Carbon Dioxide and Flowering in *Pharbitis nil* Choisy

PETER R. HICKLENTON AND PETER A. JOLLIFE

Department of Plant Science, The University of British Columbia, Vancouver, British Columbia V6T 1W5 Canada

ABSTRACT

The effects of photoperiod on floral and vegetative development of *Pharbitis nil* were modified by atmospheric CO₂ concentrations maintained during plant growth. Short day (SD) photoperiods caused rapid flowering in *Pharbitis* plants growing in 0.03 or 0.1% CO₂, while plants in long day (LD) conditions remained vegetative. At 1 or 5% CO₂ however, flower buds were developed under both the SD and LD photoperiods. Flowering was earliest in the plants exposed to SD at low CO₂ concentrations which formed floral buds at stem node 3 or 4. At high CO₂ concentrations, floral buds did not form until stem node 6 or 7. Both high CO₂ concentrations and LD photoperiods tended to enhance stem elongation and leaf formation.

The occurrence of flowering under normally noninductive LD photoperiods at 1 or 5% CO₂ is readily explained in terms of higher photosynthetic rates. Plants grown at 0.03 or 1% CO₂ in either photoperiod tended to approach maximum photosynthesis between 0.1% and 0.5% CO₂. In addition, relative growth rates were not significantly increased by growth at 1 or 5% CO₂. Possible alternative mechanisms for the interactive effects of CO₂ and photoperiod are discussed.

Photoperiodic flowering responses are known to be influenced by several environmental factors, and in some cases such interactions may be mediated by changes in photosynthesis. Early investigations (5) showed that irradiance was important in determining the degree of response to photoperiod in SDP. An involvement of photosynthesis was later supported by the finding that externally supplied carbon compounds could sometimes substitute for high irradiance (15). Temperature treatments can strongly affect photoperiodic behavior (25), although there is little direct evidence that this may occur via photosynthesis.

The photosynthetic substrate CO₂ has also been found to alter photoperiodic flowering behavior in both SDP and LDP species. The SDP *Xanthium pensylvanicum* flowered if high CO₂ concentrations were supplied near the start of a normally noninductive long day. Flowering was inhibited, however, by high CO₂ levels during a normally inductive long dark period (7). Purhoit and Tregunna (20) found that the maintenance of high CO₂ concentrations during growth depressed flowering of the SDP *Xanthium* and *Pharbitis nil* exposed to repeated SD cycles. Conversely, they also found that high CO₂ concentrations promoted flowering in the LDP *Silene armeria* under short days. Among the Lemnaceae, high CO₂ concentrations inhibit flowering in the LDP *Lemma gibba* under long days (12), and also inhibit the SDP *Lemma paucicostata* (perpusilla) under short days (19).

All of these CO₂ effects have been found using atmospheric concentrations ±1% CO₂. In most studies, the possibility has been raised that the high CO₂ concentration effects are consequences of enhanced photosynthesis. To test this possibility, we have examined whether different CO₂ concentrations exert corresponding effects on photosynthesis rate and developmental characteristics of *P. nil* plants grown under different photoperiods.

MATERIALS AND METHODS

Plant Preconditioning. Japanese morning glory (*P. nil* Choisy cv. Imperialis Japanese) seeds were obtained by Stokes Seeds Ltd., St. Catharines, Ont., and were germinated in moist mica peat for 3–4 days. Seedlings which were uniform in height and apparent development were selected and transplanted separately into 10-cm pots containing mica peat moistened to field capacity with complete nutrient solution. The solution was modified after Johnson et al. (11) by omitting KNO₃ and by replacing NH₄H₂PO₄ with KH₂PO₄. Seedlings were then maintained for two weeks in a Sherer CEL 255-6 growth chamber at 25 ± 1 C in normal air (about 0.03% CO₂) under a LD photoperiod of 16 h light, 8 h darkness. The chamber lighting was provided by a mixture (2:1 wattage ratio) of cool-white fluorescent and incandescent lamps which delivered a quantum flux density of 60 ± 10 µE m⁻² s⁻¹ (400–700 nm) (3,600 ± 600 lux) at plant level. The ratio of irradiances at 660–730 nm was 2:1.

Carbon Dioxide Treatments. Constant concentration CO₂ treatments were applied for 14 days after preconditioning. Plants were sealed within 55 × 25 × 60-cm boxes which were in turn enclosed within Sherer CEL 255-6 growth chambers which maintained temperature and illumination at the same levels as during preconditioning. The walls of the boxes were lined with 2-mm polyvinyl chloride and the tops and bottoms were 6-mm clear Plexiglas. Either SD (8 h day/16 h night) or LD (16 h day/8 h night) cycles were maintained throughout the CO₂ treatment period.

