Effects of elevated CO_2 on time of flowering in four short-day and four long-day species

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This study was undertaken to determine if the effect of elevated CO_2 on flowering phenology is a function of the photoperiodic response of the species involved. Four long-day plants, *Achillea millefolium*, *Callistephus chinensis*, *Campanula isophylla*, and *Trachelium caeruleum*, and four short-day plants, *Dendranthema grandiflora*, *Kalanchoe blossfeldiana*, *Pharbitis nil*, and *Xanthium pensylvanicum*, were grown under inductive photoperiods (9 h for short day and 17 h for long day) at either 350 or 1000 μL/L CO_2. Time of visible flower bud formation, flower opening, and final plant biomass were assessed. Elevated CO_2 advanced flower opening in all four long-day species and delayed flowering in all four short-day species. In the long-day species, the effect of CO_2 was primarily on bud initiation; all four species formed buds earlier at high CO_2. Bud development, the difference in time between flower opening and bud initiation, was advanced in only one long-day species, *Callistephus chinensis*. Mixed results were obtained for the short-day species. Elevated CO_2 exerted no effects on bud initiation but delayed bud development in *Dendranthema* and *Kalanchoe*. In *Xanthium*, bud initiation rather than bud development was delayed. Data on bud initiation and development were not obtained for *Pharbitis*. The negative effect of CO_2 upon phenology in the short-day species was not associated with negative effects on growth. Elevated CO_2 increased plant size in both long-day and short-day species.

Key words: phenology, bud initiation, flower opening, size at flowering, photoperiodism.

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L'étude a été conduite afin de déterminer si l'effet d'une teneur élevée en CO_2 sur la phénologie florale, est fonction de la réaction photopériodique de l'espèce impliquée. Quatre plantes à jours longs, l'*Achillea millefolium*, le *Callistephus chinensis*, le *Campanula isophylla*, et le *Trachelium caeruleum*, et quatre plantes à jours courts, le *Dendranthema grandiflora*, le *Kalanchoe blossfeldiana*, le *Pharbitis nil* et le *Xanthium pensylvanicum*, ont été cultivées sous des photopériodes inductrices (9 h pour les plantes à jours courts et 17 h pour les plantes à jours longs) avec soit 350 ou soit 1000 μL/L de CO_2. Ils ont observé les moments d'apparition des bourgeons floraux et de l'ouverture des fleurs et ils ont mesuré la biomasse végétale finale. L'augmentation de la teneur en CO_2 accélère l'ouverture des fleurs chez les quatre espèces à jours longs et retarde la floraison chez les quatre espèces à jours courts. Chez les espèces à jours longs, l'effet du CO_2 se manifeste surtout sur l'initiation des bourgeons; les quatre espèces ont toutes formé leurs bourgeons plus tôt avec le CO_2 élevé. Le développement du bourgeon, i.e., l'espace de temps entre l'ouverture du bourgeon et son initiation, est accéléré seulement chez une des plantes à jours longs, soit le *Callistephus chinensis*. Chez les espèces à jours courts, les résultats sont mixtes. Le CO_2 élevé demeure sans effets sur l'initiation du bourgeon mais retarde le développement chez le *Dendranthema* et le *Kalanchoe*. Chez le *Xanthium*, c'est l'initiation du bourgeon plutôt que son développement qui est retardée. Aucune donnée n'a été obtenue sur l'initiation et le développement du bourgeon chez le *Pharbitis*. L'effet négatif du CO_2 sur la phénologie des espèces à jours courts n'est pas relié à ses effets négatifs sur la croissance. Le CO_2 élevé augmente la dimension des plantes chez les plantes à jours courts aussi bien que chez les plantes à jours longs.

Mots clés : phénologie, initiation du bourgeon, ouverture de la fleur, dimension à la floraison, photopériodisme.

