The Interactive Effects of Carbon Dioxide Enrichment and Daylength on Growth and Development in *Petunia hybrida*

J. Y. C. REEKIE, P. R. HICKLENTON and E. G. REEKIE*

Agriculture Canada Research Station, Kentville, NS, B4N 1J5 and Biology Department, Acadia University, Wolfville, NS, B0P 1X0, Canada

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Plants were grown at either 350 or 1000 μ l l⁻¹ CO₂ and in one of three photoperiod treatments: continuous short days (SD), continuous long days (LD), or short switched to long days at day 41 (SD–LD). All plants received 9 h of light at 450 μ mol m⁻² s⁻¹ and LD plants received an additional 4 h of light at 8 μ mol m⁻² s⁻¹. Growth of SD plants responded more positively to elevated CO₂ than did LD plants, due largely to differences in the effect of CO₂ on unit leaf rate. High CO₂ increased height and decreased branching under SD conditions, but had no effect under LD conditions. Elevated CO₂ also increased the number of buds and open flowers, the effect for flower number being greater in short than in long days. The specific leaf area of plants grown at 1000 μ l l⁻¹ CO₂ was reduced regardless of daylength. High CO₂ also decreased leaf and increased reproductive allocation, the magnitude of these effects being greater under SD conditions. Bud formation and flower opening was advanced under high CO₂ conditions in SD plants but bud formation was delayed and there was no effect on flower opening under LD conditions. The effects of CO₂ on plants switched from SD to LD conditions were largely intermediate between the two continuous treatments, but for some parameters, more closely resembled one or the other. The results illustrate that daylength is an important factor controlling response of plants to elevated CO₂. (© 1997 Annals of Botany Company

Key words: *Petunia hybrida* Hort. ex Vilm, carbon dioxide, photoperiod, functional growth analysis, daylength, global change, development, phenology.

INTRODUCTION

In an earlier study (Reekie, Hicklenton and Reekie, 1994), we found that the effect of elevated CO₂ on flowering is species-dependent and varies with photoperiodic response. Thus, the four long day species in this study experienced an advancement in time to flowering with elevated CO₂, while the four short day species experienced a delay. Marc and Gifford (1984) reported similar results for Triticum aestivum, a long day species and Sorghum bicolor, a short day species. A number of other studies have also found that the atmospheric concentration of CO₂ can modify the photoperiodic response. Complete absence of CO₂ prevents plants from responding to a photoperiodic stimulus (Evans, 1969) and extremely high levels of CO_2 (approx. 10000–50000 μ l l⁻¹ CO₂) can reverse the photoperiodic stimulus allowing plants to flower in a non-inductive photoperiod and preventing or delaying flowering in an inductive photoperiod (Purohit and Tregunna, 1974; Hicklenton and Jolliffe, 1980).

Exposure to different photoperiods not only affects the flowering response, but also other aspects of plant growth and development including stem elongation, lateral branching, leaf expansion and chlorophyll content (Attridge, 1990; Moe and Heins, 1990; Salisbury and Ross, 1991). Elevated CO_2 is also known to affect many of the same aspects of development (Strain and Cure, 1985; Eamus and Jarvis, 1989; Bazzaz, 1990; Enoch, 1990). However, the

* For correspondence.

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effects of CO_2 on development are often variable both among species and experiments (Hunt *et al.*, 1991; Reekie and Bazzaz, 1991; Reekie, 1996). An interaction between CO_2 and the photoperiodic response might explain some of this variability.

The objective of the present study was to determine if the effect of elevated CO_2 on growth and development varies with photoperiod in *Petunia hybrida* Hort. ex Vilm. Past research on *Petunia* indicates that photoperiod plays an important role in development, such that a short photoperiod promotes branching and vegetative growth, while a long photoperiod produces taller plants and hastens flowering (Carpenter and Carlson, 1974). It has also been shown that CO_2 enrichment promotes early flowering (Tayama and Roll, 1989; Kessler and Armitage, 1993). In this study, we grew *Petunia* in two photoperiods (long day and short day) under two CO_2 concentrations (ambient and $1000 \ \mu l \ l^{-1} CO_2$). We also examined how *Petunia* plants were affected by changing from a short to a long photoperiod at both ambient and elevated CO_2 .