Four CO₂ concentrations were used: 0.03% (ambient air), 0.1%, 1.0%, or 5.0% by volume. For 0.03% CO₂ treatments the top and bottom plates of the treatment boxes were perforated with 2.5-cm holes to allow air circulation from the growth chamber past the plants. The other CO₂ treatment boxes contained adjacent inlet ports for pure CO₂ and ambient air, and the two gas streams were mixed on entry by small fans mounted near the inlet ports. CO₂ was supplied to different boxes from a cylinder of pure, compressed CO₂ at flow rates between 10 and 40 ml min⁻¹. The air stream was withdrawn from the atmosphere outside the building and pumped into the boxes at flow rates of 0.3, 1.3, or 4.6 l min⁻¹ for the 5.0, 1.0, or 0.1% CO₂ treatments, respectively. Gases escaped to the outside atmosphere from the >0.03% CO₂ treatment boxes through outlet ports. A Beckman 864 Gas Analyzer was used to verify the level and stability of the CO₂ concentrations during the treatments.

Plant pots were saturated with nutrient solution when they were placed in the treatment boxes. There was little soil drying in the
pots sealed in the >0.03% CO₂ treatment boxes. Pots in the 0.03% CO₂ boxes were watered to field capacity at about 4-day intervals during the CO₂ treatments. Plants which were not harvested immediately following the CO₂ treatments were returned to growth chambers for 7 days under conditions identical to the preconditioning period.

**Experimental Sequence.** Plant growth and development were measured in two replicate trials, each trial starting with 72 seedlings. Twenty-four seedlings per trial were harvested following preconditioning to determine leaf and total plant dry weights. The remaining 48 seedlings were randomly distributed in lots of six among eight CO₂ treatment boxes. One box per trial was maintained under each of the eight possible photoperiod × CO₂ concentration combinations. At the start of the CO₂ treatments, three seedlings per box were tagged for identification and their stem heights were measured. At the end of the CO₂ treatments, their stem heights were remeasured and their leaf lengths and floral bud development was determined. Flower bud formation on the tagged plants was again assessed 7 days following the CO₂ treatments. The remaining three plants per treatment were harvested at the end of the CO₂ treatment period to measure their leaf and total dry weights.

Plants used for the gas exchange studies were grown as a separate group of 16 plants. Plants were preconditioned and exposed to photoperiod and CO₂ treatments as in the other two trials. There were two plants per photoperiod × CO₂ concentration combination, and the gas exchange measurements were done following the CO₂ treatment period.

**Measurements of Growth and Development.** Dry weights were determined after drying harvested plant material at 90 °C for one day. Relative growth rates were calculated from the dry weights before and after the CO₂ treatments. Stem heights were measured as the length of stem between the mica peat surface and the main stem apex. Leaf plastochron index (9) was calculated from the leaf length data using a reference leaf length of 30 mm. Floral development was assessed by recording the number of floral buds per plant and the serial node on which the first floral bud formed. Floral buds were determined by visual inspection of buds larger than 2 mm diameter. Floral buds were recognized by their pointed tips and by the equality of the ligules on either side of the bud.

**Measurement of Leaf Gas Exchange.** Two types of gas exchange measurements were made on the second lowest true leaf on the main stem. The first used a simple 2-liter closed gas exchange system incorporating a leaf cuvette and a Beckman 864 Gas Analyzer to determine net photosynthesis at CO₂ concentrations from 2.5 to 0.1% CO₂. The second involved an open system containing a leaf cuvette, a Datametrics 800 VTP mass flowmeter and a Cambridge 880 Dew Point Hygrometer to measure combined leaf and boundary layer air resistances to gas exchange. The test leaves remained attached to the plant during the gas exchange measurements. Within the cuvette, incident quantum flux density was 500 μE m⁻² s⁻¹ (400–700 nm) and leaf temperature was 30 °C. The RH of the air entering the 1.5-liter cuvette during the resistance determination was 75 ± 5%. The air flow rate through the cuvette was 0.8 liter min⁻¹ and air within the cuvette was mixed by a small fan. Test leaves were removed following the gas exchange determinations and their areas were measured using a Hayashi Denko Photoelectric Planimeter. Photosynthesis rates were calculated from the rate of change of CO₂ concentration per unit leaf area within the closed system.

**RESULTS**

CO₂ and photoperiod treatments affected many aspects of plant growth and development (Table I). Stem elongation was greatly enhanced by high (1% or 5%) CO₂ concentrations and by long photoperiods. The increased elongation caused by high CO₂ concentrations was not accompanied by increased whole plant or leaf relative growth rates. Therefore, the CO₂-induced elongation represented a change in developmental pattern and it was not simply the result of accelerated growth in the presence of additional photosynthetic substrate. Up to 1% CO₂ growth rates tended to be higher under LD than SD. That response, however, may not represent a developmental shift but may reflect the longer periods of illumination of the LDP. The effect of CO₂ treatments on leaf plastochron index were similar to the stem elongation results, with more advanced leaf development occurring at 1 or 5% CO₂ under either photoperiod.

