The Effect of Dual Perturbations on the Rhythmic Flowering Response of Biloxi Soybean 1, 2

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Evidence is accumulating to indicate that an endogenous circadian rhythm is participating in the photoperiodic response of Biloxi soybean (2, 5). Circadian rhythms of activity and behavior have been found throughout the plant and animal kingdoms and most biologists are inclined to believe that these rhythms are expressions of the so-called biological clock. It is clear that the photoperiodic response involves some kind of a clock sense since the response depends on the ability of the organisms to measure the length of the day. In Biloxi soybean it appears that the endogenous circadian rhythm passes through alternate photophil and photophobe phases of sensitivity to red light (3), and that red light received during the photophobe phase of the rhythm is inhibitory to flowering. Previously, Biloxi soybean plants were exposed to 48-hour cycles, each cycle being initiated by an 8-hour photoperiod (3). When the long dark period of such cycles was interrupted briefly at different points with red light, it was found that such light was inhibitory when given between the twelfth and twenty-fourth hours of the cycle and between the 36- and 48-hour points. On the other hand, such interruptions were noninhibitory when given between the 24- and 36-hour points in the total cycle. Therefore, it appears that the 8-hour photoperiod which initiated each cycle was responsible for the basic oscillations of the endogenous rhythm and that the perturbations reacted with these basic oscillations. However, since the endogenous rhythm which controlled the photoperiodic response seemed established and maintained by the 8-hour photoperiod which began each cycle the question arose as to whether or not the light perturbations initiated any rhythms of their own which interacted with the basic oscillations. It seemed possible that a partial answer to this question could be obtained by exposing the plants to 2 perturbations during each long dark period and to study the interaction between them. If the first perturbation initiated its own rhythm then the second perturbation should react to the first perturbation as well as to the basic oscillation. It was with these thoughts in mind that the following experiments were designed.

Materials and Methods

Seed of Biloxi soybean (Glycine max L. Merr.) was obtained from Dr. H. A. Borthwick, U. S. Department of Agriculture, Beltsville, Maryland. The seeds were planted in 10-cm unglazed pots, 4 seeds to a pot. Approximately 2 weeks after emergence of the seedlings, the plants were selectively thinned to leave 2 healthy uniform plants in each pot. The plants were maintained on long-day conditions by supplementing the natural day length with incandescent light from Mazda bulbs. Light intensities of 50 ft-c at the leaf surface were maintained in this manner until 2:00 AM each morning. The plants were used for experimental treatment when the second trifoliate leaf was fully expanded. Ten plants per treatment were used in all experiments and each experimental treatment repeated 7 times. At the termination of each experiment, the plants were returned to the greenhouse and allowed to grow for 4 to 5 weeks. At this time the plants were dissected and the number of nodes bearing floral primordia on each plant was recorded. Light for the main photoperiods and also for the perturbations was provided by banks of powergroove lamps. The light intensity at the leaf surface was 2,200 ft-c.

Results

Experiment 1, The Effect of Two 30-Minute Perturbations on the Flowering of Biloxi Soybean.

Plants were exposed to a 48-hour cycle, initiated by an 8-hour photoperiod and the dark period interrupted at various times with two 30-minute perturbations. One group of plants received their first perturbation at the 16-hour point in the cycle and were then returned to darkness. At subsequent intervals, one lot of plants was exposed to a second 30-minute perturbation. The results are summarized and plotted according to the time of first perturbation in figure 1. Thirty minutes of light given at the 16-hour point in the cycle is completely inhibitory to flowering regardless of any subsequent treatment. Thirty minutes of light at the 24-hour point in the cycle and followed by a second 30-minutes at either the 28 or 32-hour points results in no stimulation of flowering above the control level. All plants were vegetative when the second perturbation was given at the 40-hour point in the cycle regardless of the time of the first perturbation.

1 Manuscript received March 18, 1964.
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very little in their effects and that such single perturbations give very little stimulation. With two 30-minute perturbations (fig 1), no significant stimulation of flowering can be achieved. It seems, therefore, that two 30-minute perturbations do not interact appreciably with one another and, regardless of how they are spaced, they do not appreciably change the basic endogenous rhythm initiated by the 8-hour photoperiod.

**Experiment 2, The Effect of Two 2-Hour Perturbations on the Flowering Response of Soybean.** The plants were again exposed to a 48-hour cycle, initiated by an 8-hour photoperiod, and the dark period interrupted at various intervals with 2 perturbations each of 2 hours duration. The results of experiment 2 are summarized and plotted to show the relationship between the time of the perturbation and the cycle time in figure 3. When the first perturbation is given 20 hours after the start of the cycle, the inhibitory effect of light at this point can-

![Figure 1](image1.png)

**Fig. 1.** Experiment 1. The effect on the flowering of Biloxi soybean of two 30-minute perturbations. The point in the cycle at which the first perturbation was given is represented by a solid square and is connected to the closest second perturbation by a dotted line. Thus a single perturbation at the 20-hour point in the cycle resulted in the production of 17 flowers per 10 plants. A second perturbation at the 24-hour point in the cycle produced 40 flowers. When the second perturbation was given at the 28-hour point in the cycle, 39 flowers were produced and so on. The control level of flowering, plants which received an uninterrupted dark period, is represented by a solid horizontal line at the point indicating the number of nodes bearing floral primordia. When the first interruption was given at the 16-hour point in the cycle, flowering was completely inhibited.

