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Soybean (*Glycine max* (L.) Merr.) growth and development response to CO_2 enrichment under different temperature regimes

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Abstract

The carbon dioxide (CO_2) concentration of the global atmosphere has increased during the last decades. This increase is expected to impact the diurnal variation in temperature as well as the occurrence of extreme temperatures. This potentially could affect crop production through changes in growth and development that will ultimately impact yield. The objective of this study was to evaluate the effect of CO_2 and its interaction with temperature on growth and development of soybean (*Glycine max* (L.) Merr., cv. Stonewall). The experiment was conducted in controlled environment chambers at the Georgia Envirotron under three different temperatures and two CO_2 regimes. The day/night air temperatures were maintained at 20/15, 25/20 and 30/25 °C, while the CO_2 levels were maintained at 400 and 700 ppm, resulting in six different treatments. Plants were grown under a constant irradiance of 850 μmoles m^{-2} s^{-1} and a day length of 12 h; a non-limiting supply of water and mineral nutrients were provided. Five growth analyses were conducted at the critical development stages V4, R3, R5, R6 and R8. No differences in start of flowering were observed as a function of the CO_2 level, except for the temperature regime 25/20 °C, where flowering for the elevated CO_2 level occurred 2 days earlier than for the ambient CO_2 level. For aboveground biomass, an increase in the CO_2 level caused a more vigorous growth at lower temperatures. An increase in temperature also decreased seed weight, mainly due to a reduction in seed size. For all temperature combinations, final seed weight was higher for the elevated CO_2 level. This study showed that controlled environment chambers can be excellent facilities for conducting a detailed growth analysis to study the impact on the interactive effect of changes in temperature and CO_2 on soybean growth and final yield.

Keywords: Growth chamber; Controlled environment; Global climate change; Growth analysis; Soybean; Temperature; CO_2; Development

1. Introduction

It is well known that the carbon dioxide (CO_2) concentration of the global atmosphere has increased during the last few decades, mainly due to energy consumption from fossil fuels. Since the start of the industrial revolution, the atmospheric CO_2 level has increased from 280 ppm to around 370 ppm, and continues to rise at approximately 1.8 ppm per year (Mendelsohn and Rosenberg, 1994; Etheridge et al., 1996; Keeling and Whorf, 2000). It is expected that the CO_2 level might reach a concentration of 600–1000 ppm by the end of this century (Cox et al., 2000). Several new studies have shown that the climate record of the 20th century cannot be explained solely by accounting for solar variability, volcanic eruptions and El Niño cycles. It appears more likely that greenhouse gases from human activities were the dominant drivers of these global-average temperature changes during the 20th century (USGCRP, 2000). Due to the increase of the CO_2 level, it is expected that the maximum, minimum and mean global temperatures will also change by 3–4 °C (Taylor and MacCracken, 1990; Watson et al., 1990). The Intergovernmental Panel on Climate Change (IPCC) expects a global surface temperature increase, ranging from 1.0 to 3.5 °C by 2100 based on the predictions of the gen-

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eral circulation models (GCM), such as GISS, UKMO, OSU and GFDL-R30 (IPCC, 2001). The interactive effects of global warming and increasing CO_2 levels could especially impact agriculture, affecting both growth and development of crops and ultimately impacting yield and food production (Cox et al., 2000; Hansen et al., 2000).

Bunce and Ziska (1996) found that total respiration for soybean increased very little with an increase in temperature, despite an increase in the relative growth rate. Temperature effects on a soybean plant are a major determinant for growth, development and yield. Sionit et al. (1987a) reported that the root to shoot ratio, leaf mass ratio and specific leaf weight of soybean decreased with increasing temperature. Thomas and Raper (1978), Flint and Patterson (1983), and Seddigh and Jolliff (1984) also found an increase in height and branching of soybeans with an increase in temperature. The photosynthetic rate is affected by temperature due to its pervasive role in the regulation of the biochemical reaction rates, morphogenetic processes, and the exchange of CO_2 and energy with the atmosphere (Hofstra, 1984; Long and Woodward, 1988; Piper et al., 1996; Wang et al., 1997). The optimum range of temperatures for growth and development of soybean has been reported to be between 20 and 30 °C (Hofstra, 1972; Hesketh et al., 1973).

Under high light intensities, the diffusion rate of CO_2 from the air to the stomata is the major limiting factor for CO_2 assimilation. As the rate of diffusion is proportional to the concentration gradient, higher concentrations of CO_2 generally stimulate the net photosynthetic rate (Cure and Acock, 1986; Allen et al., 1987; Amthor, 1995) and may also reduce transpirational losses from plants (Allen, 1990; Allen and Amthor, 1995). Elevated CO_2 can also promote a full recovery under environmental stresses (Ferris et al., 1998). The stimulation of the net photosynthetic rate is, in part, because a high CO_2 concentration increases carboxylation and reduces photorespiration (Long, 1991). Ziska and Bunce (1995) found that elevated CO_2 concentrations did not increase whole plant photosynthesis, except at the highest temperature for two soybean varieties. They concluded that the relationship between temperature and CO_2 concentration might not reflect known changes in carboxylation kinetics. According to Pritchard et al. (1999), the most significant direct effect of elevated CO_2 on plant growth is an increase in carbohydrate availability and water-use efficiency. Combined they stimulate cell proliferation promoting cell division or cell expansion, or both. According to Murray (1995), C3 species are able to utilize extra CO_2 to support faster growth, especially during the early stages of development. Sionit et al. (1987a) reported that total soybean leaf area increased in response to CO_2 enrichment among the temperature regimes. They also reported that the leaf to mass ratio remained relatively constant across the CO_2 treatments, but that the specific leaf mass varied with CO_2 and temperature regimes. Lee et al. (1997) found an increase in the leaf area index and leaf dry mass of soybean due to elevated CO_2 concentrations, but there was no effect on leaf area, plant height, total biomass or grain yield. Leadley and Reynolds (1989) also reported that final leaf area was not affected by CO_2. Jones et al. (1984) measured a faster rate in leaf area increase in soybeans grown at a high CO_2 concentration in controlled environment chambers under natural light compared to ambient CO_2 concentrations. Torbert et al. (2004) reported a significant increase in nitrogen fixation under elevated CO_2 concentrations. A meta-analysis was recently conducted by Ainsworth et al. (2002) to summarize the known effects of CO_2 on soybean physiology, growth and yield.