[Traduit par la rédaction]

Introduction

Prolonged exposure to elevated CO_2 in the range of 500 to 1500 $\mu L \cdot L^{-1}$ has been shown to have various effects upon phenology (Wittwer and Robb 1964; Goldsberry 1965; Hesketh and Helmers 1975; Paez et al. 1980; Carter and Peterson 1983; Garbutt and Bazzaz 1984; Marc and Gifford 1984; Nelson 1985; Reekie and Bazzaz 1991). Depending upon the particular species and environmental conditions, these levels of CO_2 may advance, delay, or have no effect on phenology. It is generally believed that these effects are an indirect result of the effect of CO_2 on photosynthesis and growth. Elevated CO_2 generally enhances photosynthesis and growth, but the magnitude of these effects are highly variable depending upon species, environmental conditions, and length of exposure (Strain and Cure 1985; Bazzaz 1990). However, CO_2-induced effects on phenology are not always correlated with growth (Marc and Gifford 1984; Reekie and Bazzaz 1991).

Early studies showed that exposure to extremely high levels of CO_2 (ca. 10 000 – 50 000 $\mu L \cdot L^{-1}$) can influence the photoperiodic response of some species, causing short-day (SD) plants to flower under long-day (LD) conditions, delaying flowering in SD plants under SD conditions, and inducing flowering in LD plants under SD conditions (Purohit and Tregunna 1974; Hicklenton and Jolliffe 1980). These effects do not appear to be related to photosynthetic rate enhancement since exposure to elevated CO_2 in the dark has similar effects (Campbell 1957). The results of these studies raise the question

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of whether the effect of lower levels of CO_2 (500−1000 $\mu L \cdot L^{-1}$) upon phenology are in any way related to the photoperiodic response of these species.

Changes in phenology in response to elevated CO_2 are in the range of 1 to 2 weeks (Hesketh and Helmers 1975; Paez et al. 1980; Garbutt and Bazzaz 1984; Reekie and Bazzaz 1991). Changes of this magnitude are critical for species with maturation times that approach the length of the growing season. Small delays or advancements in flowering for these species can mean the difference between successful reproduction and failure. Further, changes in time of flowering may disrupt pollinator relationships (Garbutt and Bazzaz 1984) and could alter competitive hierarchies in plant communities (St. Omar and Horvath 1983; Reekie and Bazzaz 1991). Given the current rate of increase in the atmospheric CO_2 concentration (Bolin 1986), it is important to determine if there is indeed any interaction between prolonged exposure to low levels of elevated CO_2 (ca. 1000 $\mu L \cdot L^{-1}$) and the photoperiodic response in plants. Such information may allow us to better predict how phenology in particular species may be affected by the rising atmospheric CO_2 concentration.

In this study, four SD and four LD species were grown in inductive photoperiods at ambient (350 $\mu L \cdot L^{-1}$) and elevated (1000 $\mu L \cdot L^{-1}$) CO_2. The effects of CO_2 concentration on timing of bud formation, bud development, flower opening, and plant biomass were assessed. Our objective was to determine if there is any consistent difference in the response of SD and LD plants to elevated CO_2.

Materials and methods

Plant material

The following eight plant species were examined in this study: *Achillea millefolium* L., *Callistephus chinensis* Nees, *Campanula isophylla* Moretti, *Dendranthema grandiflora* Tzvelev, *Kalanchoe blossfeldiana* Poellniz, *Pharbitis nil* Chois., *Trachelium caeruleum* L., and *Xanthium pensylvanicum* Gandoger. *Dendranthema*, *Kalanchoe*, *Pharbitis*, and *Xanthium* are qualitative SD species with critical photoperiods of approximately 9, 12, 9, and 9 h, respectively (Evans 1969; Salisbury and Ross 1978). *Campanula* and *Trachelium* are qualitative LD plants with critical photoperiods of 14 and 16 h, respectively (Larson 1980). *Achillea* will flower under long-days but displays a great deal of genotypic variation in its critical photoperiod (Clausen et al. 1948). Preliminary trials with our seed material indicated that flowering does not occur in a 9-h photoperiod but does at 17 h. *Callistephus* is a quantitative LD plant that flowers regardless of photoperiod but flowers more rapidly at long photoperiods (Withrow and Benedict 1936). All four LD plants were grown from seed obtained from Ball Superior, Mississauga, Ont. Two of the SD species, *Dendranthema* and *Kalanchoe*, were obtained as rooted cuttings from Yoder Canada Ltd., Leamington, Ont., while the other two species were grown from seed. *Xanthium* seeds were obtained from Valley Seed Service, Fresno, Calif., and *Pharbitis* seeds were obtained from Carolina Biological Supply Company, Burlington, N.C.