MATERIALS AND METHODS

Seed of *Petunia* cv. Primetime White Hybrid, obtained from Stokes Seed Ltd, Ontario, was germinated in 12 plug trays (size 288) filled with ASB Starter Mix (Greenworld Ltd, Waterloo, Ontario). Each cell was sown with one seed, germination occurred in 5 d in a fogged greenhouse. A week after emergence, trays were randomly assigned in groups of three to each of four controlled-environment growth

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cabinets (model GR-36, Enconaire Systems Ltd, Winnipeg, Manitoba).

The growth cabinets provided four environments: (1) high (1000 μ l l⁻¹) CO₂ and long days (HCLD); (2) low $(350 \ \mu l \ l^{-1}) \ CO_2$ and long days (LCLD); (3) high CO₂ and short days (HCSD); and (4) low CO₂ and short days (LCSD). All plants were given a basic photoperiod of 9 h, in addition, long day cabinets also provided a 4 h light break each night. Basic photoperiod provided a photosynthetic photon flux (PPF) of 450 μ mol m⁻² s⁻¹ (400-700 nm) supplied by a mixture of cool-white fluorescent and incandescent lamps (75% and 25% input wattage, respectively). PPF during the light break was 8 μ mol m⁻² s⁻¹ (100% incandescent). Carbon dioxide was injected into the growth cabinets from a liquid source, and its concentration monitored by an infrared gas analyser (Nova Model 421P, Hamilton, Ontario) drawing a 45 s air sample from each growth cabinet sequentially on a 180 s cycle (Lander Control Systems Inc., Orangeville, Ontario). High CO, plants were exposed to their assigned CO₂ level only during the main light period and were given ambient CO₂ for the remaining time. The air temperature within the growth cabinets was maintained at 16 ± 0.5 °C. Plants with their respective CO₂ and photoperiod treatments were switched among chambers weekly.

Fertilization began 20 d from sowing and was carried out on a weekly basis. Plants were fertilized with a mixture of 15:15:18 general purpose fertilizer (Plant Products Co. Ltd, Brampton, Ontario), calcium nitrate, and Sequestrene 330 Fe (CIBA-GEIGY, Mississauga, Ontario). This mixture contained 200 μl l^-1 nitrogen, 58 μl l^-1 calcium and 100 μl l^-1 iron. All plants were watered as required throughout the experiment. Thirty-four days after sowing, plants from all treatments were transplanted to 7.5 cm standard pots (175 ml) containing one part perlite to four parts ASB Grower Mix (Greenworld Ltd, Waterloo, Ontario). A week after transplanting, 45 plants from the high CO₂, short day growth cabinets were transferred to the high CO₂, long day growth cabinet. At the same time, an additional 45 plants were transferred from the low CO₂, short day growth cabinets to the low CO₂, long day growth cabinets. Including plants which were not transferred, there were a total of six $CO_{2} \times photoperiod$ treatments.

For growth analysis, a total of nine sequential harvests were made commencing as soon as plants were large enough to be separated into their component parts. The first harvest was 26 d after sowing and subsequent harvests were at weekly intervals thereafter. Each harvest consisted of at least five plants from each treatment. For each plant, leaf area was measured and leaves, stem, root, and reproductive parts were separated and oven dried (at 60 °C for 48 h) for weight determination. The last harvest performed on day 82 was used to assess differences in plant size and morphology among the six treatments. Plant height, number of lateral branches, and number of open flowers and buds were determined for each individual. Specific leaf area (SLA) was calculated by dividing total leaf area by leaf dry weight. Total dry weight and percentage allocation to leaf, stem, root and reproductive parts were also determined.