There are still many questions about the interactive effects of increasing temperatures and CO_2 concentrations on plant growth and development. Idso et al. (1987), Baker et al. (1989) and Newman et al. (2001) reported that CO_2 effects generally increase with increasing temperatures, whereas Coleman and Bazzaz (1992) and Tremmel and Patterson (1993) reported that CO_2 effects were greater at ambient temperatures than at high temperatures. The objective of this study was to evaluate the effect of CO_2 on soybean growth and development under different temperature regimes and to determine how the interactive effects of CO_2 and temperature impact the growth characteristics of a soybean plant.

2. Material and methods

2.1. Environment

The experiment was conducted in the controlled-environment chambers of the Georgia Envirotron, located at the College of Agricultural and Environmental Sciences—Griffin campus of the University of Georgia (Ingram et al., 1998). Six large Conviron growth chambers (model CG72), with a floor space of 8.64 m^2 and a height of 2.20 m, were used in this experiment. Carbon dioxide was automatically injected into the chambers and the level in the chambers was controlled using a CO_2 delivery system and chamber vents. An individual LICOR infrared gas analyzer (LI-800 GasHound CO_2 Analyzer, LI-COR, NE, USA) was used to monitor CO_2 levels for each chamber independently; the accuracy of the analyzer was 2% at a level of 700 ppm. All chambers also included a drip irrigation system.

Each chamber was assigned a unique temperature and CO_2 combination, resulting in six different treatments. These included a CO_2 concentration of 400 ppm and a day/night temperature 20/15 °C; a CO_2 concentration of 400 ppm and a day/night temperature of 25/20 °C; a CO_2 concentrations 400 ppm and a day/night temperature of 30/25 °C; a CO_2 concentration of 700 ppm and a day/night temperature of 20/15 °C; a CO_2 concentration of 700 ppm and a day/night temperature of 25/20 °C and a CO_2 concentration of 700 ppm and a day/night temperature 30/25 °C. The photoperiod of each chamber was set at 12 h. Photosynthetically active radiation (PAR) inside the growth chambers was 753.7 μmoles m^{-2} s^{-1}, measured at the top of the pots at planting and at the top of canopy during the course of the experiment.

The experimental unit was a large 25 l plastic container, each containing two equally spaced plants. A total of 120 containers were used in each study and 20 containers were assigned randomly to each treatment or chamber. The experimental design was completely randomized with four replicates per treatment. Twenty pots corresponding to each CO_2 versus day/night temperatures combination were placed in each

corresponding chamber. The pots were rotated biweekly until flowering to minimize border effects. The distance between pots was maintained at 20 cm; the surface area of the pots was 625 cm^2. Pots were filled with washed sand. Six seeds of soybean, cultivar Stonewall (Maturity Group VII), were sown in each pot and thinned to two plants per pot after germination. The seeds did not receive any chemical treatments and were not inoculated. Plants were watered daily with a modified half-strength of Hoagland's solution (Downs and Hellmers, 1975) and inorganic nitrogen through an automated irrigation system.

2.2. Measurements

Vegetative and reproductive development was recorded every 2 days during the growing season. Growth analysis sampling was conducted at the developmental stages V4, R3, R5, R6 and R8 (Fehr et al., 1971) for all combinations of three day/night temperatures (20/15, 25/20 and 30/25 °C) and two CO_2 concentrations (400 and 700 ppm). For each growth analysis harvest, four pots with two plants per pot from each chamber were randomly selected and used to measure the individual plant components. Total leaf area (cm^2 $plant^{-1}$), total aboveground dry mass per (g $plant^{-1}$), leaf dry mass (g $plant^{-1}$) were determined for all stages. Final grain weight, seed number per plant and seed weight per plant were measured at final harvest.

The plants in each pot, e.g., two plants, were cut at the base and the individual plant components were separated into leaves, stems, petioles and pods (greater than 5 mm). Leaf area was determined with a leaf area meter (LI 3000, LI-COR, NE, USA). The plant components, including leaflets, stems, cotyledons, petioles and pods were dried at 65 °C for a minimum of 72 h. Total aboveground dry biomass for each pot was obtained by adding all plant components. To obtain the aboveground dry biomass per plant, total pot biomass was divided by two. These values were then used for statistical and growth analysis. The specific leaf area (SLA) (cm^2 g^{-1}), leaf area ratio (LAR) (cm^2 g^{-1}) and leaf weight ratio (LWR) (g g^{-1}) were calculated for each sampling date as the ratio of leaf area to leaf biomass, leaf area to aboveground biomass and leaf biomass to total plant aboveground biomass, respectively.

2.3. Statistical analysis

2.3.1. Main effects and interactions

Three-way analysis of variance was applied on the growth analysis variables using the ANOVA procedure of SAS System, Version 6.12 (SAS Institute Inc., 1989) to evaluate the effects of CO_2 and temperature as a function of growth stage. Statistical significance of main effects and their interactions were assessed with *F*-tests.

2.3.2. Temporal analysis

The aboveground variables, such as total aboveground biomass and leaf mass, were analyzed by fitting empirical models to describe their temporal variation as a function of thermal units or degree days after emergence (DDE). Degree days were defined as the accumulation of the difference between the daily mean temperature and a base temperature. In this study, a base temperature of 10 °C was adopted (Sexton et al., 1998).

To describe total aboveground biomass growth, a sigmoid function was used, as shown in Eq. (1). Sigmoid functions have been used to describe plant growth in many studies (Swinton and Lyford, 1996; Nobrega et al., 2001 and Gava et al., 2001). This function was adopted to allow for a comparison among the growth rates of the different treatments.

$$\hat{Y}_{ijk} = \left[\cos\left[(X_{ij} + 2)\frac{\pi}{2}\right] + 1\right]^{\beta} \quad (1)$$

$\hat{Y}_{ijk}$ represents the predicted ratio between total aboveground biomass (g $plant^{-1}$) and maximum total aboveground biomass for the CO_2 concentration i (i = 400, 700 ppm), temperature regime j (j = 20/15, 25/20, 30/25 °C) and replication k (k = 1–4); X_{ij} represents the ratio between degree days after emergence (°C day) and maximum degree days after emergence for the CO_2 concentration i and temperature regime j; and β represents the biomass growth rate (slope of the linear regression model) (g °C day $plant^{-1}$). A similar model was also used to analyze leaf mass. In this case, $\hat{Y}_{ijk}$ was replaced with the predicted ratio between leaf mass (g $plant^{-1}$) and maximum leaf mass (g $plant^{-1}$).

For both models, the maximum likelihood method was applied to estimate the empirical parameters (β), using the MIXED procedure of the SAS System Version 6.12 (SAS Institute Inc., 1989), after the transformation of biomass and degree days in relative values. This was done by dividing biomass of a determined stage, CO_2 concentration (i), temperature regime (j) and replication (k) by the maximum biomass of a CO_2 concentration (i) and temperature regime (j). The same transformation was made for degree days. *t*-Tests for contrasts were used to evaluate the effect of the CO_2 concentration on β (empirical parameter) for each temperature regime.

