Why does elevated CO_2 affect time of flowering? An exploratory study using the photoperiodic flowering mutants of *Arabidopsis thaliana*

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Summary

- Evidence is accumulating that the effect of CO_2 on time of flowering involves interactions with photoperiod, but the basis for this interaction is unclear. Here, which components of the photoperiod flowering pathway account for this interaction in *Arabidopsis thaliana* were examined.
- Ten mutants deficient in particular loci in the photoperiod pathway, as well as the wild type, were grown under short and long days at either ambient or elevated CO_2. Leaf number at flowering and the number of days required for induction of flowering were determined.
- Elevated CO_2 interacted with both the photoreceptors and the subsequent transduction reactions in the photoperiod pathway. The direction and magnitude of the effects varied with photoperiod. Elevated CO_2 also affected flowering by increasing rate of leaf production.
- The net effect of elevated CO_2 on time of flowering varies because CO_2 has a complex array of effects on different elements of the developmental pathway leading to flower induction that may either hasten or delay flowering depending upon the influence of other environmental factors such as photoperiod.

Introduction

Developmental biologists have made a great deal of progress identifying the specific loci involved in various developmental pathways using tools such as loss-of-function mutants, transgenic organisms that overexpress specific gene products, and gene expression studies. Such studies are generally conducted under a relatively narrow set of carefully defined environmental conditions to facilitate comparisons among studies. However, individual genes are not necessarily expressed in all environments and often differ substantially in their phenotypic effects among environments. This has led evolutionary biologists to call for an examination of gene function in ecologically relevant settings (Weinig & Schmitt, 2004). This emerging field of ecological development aims for a mechanistic understanding of phenotypic variation (Sultan, 2005). This involves understanding how environmental signals are perceived, the subsequent transduction pathways that lead to the resulting phenotypes, and the ecological and evolutionary consequences of these phenotypic outcomes. It has been argued that understanding how plants integrate and respond to contrasting environmental signals and novel environments is critical if we are to understand and predict the response of plants to global environmental change (Ackerly & Sultan, 2006).

Atmospheric CO_2 levels have been shown to have marked, though often highly variable effects on time of flowering in a range of different species (Cleland *et al.*, 2006; Springer & Ward, 2007). As time of flowering can be crucial to reproductive success (Schemske, 1977; Augspurger, 1981; Schmitt, 1983), understanding and predicting how rising atmospheric CO_2 levels will affect flowering is an important goal.

To reach the flowering stage, plants must reach a minimum size to provide the necessary resources for flower development (Lacey, 1986; Wesselingh *et al.*, 1997). Given that elevated CO_2 stimulates plant growth, flowering time should be advanced by elevated CO_2 owing to earlier attainment of the required minimum size. Some studies have found that the

Table 1 Information on the *Arabidopsis thaliana* gene mutants used in this study

Genotype	ABRC stock number[a]	Mutagen[b]	Allele information
phyB-5	CS6213	EMS	Strong allele (Reed *et al*., 1993)
phyB-7	CS6215	EMS	Weak allele (Reed *et al*., 1993)
phyA201	CS6219	EMS	Strong allele (Nagatani *et al*., 1993)
cry2-1	CS108	EMS	Strong allele (Guo *et al*., 1998)
cry2-3	CS187	X	Strong allele (Guo *et al*., 1998)
gi-3	CS51	EMS	Strong allele (Koornneef *et al*., 1991)
gi-4	CS181	EMS	Weak allele (Koornneef *et al*., 1991)
co-4	CS177	EMS	Weak allele (Koornneef *et al*., 1991)
ft-1	CS56	EMS	Weak allele (Kardailsky *et al*., 1999)
ft-3	CS185	EMS	Strong allele (Kardailsky *et al*., 1999)
L*er*	CS8581		Wild Type

[a]ABRC, the *Arabidopsis* Biological Resource Center.
[b]EMS, ethylmethane sulfonate; N, fast neutrons; X, X-ray.

effect of elevated CO_2 on flowering time can be entirely explained by its effect upon growth (He & Bazzaz, 2003), but others have found that the effect of elevated CO_2 on growth is poorly correlated with its effect on flowering (Marc & Gifford, 1984; Reekie & Bazzaz, 1991; Reekie *et al*., 1994). An alternative explanation for the effect of CO_2 on flowering is that it has a direct impact on developmental pathways leading to flower induction. It has long been known that the presence of CO_2 is required for plants to respond to photoperiod in those species where flowering is controlled by day length (Bassi *et al*., 1975). More recently, it has been found (Reekie *et al*., 1997; Johnson & Reekie, in press) that elevating CO_2 from ambient levels to levels expected by the end of this century hastens flowering in long-day plant species, but delays or has no effect on flowering in short-day species.