Experimental design

Separate experiments were conducted for each species, and the individual experiments were run sequentially. Each experiment involved growing plants at either low (350 $\mu L \cdot L^{-1}$) or high (1000 $\mu L \cdot L^{-1}$) CO_2 in an inductive photoperiod of 9 h for SD plants and 17 h for LD plants. In the case of *Achillea*, an additional group of plants were grown from seed under a 9-h noninductive photoperiod. Half of these plants were assigned at random to the low CO_2 treatment and the other half to high CO_2. Plants were grown under these conditions for 22 days and then under a 17-h inductive photoperiod until the end of the experiment. The number of replicates per CO_2 treatment were 23, 19, 22, 20, 19, 22, 19, and 30 for *Achillea*, *Callistephus*, *Campanula*, *Dendranthema*, *Kalanchoe*, *Pharbitis*, *Trachelium*, and *Xanthium*, respectively.

Plant culture

All species except for *Pharbitis* were grown in 10-cm standard pots (375 mL) containing one part perlite to four parts of ASB grower mix (Greenworld Ltd., Waterloo, Ont.). *Pharbitis* was grown in 15-cm pots (1 L) containing turface (AIMCOR, Deerfield, Ill.). *Pharbitis* was leached with water daily, then fertilized with a mixture of 15:15:18 general purpose fertilizer (Plant Products Co. Ltd., Brampton, Ont.), calcium nitrate, and Sequestrene 330 Fe (CIBA-GEIGY, Mississauga, Ont.). This mixture contained 200 $\mu L \cdot L^{-1}$ nitrogen, 58 $\mu L \cdot L^{-1}$ calcium, and 100 $\mu L \cdot L^{-1}$ iron. The other seven species were watered as required and fertilized twice weekly with 15:15:18 general purpose fertilizer containing 200 $\mu L \cdot L^{-1}$ nitrogen.

The experiments were conducted in controlled-environment growth cabinets (model GR-36, Enconaire Systems Ltd., Winnipeg, Man.) maintained at either 350 or 1000 $\mu L \cdot L^{-1}$ CO_2. Carbon dioxide was injected into the growth cabinets from a liquid source and its concentration monitored by an infrared gas analyzer (Nova Model 421P, Hamilton, Ont.) by drawing a 45-s air sample from each growth cabinet sequentially on a 180-s cycle (Lander Control Systems Inc., Orangeville, Ont.). Plants were exposed to their assigned CO_2 level continuously throughout the entire experiment. All plants received a photosynthetic photon flux (PPF) of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400–700 nm) supplied by a mixture of cool-white fluorescent and incandescent lamps (62.5 and 37.5% input wattage, respectively) for 9 h. This was supplemented with an additional 8 h of 8.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF (60.3% input wattage fluorescent and 39.7% incandescent) of low light each day. In the case of LD plants, the 8 h of low light extension preceded the 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF 9 h of high light period to give a total photoperiod of 17 h. For the SD plants, the 8 h of low light was given within the high light photoperiod. This resulted in 8 h of approximately 308.5 and 1 h of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF for a total photoperiod of 9 h. The daily integral PPF was 9.96 $mol \cdot m^{-2}$ in all photoperiod treatments. To minimize PPF variability within growth cabinets, the area within 20 cm of the growth cabinet wall was not used. There was less than 3% variation in PPF among plant positions. Plants with their respective CO_2 and photoperiod treatments were switched among growth cabinets on a weekly basis. At the same time, position of plants within cabinets was rearranged. Air temperature within the growth cabinets was 21 ± 0.5°C.