To compare the temporal variation in leaf area for both CO_2 levels at one specific temperature regime, two leaf area growth rates were defined. The first rate, i.e., Rate1, was defined as:

$$\text{Rate1} = \left(\frac{\text{leaf\_area}_{\max} - \text{leaf\_area}_{V4}}{\text{DDE}_{\max} - \text{DDE}_{V4}}\right) \quad (2)$$

where Rate1 represents the leaf area growth rate between the beginning of stage V4 and the corresponding time to the maximum leaf area (m^2 °C day $plant^{-1}$); $\text{leaf\_area}_{\max}$ the maximum mean leaf area value during the crop cycle (m^2 $plant^{-1}$); leaf_area_{V4} the average leaf area value at the beginning of stage V4 (m^2 $plant^{-1}$); $\text{DDE}_{\max}$ the degree days at $\text{leaf\_area}_{\max}$ (°C day) and DDE_{V4} are the degree days at the beginning of stage V4 (°C day).

The second rate, i.e., Rate2, was defined as:

$$\text{Rate2} = \left(\frac{\text{leaf\_area}_{R8} - \text{leaf\_area}_{\max}}{\text{DDE}_{R8} - \text{DDE}_{\max}}\right) \quad (3)$$

where Rate2 represents the leaf area growth rate between the time corresponding to the maximum leaf area and the beginning of the R8 stage (m^2 °C day); leaf_area_{R8} the mean leaf area value at beginning of stage R8 (m^2 $plant^{-1}$), and DDE_{R8} the degree

days at the beginning of stage R8 (°C day). As this partially represents the leaf senescence period, it is expected that this rate is negative. For specific leaf area, leaf area ratio, leaf weight ratio, the mean values at each harvest date were used to represent their temporal variation.

To compare the temporal variation in pod biomass growth for both CO_2 levels at one specific temperature regime, three pod growth rates were defined as Rate1 (between V4 and R3), Rate2 (between R3 and R6) and Rate3 (between R6 and R8). To calculate these rates, the same procedures as presented for leaf area were used. The effect of temperature and CO_2 on the seed yield, seed number per plant and seed weight per plant was compared using analysis of variance (ANOVA).

3. Results and discussion

3.1. Flowering

Only the temperature regime 25/20 °C showed a difference in the start of flowering date (R1: defined as the date when 50% of the plants have at least one flower at any node (Fehr et al., 1971)) between the elevated and ambient CO_2 levels. For this temperature regime, R1 was 2 days advanced for the elevated CO_2 level (Table 1). The other two temperature regimes did not show a difference in R1 between the ambient and elevated CO_2 levels. In spite of the controversy about the effects of CO_2 on the advancement or delay of flowering, in our study, the number of days to R1 appeared to be more strongly affected by air temperature than by the CO_2 concentration. This result is in agreement with Sionit et al. (1987a,b) and Baker et al. (1989), who also found that temperature affects the development rate of soybean to a far greater extent than the CO_2 concentration. Flowering was first observed in the 30/25 temperature regime (507.5 DDE; 29 DAE for both CO_2 levels), followed by the 25/20 °C temperature regime (412.5 DDE; 33 DAE and 387.5 DDE; 31 DAE for the ambient and elevated CO_2 level, respectively) and the 20/15 °C temperature regime (450.0 DDE and 60 DAE for both CO_2 levels) (Table 1). Please note that for soybean the temperature regime of 30/25 is not supra-optimal for the flowering.

Table 1
Degree days between emergence and the start of flowering (DDEF), days between emergence and the start of flowering (DAEF), degree days between the start of flowering and physiological maturity (DDFM), days between flowering and physiological maturity (DAFM), degree days between emergence and physiological maturity (DDEM), and days between emergence and to physiological maturity (DAEM) for three temperature regimes and two CO_2 levels

Temperature (day/night) (°C)	CO_2 level (ppm)			
	400		700	
	DDEF (°C day)	DAEF	DDEF (°C day)	DAEF
20/15	450.0	60	450.0	60
25/20	412.5	33	387.5	31
30/25	507.5	29	507.5	29
	DDFM (°C day)	DAFM	DDFM (°C day)	DAFM
20/15	465	62	465	62
25/20	752.5	60	537.5	43
30/25	805	46	962.5	55
	DDEM (°C day)	DAEM	DDEM (°C day)	DAEM
20/15	915	122	915	122
25/20	1162.5	93	925	74
30/25	1312.5	75	1470	84

At ambient CO_2 levels, flowering in soybeans is mainly controlled by temperature and photoperiod. In this study, photoperiod was fixed at 12 h and was below the critical photoperiod for this genotype. We, therefore, expected to find the same degree days after emergence for development for all temperature regimes. However, there were differences in DDE for all temperature regimes (Table 1). DDE, in this study, was calculated as the difference between the daily mean temperature and a base temperature, which was defined as 10 °C. For the temperature regimes 20/15, 25/20 and 30/25 °C, 7.5, 12.5 and 17.5 °C day, respectively, were accumulated for each day after emergence. According to Wang et al. (1997), reproductive development of soybean can be modified by temperature, although it is frequently under photoperiodic control. This difference between DDE might be explained as the effect of the night temperature on soybean development as observed by Thomas and Raper (1981), Seddigh et al. (1989) and recently by Bunce (2004).

3.2. Growth analysis

Total aboveground biomass, leaf mass and leaf area, pod biomass and the fitted models representing their variation as a function of DDE for each combination of CO_2 and temperature regimes are shown in Fig. 1. The maximum likelihood estimates of the growth rate (β) for total aboveground biomass and leaf mass and the contrasts between the growth rate for the different CO_2 levels at each temperature regime are listed in Tables 2 and 3. For total aboveground biomass, the difference between ambient and elevated CO_2 levels was significant for both temperature regimes 20/15 and 30/25 °C ($p = 0.0064$ and 0.0001, respectively; Table 2). Biomass growth rate (β) for the temperature regime 20/15 °C was greater at the elevated than the ambient CO_2 level (2.03 and 1.72 g °C day plant^{-1}, respectively). Sionit et al. (1987b) also found that an increase in the CO_2 level caused soybean to grow more vigorously at lower temperatures. However, for the temperature regime 30/25 °C, the total biomass growth rate (β) was greater at the ambient than the elevated CO_2 level (1.53 and 1.03 g °C day plant^{-1}, respectively; Table 2). The fitted empirical model (Fig. 1c) for this temperature regime showed that there was an increase in β for the ambient CO_2 level and a decrease in β for the elevated CO_2 level after the R6 stage. This result was expected because the whole plant response to elevated CO_2 level may decrease over time due to biochemical limitations, e.g., a decrease in rubisco activity, ultra structural limitations, e.g., chloroplast disruption or changes at the canopy level, e.g., self-shading (Pritchard et al., 1999).