Molecular genetic studies using *Arabidopsis thaliana* have identified several genetic components in the developmental pathway responsible for sensing changes in daylength and the subsequent transduction reactions (Komeda, 2004). *Arabidopsis thaliana* is a quantitative long-day plant that flowers earlier in response to long daylengths, but will eventually flower even in short days (Levy & Dean, 1998). The genetic components within the photoperiod pathway include: the red/far-red photoreceptors, *PHYTOCHROME A* (*PHYA*) and *PHYTOCHROME B* (*PHYB*), the blue light photoreceptor, *CRYPTOCHROME 2* (*CRY2*), genes associated with the internal time-keeping mechanism (i.e. circadian rhythms), *GIGANTEA* (*GI*) and *CONSTANS* (*CO*), and the gene, *FLOWERING LOCUS T* (*FT*), which is at the intersection point between the photoperiod pathway and the autonomous pathway (Komeda, 2004). The autonomous pathway is responsible for the flower induction that ultimately occurs in the absence of an appropriate photoperiod signal in *A. thaliana* (Komeda, 2004).

The present study took advantage of the well-characterized signal transduction pathway of photoperiodism in *A. thaliana* to acquire a better understanding of how CO_2 affects flowering time and in particular, how it may interact with the photoperiodic pathway. Our objective was to determine whether CO_2 interacts with the light-sensing mechanism, or the subsequent transduction reactions by comparing the CO_2 response of mutants deficient in particular loci at various points in the photoperiodic pathway. Since perception and response to environmental stimuli depend on distinct genetic components subject to different evolutionary constraints, it is critical to distinguish between cue perception and response if we are to understand the ecology and evolution of how a plant responds to its environment (Griffith & Sultan, 2005). Our results provide insight into how the interplay between the genetic and environmental factors involved in the control of regulatory pathways affect phenotypic plasticity in a critically important trait, and provide a framework for understanding and predicting the sometimes complex effects of elevated CO_2 on flowering and other developmental processes.

Materials and Methods

Plant material

Ten lines of *A. thaliana* (L.) Heynh. gene mutants (all derived from the L*er* ecotype) and the L*er* wild type were used in the experiment (Table 1). The lines included loss of function mutants for the light sensors, *phytochrome B* (*phyB-5*, *phyB-7*), *phytochrome A* (*phyA201*), and *cryptochrome* 2 (*cry2-1*, *cry2-3*). There were also loss of function mutants for the circadian rhythm genes, *gigantea* (*gi-3*, *gi-4*) and *constans* (*co-4*), and for the gene responsible for interfacing with the autonomous pathway, *flowering locus T* (*ft-1*, *ft-3*). Wherever available, two different alleles were selected for each mutant. These alleles sometimes differed in the strength of the mutation. A strong allele was one in which all or most function was removed by the mutation; a weak allele retained partial function. All lines were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University, USA.