To describe phenological patterns, 15 plants from each of

the six treatments were observed on a daily basis. Time to first visible bud and time of first open flower were recorded for each individual. The difference in time between these two events provided an estimate of the length of time required for bud development.

Estimates of instantaneous values of relative growth rate (RGR), leaf area ratio (LAR), and unit leaf rate (ULR) for each harvest date were calculated using the regression method of Hunt and Parsons (1974). Morphological and final biomass data were analysed by analysis of variance using the GLM procedure of SAS (version 6 for personal computers). Data were analysed as a two × three factorial (i.e. two CO₂ levels crossed with three daylength treatments) in a completely random design. Phenological data were not normally distributed, therefore, the Kruskal-Wallis test was used to examine differences among the six treatment groups. Size at bud formation and flowering were estimated by fitting the mean time of these events into the regression equations fitted to the total weight-time data (i.e. the growth analysis equations). Estimates, together with their 95% confidence intervals were obtained for each treatment combination.

RESULTS

Growth analysis

Plants grown under long day (LD) conditions exhibited a higher initial relative growth rate (RGR) than plants grown in short day (SD) conditions when given the same CO_2 treatment (Fig. 1 A and B). However, this enhancement was



FIG. 1. Relative growth rate for plants grown at either 350 (LC) or 1000 µl l⁻¹ CO₂ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to long days at day 41 (SD–LD). Error bars are ± two s.e. A, ○, HCLD; ●; LCLD; B, ○, HCSD; ●, LCSD; C, ○, HCSD–HCLD; ●, LCSD–LCLD.

FIG. 2. Unit leaf rate for plants grown at either 350 (LC) or $1000 \ \mu l \ l^{-1}$ CO₂ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to long days at day 41 (SD–LD). Error bars are \pm two s.e. Legend as for Fig. 1.

 $40 \,\, 50 \,\, 60$

Days

20 30

0.12

0.08

0.04

0.00

Unit leaf rate (g dm⁻² d⁻¹)

short lived, disappearing by day 41 in both CO_2 treatments. Plants switched from SD to LD conditions at day 41 exhibited essentially the same pattern as continuous SD plants (Fig. 1C). No significant difference in RGR was found between the high and low CO_2 -grown plants when given an LD photoperiod (Fig. 1A); a higher RGR was observed in the high CO_2 plants from day 33 to 47 under SD conditions (Fig. 1B). In the treatment in which plants were switched from the SD to LD photoperiod, RGR did not differ between CO_2 regimes after the day of the switch (Fig. 1C).

Unit leaf rate (ULR) of plants grown in different daylengths did not differ until day 41 when it became higher under SD conditions, but after day 54 differences among treatments disappeared again (Fig. 2A and B). Plants switched from SD to LD conditions again exhibited a pattern very similar to that of continuous SD plants (Fig. 2C). When plants were grown under the same photoperiodic conditions, high CO₂ plants exhibited a higher ULR than low CO₂ plants (Fig. 2). This effect was most pronounced under short day conditions where enhancement occurred between day 33 and 68. Under LD conditions, enhancement only occurred from day 41 to day 54. In the SD to LD switch treatment, high CO₂ enhanced ULR from the time of the switch on day 41 to day 68 (Fig. 2C).

Initially, leaf area ratio (LAR) was higher in plants grown in the LD photoperiod (Fig. 3). This effect disappeared over time, and by day 54 in the low CO_2 treatment the difference between SD and LD plants was reversed. In the LD photoperiod, the level of CO_2 did not affect LAR until day 40 when low CO_2 plants showed a higher LAR than high CO_2 plants (Fig. 3A). The same trend was observed in plants grown in the SD photoperiod, but low CO_2 plants did not exhibit a higher LAR until day 48 (Fig. 3B). Plants switched from the SD to LD photoperiods exhibited essentially the same pattern as plants in continuous short days; i.e. a higher LAR in the low CO_2 regime from day 48 onward (Fig. 3C).