Table 2
Biomass growth rate estimates (β) for the models describing total aboveground biomass as a function of degree days after emergence (DDE) for each temperature and CO_2 level

Temperature (day/night) (°C)	CO_2 (ppm)	$\beta \pm$ standard error (g °C day plant^{-1})	r^2[a]	[b]β diff.	[c]p-Value
20/15	400	1.72814 ± 0.0779	0.97	−0.3063	<0.01[d]
20/15	700	2.03448 ± 0.0779	0.97		
25/20	400	1.71410 ± 0.0600	0.98	0.1044	0.27ns
25/20	700	1.60968 ± 0.0738	0.97		
30/25	400	1.57796 ± 0.0636	0.99	0.5440	<0.01[d]
30/25	700	1.03391 ± 0.0569	0.87		

ns: not significant at the 0.05 probability level.
[a] Determination coefficient.
[b] β diff.: difference between β for 400 and 700 ppm CO_2.
[c] p-Value associated to t-test for contrast between β parameters from the same temperatures and different CO_2 levels.
[d] Significant at the 0.05 probability level.

There was no significant difference ($p = 0.275$) for β to ambient and elevated CO_2 level for the temperature regime 25/20 °C (Table 2). The increase in temperature caused a decrease in the plant biomass weight (Fig. 1a–c) due to a decrease of the soybean cycle as shown in Table 1.

For leaf biomass, the growth rate (β) parameter was also significantly different for the temperature regimes 20/15 and 30/25 °C, while for the temperature regime 25/20 °C, there was no significant difference between the elevated and ambient CO_2 level ($p = 0.133$) (Table 3). This is the same trend that was found for the total biomass growth rate.

The variation in leaf area as a function of DDE is shown in Fig. 1g–i. For Rate1 (m^2 °C day), defined as the leaf area growth rate between the beginning of the V4 stage and the time corresponding to maximum leaf area, there was no significant difference between the ambient and elevated CO_2 levels for the temperature regimes 20/15 and 30/25 °C ($p = 0.0719$ and 0.2669, respectively). A significant difference between the ambient and

Table 3
Leaf growth rate estimates (β) for the models describing leaf mass as a function of degree days after emergence (DDE) for each temperature and CO_2 level

Temperature (day/night) (°C)	CO_2 (ppm)	$\beta \pm$ standard error (g °C day plant^{-1})	r^2	[a]β diff.	[b]p-Value
20/15	400	1.42143 ± 0.1002	0.94	−0.3030	0.03[c]
20/15	700	1.72451 ± 0.1002	0.94		
25/20	400	1.17244 ± 0.0771	0.90	−0.1851	0.13ns
25/20	700	1.35754 ± 0.0949	0.95		
30/25	400	1.23398 ± 0.0818	0.96	0.4248	<0.01[c]
30/25	700	0.80918 ± 0.0731	0.77		

ns: not significant at the 0.05 probability level.
[a] β diff.: difference between β for 400 and 700 ppm CO_2.
[b] p-Value associated to t-test for contrast between β parameters from the same temperatures and different CO_2 levels.
[c] Significant at the 0.05 probability level.

Table 4
Estimates of leaf area growth rates for three different temperature regimes and two CO_2 levels

Temperature (day/night) (°C)	CO_2 (ppm)	Rate1 (m^2 °C day)	[a]Diff1 (m^2 °C day)	[b]p-Value	Rate2 (m^2 °C day)	[b]Diff2 (m^2 °C day)	[c]p-Value
20/15	400	0.003879	0.002922	0.07ns	−0.000694	0.000456	<0.01[d]
20/15	700	0.003310			0.000238		
25/20	400	0.001968	0.000710	0.03	−0.000218	0.000084	0.78ns
25/20	700	0.001258			−0.000134		
30/25	400	0.000862	0.000341	0.26ns	−0.000045	0.000294	0.34ns
30/25	700	0.001203			−0.000339		

Rate1 represents leaf area growth between the beginning of the V4 stage and the time corresponding to the maximum leaf area and Rate2 represents leaf area growth between the time corresponding to the maximum leaf area and the beginning of the R8 stage; ns: not significant at the 0.05 probability level.
[a] Diff1: difference of the mean Rate1 between 400 and 700 ppm CO_2.
[b] Diff2: difference of the mean Rate2 between 400 and 700 ppm CO_2.
[c] p-Value: value associated to t-test for contrasts between Rate1 for the same temperature regime and different CO_2 levels.
[d] Significant at the 0.05 probability level.

