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REGARDLESS OF WHETHER RISING ATMOSPHERIC CARBON DIOXIDE LEVELS INCREASE AIR TEMPERATURE, FLOWERING PHENOLOGY WILL BE AFFECTED

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Many species, particularly those that flower in spring, are flowering 3–5 d earlier than they did in the first half of the twentieth century. This has been interpreted as a consequence of rising temperatures. However, correlated with this rise in temperature is an increase in atmospheric CO_2, which may also modify phenology. The effects of elevated CO_2 and temperature on time of flowering were examined in 22 Asteraceae species. Plants were exposed to three treatments, ambient CO_2 with ambient temperature, elevated CO_2 (700 μL L^{-1}) with ambient temperature, and elevated CO_2 with elevated temperature (ambient + 1.5°C). On average, elevated CO_2 by itself advanced flowering by 4 d; increasing temperature as well as CO_2 advanced flowering by an additional 3 d. There were marked differences among species in response to treatment. In elevated-CO_2 conditions, this was related to photoperiodic response: CO_2 was more likely to hasten phenology in long- than in short-day species. Early- and late-flowering species did not differ in response to elevated CO_2, but the combined effect of elevated CO_2 and temperature hastened flowering more in early- than in late-flowering species. The direct effect of CO_2 on phenology may be as important as its indirect effect through climate change.

Keywords: Asteraceae, time of flowering, photoperiodic response, long-day plants, short-day plants.

Introduction

There have been marked changes in plant phenology over the past century (Abu-Asab et al. 2001; Fitter and Fitter 2002; Primack et al. 2004; Defila and Clot 2005; Hu et al. 2005; Menzel et al. 2006). In general, species are flowering earlier today than previously, but there are exceptions. For example, Abu-Asab et al. (2001) examined changes in time of flowering in 100 species over three decades in the Washington, DC, area. Of these, 89 flowered earlier, and 11 species flowered later. Such differences appear to be at least partially associated with flowering season. Plants that flower in spring show a greater tendency to earlier flowering than those that flower in the latter half of the growing season (Bradley et al. 1999; Fitter and Fitter 2002; Dunne et al. 2003).

These changes in phenology have been interpreted as a consequence of the increase in temperature that has been observed over this time. Temperature, through its effects on the rate of enzyme-mediated reactions, has profound effects on the rate of development (Minorsky 2002), and cumulative annual temperature sum is a good predictor of flowering time in many species (Went 1953). Experimental warming also shows that increased temperature has a marked effect on flowering time in many, but not all, species (Price and Wasser 1998; Cleland et al. 2006; Lambrecht et al. 2007). Further, at least one experimental study has shown that elevated temperature has differential effects on flowering in early- and late-flowering species, hastening it in tallgrass prairie species that flower before the seasonal peak in temperature and delaying it in species that flower after the peak (Sherry et al. 2007). Because development is more likely to be limited by temperature early in the growing season when ambient temperatures are relatively low, whereas temperature increases in the latter part of the season may exceed the optimal range, differential effects of temperature increases for early- versus late-flowering species are to be expected. Although the evidence that the changes in phenology in the recent past are a function of temperature change is strong, it is important to remember that changes in a number of other environmental factors, including atmospheric CO_2, nitrogen deposition, and precipitation, correlate with the rise in temperature and also affect phenology (Cleland et al. 2006).

Although it is not as well studied as temperature effects, the concentration of atmospheric CO_2 may also directly affect time of flowering, even in the absence of temperature change. Elevated CO_2 hastens phenology in some species, delays it in others, and appears to have no effect in still others (St. Omer and Horvath 1983; Garbutt and Bazzaz 1984; Reekie and Bazzaz 1991). The mechanistic basics for these direct effects of CO_2 on phenology and development are still unclear. Through its positive effects on photosynthesis, elevated CO_2 generally enhances growth rates (Norby and O'Neill 1989; Poorter and Navas 2003), which may affect time of flowering by decreasing the time required to reach the minimum critical size for floral induction (Reekie and Bazzaz 1991; He and Bazzaz 2003). However, in at least some species, the effect of CO_2 on phenology is not closely related to effects on growth (Reekie and Bazzaz 1991). Purohit and Tregunna (1974) and Hicklenton and Jolliffe (1980) have shown that very concentrated CO_2 (from 30 to 150 times current ambient) can alter a plant's photoperiodic response in that long-day plants become short-day plants and vice versa. More moderate CO_2 enrichment, reflective of predicted atmospheric changes, does

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not reverse the photoperiodic response, but flowering in long-day plants is hastened by elevated CO_2, whereas it is either delayed or unaffected in short-day plants (Reekie et al. 1994). Because long-day species tend to flower in the spring and early summer and short-day species flower in late summer and autumn, the effect of elevated CO_2 is likely to reinforce the differential effects of temperature.

Although these studies, on relatively few species, have demonstrated that elevated CO_2 can directly affect phenology and that this effect can vary depending on the daylength response, the generality and ecological significance are not clear. With the exception of one study with *Achillea millifolium*, previous studies have been done under constant photoperiod and temperature. In *A. millifolium*, growth at elevated CO_2 under a noninductive photoperiod has been shown to modify response to a subsequent inductive photoperiod (Reekie et al. 1994). Further, under natural conditions, where temperature and photoperiod vary seasonally, any interaction between CO_2 and photoperiod on flowering may be overwhelmed by the effect of temperature on development.