FIG. 3. Leaf area ratio of plants grown at either 350 (LC) or 1000 μ l l⁻¹

 CO_2 (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to

long days at day 41 (SD-LD). Error bars are \pm two s.e. Legend as

for Fig. 1.

Growth and morphology at final harvest

Plants grown in continuous LD were taller and had fewer lateral branches than those in SD (Table 1). Switching plants on day 41 from SD to LD conditions changed morphology to the LD form; plants grew taller and had less lateral branching. There was no overall effect of CO_2 , but there were significant $CO_2 \times$ daylength interactions for both plant height and number of lateral branches. When high CO_2 was applied under SD conditions, plants were taller and had less lateral branching compared with their low CO_2 counterparts. On the other hand, high CO_2 combined with LD conditions had no effect on plant height or lateral branching. High CO_2 increased the number of branches in plants switched from the SD to LD photoperiod, but did not affect plant height.

Continuous LD conditions increased the number of open flowers relative to continuous SD conditions (Table 1). Plants which were switched from SD to LD conditions on day 41 were intermediate between the two continuous photoperiod treatments. High CO_2 increased the number of open flowers, the magnitude of this effect being greater in the SD and SD–LD treatments than in the continuous LD



70 80 90



Treatment	Number of branches	Plant height (cm)	Number of buds	Number of flowers	Specific leaf area (dm ² g ⁻¹)	
HCLD	7.2 ± 0.43	12.6 ± 0.38	13.2 ± 1.20	7.6 ± 0.44	2.22 ± 0.09	
HCSD	9.0	8.4	14.2	5.4	1.68	
LCLD	6.6	12.6	11.6	5.2	2.6	
LCSD	12.2	6.9	8.6	0.2	2.17	
$HCSD \rightarrow HCLD$	7.8	10.6	13.4	7.2	1.57	
$LCSD \rightarrow LCLD$	6.4	11.3	10.2	1.8	1.99	
Daylength	*	*	n.s.	*	*	
CO ₂	n.s.	n.s.	*	*	*	
$\bar{\text{Daylength}} \times \text{CO}_2$	*	*	n.s.	*	n.s.	

TABLE 1. The effects of day-length and level of CO_2 on morphology of plants harvested 82 d after sowing

Plants were grown at either 350 (LC) or 1000 μ l l⁻¹ CO₂ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD) or switched from short to long days at day 41 (SD–LD). Values represent the treatment means \pm s.e. Whether an effect was significant (*), or not (n.s.) at the P = 0.05 level is indicated at the bottom of the table.

TABLE 2. The effects of day-length and level of CO_2 on total dry weight and percentage allocation to leaf, stem, root and flowering parts at day 82

Treatment	Total dry weight (g)	% Leaf	% Stem	% Root	% Flower parts
HCLD	2.92 ± 0.18	37.0 ± 1.1	27.9 ± 1.1	12.6 ± 1.0	22.5 ± 1.6
HCSD	3.14	41.2	27.8	12.7	18.4
LCLD	2.20	37.9	31.8	10.3	20.0
LCSD	1.81	56.6	26.1	13.9	3.4
$HCSD \rightarrow HCLD$	3.32	39.3	30.1	10.6	20.0
$LCSD \rightarrow LCLD$	2.02	46.5	28.5	11.2	13.8
Daylength	n.s.	*	n.s.	*	*
CO,	*	*	n.s.	n.s.	*
$Daylength \times CO_2$	*	*	n.s.	n.s.	*

Plants were grown at either 350 (LC) or 1000 μ l l⁻¹ CO₂ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to long days at day 41 (SD–LD). Values represent the treatment means \pm s.e. Whether an effect was significant (*), or not (n.s.) at the P = 0.05 level is indicated at the bottom of the table.

treatment. High CO_2 uniformly increased the number of buds regardless of daylength.