Experimental measurements

Time of first visible bud and time of first open flower were recorded for each individual from daily observations. The difference in time between these two events provided an estimate of the length of time required for bud development. Owing to the similarity of vegetative and flower buds in *Pharbitis*, only time to first open flower was determined. Except for *Callistephus* and *Trachelium*, all plant species were harvested and their vegetative and reproductive parts dried and weighed. Root biomass was collected only in *Dendranthema* and *Kalanchoe*. Total plant leaf area was measured by means of a leaf area meter (LI-COR model LI-300, Lincoln, Nebr.) and specific leaf area (leaf area per unit dry weight) calculated for *Dendranthema*, *Kalachoe*, *Pharbitis*, and *Xanthium*. In *Campanula*, specific leaf area was calculated from a subsample of leaves. For *Kalanchoe* and *Dendranthema*, plants were harvested when all individuals of a given species had at least one open flower. For the remaining species, plants were harvested at a predetermined developmental stage. For *Pharbitis*, *Xanthium*, and *Achillea*, individual plants were harvested as they reached anthesis. For *Campanula*, individual plants were harvested at senescence of the first flower.

Data analysis

Phenology data for all species and biomass data for *Dendranthema* were not normally distributed and the Wilcoxon rank sum test was used to determine the significance of differences between CO_2 treatments. To determine whether phenological response to CO_2 treatment was contingent upon photoperiodic response of the species, a 2 × 2

TABLE 1. Flowering phenology in four long-day and four short-day species grown at either low (350 $\mu L \cdot L^{-1}$) or high (1000 $\mu L \cdot L^{-1}$) CO_2

	Days to first bud			Bud development			Days to first flower		
	Low	High	*p*	Low	High	*p*	Low	High	*p*
Long-day spp.									
Achillea									
17 h	59.1	51.7	0.02	21.2	22.9	0.11	80.2	74.6	0.02
9:17 h	53.4	47.8	0.69	23.3	22.6	0.72	76.7	70.4	0.48
Callistephus	77.7	72.6	0.01	10.2	7.2	0.01	87.9	79.8	0.01
Campanula	89.9	76.3	0.01	28.5	28.5	0.92	118.4	104.8	0.01
Trachelium	88.8	76.9	0.01	29.6	31.1	0.36	118.4	108.0	0.08
Short-day spp.									
Dendranthema	23.0	23.0	1.00	29.7	30.6	0.07	52.7	53.6	0.07
Kalanchoe	31.0	31.0	1.00	32.5	36.1	0.01	63.5	67.1	0.01
Pharbitis	—	—	—	—	—	—	40.2	45.1	0.01
Xanthium	20.0	21.5	0.01	10.5	10.6	0.98	30.5	32.1	0.01

NOTE: Bud development time is the number of days between the appearance of the first bud and opening of the first flower. *Achillea* was grown either in continuous long days (17 h) or grown in short days (9 h) for 22 days and then switched to long days. The *p*-value represents the probability that differences between CO_2 levels could have been obtained by chance.

TABLE 2. Effect of low (350 $\mu L \cdot L^{-1}$) versus high (1000 $\mu L \cdot L^{-1}$) CO_2 upon plant size in two short-day species

	Dendranthema			*Kalanchoe*		
	Low	High	*p*	Low	High	*p*
No. of main stem nodes	24.9	23.2	0.26	12.9	14.3	0.01
Leaf area (cm^2)	994	1160	0.01	483	756	0.01
Specific leaf area ($cm^2 \cdot g^{-1}$)	360	360	0.75	133	126	0.02
Vegetative biomass (g)	6.90	9.65	0.01	5.14	8.33	0.01
Reproductive biomass (g)	5.00	6.80	0.01	2.75	5.32	0.01
Total biomass (g)	11.90	16.45	0.01	7.89	13.65	0.01

NOTE: All individuals of a given species were harvested on the same day. Plants were harvested when the last individual to form buds opened its first flower. The *p*-values represent the probability that the differences between CO_2 levels could have been obtained by chance.

contingency table (i.e., SD vs. LD species × advanced vs. delayed flowering) was used. Due to the small number of species, the Fisher exact test was used to calculate probabilities. Unless otherwise stated, all tests of significance were based on the 0.05 level of probability.