This study addressed these concerns by examining the effects of elevated CO_2 by itself and the combined effect of elevated CO_2 and temperature on the flowering phenology of 22 species in the family Asteraceae grown under natural, seasonally varying temperature and daylength. These two global change scenarios were chosen because the rise in CO_2 concentration is global in distribution, whereas its effect on temperature varies widely among regions. As a consequence, many regions may experience an increase in CO_2 concentration with little or no change in temperature (Menzel et al. 2006). The chosen species flower over the entire growing season, include short-day, long-day, and day-neutral species, and allow us to address several questions. How important is the effect of elevated CO_2 on flowering phenology relative to the combined effects of elevated CO_2 and temperature? Do long- and short-day species still differ in their response to elevated CO_2 when temperature and photoperiod vary seasonally? Do early- and late-flowering plants differ in their response to the combined effect of elevated temperature and CO_2, and if so, to what extent is this a function of their response to CO_2 versus temperature?

Material and Methods

Plants

The species used in this experiment and specific information on mode of propagation, date of planting, life history, and photoperiodic response are listed in table 1. These particular species were chosen on the basis of our knowledge of their photoperiodic response and to maximize the range in seasonal flowering time. This list includes both native and nonnative species; all species, however, commonly grow within Nova Scotia, Canada, in either natural or cultivated ecosystems. All are insect pollinated: 4 are annuals and 18 are perennials, and 11 are classified as long-day, 9 as short-day, and 2 as day-neutral plants. For two of the species, *Tanacetum vulgare* and *Solidago uliginosa*, the photoperiodic response was unknown, and we assumed that they had the same response as others in the genus that flowered at the same time of year. To determine whether this assumption may have influenced our conclusions, we analyzed the data both with and without these two species (see "Results"). Species that were propagated vegetatively were grown from single ramet rhizome or stem cuttings from plants grown in a uniform experimental garden the previous year and overwintered naturally. All species were planted in May (30 individuals per species), but because of different growth patterns and the availability of seed, the different species were not all planted on the same day. However, all individuals of a given species were planted on the same day regardless of CO_2 or temperature treatment.

Growth Conditions

Five individual glasshouse compartments in the K. C. Irving Environmental Science Centre (Acadia University, Wolfville, Nova Scotia) were used for this study. The compartments (3.6 m × 4.3 m) were independently cooled by geothermal heat exchange, providing a high degree of control over temperature. Two compartments were maintained at ambient CO_2 (370 μL L^{-1}) and temperature (ACAT), two were maintained at elevated CO_2 (700 μL L^{-1}) and ambient temperature (ECAT), and one was maintained at elevated CO_2 (700 μL L^{-1}) and elevated temperature (ambient temperature + 1.5°C; ECET). The elevated CO_2 concentration is that predicted for the middle of the next century, assuming that the current rate of increase remains constant (Intergovernmental Panel on Climate Change 2007). We chose a 1.5°C increase in temperature because this is the predicted increase for our research site (Nova Scotia; Canadian Institute for Climate Studies 2005). External air temperature was continuously monitored 1.5 m above the ground surface with a thermistor installed in a standard Stevenson screen located in the Harriet Irving Botanical Gardens (Acadia University, Wolfville, Nova Scotia). External temperature was used as the set point for the glasshouse control system (Argus Controls, White Rock, British Columbia) in the ambient-temperature compartments, while the set point in the elevated-temperature treatment was 1.5°C above it. Carbon dioxide in each of the high-CO_2 compartments was monitored by a single infrared gas analyzer (Vaisala GMM1 1A) interfaced with the Argus glasshouse control system. The infrared analyzer sampled air from each of the compartments once every 12 min. If the measured CO_2 was below the required set point, pure CO_2 (Praxair, Mississauga, Ontario) was injected. Relative humidity in all compartments was maintained at a minimum of 65% by high-pressure fogging when it was below this value. Plants received natural light and photoperiod during the experiment, which was done between May 5 and October 30, 2004 (days of the year 125–303). Mean daily temperatures in the ambient treatment varied between 12° and 26.5°C over the experiment (fig. 1) and averaged 17.1° and 18.6°C in the ambient and elevated temperature treatments, respectively, over the entire experimental period. Photoperiod varied between 11.3 and 16.9 h (fig. 1).

Ten individuals per species-treatment combination were planted (i.e., 30 individuals per species). However, because of mortality during establishment or failure to flower, the actual number of plants per species/treatment combination varied between 5 and 10 (mean = 8.4). All individuals were grown in 2.83-L pots (Classic 300, Nursery Supplies, Fairless Hills, PA) with

Table 1

Method of Propagation (MP), Life History (LH), Date of Planting (DP), and Photoperiodic Response (PP) of Species Used in This Study