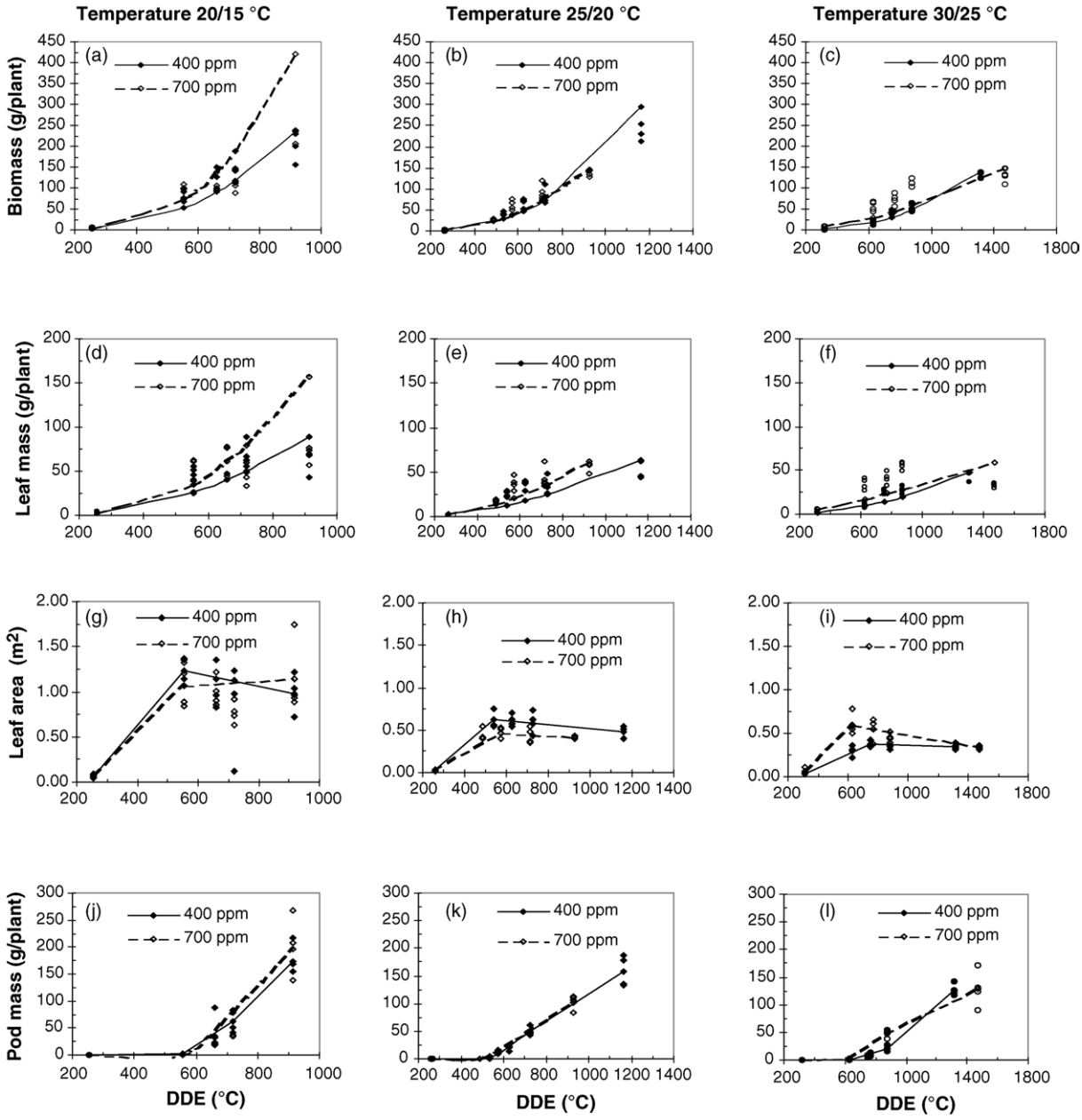

Fig. 1. Total aboveground biomass (g plant^{-1}) (a–c), leaf mass (g plant^{-1}) (d–f), leaf area (m^2) (g–i) and pod growth at elevated CO_2 (- - -) and ambient CO_2 (—) as a function of degree days after emergence (DDE). The open symbols represent the elevated CO_2 measurements and the solid symbols represent the ambient CO_2 measurements for each harvest stage. The lines represent the model predicted values for biomass, leaf mass and leaf area.

elevated CO_2 levels for Rate1 was only found for temperature regime 25/20 °C ($p = 0.0282$) (Table 4). For this temperature regime, Rate1 was greater for the ambient than the elevated CO_2 level. For Rate2 (m^2 °C day), defined as the leaf area variation rate between the time corresponding to the maximum leaf area and the beginning of the R8 stage, there was no significant difference between the ambient and elevated CO_2 level for the temperature regimes 25/20 and 30/25 °C ($p = 0.7839$ and 0.3452, respectively), while for temperature regime 20/15 °C Rate2 was significantly different ($p = 0.0001$) (Table 4).

The variation in pod biomass growth as a function of DDE is shown in Fig. 1j–l. The pod growth rate was divided into three rates, e.g., Rate1: V4–R3, Rate2: R3–R6 and Rate3: R6–R8. For Rate1 (g °C day plant^{-1}) and temperatures regimes 25/20 and 30/25 °C, there was a significant difference between the ambient and elevated CO_2 levels ($p = 0.0032$ and 0.0128, respectively; Table 5). For the 30/25 °C temperature regime, Rate1 was greater for the elevated CO_2 than for the ambient level (0.0051 and 0.0005 g °C day plant^{-1}, respectively). However, for the 25/20 °C temperature regime, Rate1 was greater at the ambient than at the elevated CO_2 level (0.0076 and 0.0019 g °C day plant^{-1}, respectively). For Rate2 (g °C day plant^{-1}), only the temperature regime 30/25 °C showed a significant difference between the ambi-

Table 5
Estimates of pod biomass growth rates for three different temperature regimes and two CO_2 levels

Temperature (day/night) (°C)	CO_2 (ppm)	Rate1 (m^2 °C day)	[a]Diff1 (m^2 °C day)	[d]*p*-Value	Rate2 (m^2 °C day)	[b]Diff2 (m^2 °C day)	[d]*p*-Value	Rate3 (m^2 °C day)	[c]Diff3 (m^2 °C day)	[d]*p*-Value
20/15	400	0.0046	0.0010	0.53ns	0.3747	0.0793	0.13ns	2.3573	1.6088	0.14ns
20/15	700	0.0036	–	–	0.2954	–	–	0.7485	–	–
25/20	400	0.0076	0.0056	<0.01[e]	0.2484	0.0299	0.56ns	0.2515	0.0022	0.99ns
25/20	700	0.0019	–	–	0.2185	–	–	0.2493	–	–
30/25	400	0.0005	0.0045	0.01[e]	0.0775	0.1137	0.03[e]	0.8711	0.7354	0.49ns
30/25	700	0.0051	–	–	0.1912	–	–	0.1357	–	–

Rate1 represents pod biomass growth between the beginning of the V4 and R3 stages; Rate2 represents pod biomass growth between R3 and R6 and Rate3 represents pod biomass growth between R6 and R8; ns: not significant at the 0.05 probability level.
[a] Diff1: difference of the mean Rate1 between 400 and 700 ppm CO_2.
[b] Diff2: difference of the mean Rate2 between 400 and 700 ppm CO_2.
[c] Diff3: difference of the mean Rate3 between 400 and 700 ppm CO_2.
[d] *p*-Value: value associated to *t*-test for contrasts between Rate1 (or Rate2 or Rate3) for the same temperature regime and different CO_2 levels.
[e] Significant at the 0.05 probability level.

ent and elevated CO_2 level ($p = <0.03$). For this temperature regime, Rate2 was greater at the elevated CO_2 level (0.0775 and 0.1912 g °C day plant^{-1}, respectively). For Rate3, none of the temperature regimes (20/15, 25/20 and 30/25 °C) showed a significant difference between the ambient and elevated CO_2 level ($p = 0.1474$, 0.9984 and 0.4978, respectively; Table 5). The greatest impact of the increased CO_2 level on pod biomass growth was found for the highest temperature regime 30/25 °C. For this temperature regime, the pod biomass growth rate at the elevated CO_2 level was greatest for Rate1 and Rate2. According to Nakamoto et al. (2004), the response of seed yield to CO_2 enrichment is mainly attributed to the response during the reproductive period. Based on Nakamoto et al. (2004) observations and the results of this study, it is possible suggest that the effect of elevated CO_2 on pod growth rate mainly occurs between R1 and R6, e.g., corresponding to Rate1 and Rate2.