Results

The four LD species in this study all produced flower buds earlier when grown under high CO_2 (Table 1). Mean advancement was 7 days for *Achillea*, 5 days for *Callistephus*, 14 days for *Campanula*, and 12 days for *Trachelium*. However, the length of time required for bud development (i.e., time from first visible bud to first open flower) did not differ significantly between CO_2 levels, except for *Callistephus* for which elevated CO_2 hastened bud development by 3 days. In all four LD species, time from seed germination to opening of the first flower was shortened by elevated CO_2. This effect was significant at the 2% level for *Achillea*, at the 1% level for *Callistephus* and *Campanula*, and at the 8% level for *Trachelium*. The result of the separate experiment performed on *Achillea* involving switching plants from a noninductive photoperiod are shown in Table 1. There were no significant differences between CO_2 levels in days to bud formation, length of bud development, or days to first flower.

Within the SD species group, time of bud formation was not affected by CO_2 treatment in *Dendranthema* and *Kalanchoe*; all plants within the same species formed buds on the same day regardless of CO_2 level. No data on bud formation was collected for *Pharbitis*. *Xanthium* showed a significant 2-day delay in bud formation at high CO_2. The length of time required for bud development increased with elevated CO_2 in *Dendranthema* by 1 day and in *Kalanchoe* by 4 days. Bud development in *Xanthium* was not affected by CO_2 levels. Elevated CO_2 delayed time of flower opening in all four SD species. This effect was significant at the 7% level for *Dendranthema* and at the 1% level for *Kalanchoe*, *Pharbitis*, and *Xanthium*. A 2 × 2 contingency table constructed with photoperiodic response (i.e., SD versus LD) as the two columns and the CO_2 effect (i.e., delayed versus advanced flowering) as the two rows revealed that the probability of getting a result this extreme (i.e., all LD plants responding one way and all SD plants the other) by chance is remote ($p < 0.02$). There is a significant association between photoperiodic response and CO_2 effect on phenology.

Individuals of the two SD species, *Dendranthema* and *Kalanchoe*, were harvested at a single time. Time of harvest was determined by the opening of the first flower on the last individual to flower. These data, shown in Table 2, compare plant size between CO_2 treatments. In *Dendranthema*, high CO_2 increased vegetative (i.e., root, stem, and leaf) as well as reproductive (i.e., flower, bud, and peduncle) biomass. Leaf

in two short-day species

	Pharbitis			*Xanthium*		
	Low	High	*p*	Low	High	*p*
No. of main stem nodes	—	—	—	9.5	9.1	0.15
Leaf area (cm^2)	555	826	0.02	268	292	0.10
Specific leaf area ($cm^2 \cdot g^{-1}$)	299	271	0.01	354	285	0.01
Vegetative biomass (g)	3.12	4.67	0.02	1.13	1.61	0.01
Reproductive biomass (g)	0.51	0.66	0.03	0.42	0.66	0.01
Total biomass (g)	3.64	5.32	0.01	1.56	2.27	0.01

NOTE: Individual plants were harvested at anthesis. Due to differences in phenology, day of harvest varied among individuals. The *p*-values represent the probability that the differences between CO_2 levels could have been obtained by chance.