Species	MP[a]	LH[b]	DP[c]	PP[d]	Source[e]
Achillea millefolium	V	P	126	LD	Dole and Wilkins 2005
Aster cordifolius	V	P	127	SD	Dole and Wilkins 2005
Aster lateriflorus	V	P	128	SD	Dole and Wilkins 2005
Aster macrophyllus	V	P	126	SD	Dole and Wilkins 2005
Aster novi-belgii	V	P	126	SD	Dole and Wilkins 2005
Calendula officinalis	S	A	138	LD	Dole and Wilkins 2005
Callistephus chinensis	S	A	139	LD	Dole and Wilkins 2005
Centaurea nigra	V	P	127	LD	Dole and Wilkins 2005
Coreopsis grandiflora	S	P	139	LD	Dole and Wilkins 2005
Cosmos bipinnatus	S	A	138	SD	Dole and Wilkins 2005
Euthamia gramnifolia	V	P	126	SD	Dole and Wilkins 2005
Gazinia rigens	S	P	138	LD	Dole and Wilkins 2005
Helianthus annuus cv. Sunspot	S	A	139	SD	Dole and Wilkins 2005
Helianthus tuberosus	V	P	141	DN	Salisbury and Ross 1978
Heliopsis helianthoides	S	P	138	LD	Pyle 2002
Hieracium floribundum	V	P	127	LD	Halevy 1985
Rudbeckia hirta	S	P	139	LD	Dole and Wilkins 2005
Solidago canadensis	V	P	126	SD	Dole and Wilkins 2005
Solidago uliginosa	V	P	127	SD	Hurlbert 1970
Tanacetum parthenium	S	P	138	LD	Dole and Wilkins 2005
Tanacetum vulgare	V	P	128	LD	Dole and Wilkins 2005
Taraxacum officinale	S	P	149	DN	Stewart-Wade et al. 2002

[a] V = vegetative, S = seed. Plants for species propagated vegetatively and seeds for *T. officinale* were collected from natural populations in Nova Scotia, Canada. Seed for the remaining species was obtained from the following commercial suppliers: Unwins-Histon, Cambridge, England (*C. bipinnatus*, *H. helianthoides*, *T. parthenium*); McKenzie, Brandon, Manitoba (*C. chinensis*); OSC, Waterloo, Ontario (*C. grandiflora*, *H. annuus*, *R. hirta*); Suttons Seeds, Torbay, England (*C. officinalis*, *G. rigens*).

[b] P = perennial, A = annual.

[c] Day of the year.

[d] LD = long day, SD = short day, DN = day neutral.

[e] Cited reference is for the specific species named with the following exceptions. The reference for *Aster* species is for the genus as a whole. The reference for *H. annuus* is for that specific cultivar. No specific information was available for *S. uliginosa* or *T. vulgare*, and the references are for other members of the same genus with similar flowering times.

ASB Greenworld Original Grower Mix (Pointe Sapin, New Brunswick) as a growing medium. Plants were watered as required and fertilized with 18-9-27 hydroponic fertilizer (Plant Products, Brampton, Ontario) on day 184. Pots were placed on rolling carts, and plants with their respective treatments were rotated among the compartments on a monthly basis to avoid confounding potential differences among compartments with particular treatments.

Data Collection and Analysis

Plants were observed daily, and date of flowering of individuals was recorded as the first day ray florets on the first capitulum were completely open, after which all aboveground parts were harvested. Shoot dry weight was determined after drying to constant mass at 55°C.

All statistical analyses were performed with SAS (Windows ver. 9.1, 2002–2003; SAS Institute, Cary, NC). A split-plot 3×22 factorial ANOVA was used to examine differences among the three environmental treatments and 22 species in time of flowering and size at flowering. There were five main plots in this analysis, represented by the groups of plants that were rotated among glasshouse compartments. Each group contained multiple individuals of all 22 species. Because the individual glasshouse compartments represented the experimental units for the CO_2 and temperature treatments, the significance levels of these effects were determined by comparison with the main plot error term. The significance of differences among species and the interaction between species and environmental treatment were tested via the residual error term (i.e., the error term for individual plants). It should be noted, however, that there was no significant difference between the main plot error term and the residual error term for either of the dependent variables in this experiment (table 2). The plant size data were transformed before analysis by taking the natural logarithm of the plant mass to improve normality.

The effect of elevated CO_2 on flowering was determined by comparing the elevated CO_2 and ambient temperature treatment to the ambient CO_2 and temperature treatment, i.e., the difference between the ACAT and ECAT treatments. The effect of elevated temperature was determined by taking the difference between the ECET and ECAT treatments, and the combined effect of elevated CO_2 and temperature was assessed as the difference between the ACAT and ECET treatments. Our