TABLE 3. The effects of daylength and level of CO_2 on reproductive phenology

Plants in continuous LD conditions had a higher SLA than plants in continuous SD conditions (Table 1). Plants switched at day 41 from SD to LD conditions had a lower SLA than either of the two continuous treatments. High CO_2 decreased SLA regardless of daylength.

Daylength had no overall effect on total plant dry weight (Table 2). High CO_2 increased total weight in all daylength treatments but this effect was greater under SD than LD conditions. The CO_2 enhancement ratio was 1.73 and 1.33 for plants in constant SD and LD conditions, respectively. Plants switched from SD to LD conditions at day 41 had an intermediate CO_2 enhancement ratio of 1.64.

Continuous LD conditions decreased root and leaf allocation and increased reproductive allocation relative to continuous SD conditions (Table 2). Plants switched from SD to LD conditions were intermediate between the continuous daylength treatments in leaf and reproductive allocation, and similar to the LD treatment in root allocation. High CO_2 decreased leaf and increased reproductive allocation. The magnitude of these effects was much greater under SD conditions.

Treatment	Days to bud formation	Bud development (d)	Days to flower opening
HCLD	46·7 ^b	17·8 ª	64·5 ª
HCSD	49·1 °	22·8 b	71·9 ^b
LCLD	46·2 ª	18·7 ^a	64·9 ^a
LCSD	56·6 ^e	30·0 °	86.6 d
$HCSD \rightarrow HCLD$	49·1 °	20·9 ^b	70·0 ^b
$LCSD \rightarrow LCLD$	51·1 ^d	28·4 °	79·5 °

Plants were grown at either 350 (LC) or $1000 \ \mu l^{-1} CO_2$ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to long days at day 41 (SD–LD). Within any one column, means followed by the same superscript were not significantly different from each other at P = 0.05.

Reproductive phenology

Long day conditions promoted early bud formation (Table 3). Comparing the constant LD to the constant SD treatment, advancement was 10.4 d at low CO₂ and 2.4 d at

Treatment	Size at bud formation (g)	95% Confidence interval	Size at flower opening (g)	95% Confidence interval
HCLD	0.39	0.37-0.42	1.68	1.56–1.82
HCSD	0.39	0.37-0.41	2.57	2.36-2.80
LCLD	0.21	0.19-0.23	1.03	0.93-1.15
LCSD	0.42	0.39-0.46	1.71	1.30 - 2.26
$HCSD \rightarrow HCSD$	0.37	0.34-0.40	2.20	1.99-2.43
$LCSD \rightarrow LCLD$	0.27	0.24-0.29	1.81	1.57 - 2.08

TABLE 4. The interactive effects of day-length and level of CO_2 on total plant dry weight at time of bud formation and flower opening

Plants were grown at either 350 (LC) or 1000 μ l l⁻¹ CO₂ (HC), and in one of three daylength treatments: continuous short days (SD), continuous long days (LD), or switched from short to long days at day 41 (SD–LD). Values were estimated using the mean time of bud formation and flower opening for each treatment, and the fitted relationships between plant weight and time from growth analysis.

high CO₂. Switching LCSD plants to LCLD at day 41 advanced bud formation by 5.5 d relative to plants maintained in continuous LCSD conditions. The treatment that involved switching plants from HCSD to HCLD on day 41 probably did not provide sufficient time for the treatments to take effect; plants formed buds on day 49, the same day as plants maintained continuously in HCSD conditions. High CO₂ had contrasting effects on bud formation depending on the photoperiod, advancing bud formation in SD and delaying it in LD conditions. While plants in the HCSD treatment formed buds 7.5 d earlier than LCSD plants, bud formation in the HCLD treatment was 0.5 d slower than the LCLD treatment. The switch treatment was intermediate between the constant SD and LD treatments in terms of the CO₂ effect; elevated CO₂ advanced bud formation by 2 d.