The temporal trends for SLA, LAR and LWR as a function of DDE are shown in Fig. 2. For all temperature regimes, SLA at the V4 stage was greater at the ambient than at the elevated CO_2 level. The specific leaf area also decreased due an increase in temperature for both CO_2 levels. This could be caused by an increase in the accumulation of total nonstructural carbohydrates in the leaves (TNC), which occurs when *C* fixation exceeds *C* utilization (Pritchard et al., 1999). Leaf initiation, represented here as LAR, was reduced at the elevated CO_2 level for all temperature regimes (Fig. 2d–f), similar to the observations made by Pritchard et al. (1999). The leaf weight ratio (LWR) decreased as a function of DDE for all temperature regimes (Fig. 2g–i) due to plant development and senescence.

3.3. Seed weight

The significance of the main effects and interactions among CO_2 and temperature on seed weight per plant, seed number per plant and individual seed mass at maturity are shown in Tables 6 and 7. There was a significant positive interaction between temperature and CO_2 for seed weight and seed number per plant. Seed weight increased by an average of 7.5% for temperature regimes 20/15 and 30/35 °C under the elevated CO_2 level. However, the increase was smaller as the temperature regime increased. The number of seeds per plant only increased for the elevated CO_2 level and the lowest temperature regime; the increase was also smaller as the temperature increased. There was no significant interaction between CO_2 and temperature for individual seed size, so the effects of ambient and elevated CO_2 were similar. The ANOVA of the individual seed size (Table 6) confirmed that the seeds were significantly larger under high temperature treatments, indicating an acceleration of early seed growth for the higher temperature. In an earlier study, Egli and Ward-

Table 6
Analysis of variance for the effects of CO_2 and temperature on final seed weight, individual seed size, and seed number at final harvest

Source of variation	d.f.	Seed weight (g plant^{-1})	Seed size (g)	Seed number (# plant^{-1})
		p-Value		
Replicate	3	0.05	0.26	0.10
CO_2 (*C*)	1	0.17ns	0.75ns	0.12ns
Temperature (*T*)	2	<0.01[a]	<0.01[a]	<0.01
$C \times T$	2	0.01[a]	0.06ns	0.02[a]
Error	15			
Total	23			

ns: not significant at the 0.05 probability level.
[a] Significant at the 0.05 probability level.

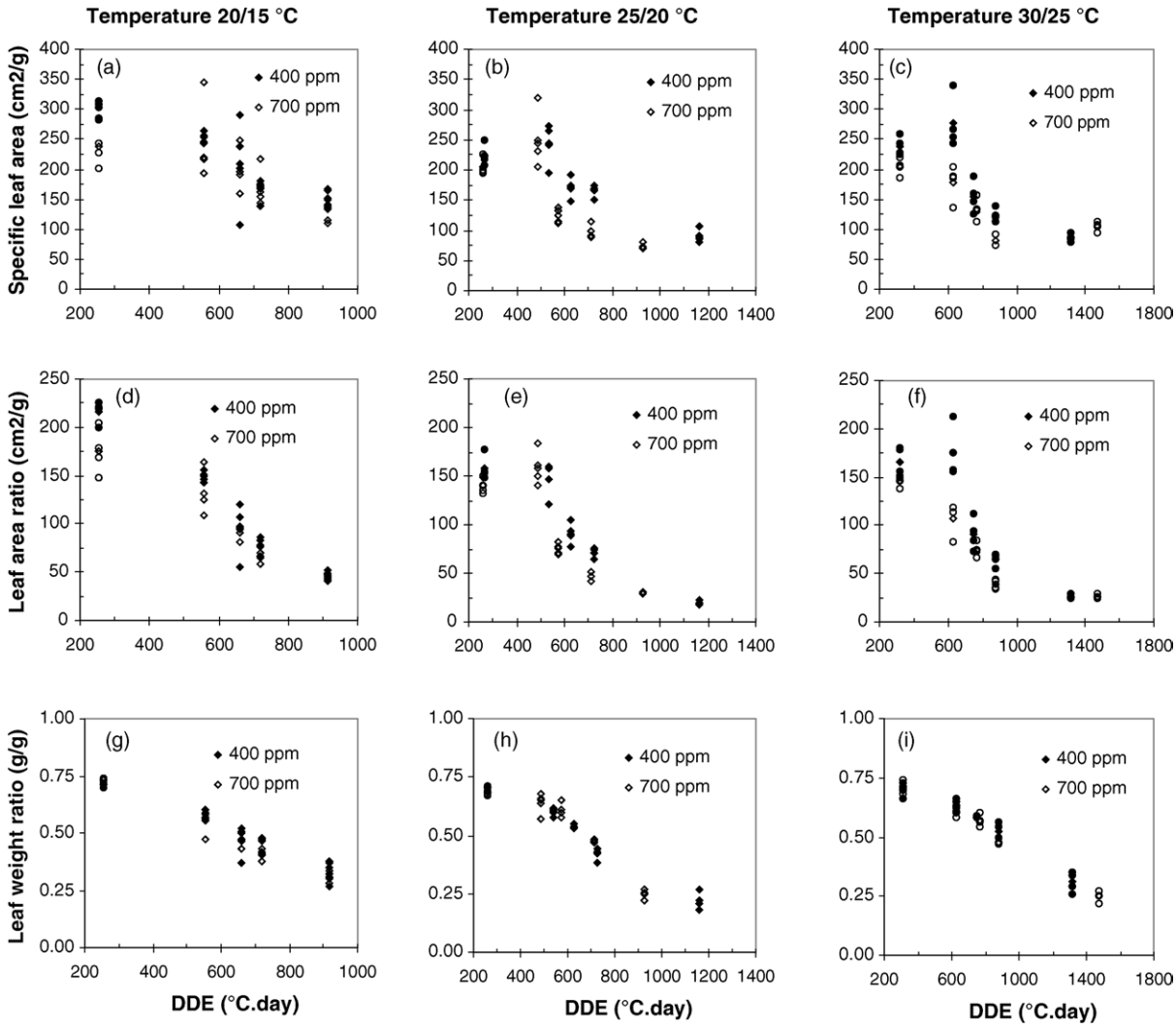

Fig. 2. Specific leaf area (ratio of leaf area to leaf biomass, $m^2\ g^{-1}$) (a–c), leaf area ratio (ratio of leaf area to aboveground biomass, $m^2\ g^{-1}$) (d–f) and leaf weight ratio (ratio of leaf biomass to total plant aboveground biomass, $g\ g^{-1}$) (g–i) at elevated CO_2 and at ambient CO_2 as a function of degree days after emergence (DDE). The open symbols represent the elevated CO_2 measurements and the solid symbols represent the ambient CO_2 measurements for each harvest stage.

law (1980) found that for soybean an increase in both the day and night temperature increased the individual seed growth rate.