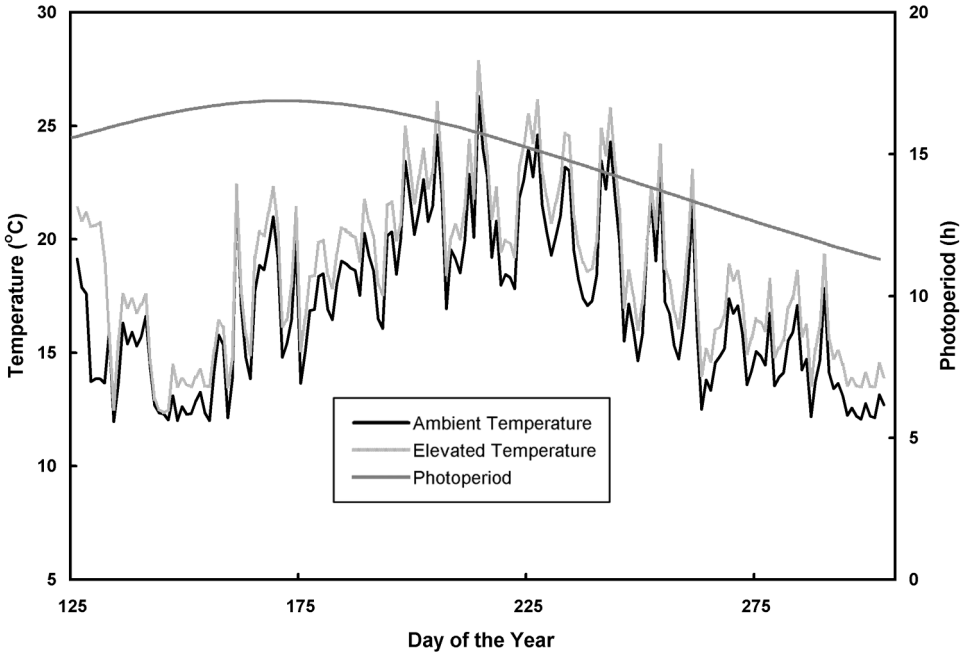

Fig. 1 Mean daily temperature for the ambient- and elevated-temperature treatments and photoperiod for May 5–October 30, 2004 (days of the year 125–303), in Wolfville, Nova Scotia, Canada.

estimates of the "CO_2 effect" and the "temperature effect" assume that the effects of CO_2 and temperature on flowering are additive (i.e., no $CO_2 \times$ temperature interaction). If such an interaction exists, our estimate of the CO_2 effect is applicable only to ambient temperature conditions, and our estimate of the temperature effect is applicable only to plants grown at elevated CO_2. A priori paired comparisons were made to compare the effect of CO_2 and temperature on long- versus short-day and on early- versus late-blooming species. For the latter analysis, species were assigned to categories on the basis of their flowering time under ambient conditions. The early-blooming group was *Hieracium floribundum*, *Centaurea nigra*, *Calendula officinalis*, *Gazinia rigens*, *Solidago canadensis*, *Achillea millefolium*, *Rudbeckia hirta*, *S. uliginosa*, *Aster macrophyllus*, *Tanacetum parthenium*, and *T. vulgare*. The late-blooming group was *Callistephus chinensis*, *Cosmos bipinnatus*, *Euthamia gramnifolia*, *Heliopsis helianthoides*, *Coreopsis grandiflora*, *Helianthus annuus*, *Aster lateriflorus*, *Aster cordifolius*, *Aster novi-belgii*, *Helianthus tuberosus*, and *Taraxacum officinale*.

Table 2

Results of a Split-Plot ANOVA Examining the Effect of Treatment and Species on Date and Size at Flowering

Source	df	Mean square	*F*	*P*
Date of flowering:				
Treatment	2	3045	34.67	.0280
Main plot error	2	88	1.46	.2333
Species	21	12,472	207.19	<.0001
Species × treatment	42	101	1.69	.0057
Residual error	486	60		
Size at flowering:				
Treatment	2	13.121	35.80	.0272
Main plot error	2	.366	2.21	.1105
Species	21	13.124	79.24	<.0001
Species × treatment	42	.323	1.95	.0005
Residual error	486	.166		

Note. The effects of CO_2 and temperature treatments were tested using the main plot error term, while the effects of species and the interaction between species and treatment were tested using the residual error term. The main plot error term was also tested against the residual error term to determine whether there were significant differences among compartments aside from treatment effects. Probability values of <0.05 are underlined.

Results

The species differed markedly in both the time of flowering and the size at which they flowered (table 2). There was an almost-3-mo difference in the mean date of flowering between the first- and last-flowering species. Under ambient conditions, the earliest was *Hieracium floribundum*, on day 181, and the latest was *Helianthus tuberosus*, on day 263 (table 3). Differences among species in time of flowering were associated with size, with early species flowering at a smaller size than late species. For example, *H. floribundum* was the earliest and smallest, and *H. tuberosus* the latest and largest (table 3). In general, long-day species flowered earlier than short-day species (fig. 2). The only two day-neutral species in the study flowered relatively late.

There were also differences among treatments in time and size at flowering (table 2). In general, plants flowered first in the ECET treatment, then in the ECAT, and finally in the ACAT (table 3). Averaged across all species, the mean flowering times in the ECET, ECAT, and ACAT treatments were days 212, 215, and 219 (standard error 0.6), respectively. Paired comparisons (ACAT-ECAT) indicated that elevated CO_2 significantly accelerated flowering by an average of 4 d ($P < 0.0001$). Similarly, elevated temperature (comparison ECAT-ECET) accelerated flowering by 3 d ($P = 0.0007$). Mean size at flowering was 14.01, 15.80, and 9.30 g for ECET, ECAT, and ACAT, respectively (back-transformed values). Elevated CO_2 increased size at flowering ($P < 0.0001$), while elevated temperature decreased it ($P = 0.0127$). Although both effects were significant at the 0.05 level, the increase in plant size at elevated CO_2 (ECAT-ACAT = 6.50 g) was much greater than the decrease in plant size at elevated temperature (ECET-ECAT = −1.79 g).