Growth conditions

Four treatment combinations were imposed: short daylength, ambient CO_2; short daylength, high CO_2; long daylength, ambient CO_2; long daylength, high CO_2. Each of the four treatments was supplied in one of four identical growth chambers (Conviron, Controlled Environments Ltd., Winnipeg, MB, Canada) located in the Irving Environmental Research Center, Acadia University, Canada. Growth chambers for the ambient and high CO_2 treatments were programmed to deliver CO_2 levels of 380 µl l^{-1} (equivalent to the current atmospheric CO_2 level) and 1000 µl l^{-1}, respectively. The short daylength (SD) treatment received 9 h of illumination daily and the long daylength (LD) treatment received 18 h of illumination daily. Light was provided using a combination of fluorescent and incandescent light bulbs. To separate the effects of photoperiod on the developmental pathways leading to flowering from its effects upon growth, we adjusted the number of light bulbs to produce a photosynthetic photon flux (PPF) density of 500 µmol m^{-2} s^{-1} in SDs and 250 µmol m^{-2} s^{-1} in LDs, so that plants grown in both daylengths received the same daily photosynthetic photon flux. Red : far-red light ratios were uniform at 1.60 across all four growth chambers. To ensure that light conditions remained constant over the course of the experiment, weekly inspection was performed using a LI-189 Light Meter (Li-Cor Inc., Lincoln, NE, USA) to monitor PPF and a Skye SKR 110 R:FR sensor (Skye Instruments, Powys, UK) to monitor red : far-red ratio. The height of the light canopy above the plants was adjusted as necessary to maintain constant light levels.

Two independent trials with identical procedures were conducted at different times. For each trial, 264 pots (3.5 × 3 inch (7.6 × 8.9 cm)) were filled with commercial potting soil (Original Grower Mix; ASB Greenworld, Pointe Sapin, NB, Canada). After seeds were sown on the surface of the soil, the pots were placed in a cold room in the dark at 4°C for a period of 5 d to break dormancy. Thereafter, the pots containing the same line of seeds were randomly assigned to one of the four growth chambers, such that each line was represented by six replicate pots within each chamber/treatment. Seedlings were thinned to one per pot 5 d after emergence. All the growth chambers were programmed to provide a temperature of 23°C and 60% relative humidity. Water was applied to the plants every second day and a 1 : 1000 diluted liquid fertilizer 10–6–16 (Plant Products Co. Ltd., Brampton, ON, Canada) was applied once a week. All pots were randomly rotated within chambers every 3 d to minimize position effects. Pots and their corresponding photoperiod and CO_2 treatments were also rotated among chambers on a weekly basis to minimize possible chamber effects.

Data collection

Time of flowering can be measured either in terms of days (chronological age) or in terms of leaf number (developmental age). Chronological age at flowering is the product of developmental age at flowering and average growth rate (i.e. number of leaves produced per day). As a result, the two measures are closely correlated provided that growth rates are uniform across the entities being compared (Koornneef *et al.*, 1991). From the point of view of understanding the effect of mutations in the genetic pathways controlling flowering, developmental age is more useful than chronological age as flowering mutations can have pleiotropic effects on growth. Consequently, our knowledge of the developmental pathways controlling flowering in *A. thaliana* are based primarily on analyses of leaf number (Callahan & Pigliucci, 2005). From an ecological perspective, however, chronological age at flowering is more useful as it can be related directly to the length of the growing season and to other aspects of the environment such as pollinator availability. As the focus of our study was the effect of CO_2 on reproductive timing and CO_2 is known to effect growth rate, we measured both chronological and developmental age at flowering in the wild type. Comparison of these two measures allowed us to determine to what extent the effect of CO_2 on reproductive timing was the result of its effects on growth rate versus developmental processes. However, in comparing time of flowering in the wild type with that in the various mutants, we restricted our comparisons to leaf number at flowering to avoid confounding the effect of these mutations on development with possible effects on growth rate.

Time of flowering can be assessed using a wide range of different reproductive stages, including first flower bud, first open flower, peak flowering, etc. As this study focused on the initiation of flowering by the photoperiodic pathway rather than subsequent events, the first visible sign that flowering had been induced was used. Time of flowering was determined by visually inspecting each plant on a daily basis and noting both the number of leaves and the number of days since germination when the apical meristem changed in size, color and shape, signaling the shift to the reproductive state.