The usual effects of photoperiod on flowering in Pharbitis were upset by high CO₂ concentrations during growth. Under normally noninductive long photoperiods, floral buds developed on some of the plants exposed to 1 or 5% CO₂ (Table I). Over the two replicate experimental trials, half of the plants given the LD × high (≥1%) CO₂ concentration treatments formed flower buds starting at about node seven from the stem base. No flower buds formed on plants grown under LD at 0.03 or 0.1% CO₂. Among plants grown under SD, CO₂ treatments affected the position of the first floral node but not the over-all number of flower buds

Table 1. Effects of CO₂ and Photoperiod Treatments on Some Characteristics of Growth and Development in P. nil

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CO₂ Concentration (%)</th>
<th>SD Treatment</th>
<th>LD Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.03</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Stem elongation during CO₂ treatments (m)</td>
<td>0.34a</td>
<td>0.50b</td>
<td>0.70c</td>
</tr>
<tr>
<td>Relative growth rate (whole plant) g g⁻¹ day⁻¹</td>
<td>0.164a</td>
<td>0.159b</td>
<td>0.165c</td>
</tr>
<tr>
<td>Relative growth rate (leaves) g g⁻¹ day⁻¹</td>
<td>0.185a</td>
<td>0.181b</td>
<td>0.188c</td>
</tr>
<tr>
<td>Plastochron index</td>
<td>5.63a</td>
<td>5.69a</td>
<td>7.47a</td>
</tr>
<tr>
<td>Floral buds formed per plant, 1 week after end of CO₂ treatments</td>
<td>4.72a</td>
<td>3.50b</td>
<td>4.50c</td>
</tr>
<tr>
<td>Mean node of first floral bud</td>
<td>3.50a</td>
<td>3.25b</td>
<td>7.00c</td>
</tr>
</tbody>
</table>

1 Figures in parentheses indicate percentage of plants bearing floral buds.

Downloaded from on January 6, 2018 - Published by www.plantphysiol.org
Copyright © 1980 American Society of Plant Biologists. All rights reserved.
formed. Treatments with 1 or 5% CO₂ delayed the appearance of flower buds by about three or four nodes under the inductive SD photoperiods. We would like to emphasize that although flower buds formed in several different treatments, flowering was by far the most vigorous in plants grown under SD at either 0.03 or 0.1% CO₂.

Table I supports the existence of a threshold CO₂ concentration between 0.1 and 1.0% CO₂. At concentrations below that threshold, stem elongation and leaf development were relatively slow and flowering followed the normal SDP pattern. Above the threshold, stem elongation and leaf formation were rapid, and normal flowering responses were partly reversed. Figure 1 allows a visual comparison to be made of plants and flower buds produced under several of the treatments regimes.

*Pharbitis* leaves approached photosynthetic CO₂ saturation between 0.1 and 0.5% CO₂ irrespective of the photoperiod or CO₂ concentration during growth (Fig. 2). The irradiance the leaves received during the photosynthesis determinations (500 μE m⁻² s⁻¹) was considerably greater than the irradiance available during plant growth (60 μE m⁻² s⁻¹). It is likely that photosynthesis was saturated, or very nearly saturated, with CO₂ during the course of plant growth in 0.1% CO₂ and higher concentrations.

At test concentrations above 0.5% CO₂, photosynthetic rates of plants which had been grown in 0.03% CO₂ were always higher than those grown in 1.0% CO₂ (Fig. 2). This effect was not due to the leaf stomata. Measurements of stomatal resistance were carried out at the same time as the CO₂ exchange determinations and yielded no significant difference between the growth regimes. Stomatal response to test CO₂ concentration was not large; combined stomatal and boundary layer resistances ranged from about 4 s cm⁻¹ at 0.03% CO₂ to about 5.5 s cm⁻¹ at 2.5% CO₂.

**DISCUSSION**

These investigations have confirmed (20) that ≥1% CO₂ delays flowering in *Pharbitis* under short days, and they have demon-
stratified that such CO₂ levels promote flowering under normally noninductive long photoperiods. The results parallel the findings of Campbell (7) who studied the effects of relatively brief treatments with up to 22.1% CO₂ on flowering of Xanthium. Comparable effects of high CO₂ concentrations on flowering have been observed in other studies on Silene (20) and species of Lemma (12, 19). In all cases known to us, the presence of ≥1% CO₂ has acted to oppose normal photoperiodic flowering responses.