Despite these general patterns, there were differences among species in their response to CO_2 and temperature, as indicated by the significant species × treatment interactions for both time and size at flowering (table 2). Although elevated CO_2 generally advanced the time of flowering, the effects for individual species varied from nonsignificant for *Aster lateriflorus*, *Aster cordifolius*, *Centarea nigra*, and *Solidago uliginosa* to a 20-d advancement for *H. floribundum* (table 3). Similarly, there was considerable difference in the response of flowering to temperature, from a 5-d delay for *S. uliginosa* to a 13-d advancement for *Gazinia rigens*. On average, elevated CO_2 increased size at flowering, but the magnitude of this increase varied among species: For 6 of the 22 species—*Achillea millefolium*, *G. rigens*, *Helianthus annuus*, *H. floribundum*, *H. tuberosus*, and *Taraxacum officinale*—there was no significant difference between the ACAT and ECAT treatments. Elevated temperature had much less effect on size at flowering than did CO_2, so the difference between the ECET and ECAT was significant for only 3 of the 22 species: *G. rigens*, *S. uliginosa*, and *Tanacetum parthenium*.

Paired comparisons indicated that much of the variation among species in the response of flowering time to CO_2 treatment was associated with a significant difference between long- and short-day species (ACAT-ECAT; $P = 0.0068$). There was no significant difference between the ambient- and elevated-CO_2 treatments for short-day species, but long-day species flowered 6.4 d earlier, on average (fig. 2). Both day-neutral

Table 3

Mean (±SE) Date and Size at Flowering in 22 Asteraceae Species

	Date of flowering (day of year)			Size at flowering (ln transformed, g)		
Species	ACAT	ECAT	ECET	ACAT	ECAT	ECET
Achillea millefolium	207 ± 3.5	192 ± 2.5	190 ± 2.5	2.16 ± .18	2.36 ± .13	2.32 ± .13
Aster cordifolius	248 ± 2.6	249 ± 2.6	252 ± 2.7	2.96 ± .14	3.49 ± .13	3.39 ± .14
Aster lateriflorus	242 ± 2.6	244 ± 2.5	241 ± 2.5	2.66 ± .14	3.51 ± .14	3.24 ± .13
Aster macrophyllus	210 ± 2.5	206 ± 2.6	198 ± 2.5	2.51 ± .13	3.01 ± .14	3.13 ± .13
Aster novi-belgii	262 ± 2.5	258 ± 2.5	258 ± 2.6	2.54 ± .13	2.95 ± .13	2.93 ± .14
Calendula officinalis	191 ± 2.5	187 ± 2.5	186 ± 2.5	1.88 ± .13	2.30 ± .13	2.22 ± .13
Callistephus chinensis	213 ± 2.5	208 ± 2.5	207 ± 2.5	1.36 ± .13	2.24 ± .13	2.19 ± .13
Centaurea nigra	190 ± 3.5	192 ± 3.2	187 ± 2.9	1.41 ± .13	2.06 ± .17	1.70 ± .15
Coreopsis grandiflora	221 ± 3.2	211 ± 2.7	208 ± 2.5	1.03 ± .17	1.95 ± .14	1.73 ± .13
Cosmos bipinnatus	216 ± 2.5	212 ± 2.5	206 ± 2.6	3.07 ± .14	3.56 ± .14	3.23 ± .14
Euthamia gramnifolia	218 ± 2.5	214 ± 2.5	215 ± 2.5	2.53 ± .13	3.34 ± .13	3.36 ± .13
Gazinia rigens	204 ± 2.7	203 ± 2.7	190 ± 3.5	1.51 ± .14	1.40 ± .15	.92 ± .18
Helianthus annuus	227 ± 2.7	223 ± 2.6	218 ± 2.5	3.24 ± .14	3.47 ± .14	3.56 ± .13
Helianthus tuberosus	263 ± 2.9	256 ± 2.9	262 ± 3.2	4.08 ± .15	4.31 ± .15	4.35 ± .17
Heliopsis helianthoides	218 ± 2.5	212 ± 2.5	207 ± 2.5	2.49 ± .13	3.40 ± .13	3.24 ± .13
Hieracium floribundum	181 ± 5.5	161 ± 5.5	158 ± 4.5	.14 ± .29	.42 ± .29	.68 ± .24
Rudbeckia hirta	207 ± 2.5	206 ± 2.5	202 ± 2.5	2.93 ± .13	3.36 ± .13	3.08 ± .13
Solidago canadensis	206 ± 2.6	206 ± 2.6	204 ± 2.5	2.12 ± .14	2.86 ± .14	2.90 ± .13
Solidago uliginosa	209 ± 2.6	210 ± 3.5	215 ± 2.7	2.44 ± .14	3.23 ± .17	2.64 ± .14
Tanacetum parthenium	211 ± 2.5	207 ± 2.5	202 ± 2.5	1.67 ± .13	2.04 ± .13	1.58 ± .13
Tanacetum vulgare	211 ± 2.7	203 ± 2.5	194 ± 2.5	2.17 ± .14	2.95 ± .13	2.92 ± .13
Taraxacum officinale	267 ± 4.5	262 ± 3.2	260 ± 4.5	2.11 ± .20	2.45 ± .17	2.87 ± .24

Note. *n* varied between 5 and 10, depending on species/treatment combination. Plants were grown at ambient CO_2 and temperature (ACAT), elevated CO_2 and ambient temperature (ECAT), or elevated CO_2 and elevated temperature (ECET).