TABLE 4. Effect of low (350 $\mu L \cdot L^{-1}$) versus high (1000 $\mu L \cdot L^{-1}$) CO_2 upon plant size in two long-day species

	Low	High	*p*
Achillea (17 h)			
Vegetative biomass (g)	12.65	16.69	0.01
Reproductive biomass (g)	1.77	3.03	0.01
Total biomass (g)	14.42	19.72	0.02
Achillea (9:17 h)			
Vegetative biomass (g)	11.19	16.35	0.01
Reproductive biomass (g)	2.32	3.18	0.04
Total biomass (g)	13.51	19.53	0.01
Campanula			
No. of main stem nodes	11.3	11.3	0.77
Specific leaf area ($cm^2 \cdot g^{-1}$)	288	229	0.01
Vegetative biomass (g)	5.19	5.20	0.98
Reproductive biomass (g)	1.79	2.76	0.01
Total biomass (g)	6.98	7.96	0.13

NOTE: Individuals of *Achillea* and *Campanula* were harvested at anthesis and flower senescence, respectively. Owing to differences in phenology, day of harvest varied among individuals. *Achillea* was grown either in continuous long days (17 h) or grown in short days (9 h) for 22 days and then switched to long days. The *p*-values represent the probability that the differences between CO_2 levels could have been obtained by chance.

area increased with CO_2, but specific leaf area and the number of main stem nodes were not affected by CO_2 treatment. In *Kalanchoe*, high CO_2 increased leaf area and both vegetative and reproductive biomass. Specific leaf area decreased with high CO_2, indicating an increase in leaf thickness or density with high CO_2. The number of main stem nodes to the terminal inflorescence was also increased by high CO_2.

The two SD species, *Pharbitis* and *Xanthium*, and the two LD species, *Achillea* and *Campanula*, were harvested as each individual reached a certain stage (i.e., anthesis for *Pharbitis*, *Xanthium*, and *Achillea*, and flower senescence for *Campanula*) in its life cycle. These data, therefore, examine the effect of CO_2 treatment on plant size at a given growth stage. High CO_2 increased vegetative and reproductive biomass in both *Pharbitis* and *Xanthium* (Table 3). Leaf area also increased in *Pharbitis* but not in *Xanthium*, while specific leaf area decreased in both species. Level of CO_2 had no effect on the number of main stem nodes in *Xanthium*. Regardless of photoperiodic treatment, *Achillea* showed a significant increase in both vegetative and reproductive biomass with high CO_2 (Table 4). Although high CO_2 increased reproductive biomass in *Campanula*, vegetative and total biomass did not differ significantly between CO_2 treatments (Table 4). Specific leaf area decreased with CO_2. The number of main stem nodes was the same regardless of CO_2 treatment.

Discussion

We found elevated CO_2 advanced flowering in the four LD species and delayed flowering in the four SD species (Table 1). There are few published reports on the effect of CO_2 on flowering or photoperiodism. Where information exists it is apparent that LD species generally display an advancement in time of flowering (Wittwer 1967; Marc and Gifford 1984; Nelson 1985; Mortensen 1987), and SD species often display a delay in flowering (Posner 1971; Wittwer 1967; Marc and Gifford 1984) at high CO_2 (ca. 600–1500 $\mu L \cdot L^{-1}$). However, there are exceptions to this pattern, especially with regard to SD species (Goldsberry 1965; Mortensen 1985, 1987). The mixed results may be due in part to differences in photoperiodic conditions during CO_2 fumigation. In our study, *Achillea* showed a different flowering response to CO_2 depending on whether plants were grown continuously under an inductive photoperiod or switched from noninductive to inductive conditions (Table 1). In studies in which plants have been maintained continuously under photoperiodically inductive conditions, results conformed closely to those in our study. Marc and Gifford (1984) found that when *Triticum aestivum* (LD) was grown from seed under LD conditions, floral initiation was advanced, whereas *Sorghum bicolor* (SD) grown under SD conditions showed a delay in floral development.