Statistical analyses

In the analysis, data were blocked over time (i.e. the two trials represented blocks in a randomized complete block design). The leaf number data were analysed using a split-plot ANOVA performed using the GLM procedure of SAS version 9.1 (SAS Inc., Cary, NC, USA). The growth chambers (four chambers × two trials = 8 plots) were the main plots and individual plants, which were nested inside of the main plots, were the subplots. Block, CO_2 level and photoperiod were main plot factors and line was a subplot factor. Block, the main plot error term and the residual error term were random factors, and photoperiod, CO_2 and line were fixed factors. A natural logarithm transformation was used to homogenize variances and achieve a normal distribution. The effect of block, and the main effects of CO_2, photoperiod and their interaction were tested using the main plot error term. The effects of lines

Table 2 Results of a split-plot ANOVA evaluating effects of CO_2, day length, line, and their interactions on leaf number at flowering in *Arabidopsis thaliana*

Source of variation	Leaf number at flowering			
	df	Sum of squares	*F* value[a]	Significance
Main plots	7			
Block	1	0.16	4.44	0.0357
CO_2	1	0.38	10.24	0.0015
Daylength	1	177.52	4795.01	< 0.0001
CO_2 × daylength	1	0.43	11.58	0.0007
Main plot error	3	0.19	1.72	0.1624
Subplots	509			
Line	10	96.17	259.77	< 0.0001
CO_2 × line	10	4.58	12.38	< 0.0001
line × daylength	10	22.82	61.65	< 0.0001
CO_2 × line × daylength	10	3.77	10.17	< 0.0001
Residual error	469	17.36		

[a]The effects of block, CO_2, daylength and CO_2 × daylength were tested using the main plot error term, (i.e. among chamber error). The remaining effects were tested using the residual error term.

and the interactions between lines and photoperiod/CO_2 were tested using the residual error term. It should be noted, however, that the main plot error term was not significantly different from the residual error term in this study. A priori paired contrasts or planned individual degree of freedom comparisons (Sokal & Rohlf, 1995) were used to determine the level of significance for differences between mutants and the wild type, and between low and high CO_2 levels for each line. The 0.05 level of significance was used for all comparisons.

A similar analysis was used to examine the effect of CO_2 and daylength on the number of days to flowering except that here, the wild type was the only line included in the analysis. The natural logarithm transformation was again used to homogenize variances among treatments and achieve a normal distribution.

Results

The effect of daylength and the various mutations on developmental age at flowering were as expected. Plants generally flowered at a higher leaf number in SDs than LDs (Table 2, Fig. 1). Under LDs and ambient CO_2 levels, all mutants with the exception of those involving *phytochrome*, initiated flowering with more leaves than the wild type (Table 2, Fig. 1). The two *phyB* mutants initiated flowering at a smaller leaf number than the wild type, while the *phyA* mutant was not significantly different from the wild type. Under SDs and ambient CO_2 conditions, there was no significant difference between any of the *phytochrome* mutants and the wild type; the other mutants all flowered at a higher leaf number than the wild type (Table 2, Fig. 1).

The effect of CO_2 on leaf number at flowering was highly variable depending upon daylength and genetic line; the