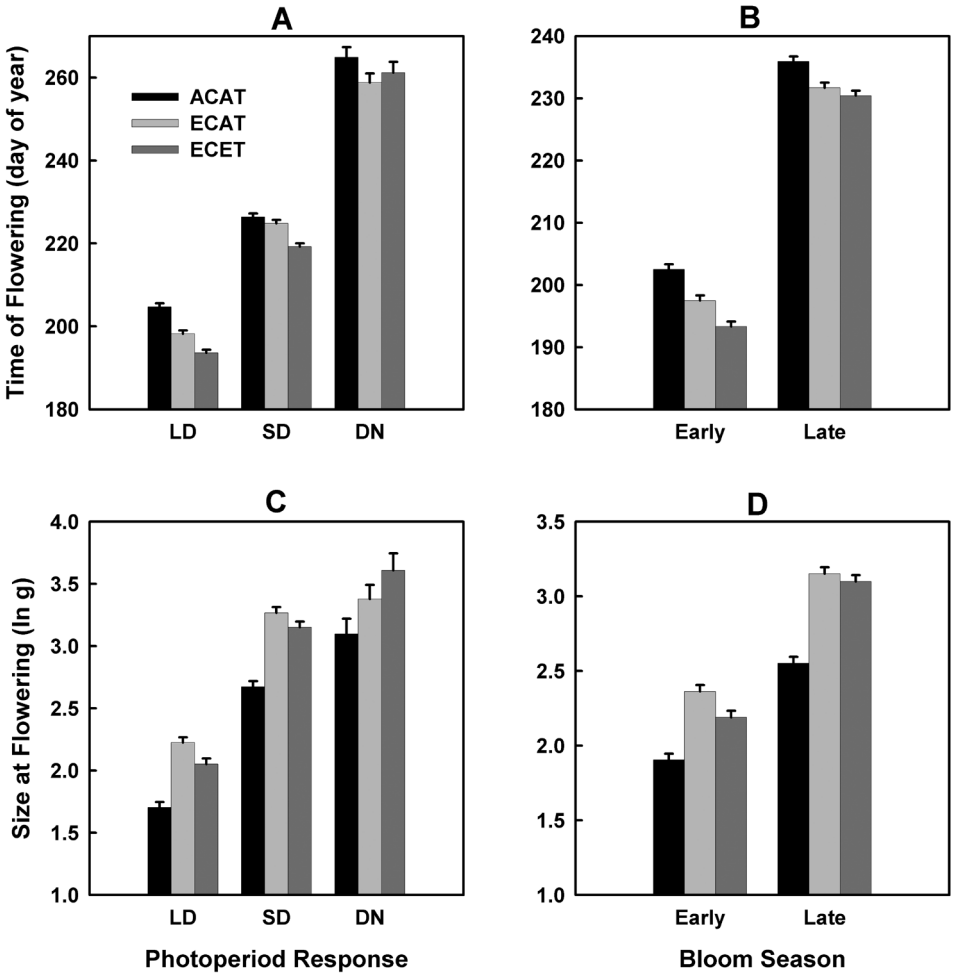

Fig. 2 Time of flowering (*A*, *B*) and size at flowering (*C*, *D*) in long-day (*LD*), short-day (*SD*), and day-neutral (*DN*) species (*A*, *C*) or in early versus late-blooming species (*B*, *D*). Plants were grown at ambient CO_2 and temperature (*ACAT*), elevated CO_2 and ambient temperature (*ECAT*), or elevated CO_2 and elevated temperature (*ECET*).

species, *H. tuberosus* and *T. officinale*, were similar to the long-day species in their response to elevated CO_2, flowering 6.2 d earlier at ambient temperature, on average (fig. 2). Although daylength response had an effect on the response of flowering to elevated CO_2, there was no significant difference between long- and short-day species in the effect of temperature (ECAT-ECET) on time of flowering ($P = 0.1264$). On average, long-day species flowered 4.6 d earlier at elevated temperature, compared with 2.6 d earlier for short-day species (fig. 2). Temperature had no significant effect on time of flowering in the day-neutral species (fig. 2). These conclusions concerning the differences between long- and short-day species were not affected by including the two species *S. uliginosa* and *Tanacetum vulgare* for which we had to infer photoperiodic response from close relatives. Deleting these two species from the analysis modified the means slightly but did not change any of the conclusions regarding which differences were significant.

The effect of CO_2 on size at flowering (ACAT-ECAT) did not differ between long- and short-day plants ($P = 0.4498$): both flowered at a larger size in elevated CO_2. Day-neutral species behaved similarly, but there was no significant difference between ACAT and ECAT plants (fig. 2). The effect of elevated temperature on size at flowering was the same in both long- and short-day species ($P = 0.5167$): both flowered at a smaller size. The slightly larger size of day-neutral species at elevated temperature was not significant (fig. 2).