Seed weight per plant was positively correlated (88%) to the number of seeds per plant for all treatments, while there was no significant relationship between the seed weight per plant at harvest maturity and the individual seed size or mass for each individual treatment (p-value = 0.24). Thus, it was possible to explain 88% of the variation in seed weight per plant by the differences in seed number rather than in seed size for the various temperature and elevated CO_2 treatments. The relationship between seed weight and seed number per plant for soybean has also been shown in previous studies (Ferris et al., 1999; Egli and Yu, 1991).

Table 7
Average values of final seed weight, seed size and seed number at final harvest

CO_2 (ppm)	Temperature (°C)	Seed weight (g plant^{-1})	Seed mass (g)	Seed number (# plant^{-1})
400	20/15	55.39	0.28	198.30
400	25/20	52.71	0.28	190.77
400	30/25	35.85	0.19	187.45
700	20/15	60.12	0.26	227.34
700	25/20	29.06[a]	0.24	125.30
700	30/25	38.70	0.24	163.45

[a] Data obtained at R7 (physiological maturity) due to a problem with the operation of the chamber.

3.4. Interactive effects of CO_2 and temperature

The temporal analysis of the individual growth characteristics and traits showed that the response of soybean to elevated CO_2 is temperature dependent. The lowest temperature regime (20/15 °C) showed a higher biomass growth rate (β) and leaf growth rate (β) for the elevated CO_2 level than the normal CO_2 level (Tables 3 and 4). These results are similar to those found by Tremmel and Patterson (1993), Baker et al. (1989) and Coleman and Bazzaz (1992). For final yield, there was a strong interaction of CO_2 and temperature on final seed weight per plant and seed number per plant.

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References

Ainsworth, E.A., Davey, P.A., Bernacchi, C.J., Dermody, O.C., Heaton, E.A., Moore, D.J., Morgan, P.B., Naidu, S.L., Yoo Ra, H., Zhu, X., Curtis, P.S., Long, S.P., 2002. A meta-analysis of elevated CO_2 effects on soybean physiology, growth and yield. Global Change Biol. 8, 695–709.

Allen Jr., L.H., 1990. Plant responses to rising carbon dioxide and potential interactions with air pollutants. J. Environ. Qual. 19, 15–34.

Allen Jr., L.H., Boote, K.J., Jones, J.W., Jones, P.H., Valle, R.R., Acock, B., Rogers, H.H., Dahlman, R.C., 1987. Response of vegetation to rising carbon dioxide: photosynthesis, biomass, and seed yield of soybean. Global Biogeochem. Cycles 1, 1–14.

Allen, L.H., Amthor Jr., J.S., 1995. Plant physiological responses to elevated CO_2, temperature, air pollution, and UB-V radiation. In: Woodwell, G.M., Mackenzie, F.T. (Eds.), Biotic Feedbacks in the Global Climate System: Will the Warning Feed the Warning? Oxford University Press, New York, pp. 51–84.

Amthor, J.S., 1995. Terrestrial higher-plant response to increasing atmospheric [CO_2] in relation to the global carbon cycle. Global Change Biol. 1, 243–274.

Baker, J.T., Allen Jr., L.H., Boote, K.J., Jones, P., Jones, J.W., 1989. Response of soybean to air temperature and carbon dioxide concentration. Crop Sci. 29, 98–105.

Bunce, J.A., 2004. A comparison of the effects of carbon dioxide concentration and temperature on respiration, translocation and nitrate reduction in darkened soybean leaves. Ann. Bot. 93 (6), 665–669.

Bunce, J.A., Ziska, L.H., 1996. Responses of respiration to increase in carbon dioxide concentration and temperature in three soybean cultivars. Ann. Bot. 77, 507–514.

Coleman, J.S., Bazzaz, F.A., 1992. Effects of CO_2 and temperature on growth and resource use of co-occurring C3 and C4 annuals. Ecology 73, 1244–1259.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, I.J., 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature 408, 184–187.

Cure, J.D., Acock, B., 1986. Crop responses to carbon dioxide doubling: a literature survey. Agric. Forest Meteorol. 38, 127–145.

Downs, R.J., Hellmers, H., 1975. Environment and the Experimental Control of Plant Growth. Academic Press Inc., New York, NY.

Egli, D.B., Yu, Z., 1991. Crop growth rate and seeds per unit area in soybean. Crop Sci. 31, 439–442.

Egli, D.B., Wardlaw, I.F., 1980. Temperature response of seed growth characteristics of soybeans. Agron. J. 72, 560–540.

Etheridge, D.M., Steele, L.P., Langenfelds, R.L., Francey, R.J., Barnola, J.M., Morgan, V.A., 1996. Natural and anthropogenic changes in atmospheric CO_2 over the last 1000 years from air in Antartic ice and firn. J. Geophys. Res. 101, 4115–4128.

Fehr, W.R., Caviness, C.E., Burmood, D.T., Pennington, J.S., 1971. Stage of development descriptions of soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11, 929–931.

Ferris, R., Wheeler, T.R., Ellis, R.H., Hadley, P., 1999. Seed yield after environmental stress in soybean grown under elevated CO_2. Crop Sci. 39, 710–718.

Ferris, R., Wheeler, T.R., Hadley, P., Ellis, R.H., 1998. Recovery of photosynthesis after environmental stress in soybean grown under elevated CO_2. Crop Sci. 38, 948–955.

Flint, E.P., Patterson, D.T., 1983. Influence and temperature effects on growth in soybean (*Glycine max*) and associated C3 and C4 weeds. Weed Sci. 31, 193–199.

Gava, G., J de, C., Trivelin, P.C.O., de Oliveira, M.W., Penatti, C.P., 2001. Growth and accumulation of nitrogen by sugarcane cultivated in soil covered with cane trash. Pesq. Agropec. Bras. 36 (11), 1347–1354.

Hansen, W.J., Sato, M., Ruedy, R., Lacis, A., Oinas, V., 2000. Global warming in the twenty-first century: an alternative scenario. Proc. Natl. Acad. Sci. U.S.A. 97, 9875–9880.

Hesketh, J.D., Myhre, D.L., Willey, C.R., 1973. Temperature control of time intervals between vegetative and reproductive events in soybean. Crop Sci. 15, 250–254.

Hofstra, G., 1972. Response of soybeans to temperature under high light intensities. Can. J. Plant Sci. 52, 535–543.