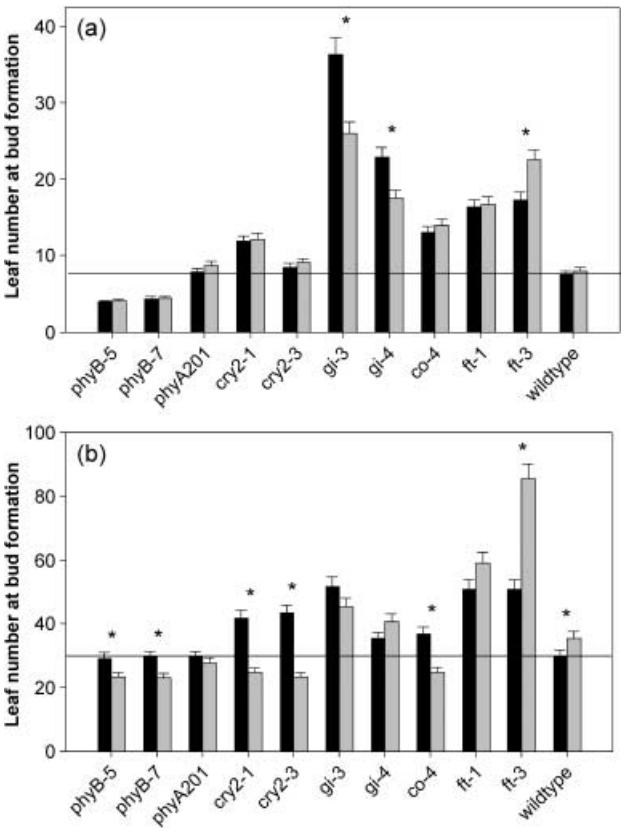

Fig. 1 Leaf number at flowering in different lines of *Arabidopsis thaliana* grown at ambient (closed bars) versus high CO_2 (tinted bars) in (a) long versus (b) short day lengths. An asterisk (*) indicates a significant difference ($P < 0.05$) between ambient and high CO_2 treatments within a given line. Error bars denote 1 SE. A line representing average leaf number at flowering in the wild type at ambient CO_2 level is drawn across each panel to facilitate comparisons. Note the change in scale between panels.

interactions between CO_2 and daylength, CO_2 and line, and CO_2, line and day length were all highly significant (Table 2). The wild type showed no significant response to CO_2 under LDs, but elevated CO_2 increased the number of leaves at flowering in SDs. The various mutant lines fell into one of three categories with regard to their response to CO_2 depending upon whether CO_2 had an effect in SDs or LDs.

The first category consists of the photoreceptor mutants and the circadian rhythm mutant, *co*. Elevated CO_2 reduced the number of leaves at flowering in the two *phyB* mutants, the two *cry2* mutants and the *co* mutant in SDs, but had no effect in LDs. The only photoreceptor mutant that differed from this pattern was *phyA*. Like the other photoreceptor mutants, CO_2 had no effect on *phyA* in LDs, but unlike the other photoreceptor mutants, there was no significant effect in SDs either. However, the *phyA* mutant did differ significantly from the wild type in its response to CO_2 in SDs (elevated CO_2 increased number of leaves at flowering in the wild type, Fig. 1) indicating that elevated CO_2 reduced the number of leaves at flowering in the *phyA* mutant relative to the wild type. In essence, the *phyA* mutant responded to CO_2 in the same manner as the other receptor mutants and *co*, but the effect in *phyA* was much weaker than in the other mutants in this category. Based upon the extent to which elevated CO_2 reduced the number of leaves at flowering in SDs, the various mutants in this category would ranked from the strongest to the weakest effect as follows: *cry2-3*, *cry2-1*, *co-4*, *phyB-7*, *phyB-5* and *phyA201*.

The second category consists of the two *gi* mutants. In contrast to the previous group of mutants, elevated CO_2 had no effect on leaf number at flowering in SDs, but reduced leaf number in LDs (Fig. 1). However, it should be noted that the two *gi* mutants did differ from the wild type in their response to CO_2 in SDs. This indicates that the tendency for elevated CO_2 to reduce leaf number at flowering that was observed in LDs was carried over to a sufficient extent in SDs to nullify the tendency for elevated CO_2 to increase leaf number in the wild type.

The third category consists of the two *ft* mutants. The strong *ft* mutant (*ft-3*) was the only line that responded to CO_2 in both long and short days; elevated CO_2 increased leaf number at flowering in both daylengths. The weak ft mutant (*ft-1*) did not display any significant response to CO_2 in either long or short days. However, its pattern was similar to that of the strong allele (*ft-3*) with regard to CO_2 effects (Fig. 1); the differences between CO_2 concentrations in the case of the weak allele were just not significant at the 0.05 level of probability ($P = 0.0592$ in SDs and $P = 0.7289$ in LDs). Given that the *ft-1* allele is a very weak mutation in which transcription of *FT* is largely normal (Yoo *et al.*, 2005), this is to be expected.

The wild type required fewer days to initiate flowering (i.e. flowered at a younger chronological age) in LDs ($F_{1,3} = 169.3$, $P = 0.0010$) and at elevated CO_2 ($F_{1,3}$, $P = 0.0249$), but there was no significant interaction between these two factors ($F_{1,3} = 0.80$, $P = 0.4373$) (back-transformed 95% CI for LDs was 19.6–21.5 and 17.2–18.8 for ambient and high CO_2, respectively, compared with 35.7–39.2 and 28.7–31.4 in SDs).