Compared with the effect of photoperiodic response, there was relatively little difference between early- and late-blooming species in the effect of CO_2 and temperature treatments on time of flowering (fig. 2). There was no significant difference between these two groups in the effect of either CO_2 (ACAT-ECAT, $P = 0.6334$) or temperature (ECAT-ECET, $P = 0.0784$). However, the combined effect of elevated CO_2 and temperature (ACAT-ECET) was significantly different between early- and late-blooming species ($P = 0.0283$). Relative to ambient conditions, the early group flowered 3.9 d sooner under elevated CO_2 and temperature than did the late group. The difference between early- and late-blooming species in size at flowering was similar to the difference in time of flowering: the separate effects of CO_2 ($P = 0.1336$) and temperature ($P = 0.1768$) were not significant, whereas the combined effect of

CO_2 and temperature (ECET-ACAT) was ($P = 0.0048$); size at flowering was larger in late- than early-blooming species.

Discussion

Increasing atmospheric CO_2 to 700 μL L^{-1} without any increase in temperature hastened flowering by an average of 4 d, while increasing temperature by 1.5°C in addition to increasing CO_2 hastened flowering by an additional 3 d. These results suggest that future changes in phenology with rising CO_2 levels may be as closely associated with the direct effects of CO_2 as they are with indirect effects through global warming. This finding also has implications for understanding changes in phenology that have been observed in the recent past (Fitter and Fitter 2002; Parmesan and Yohe 2003; Root et al. 2003; Menzel et al. 2006). Although most studies of changes in flowering over several decades have attributed them to increased temperature, other correlated changes, such as the rise in atmospheric CO_2, may have been just as important. Menzel et al. (2006) addressed this issue by correlating changes in phenology between different regions with warming and found a strong correlation between the observed change in spring phenology and the extent of warming across 19 European countries, indicating that temperature change is clearly an important driver of these phenological changes. However, less than 50% of the variation was accounted for, suggesting that temperature may not be the only factor involved. The results of our study, as well as those of Cleland et al.'s (2006), suggest that increasing CO_2 may be just as important as the effect of temperature in explaining past phenological shifts.

Given that the increase in atmospheric CO_2 and warming are correlated on a global scale, it might be argued that separating the effects of these two factors is not necessary for accurately predicting the effects of global change on phenology. However, these two global change factors are not necessarily well correlated regionally. Increases in atmospheric CO_2 will be more or less evenly distributed across the surface of the earth because of rapid mixing within the atmosphere, but increases in temperature can vary dramatically from region to region, as illustrated by recent temperature changes (Menzel et al. 2006). Accurately predicting how flowering phenology will change in a specific region will require that we decouple the effects of elevated CO_2 and temperature on flowering. Furthermore, our and previous studies clearly show that the effects of both temperature and CO_2 (Reekie and Bazzaz 1991; Reekie et al. 1997; Abu-Asab et al. 2001; Fitter and Fitter 2002; Cleland et al. 2006; Sherry et al. 2007) vary widely among species. Understanding the mechanism by which these factors influence phenology will be necessary if we are to predict how individual species will respond. For example, the effect of CO_2 (ECAT-ACAT), but not that of temperature (ECET-ECAT), varied with the photoperiodic response of the species (fig. 2). This knowledge allows us to start to make predictions regarding how particular species will respond to global change that would not be possible if the effects of CO_2 were not separated from those of temperature. However, given the large differences in the response of species to both CO_2 and temperature (table 3), it is clear that further knowledge of the mechanisms responsible for these changes in phenology is required.

One very simple mechanism by which both elevated CO_2 and temperature may influence time of flowering is by modifying growth rate. Many plants must reach a minimum critical size before flowering can be induced by an external signal such as daylength (Rathcke and Lacey 1985). Therefore, increases in the rate of growth allow plants to reach the minimum size required for flowering earlier. If this effect were responsible for the observed differences in time of flowering, plants in the different treatments would flower at the same size regardless of when they flowered. In general, elevated CO_2 increased and elevated temperature decreased size at flowering (table 3). However, there were significant differences among species in the effects of both CO_2 and temperature on size at flowering, so it is necessary to examine this hypothesis for individual species. Elevated CO_2 significantly increased size at flowering in 16 of the 22 species examined and had no effect on the others (ACAT-ECAT comparison). Elevated temperature, however, significantly decreased size at flowering in only 3 of the 22 species; in the other 19 species, there were no significant differences between the ECAT and ECET plants. These results suggest that the effect of temperature on time of flowering can be attributed to its effect on growth in most species, while the effect of CO_2 on time of flowering is independent of size in most, but not all, species.

Given that the effect of CO_2 on time of flowering is largely independent of its effect on size, an alternative explanation for its effect on flowering is required. In addition to its effect on air temperature through climate change, elevated CO_2 also increases leaf temperature through reductions in stomatal conductance and, consequently, evaporative cooling (Lawlor and Mitchell 1991). The magnitude of this effect varies with environmental conditions such as humidity, air temperature, air movement, and irradiance but ranges from no difference at night to a maximum of 1°C or 2°C at midday under the appropriate environmental conditions (Triggs et al. 2004). This effect could help explain why elevated CO_2 hastens flowering. However, it is unlikely to be the primary explanation for the effect of CO_2 because it cannot explain why the response differed between long- and short-day species, as the effect of temperature did not differ with photoperiodic response (fig. 2).