Bud development was advanced 11·3 and 5·0 d by LD compared with SD conditions in ambient and high CO_2 conditions, respectively (Table 3). Switching plants from SD to LD conditions did not affect bud development relative to plants maintained in SD conditions. There was no CO_2 effect on bud development under constant LD conditions, but under SD conditions, high CO_2 advanced bud development. Elevated CO_2 also advanced bud development to a lesser extent in the SD–LD switch treatment.

Constant LD conditions promoted flower opening; plants in the HCLD treatment flowered 7·4 d earlier than those in the HCSD, and the LCLD treatment flowered 21·7 d before the LCSD treatment (Table 3). Plants in the HCLD and LCLD treatments were the fastest to flower, flowering 5·1 d earlier than the next group. Switching HCSD plants to the HCLD treatment had no effect on flower opening. Switching LCSD plants to the LCLD treatment advanced flowering by 7·1 d. Thus, while CO_2 played no role in flower opening under constant LD conditions, high CO_2 advanced flower opening by 14·7 d under constant SD conditions and by 9·5 d in the switch treatment.

Size at bud formation and flower opening

There was a strong interaction between daylength and CO_2 on size at bud formation such that the effect of daylength varied depending on the level of CO_2 and the

effect of CO_2 varied depending on the daylength (Table 4). Plants grown under low CO_2 were lighter at bud formation under LD conditions; however, at high CO_2 there was no effect of photoperiod. Elevated CO_2 increased plant weight at the time of bud formation in long days but not short days. At low CO_2 , plants in the switch treatment produced buds at the same plant weight as LD plants. At high CO_2 , there was no difference in size at bud formation among any of the three daylength treatments.

Plants grown in continuous SD were heavier at flower opening than those grown in continuous LD at both low and high CO_2 (Table 4). Plants in the switch treatment flowered at the same weight as plants in the continuous SD treatment at the same CO_2 level. Elevated CO_2 increased weight at flower opening in the continuous SD and LD treatments, but not in the switch treatment.

DISCUSSION

An increase in the level of CO₂ had different effects on growth depending upon daylength (Fig. 1, Table 2). It has been shown in a number of previous studies that the effect of elevated CO₂ on growth depends upon available light (Sionit, Hellmer and Strain, 1982; Pearcy and Bjorkman, 1983; Sionit and Patterson, 1984; Bazzaz, 1990). Given that both CO₂ and photon flux density directly affect photosynthesis, there are a number of possible physiological mechanisms which might explain such an interaction. However, it is unlikely that the effect of daylength in the present study was related to differences in the total photon flux received in the two photoperiod regimes. The additional light given during the night break in the long photoperiod amounts to less than 1% of the total light given during the regular 9 h photoperiod and was below the light compensation point for photosynthesis. Such changes will not have a direct impact upon carbon gain. Instead we conclude that the differences between LD- and SD-grown plants in the present study are due specifically to the effect of daylength on development.

Long day plants were taller, less branched, had a lower root and leaf allocation, and a higher reproductive allocation, LAR and SLA than SD plants (Fig. 3; Tables 1 and 2). Similar responses to daylength have been observed in a wide range of species (Attridge, 1990; Moe and Heins, 1990). Under the conditions of the present experiment these traits had little impact on RGR (Fig. 1) and no effect upon final biomass (Table 2). However, these developmental changes apparently affected plant response to CO₂. The increase in RGR with CO₂ which was observed under SD (Fig. 1B) but not LD (Fig. 1A) conditions, is due largely to the more positive response of ULR to elevated CO_2 in the SD treatment (Fig. 2A and B). Due to the positive impact of CO₂ on photosynthesis, an increase in ULR is to be expected (e.g. Bazzaz et al., 1989), however the reasons why this effect is greater under SD conditions is less clear. We can envisage three possible explanations: it has previously been demonstrated that modifying the demand for carbon by manipulating the number or size of actively growing meristems can substantially alter the beneficial effects of CO₂ upon photosynthesis. An increase in the number of meristems or 'sink strength' increases the response to elevated CO₂ (e.g. Arp, 1991; Reekie, 1996). Increases in the demand for carbon increase the export of carbon from leaves and prevent end product inhibition of photosynthesis or, in extreme cases, damage to the photosynthetic apparatus by the accumulation of large starch granules in the chloroplasts (Cave, Tolley and Strain, 1981; Wulf and Strain, 1982). In this study SD plants had more actively growing meristems, and a larger sink strength, than LD plants because of their increased branching. This may result in a more positive photosynthetic response to increases in CO₂ concentration.