Discussion

Several previous studies have examined the effect of elevated CO_2 on time of flowering in *A. thaliana*. Results have been mixed; elevated CO_2 sometimes hastens flowering (Zhang & Lechowicz, 1995; Bidart-Bouzat *et al.*, 2004), but several studies have shown no effect (Zhang & Lechowicz, 1995; VanderKooij & DeKok, 1996; Ward & Strain, 1997). To date, no explanation has been offered for the contrasting results of these studies. In the present study, elevated CO_2 had no effect on leaf number at flowering under LD conditions and increased leaf number at flowering in SD conditions. However, it reduced number of days to flowering under both conditions because of the positive effect of elevated CO_2 on growth rate (i.e. plants produced leaves more rapidly at elevated CO_2). It would appear that the effect of elevated CO_2 on time of flowering in *A. thaliana* is the net result of the positive effect of elevated CO_2 on growth and a negative effect resulting from its tendency to increase leaf number at flowering. Given that the effect of elevated CO_2 on growth varies widely depending upon a variety of environmental conditions (Mckee & Woodward, 1994; Hattenschwiler & Korner, 2000), and daylength affects the effect of elevated CO_2 on development (Fig. 1), it is to be expected that the net effect on time of flowering will vary widely.

The effect of CO_2 on developmental age at flowering (i.e. leaf number) was a function of several different effects acting at different points in the developmental pathway (Fig. 1). The interaction between CO_2 and the photoreceptors in short days indicates that CO_2 can affect the perception of light signals. The interaction between CO_2 and *CO* in short days could be a function of the effect of CO_2 on the photoreceptors as *CO* is downstream of the photoreceptors (Komeda, 2004), or it may represent an independent effect of CO_2 on the time-keeping mechanism. However, the interaction between CO_2 and *GI* in long days, indicates that CO_2 does affect the internal time-keeping mechanism at least under long days. The interactions between CO_2 and *FT* in both short and long photoperiods could represent: the upstream effects on the photoreceptors and the time-keeping mechanism; a direct effect on *FT*; or an effect any point upstream in the autonomous pathway since *FT* is the intersection point between the photoperiod and autonomous pathways (Komeda, 2004).

The pervasive impact of CO_2 on both the perception and subsequent transduction of photoperiod signals may seem surprising, but there is evidence from a variety of sources to support this conclusion. Elevated CO_2, through its effects on photosynthesis, has marked effects on sugar levels within the plant. For example, in *A. thaliana* a recent study reports that total soluble sugar content in leaves increase by > 70% at elevated CO_2 (Teng *et al.*, 2006). It is increasingly clear that

sugars such as sucrose, glucose and fructose have profound effects on all aspects of development. Sugar-mediated signaling cascades can lead to various cellular responses including altered gene expression and enzymatic activities (Smeekens, 2000). Some light-mediated physiological events, such as the de-etiolation response, are modified by sugar levels, indicating a possible interaction between sugar and light receptors (Smeekens, 2000). Moreover, sugar has been reported to directly affect flowering time in *A. thaliana* (Zhou *et al.*, 1998; Roldan *et al.*, 1999). A recent microarray study (Li *et al.*, 2006), also revealed that *CRY2* transcription is regulated by glucose in *A. thaliana*. *PHYB*, *CO* and, indirectly, *PHYA* are under the regulation of *CRY2* (Lin, 2000; Valverde *et al.*, 2004), thus any effect of CO_2 on *CRY2* would also affect the other photoreceptors and *CO*. Exposure of *A. thaliana* to elevated CO_2 increases gibberellic acid levels in leaves (Teng *et al.*, 2006). Both gibberellic acid and the circadian rhythm gene *GI* share an important transduction component, *SPINDLY* (Tseng *et al.*, 2004), thus it is possible that the observed CO_2 effect on *GI* may be linked to the effect of CO_2 on levels of gibberellic acid. Finally, it has recently been shown (Springer *et al.*, 2008) that elevated CO_2 affects the expression of *FLOWERING LOCUS C* (*FLC*), an inhibitor of *FT*. Genes in the autonomous pathway promote *FT* through the suppression of *FLC* (Komeda, 2004; Yoo *et al.*, 2005). Given that FLC is part of the autonomous pathway, this may explain why the effect of CO_2 on *FT* was largely independent of photoperiod (Fig. 1).

