a	0.60 b
35	35	0.47 a	0.17 b	0.82 a	0.67 a
	70	0.29 b	0.09 c	0.80 a	0.61 b

Table 2. Influence of growth temperature (26 or 35 °C) and CO₂ partial pressure [*p*CO₂ (35 or 70 Pa)] on leaf nitrogen (N), glucose, and starch concentrations of bean plants during vegetative [24 d after planting (DAP)] and reproductive (54 DAP) growth phases. Means followed by the same letter(s) within a column are not significantly different at *P* < 0.05 (Duncan's multiple range test, *n* = 12 to 13).

Temp (°C)	<i>p</i> CO ₂ (Pa)	N (mg·g ⁻¹)		Glucose (mg·g ⁻¹)		Starch (mg·g ⁻¹)	
		24 DAP	54 DAP	24 DAP	54 DAP	24 DAP	54 DAP
26	35	43.9 a	33.7 a	1.7 bc	2.7 c	67.4 bc	105.9 c
	70	32.8 b	25.5 b	2.2 ab	3.7 b	96.3 a	144.7 b
35	35	45.7 a	26.5 b	1.3 c	3.4 bc	57.4 c	102.9 c
	70	32.3 b	17.1 c	2.4 a	4.9 a	77.3 b	182.9 a

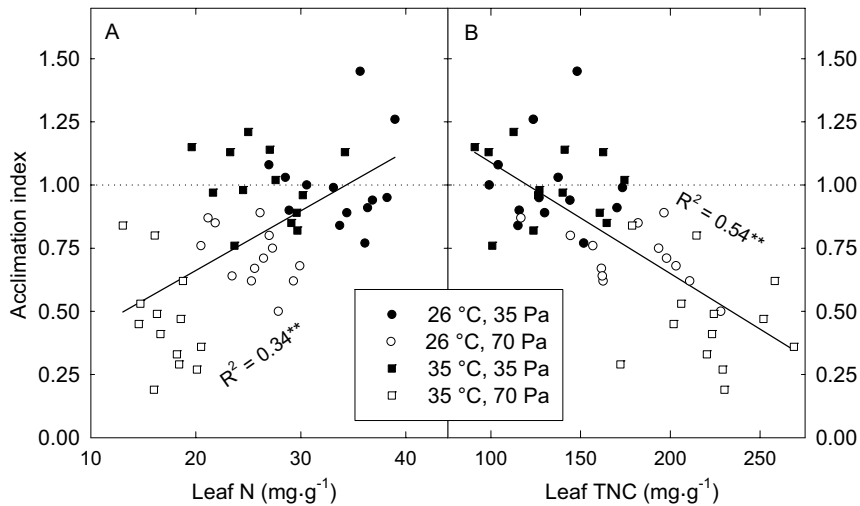


Fig. 2. Effects of (A) leaf nitrogen (N) or (B) total nonstructural carbohydrate [TNC (glucose, sucrose, and starch)] concentration on photosynthetic acclimation to elevated CO₂ in leaves of bean plants grown in a factorial combination of air temperature (26 or 35 °C) and air CO₂ partial pressure [*p*CO₂; ambient (35 Pa) and twice ambient (70 Pa)]. The acclimation index is the ratio $A_{n35E}:A_{n35A}$, where A_{n35E} is the net leaf CO₂ assimilation rate of plants grown at eCO₂ and measured at 35 Pa, and A_{n35A} is the net leaf CO₂ assimilation rate of plants grown and measured at 35 Pa. Each point represents a single leaf measurement. R² = coefficient of determination; ***P* ≤ 0.001.

Table 3. Influence of growth temperature (26 or 35 °C) and CO₂ partial pressure [*p*CO₂ (35 or 70 Pa)] on leaf area (LA), specific leaf area (SLA) and leaf area ratio (LAR) of bean plants during vegetative [24 d after planting (DAP)] and reproductive (54 DAP) growth phases. Means followed by the same letter(s) within a column are not significantly different at *P* < 0.05 (Duncan's multiple range test, *n* = 12 to 13).

Temp (°C)	<i>p</i> CO ₂ (Pa)	LA (m ²)		SLA (m ² ·kg ⁻¹)		LAR (m ² ·kg ⁻¹)	
		24 DAP	54 DAP	24 DAP	54 DAP	24 DAP	54 DAP
26	35	0.22 c	0.59 a	33.7 a	22.0 a	18.7 a	5.6 ab
	70	0.31 ab	0.71 a	30.8 a	17.5 b	17.1 a	4.4 c
35	35	0.28 bc	0.58 a	31.3 a	19.5 ab	16.3 a	6.6 a
	70	0.36 a	0.61 a	29.4 a	16.3 b	16.1 a	5.1bc

but only at 54 DAP. Temperature effects on SLA and LAR were not significant (*P* > 0.13).