A second possible explanation for the strong interaction between daylength and CO_2 on ULR is also related to the increased branching of SD plants. It is commonly observed that the response to elevated CO_2 declines with plant age (e.g. Jolliffe and Ehret, 1985; Bazzaz *et al.*, 1989). One explanation for this effect is the increased amount of selfshading which occurs as plant size increases. Elevated CO_2 increases growth and, therefore, shading occurs earlier and results in negative feedback upon growth. The increased branching of SD plants, however, would decrease the potential for self-shading as there would be less leaf overlap with multiple axes. Therefore, increases in growth with CO_2 would be less likely to result in negative feedback through increased self-shading in SD plants.

A third possible explanation for the interaction between CO_2 and daylength on ULR is related to the low SLA of SD plants (Table 1). A low SLA implies that the leaves are thicker and denser (Rogers, Thomas and Bingham, 1983). This difference between SD and LD plants represents a change in the number of cell layers or in individual cell size. Both of these changes would have negative effects on the internal CO_2 concentration. As CO_2 would have to diffuse through larger cells or more cell layers to reach the chloroplasts in SD plants, these leaves are more likely to be limited by CO_2 availability. Therefore, they would respond positively to increases in CO_2 concentration.

Although differences in CO_2 enhancement of ULR can explain much of the difference in growth response among daylength treatments, differences in the way CO_2 affects LAR (Fig. 3) were also involved. Elevated CO_2 reduced LAR due to a decrease in both SLA (Table 1) and leaf allocation (Table 2). Such effects are often observed in longterm CO_2 fumigation experiments (Bazzaz, 1990). The reduction in LAR will tend to counteract the increase in ULR at elevated CO_2 . Under SD conditions, however, LAR did not vary between high and low CO_2 -grown plants until day 48, over 7 d later than under LD conditions. The reasons for this delay are unclear.

The effects of CO₂ upon morphology (Table 1), allocation (Table 2), and phenology (Table 3) also interacted strongly with daylength. It is possible that these interactions are simply a reflection of growth effects. For example, the lack of any CO₂ effect upon plant height, bud development time, number of buds and SLA in LD plants, and the marked enhancement observed in SD plants may simply be a consequence of the much greater CO₂ enhancement of growth under SD conditions. The more marked effects of CO₂ upon allocation patterns under SD, as compared to LD conditions (Table 2) could be explained in a similar manner. Other effects are more difficult to explain in this fashion. The number of branches showed a slight, but non-significant, increase with elevated CO₂ in LD conditions, an increase which is in accord with the relatively low CO₂ enhancement of growth under LD conditions. However, the number of branches decreased significantly with the level of CO₂ under SD conditions, even though CO₂ enhanced growth substantially. Another interaction which is difficult to explain in terms of a simple growth effect, is the effect of CO₂ on the number of days to bud formation. Elevated CO₂ caused a slight, but significant, delay in days to bud formation in LD conditions and hastened bud formation in SD conditions (Table 3). Why should CO₂ enhancement of growth have different effects on the number of days to bud formation in long vs. short day conditions? There were also apparent changes in the size of plants at bud formation and flower opening among CO2 levels (Table 4). If CO2 changed phenology simply by modifying growth rate, plant size at bud formation and flower opening should be the same.