Our study has revealed that the effect of elevated CO_2 on time of flowering in *A. thaliana* is complex, involving several different effects on the developmental pathway that is involved in sensing, interpreting and transmitting photoperiod signals. Since distinct genetic elements are responsible for sensing and transmitting these light signals, and at least some of these genes have been shown to vary both within and among species (Smith, 2000), this may explain why the effect of elevated CO_2 on flowering varies so widely (Johnson & Reekie, in press). Predicting the effect of elevated CO_2 on flowering in individual species or genetic lines may well require knowledge of the mechanisms by which they perceive and transmit light signals.

The complex intertwining of developmental pathways that sense and transmit multiple environmental signals appears to be a common theme emerging from studies of ecological development. It has been shown, for example, that the developmental pathways involved in increasing stem elongation in response to flooding share many of the same elements involved in increasing stem elongation in response to shade, though the sensors used to perceive the environmental signal differ (Voesenek *et al.*, 2004; Ackerly & Sultan, 2006). Conversely, at least one of the genes that senses mechanical perturbation and initiates the thigmomorphogenic response in plants, is also known to be stimulated by darkness and both heat and cold shock (Iliev *et al.*, 2002). Given that plants must respond to, and integrate, multiple environmental signals if they are to survive and successfully reproduce in nature, this intertwining of developmental pathways is perhaps to be expected. However, it has caused some researchers to question the extent to which plants may evolve to respond appropriately to independent environmental signals (Ackerly & Sultan, 2006). This is a particular concern with regard to a novel environmental signal such elevated CO_2. Present day populations may not contain the necessary variation to allow the evolution of an appropriate flowering response to elevated CO_2 without seriously disrupting response to photoperiod. Further, the fact that the same light sensors and signal transduction genes that CO_2 interacts with to affect flowering are also involved with a variety of other developmental processes, including germination of photoblastic seeds, the etiolation response in germinating seedlings, leaf morphology, branching patterns and stem elongation (Taiz & Zeiger, 2005), suggests this disruption may be much more widespread.

There is indeed evidence that elevated CO_2 disrupts a variety of other developmental processes in addition to flowering. Elevated CO_2 is known to affect the germination of some species, particularly those with small seeds (Ziska & Bunce, 1993). Since it is generally small seeds that are photoblastic, this suggests an interaction between CO_2 and the perception/transduction of light signals. The phytochrome and cryptochrome light sensors involved in detecting changes in photoperiod are also involved in sensing the presence or absence of light for germinating seedlings and so are involved in regulating hypocotyl elongation in the etiolation response (Taiz & Zeiger, 2005). Elevated CO_2 has been shown to affect hypocotyl elongation in several different species, the magnitude and pattern of the effect varying markedly with the presence or absence of light (Zebian & Reekie, 1998). Further, brief periods of exposure to low intensity light elicit the same response to elevated CO_2 as prolonged exposure to high-intensity light indicating that this is not a simple growth response. In *Petunia hybrida*, elevated CO_2 increases height and number of branches in short, but not long days (Reekie *et al.*, 1997). *PHYB*, by detecting the ratio of red to far-red light, is the primary light sensor involved in detecting differences in light quality caused by competitors and is therefore critical to the shade avoidance response (Smith, 1995). Changes in the red : far-red ratio have a marked impact on the response of the shade intolerant herb *Sinapis alba* to elevated CO_2 even when total photosynthetic photon flux is held constant (Cowan & Reekie, 2008).

The studies cited illustrate that CO_2 interacts strongly with the presence or absence of light, photoperiod, and light quality to affect a variety of traits known to be under the control of light sensors and/or components of the signal transduction pathway. To date, these effects have been largely examined in isolation and without reference to underlying mechanisms, and it has been difficult to draw generalizations regarding the direction and magnitude of CO_2 effects (Springer & Ward, 2007). Given that we now know that CO_2 interacts with both the sensing and subsequent transduction of light signals, we argue that to accurately predict the effects of elevated CO_2

on plant development, it will be necessary to take a more mechanistic approach that explicitly considers the underlying developmental processes. Such an approach has the potential to not only help us understand the effects of elevated CO_2 on flowering, but also on many other aspects of development, and will enhance our ability to predict the often complex and varied effects of elevated CO_2 on plant growth.

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