GROWTH. Relative growth rates (RGR) ranged from 0.03 to 0.16 g·g⁻¹·d⁻¹ and differed significantly among treatments and between growth stages [*P* < 0.01, (Fig. 3, Table 4)]. During early vegetative growth stages (14–31 DAP), RGR was stimulated by eCO₂ at the warm compared to cool temperature (Fig. 3). This rapid development was manifested as increased branching and larger plant canopies rather than earlier flowering. At 24 DAP, the average eCO₂ stimulation of total biomass accumulation was 48% and 72% at 26 and 35 °C, respectively.

In contrast, during the reproductive growth stages (31–68 DAP), this growth trend was reversed as RGR was significantly reduced and reproductive development impaired by high temperature (Table 4). The responses of RGR, pod development, and harvest index to eCO₂ at high temperature were generally negative at the later developmental stages. Harvest index was unaffected by growth *p*CO₂ (*P* > 0.8), but was significantly reduced by growth at 35 °C (*P* = 0.01). At maturity (68 DAP), the average CO₂ stimulation of vegetative dry weight (roots + stems + leaves) production was 27% and 14% at 26 and 35 °C, respectively (Table 5). CO₂-enrichment increased the number of pods initiated at both temperatures (60% and 92% at 26 and 35 °C, respectively), but the majority of pods initiated at 35 °C were parthenocarpic. Actual seed yield was increased by eCO₂ at 26 °C (34%) but reduced at 35 °C (–18%).

Discussion

A greater eCO₂ stimulation of A_n at high temperature compared to optimum temperature is generally anticipated because of a proportionally greater CO₂ inhibitory effect on photorespiration of C₃ plants as temperatures rise (Long, 1991; Sage et al., 1995). Metabolism and sink demand for photosynthates also tend to increase at higher temperatures, so that there is a reduced tendency for leaf carbohydrate accumulation and negative feedback effects on A_n (Crawford and Wolfe, 1999). This is essentially what we observed at early growth stages (e.g., 24 DAP), where CO₂ stimulation of both photosynthesis [A_n (Fig. 1A)] and growth [RGR (Table 4)] were greater at 35/21 °C compared to 26/15 °C. These results are in agreement with other studies that have also focused on that early, rapid growth stage. For instance, Idso et al. (1987), Idso and Idso (1994), Kimball (1983), and Kimball et al. (1993) analyzed numerous previous studies (many of which focused on the vegetative growth stages) and found that the ratio of plant biomass at elevated CO₂ to the biomass at standard CO₂ [or the biomass growth modification ratio (BGMR)] increased with temperature by a factor of 0.083 g·g⁻¹ per °C over a 12–34 °C temperature range.

In contrast to the synergistic effects of high temperature and eCO₂ during early growth, at later growth stages, high temperature negatively affected reproductive development, which subsequently affected CO₂ responses at both the leaf and whole-plant levels. The decrease in CO₂-stimulation of A_n at high temperature in the late developmental stages (31–54 DAP) was accompanied by higher leaf carbohydrate accumulation concentrations (Table 2), lower leaf N levels, and significantly reduced RGR (Table 4). Increased carbohydrate accumulation in source leaves indicated a limited whole-plant carbon sink capacity or a limited capacity for phloem loading and translocation of soluble carbohydrates (Arp, 1991; Baker and Allen, 1993). Although

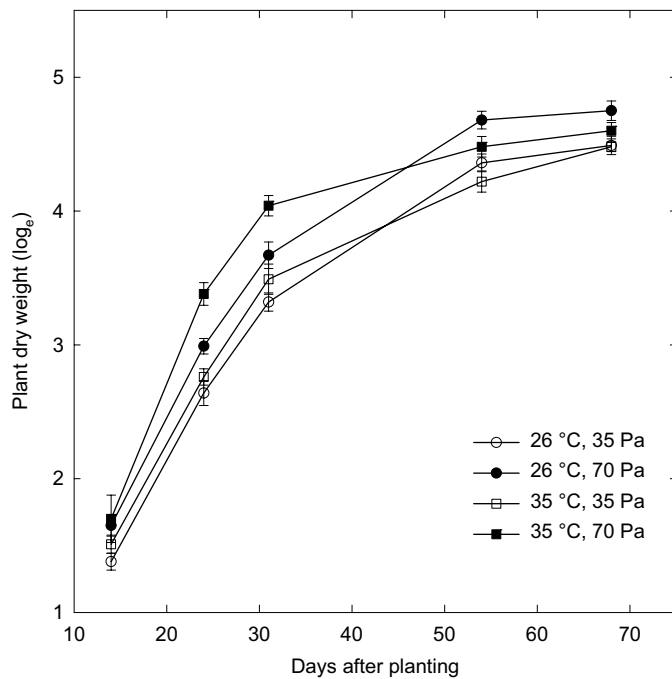


Fig. 3. Time course of total dry weight per plant, plotted on a natural logarithm (\log_e) scale, of bean plants grown in a factorial combination of air temperature (26 or 35 °C) and air CO_2 partial pressure [$p\text{CO}_2$; ambient (35 Pa) and twice ambient (70 Pa)]. Each point is the mean \pm SE of four plants from one of three repetitions of the study.