Hofstra, G., 1984. Response of source-sink relationship in soybean temperature. Can. J. Bot. 62, 166–169.

Idso, S.B., Kimball, B.A., Anderson, M.G., Mauney, J.R., 1987. Effects of CO_2 enrichment on plant growth: the interactive role of air temperature. Agric. Ecosyst. Environ. 20, 1–10.

Ingram, K.T., Hoogenboom, G., Liu, L. 1998. A multidisciplinary research facility for the study of interacting environmental stresses on plants, ASAE Paper No. 984151, St. Joseph, American Society of Agricultural Engineers.

IPCC, 2001. Climate change 2001: impact, adaptation, and vulnerability. A report of working Group II of the intergovernmental panel on climate change, 18 pp.

Jones, P., Allen Jr., L.H., Jones, J.W., Boote, K.J., Campbell, W.J., 1984. Soybean canopy growth, photosynthesis, and transpiration response to whole season carbon dioxide enrichment. Agron. J. 76, 633–637.

Keeling, C.D., Whorf, T.P., 2000. Atmospheric CO_2 records from the SIO air sampling network. In: Trends: A Compendium of Data on Global Change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, TN.

Leadley, P.W., Reynolds, J.F., 1989. Effect of carbon dioxide enrichment on development of the first six main stem leaves in soybean. Am. J. Bot. 76 (10), 1551–1555.

Lee, E.H., Pausch, R.C., Rowland, R.A., Mulchi, C.L., Rudorff, B.F.T., 1997. Responses of field-grown soybean (cv. Essex) to elevated SO_2 under two atmospheric CO_2 concentrations. Environ. Exp. Bot. 37, 85–93.

Long, S.P., 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: has its importance been underestimated? Plant Cell Environ. 14, 729–739.

Long, S.P., Woodward, F.I., 1988. Plants and Temperature, Symposium of the Society for Experimental Biology. Cambridge University Press, 406 pp.

Mendelsohn, R., Rosenberg, N.J., 1994. Framework for integrated assessments of global warming impacts. Clim. Change 28, 15–44.

Murray, D.R., 1995. Plant responses to carbon dioxide. Am. J. Bot. 82 (5), 690–697.

Nakamoto, H., Zheng, S.H., Tanaka, K., Yamazaki, A., Furuya, T., Iwaya-Inoue, M., Fukuyama, M., 2004. Effects of carbon dioxide enrichment

during different growth periods on flowering, pod set and seed yield in soybean. Plant Prod. Sci. 7 (1), 11–15.

Newman, Y.C., Sollenberger, L.E., Boote, K.J., Allen Jr., L.H., Littell, R.C., 2001. Carbon dioxide and temperature effects on forage dry matter production. Crop Sci. 41, 399–406.

Nobrega, J.Q., Rao, T.V.R., Beltrão, N.E., de, M., 2001. Growth analysis of a bean crop submitted to four levels of soil humidity. Revista Brasileira de Engenharia Agrícola e Ambiental 5 (3), 437–443.

Piper, E.L., Smit, M.A., Boote, K.J., Jones, J.W., 1996. The role daily minimum temperature in modulating the development rate to flowering in soybean. Field Crops Res. 47, 211–220.

Pritchard, S.G., Rogers, H.H., Prior, S.A., Peterson, C.M., 1999. Elevated CO_2 and plant structure: a review. Global Change Biol. 5, 807–837.

SAS Institute Inc., 1989. User's Guide, Version 6, fourth ed. SAS Institute Inc., Cary, NC, 846 pp.

Seddigh, M., Jolliff, G.D., 1984. Night temperature effects on morphology, phenology, yield and yield components of indeterminate field grown soybean. Agron. J. 76, 824–828.

Seddigh, M., Jolliff, G.D., Orf, J.H., 1989. Night temperature effects on soybean phenology. Crop Sci. 29, 400–406.

Sexton, P.J., Batchelor, W.D., Boote, K.J., Sibles, R., 1998. Evaluation of CROPGRO for prediction of soybean nitrogen balance in a Midwestern environment. Trans. ASAE 41 (5), 1543–1548.

Sionit, N., Strain, B.R., Flint, E.P., 1987a. Interaction of temperature and CO_2 enrichment on soybean: growth and dry matter partitioning. Can. J. Plant Sci. 67, 59–67.

Sionit, N., Strain, B.R., Flint, E.P., 1987b. Interaction of temperature and CO_2 enrichment on soybean: photosynthesis and seed yield. Can. J. Plant Sci. 67, 629–636.

Swinton, S.M., Lyford, C.P., 1996. A test for choice between hyperbolic and sigmoidal models of crop yield response to weed density. J. Agric. Biol. Environ. Stat. 1, 97–106.

Taylor, K.E., MacCracken, M.C., 1990. Projected effects of increasing concentrations of carbon dioxide and trace gases on climate. In: Kimball, B.A., Rosenberg, N.J., Allen Jr., L.H. (Eds.), Impact of Carbon Dioxide, Trace Gases, and Climate Change on Global Agriculture. ASA Special Publication No. 53. American Society of Agronomy Inc., Madison, WI, USA, pp. 1–17.

Thomas, J.F., Raper, C.D., 1978. Day and night temperature influence on carpel initiation and growth in soybeans. Bot. Gazz. 142, 183–187.

Thomas, J.F., Raper, C.D., 1981. Day and night temperature influence on carpel initiation and growth in soybeans. Bot. Gazz. 142, 183–187.

Torbert, H.A., Prior, S.A., Rogers, H.H., Runion, G.B., 2004. Elevated atmospheric CO_2 effects on N fertilization in grain sorghum and soybean. Field Crops Res. 88, 47–57.

Tremmel, D.C., Patterson, D.T., 1993. Responses of soybean and five weeds to CO_2 enrichment under two temperature regime. Can. J. Plant Sci. 73, 1249–1260.

USGCRP, 2000. Our Changing Planet. The FY 2001 U.S. Global Change Research Program, USGCRP, Washington, DC, 74 pp.

Wang, Z., Reddy, V.R., Quebedeaux, B., 1997. Growth and photosynthetic responses of soybean to short-term cold temperature. Environ. Exp. Bot. 37, 13–14.

Watson, R.T., Rodhe, H., Oescheger, H., Siegenthaler, U., 1990. Greenhouse gases and aerosols. In: Houghton, J.T., Jenkins, G.J., Ephraums, J.J. (Eds.), Climate Change: The IPCC Scientific Assessment. Cambridge University Press, Cambridge, pp. 1–40.

Ziska, L.H., Bunce, J.A., 1995. Growth and photosynthetic response of three soybean cultivars to simultaneous increases in growth temperature and CO_2. Phys. Plant. 94, 575–584.