These results are similar to those of a number of previous studies which have found poor correlation between the effect of CO₂ upon growth and its effect upon phenology (Marc and Gifford, 1984; Reekie and Bazzaz, 1991). This lack of correlation suggests that CO₂ affects phenology and morphology by some other mechanism(s), possibly an interaction between CO2 and phytochrome. Early physiological studies suggested such an interaction to explain the effects of very high levels of CO₂ on the photoperiodic response of flowering (Purohit and Tregunna, 1974). Phytochrome, as well as affecting flowering also affects a wide range of developmental processes including seed germination, branching, leaf development, and stem elongation (Salisbury and Ross, 1991). Therefore an interaction between CO₂ and phytochrome could explain the marked effect of CO₂ upon morphology as well as phenology in the present study.

The mechanism by which CO_2 may interact with phytochrome is unclear. Previous studies have suggested that through its effect on photosynthesis CO_2 increases the production of assimilates and that the level of assimilates modifies phytochrome action (Evans, 1969; Hicklenton and Jolliffe, 1980). However, Campbell (1957) reports that CO_2 fumigation in complete darkness and, therefore, in the absence of any effect on photosynthesis, also modifies the photoperiodic response. Similarly, Zebian and Reekie (unpubl. res.) found that the level of CO₂ modifies another phytochrome-mediated response, etiolation in Sinapis alba, in the absence of light. Another possible mechanism for a $CO_{3} \times phytochrome interaction is through modification of$ hormonal levels. Carbon dioxide is a competitive inhibitor of ethylene production, decreasing the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene (Cheverry et al., 1988). Ethylene affects a variety of developmental processes including flowering, fruit ripening, senescence, seed germination and stem elongation (Salisbury and Ross, 1991). However, the levels of CO₂ necessary to inhibit ethylene production (50000–100000 μ l l⁻¹ CO₃) are much higher than those used in the present study. Furthermore, it is unclear how CO₂ effects on the production of ethylene might account for the strong interaction observed between the level of CO₂ and photoperiod. Multifactorial experiments generally suggest that the effects of phytochrome on morphology are independent of the effects of hormones (Mohr and Schopfer, 1995).

There has been a great deal of emphasis in the recent literature on interactions between elevated CO_2 and other environmental factors, including temperature, water availability, nutrient availability, irradiance and various pollutants (Bazzaz, 1990; Bassow, McConnaughay and Bazzaz, 1994; Kirschbaum, 1994; Kirschbaum, *et al.*, 1994; Nobel and Israel, 1994; Oechel *et al.*, 1994). There has been a realization that the direct effects of the rise in atmospheric CO_2 on plants will be highly variable due to such interactions. Our study demonstrates that photoperiod is another factor which interacts strongly with the level of CO_2 .

In the field, plants are exposed to changing daylengths over the course of the growing season. The switch treatment suggests that it is not necessarily possible to predict the CO_2 response of a plant under changing daylength conditions based upon its response to continuous short vs. continuous long days. Although plants in the switch treatment were intermediate between the continuous short and long day treatments for many of the measured traits, they often more closely resembled one or other of the continuous treatments depending upon the particular parameter. Furthermore, in the case of branching, the effect of elevated CO_2 in the switch treatment was different to that in either of the two continuous treatments (Table 1).

Photoperiod is a factor which has been largely ignored by studies addressing the environmental impact of the rise in atmospheric CO_2 . Nevertheless, it is a factor which could explain some of the wide variation that has been observed in the responsiveness of plants to elevated CO_2 . Many studies for example, have noted that the responsiveness of plants changes with time (e.g. Bazzaz *et al.*, 1989). While there are probably several explanations for this effect including changes in resource availability (McConnaughay, Bernston and Bazzaz, 1993), changes in daylength over the course of the season could be very important. Future studies should be careful to document daylength conditions and consider how changes in photoperiod may influence the results.

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