Table 4. The effects of growth temperature (26 or 35 °C) and CO_2 partial pressure [$p\text{CO}_2$ (35 or 70 Pa)] on whole-plant relative growth rates (RGR), number of pods per plant, and harvest index (HI) of red kidney beans. RGR was determined during vegetative [17–31 d after planting (DAP)] and reproductive (31–68 DAP) growth phases, whereas pod yield and HI were measured at final harvest (68 DAP). Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Duncan's multiple range test, $n = 12$ to 13).

Temp (°C)	$p\text{CO}_2$ (Pa)	RGR ($\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$)		Pods (no./plant)	HI ($\text{g}\cdot\text{g}^{-1}$)
		17–31 DAP	31–68 DAP		
26	35	0.13 c	0.06 ab	23.0 b	0.26 a
	70	0.14 b	0.07 a	36.8 a	0.24 a
35	35	0.14 bc	0.05 b	6.0c	0.04 b
	70	0.16 a	0.03 c	5.3c	0.03 b

Table 5. The effects of growth temperature (26 or 35 °C) and CO_2 partial pressure [$p\text{CO}_2$ (35 or 70 Pa)] on the dry weight partitioning of red kidney beans at 68 d after planting. Means followed by the same letter(s) within a column are not significantly different at $P < 0.05$ (Duncan's multiple range test, $n = 4$).

Temp (°C)	$p\text{CO}_2$ (Pa)	Roots (g)	Stems (g)	Leaves (g)	Seeds (g)
26	35	16.1 c	24.6 b	22.8 b	21.5 b
	70	21.0 bc	29.6 b	30.1 a	30.1 a
35	35	24.5 ab	39.6 a	19.6 b	3.5 c
	70	28.9 a	47.4 a	19.5 b	2.8 c

there was some compensatory increase in stem and root dry weight allocation at high temperature [compared with plants at 26 °C (Table 5)], this was apparently not sufficient to mitigate the reduced whole-plant capacity for carbohydrate utilization and consequent downregulation of A_n . Also, the cultivar of bean used in the present study ('Redcloud') is strongly determinate (Wallace et al., 1993) and therefore, did not have the plasticity to expand alternative sinks after anthesis. Feedback inhibition of A_n associated with TNC accumulation in source leaves has been reported in several studies of CO_2 responses including beans (Ehret and Joliffe, 1985; Socias et al., 1993). Carbohydrate-mediated repression of photosynthetic gene expression and a decrease in quantity and/or activity of Rubisco (often reflected in lower leaf N levels) have been shown to contribute to downregulation of A_n at $e\text{CO}_2$ (Moore et al., 1999; Stitt, 1991).

The downregulation of A_n at $e\text{CO}_2$ observed in the current study could also be associated, in part, with an acceleration of the normal decline in leaf N and A_n observed during leaf senescence (Gepstein, 1988), as suggested by Miller et al. (1997). Ludwig and Sonnewald (2000) observed this in an $e\text{CO}_2$ study using transgenic tobacco (*Nicotiana tabacum* L.) plants over-expressing a bacterial isopentenyl transferase which catalyses the rate-limiting step in cytokinin biosynthesis. We did not focus on indicators of senescence, but any such ontogenic effects would have occurred after anthesis since treatments did not affect flowering date.

At final harvest, the CO_2 stimulation of total biomass was smaller at 35 °C (14%) compared to that at 26 °C (31%). In fact, $e\text{CO}_2$ had a significant negative effect on RGR between 31–68 DAP at high temperature, and $e\text{CO}_2$ could not compensate for negative effects of high temp on pod development or HI (Table 4). Responses of total biomass and seed yield to $e\text{CO}_2$ in soybean [*Glycine max* (L.) Merrill] have also been shown to decline with increasing temperature by factors of -0.026 and $-0.031 \text{ g}\cdot\text{g}^{-1}$ per °C, respectively (Allen et al., 1996). Prasad et al. (2002, 2003) reported that $e\text{CO}_2$ could not compensate for deleterious effects of high temperature on reproductive processes. Our results document developmentally dependent responses to $e\text{CO}_2$ and a secondary outcome of high temperature-induced reduction in pod set—the exacerbated downward photosynthetic acclimation to $e\text{CO}_2$.

To summarize, the beneficial effects of $e\text{CO}_2$ and high temperature on A_n and growth during early developmental stages were offset by the long-term, high-temperature-induced inhibition of seed set. Reduced reproductive sink development at high temperature coincided with an increase in leaf TNC, a decrease in leaf N, and more substantial downward acclimation of A_n capacity at $e\text{CO}_2$. These results underscore the importance of crop phenology and reproductive sink plasticity in determining $\text{CO}_2 \times$ temperature interactions. In addition, the results suggest that high temperature-induced limitations on reproductive development in beans and other heat-sensitive crops could reduce the potential for increased productivity in a warmer CO_2 -rich climate.

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