In the present work with Pharbitis, the responses of plants treated with high CO₂ concentrations apparently showed little resemblance to normal photoperiodic behavior. Pharbitis can be induced to flower by only one SD cycle, yet at 1 or 5% CO₂ plants showed a marked delay in the formation of flower buds despite 14 consecutive SD. Their flowering pattern was, in fact, very similar to that of plants maintained at the same CO₂ concentrations under normally noninductive, LD conditions.

Our observations on flowering of Pharbitis coincide with those of Purohit and Tregunna (20), but our results on stem and leaf development differ. We found increased stem and leaf development at high CO₂ concentrations (Table I), but they obtained the opposite result. Such differences could be due to different levels of irradiance used during plant growth, and the causes of such variations in plant response ought to be investigated further. Several other studies have found that CO₂ concentration can influence elongation in coleoptiles (6, 10) and roots (22, 23). One interesting aspect of our results is that flower buds developed on both the shortest and tallest groups of plants, but not on some intermediate groups (Table I). Flowering was, therefore, divorced from stem elongation, as has been demonstrated in the bolting and flowering of Silene (24).

This research does not implicate changes in photosynthesis rate as the cause of the high CO₂ concentration effects. Relative growth rates, which are partly dependent on photosynthetic activity, were not increased by high CO₂ concentrations (Table I). More directly, photosynthesis rate approached CO₂ saturation between 0.1 and 0.5% CO₂ (Fig. 2) but developmental effects of high CO₂ concentrations did not appear until 1.0 or 5.0% CO₂. It is possible that the composition of photosynthetic end products may change at >0.1% CO₂ without concomitant changes in photosynthesis rate. Such end products could have a critical role in determining the effects of high CO₂ concentrations as proposed by Purohit and Tregunna (20). The studies of Campbell (7), however, tend to exclude the involvement of photosynthesis, since high CO₂ concentrations were found to affect flowering in Xanthium when the gas treatments were applied during darkness.

If photosynthesis is not involved, there are several possible alternative sources of high CO₂ concentration effects which should be considered. Dark CO₂ fixation reactions may be driven by high CO₂ concentrations, and such reactions could influence developmental patterns via the types of fixation products (6), or by alterations in tissue pH (8). Nakayama (17) showed that flowering in Pharbitis depends on respiratory activity during a long, inductive dark period. It is possible that very high external CO₂ concentrations may suppress respiratory processes (13) involved in flowering, or that they may alter the response of respiration to photoperiod (16). The interaction of CO₂ with endogenous growth regulators represents another possible control mechanism. The activities of ethylene and abscisic acid can be strongly affected by CO₂ concentration (1, 21), and both growth regulators may influence flowering of Pharbitis (26).

The action of high CO₂ concentrations on development is not necessarily by one mechanism alone but could be achieved by several effects operating in tandem. One difficulty is to reconcile the partial suppression of flowering by high CO₂ levels in SD conditions with the promotive effects of such CO₂ treatments in LD conditions. A similar paradox is evident in the effects of low temperatures on flowering of Pharbitis (17) and Xanthium (7). In the present study, high CO₂ concentrations during growth tended to produce a general similarity in plant appearance between the SD and LD treatments. In both photoperiods, flowering was weak and stem elongation was rapid at 1% CO₂ or above. This similarity may indicate that in some way high CO₂ concentrations release development from domination by photoperiodic control. The possibility of such a release has previously been shown by the induction of floral primordia in some SDP and LDP under conditions of continuous darkness (18).

It is important to distinguish between the effects of high (≥1%) CO₂ concentrations, such as reported in this paper, and the effects of low CO₂ concentration treatments which have been described in some other studies on flowering. In some cases, CO₂ removal during night interruption treatments has been found to block flowering responses to the interruptions (2). Also, CO₂ removal during part of the main light period can sometimes prevent flowering (3, 4, 14). Because of the many physiological roles of CO₂, it is premature to believe that the different effects of CO₂ on flowering arise from a common mechanism.

LITERATURE CITED

5. BORTHWICK H, MW PARKER 1938 Photoperiodic perception in Biloxi soybeans.

Bot Gaz 100: 374–387

Fig. 2. Effects of CO₂ concentration on net photosynthesis rates of leaves from plants grown under SD (A) or LD (B) photoperiods and either 0.03% CO₂ (●) or 1.0% CO₂ (○). Lines drawn are hyperbolas transformed from best fit linear regressions of double reciprocal plots of photosynthesis rate against CO₂ concentration.
CARBON DIOXIDE AND FLOWERING

CORRECTION

Vol. 66: 13-17, 1980
Peter R. Hicklenton and Peter A. Jolliffe. Carbon Dioxide and Flowering in Pharbitis nil Choisy.
On page 13 the first sentence of the second paragraph of the abstract should read: The occurrence of flowering under normally noninductive LD photoperiods at 1 or 5% CO₂ is not readily explained in terms of higher photosynthetic rates.