Short Communication

Carbon Dioxide Requirements for Phytochrome Action in Photoperiodism and Seed Germination

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ABSTRACT

The effect of interrupting darkness with red light in the presence or absence of 0.03% CO₂ was studied in relation to flowering of Xanthium pennsylvanicum and germination of light-sensitive lettuce seeds. The results indicate that CO₂ is essential for red light to be effective in either process.

There are reports that removal of CO₂ during the dark period inhibited flowering of short day plants in some experiments (5, 6), but not in others (2, 4). A requirement for CO₂ and ethylene to break thermomorphy of lettuce seeds has also been reported (8). Since phytochrome is involved in the control of flowering and seed germination, we asked if CO₂ is also essential during illumination for these two classical effects of red light: promotion of seed germination and inhibition of flowering when given to short day plants in the middle of long dark periods. The results of these studies are reported.

MATERIALS AND METHODS

Experiments 1 and 2. The Xanthium pennsylvanicum plants used in these experiments were grown in long days (16 hr light alternating with 8 hr of dark) at 25°C until they were at the seven to eight leaf stage. They were divided into groups of eight plants each and transferred to short day treatment (8 hr light of 2000 µmole cm⁻² sec⁻¹ alternating with 16 hr of dark) in three 22 X 22 X 5 cm Plexiglas chambers. The chambers were illuminated from the top through a 6 cm thick water layer with cool white fluorescent tubes. The temperature in the chamber was 23°C. The plants in these chambers were exposed to 8 short day cycles. In two of these chambers, each long dark period was interrupted in the middle for 6 min with red light obtained from a bank of cool white fluorescent tubes and filtered through a CBS (Carolina Biological Supply) red filter 630. The intensity of red irradiation at the leaf surface was about 230 µmole cm⁻² sec⁻¹. While normal air was pumped through one of these two chambers during the red light treatment, the other was flushed with medical grade CO₂-free air for 35 min, beginning 15 min before and ending 16 min after the light treatment. The flow rate of CO₂-free air was more than 5 liters min⁻¹.

After eight such cycles, the plants from all of the treatments were transferred to 16-hr photoperiods (noninductive conditions) in normal air. The shoot apices of the plants were dissected after 10 and 20 days from the start of the experiment and observed for the stage of development of floral buds according to Salisbury's classification (9).

In another experiment, CO₂ was removed for 35 min without interrupting the dark period with red light. Other details of the experiment were the same as described for experiment 1.

Experiment 3. Grand Rapids lettuce seeds were placed on wetted blotters in 4-cm diameter Petri plates and immediately covered and placed in darkness at 20°C. One set of four Petri plates with 50 seeds in each was kept in darkness. In a second set, seeds were irradiated with red light for 5 min after 15 min of illumination with normal air pumped through the chamber. The third set of Petri plates was exposed to CO₂-free air for 25 min as described for experiment 1. After 5 min in the CO₂-free air, the seeds were exposed to red light for 5 min and then maintained in CO₂-free air for another 15 min. The air was saturated with water vapor in all the treatments. Subsequently, the Petri plates from both treatments were kept in normal air in darkness. The source and intensity of red irradiation were the same as described in the previous experiments. Germination counts were taken after 24 and 48 hr from the start of the experiment.

RESULTS

The results of experiment 1, which confirm the observations of preliminary investigations, are shown in Figure 1. While all plants exposed to short days reached stage 4.5 and 7.5 after 10 and 20 days, respectively, those exposed to the same number of short day cycles with red interruption in the middle of the dark period in normal air remained vegetative. In contrast to this result, when the interruption was given in the absence of CO₂, it was ineffective in inhibiting the flowering response. Removal of CO₂ from the chamber for the same duration of time in an uninterrupted dark period had no effect on the development of floral buds.

Seed germination was promoted by red light as expected (Table 1). When red light of the same intensity and duration was given in the absence of CO₂, seed germination was considerably lower. The results in Table 1 are the mean value from two experiments. The results were all different at the 1% level of significance.

DISCUSSION

These experiments demonstrate that CO₂ is essential for red light to be effective in these two processes. The small effect of red light in delaying the flowering response in the first experiment and promoting seed germination as compared to dark controls

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in the third experiment may be due to an incomplete removal of CO₂ which is constantly being produced by respiration in both systems. The question of the role of CO₂ in these two processes cannot be answered at present, but we propose that CO₂ is an essential requirement for phytochrome to act in the two responses studied in these experiments. CO₂ has also been shown to enhance other morphogenetic responses like cell elongation, and a synergistic relationship between IAA and CO₂ in stimulating growth has been demonstrated (1). Recently, Kirkland and Posner (3) have demonstrated that DCMU can supplement far red light in nullifying the inhibitory effect of red irradiation on flowering.

Moshkov and Odumanova-Dunaeva (7) reported that CO₂ is essential during the light interruption period in *Brassica crenata*, a long day plant, for causing induction in otherwise noninductive conditions but not in *Perilla ocymoides*, a short day plant. Our results in *Xanthium* are contradictory to their hypothesis that nyctophytic (short day) plants do not require CO₂ for the light interruption to be effective. Further work is in progress to find out the qualitative and quantitative differences in CO₂ fixation during the red light interruption and to study the reversibility of these differences by far red light.

**LITERATURE CITED**