In general, elevated CO_2 hastened flowering in the long-day species and had no significant effect in the short-day species. This supports an earlier suggestion based on a much smaller number of species grown under constant photoperiod and temperatures that CO_2 interacts with the photoperiodic response (Reekie et al. 1994). It should be noted, however, that in our study there were exceptions to this general pattern. *Centaurea nigra*, for example, is a long-day species unaffected by CO_2. Any explanation of how CO_2 affects the photoperiodic response should be able to account for these exceptions. Song (2007) examined the effect of CO_2 on time and size at flowering under both short and long photoperiods in flowering mutants of the facultative long-day species *Arabidopsis thaliana*. It was concluded that elevated CO_2 has both negative and positive effects on size at flowering because of interactions with several different components of the flowering pathway; some of these occur only under short days, others occur only under long days, and others are independent of photoperiod. In addition to these developmental effects, elevated CO_2 had a positive effect on time of flowering through its effect on growth.

The net effect of elevated CO_2 on time of flowering in *Arabidopsis* is therefore the sum of these opposing effects. Because the relative strengths of these effects vary depending on photoperiod and any other environmental factor that influences the effect of CO_2 on growth, the observed effect of CO_2 on time of flowering in *Arabidopsis* varies depending on environment. Similar mechanisms, if they are responsible for the effect of elevated CO_2 on time of flowering in the species examined in our study, would explain the exceptions to the general pattern of early flowering in long-day species. The species will differ in the extent to which elevated CO_2 hastens flowering and indeed in whether flowering is hastened because they flower at different times in the season and therefore differ in the length of exposure to inductive and noninductive photoperiods or because they differ in the effect of elevated CO_2 on growth. Numerous studies have documented that species often differ widely in the extent to which elevated CO_2 stimulates growth (see literature reviewed in Poorter and Navas 2003).

Although the effect of elevated temperature on time of flowering seems to be closely related to its effect on growth in most of the species studied, there were three species in which flowering occurred at a smaller size, requiring an alternate hypothesis. *Solidago uliginosa* flowered significantly later at elevated temperature, *Gazinia rigens* flowered earlier, and *Tanacetum parthenium* was not significantly affected by temperature. Aside from temperature's direct effects on rate of growth and development, its best-known effect on flowering is the role that near-freezing temperatures play in vernalization (Rathcke and Lacey 1985). Given that all perennial species were vernalized under uniform conditions before the temperature treatments, which did not go below 12°C, it is unlikely that differences in vernalization can explain the results. Because elevated temperature either delayed or had no effect on time of flowering in *S. uliginosa* and *T. parthenium* but decreased size at flowering, it is clear that high temperature had a negative effect on growth in both of these species. Time of flowering in *T. parthenium* was unaffected, presumably because flowering was controlled by some factor other than size, possibly photoperiod. For *S. uliginosa*, however, elevated temperature slowed flowering, independent of its effect on growth. This effect, and the positive effect of temperature on time of flowering in *G. rigens*, may be related to interactions between temperature and the photoperiodic response. Both high and low temperatures have been shown to eliminate the requirement for a specific photoperiod in a number of species and, in a few cases, to replace a long-day requirement with a short-day requirement or vice versa (Halevy 1985).

It has been observed that early-flowering species are more responsive to environmental change than late-flowering species (Fitter and Fitter 2002). This pattern can be explained by low temperature, which may limit early-flowering species more than it does late-flowering ones. We hypothesized that the increase in CO_2 concentration associated with the rise in temperature may also be involved, as elevated CO_2 hastens flowering more in long- than in short-day plants, and long-day species tend to flower before short-day species. The results of this study support this hypothesis in that, separately, the effect of neither temperature (ECET-ECAT) nor CO_2 (ECAT-ACAT) varied between early- and late-flowering species, but the early-season species were more responsive to the combined effects of these two factors (ECET-ACAT). Given that there was a relatively large difference in the response of long- and short-day species to elevated CO_2, we were surprised that the difference between early- and late-flowering species was not greater. This can be attributed to the relatively weak association between photoperiodic response and flowering season. Although the long-day species did tend to flower before the short-day species (fig. 2), flowering overlapped between the two groups. Part of this overlap resulted from the fact that some of the perennial species in this study were propagated from seed (table 1). Perennial species propagated from seed flowered later in the season than would be considered normal for plants that had already reached reproductive maturity.

In conclusion, increasing CO_2 without any temperature increase advanced phenology by an average of 4 d, while increasing temperature as well as CO_2 advanced phenology by an additional 3 d in the 22 Asteraceae species we examined. The effect of elevated CO_2 was related to both its effects on growth and its interactions with the photoperiod. The effect of temperature was largely associated with its effects on growth, but for some species, interactions with the photoperiodic response might be involved. The combined effects of elevated CO_2 and temperature were greater for early-flowering than for late-flowering species because of both the greater effect of elevated CO_2 on long-day species and the greater effect of temperature increases early in the growing season. It is evident that if we are to predict accurately how flowering phenology of individual species and regions will shift in response to global change, we must consider the separate and combined effects of all the individual environmental factors associated with this change.

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