Another factor that may contribute to the varied patterns described in the literature is the light environment in which plants are grown. Even the same species can have a different flowering response to elevated CO_2 when given different light conditions. For example, flowering was advanced by 4.6 days in the SD plant *Euphorbia pulcherrima* when it was subjected to 1000 $\mu L \cdot L^{-1}$ CO_2, but when given an additional 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF, time to flowering was delayed 6.6 days (Mortensen 1985). Other studies on *Dendranthema*, *Begonia* ×*hiemalis*, and *Euphorbia pulcherrima* all show that time to flowering varies depending on the interaction between PPF and elevated CO_2 (Mortensen 1985; Mortensen and Ulsaker 1985). Therefore, it is not possible to compare the flowering response of different species to elevated CO_2 unless they are given uniform experimental conditions.

Our results suggest that different aspects of the flowering process are affected by elevated CO_2 concentration and that the specific effect may depend upon the photoperiodic response of the particular species. In the LD species, the advancement

of flowering was due mainly to a hastening of bud formation. This agrees with the work of Marc and Gifford (1984), in which *Triticum aestivum* (LD) showed an advancement in bud initiation when grown under continuous elevated CO_2. In our study, elevated CO_2 delayed bud development in two of the SD species, *Dendranthema* and *Kalanchoe*. Similar results were reported for *Sorghum bicolor* (SD) grown continuously under elevated CO_2 (Marc and Gifford 1984). *Xanthium* showed a different pattern. Bud formation was delayed, but bud development was unaffected by elevated CO_2. However, bud formation in this study was based on the appearance of macroscopic buds rather than microscopic dissection of the apex (Marc and Gifford 1984). Buds in *Xanthium* grown at high and low CO_2 could have been initiated simultaneously but became visible earlier in the low CO_2 treatment owing to faster bud development.

One possible explanation for the effect of CO_2 upon phenology is that it modifies growth rate and therefore changes the time necessary to attain the minimum size required for flowering. In *Campanula*, individual plants flowered on the same main stem node regardless of CO_2 regime, indicating the attainment of the same physiological age when flowering occurred. Vegetative biomass at flowering was similar between treatments. The difference in total biomass was solely due to the contribution of a larger reproductive biomass with high CO_2. It is plausible that advancement of flowering in *Campanula* was due to accelerated development, i.e., earlier attainment of flowering competency. On the other hand, *Achillea* (LD) showed significant increase in vegetative biomass at flowering with high CO_2. This increment in size with elevated CO_2 was accompanied by an advancement in flowering under continuous LD and had no effect on flowering under SD switched to LD photoperiod. This suggests that differences in size between CO_2 treatments may have been important in the flowering process but are not the only factor involved.

Although the effect of CO_2 on plant size may partially explain its effect on phenology in one of our LD species, it cannot easily account for its effect on phenology in the SD species. Elevated CO_2 delayed flowering, yet increased both size at flowering in *Pharitis* and *Xanthium* and size at a predetermined growth stage in *Dendranthema* and *Kalanchoe*. If the effect of CO_2 on phenology is mediated through its effect on growth, then growth enhancement must have contrasting effects on flowering in SD versus LD species.

Previous studies also found poor correlation between the CO_2 effect upon growth and its effect upon phenology. For example, Reekie and Bazzaz (1991) found that elevated CO_2 affects both growth and phenology to varying degrees depending upon species and environmental conditions. However, treatments in which CO_2 had significant effects upon growth were generally different from those in which phenology was affected. Similarly, Marc and Gifford (1984) showed that the delay in bud development in *Sorghum bicolor* (SD) at high CO_2 was not accompanied by any difference in shoot dry weight. Further, in a short-term CO_2 enrichment experiment, it was shown that the CO_2 effect on floral development in the LD species *Triticum aestivum* varied depending on when CO_2 enrichment was applied, despite the fact that timing of CO_2 enrichment had no effect upon growth. Growth was promoted uniformly regardless of when CO_2 was applied.

In conclusion, elevated CO_2 advanced flowering in the LD species and delayed flowering in the SD species. These effects cannot be explained by the effect of CO_2 upon growth without assuming that the SD and LD species responded differently to growth enhancement. It is possible that CO_2 was exerting an influence that was independent of its effects on photosynthesis and growth.

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