Figure 2 shows the response of plants to a single 30-minute and 1-hour perturbation given in a 48-hour cycle. From these curves it seems apparent that single perturbations of 30 minutes or 1 hour differ

![Figure 2](image2.png)

**Fig. 2.** The effect on the flowering of Biloxi soybean of 30-minute and 1-hour perturbations given in a 48-hour cycle. The control level of flowering is represented by the solid line. Data for 1-hour perturbations from Nanda and Hamner (6). Thirty-minute perturbations taken from Carpenter and Hamner (3).

![Figure 3](image3.png)

**Fig. 3.** Experiment 2. The effect of two 2-hour perturbations given at various times in the dark period of a 48-hour cycle on the flowering response of Biloxi soybean. In each instance the first single perturbation is represented by a solid square. A dotted line connects this single perturbation with the following second perturbation. Two hours of light at the 20-hour point in the cycle resulted in the production of 5 flowers. When this was followed by a second perturbation at the 24-hour point in the cycle, 26 flowers were produced. The control level of flowering, plants which received an uninterrupted dark period, is represented by a solid horizontal line at the point corresponding to the number of flowers produced.
not be overcome by an additional 2 hours of light given later in the cycle. Two hours of light at the 24-hour point in the cycle results in some stimulation of flowering above the control level. When this perturbation is followed by a second 2-hour perturbation at the 28-hour point in the cycle, the level of flowering is more than twice that of the control plants. As in the first experiment, when the second perturbation was given at the 40-hour point in the cycle, little or no flowering was observed with any combination of light perturbations (fig 3). In this experiment there is significant interaction between the 2 perturbations and therefore it seems likely that each 2-hour perturbation produces weak rhythms which interact with each other but these weak rhythms are not strong enough to cancel out the basic rhythm caused by the 8-hour light period.

Experiment 3, Effect of Two 2-Hour Perturbations in a 72-Hour Cycle. The results of the previous experiment indicate that a second peak of flowering may occur at the 48-hour point in a cycle if the 2 perturbations are properly spaced. In an attempt to determine this second peak of flowering, the plants were exposed to a 72-hour cycle initiated by an 8-hour photoperiod and the dark period interrupted with 2 perturbations each of 2 hours duration. The response of the plants to 7 repetitions of the experimental treatment are plotted in figure 4. As the first 2-hour perturbation is given nearer to the 48-hour point in the cycle, the second peak of flowering is shifted from 48 to the 52-hour point and ultimately to the 56-hour point in the cycle. Moreover, when the perturbations are separated by 12 hours, a decrease in the flowering response is obtained. It appears that the first perturbation may initiate a weak rhythm that is interacting with the second perturbation to some extent.

Experiment 4, Effect of Two Perturbations of

![Graph](https://example.com/graph.png)

Fig. 4. Experiment 3. The effect of two 2-hour light perturbations given during the dark period of a 72-hour cycle on the flowering response of Biloxi soybean. A solid triangle represents the first perturbation in each instance. It is connected to the point of the first following perturbation by a dotted line. The solid horizontal line represents the control level of flowering, plants which were given an uninterrupted dark period. The bars at the top of the graph represent the photophil and photophobe phases of the basic rhythm. The dark areas correspond to the photophobe phases. Graph A shows the response of the plants when the first perturbation was given at the 20-hour point in the cycle. Graph B shows the response when the first perturbation was given at the 24-hour point. The remainder of the graphs show the response of the plants when the first perturbation was given at the indicated times, 4-hour intervals from 24 to 60 hours.
Different Durations on the Flowering Response of Soybean. Experiment 1 indicated that 30-minute perturbations are primarily inhibitory to flowering in Biloxi soybean. The results of experiments 2 and 3 indicated that 2-hour perturbations may be highly stimulatory to flowering. The present experiment was designed to attempt to determine the extent of the interaction between the 2 perturbations. The first perturbation was given as a 3-minute interruption and the second as a 4-hour perturbation; thus the total amount of light to which the plants were exposed was 3 minutes longer in this experiment than in the previous 2 experiments. The plants were subjected to a 48-hour cycle, initiated by an 8-hour photoperiod and the dark period was interrupted at various times. The results of experiment 4 are plotted in figure 5 to show the relationship of the perturbation to the time in the cycle that the light was given. A 3-minute perturbation at the 16-hour point in the cycle is completely inhibitory; however, this inhibition may be partially overcome by a 4-hour perturbation at either the 24- or 28-hour points in the cycle. When the second perturbation was given 12 hours before the end of the cycle (36-hour point in experiments 1, 2 and 4, and the 60-hour point in experiment 3), the level of flowering is markedly decreased below the controls and in many cases the level of flowering is zero. The degree of stimulation in this experiment is much less than in experiment 2, when the 2 perturbations were given at the same points in the cycle, even though the total amount of light given to the 2 groups of plants in the separate experiments was approximately the same.

Discussion

Light perturbations given during long dark periods may stimulate or inhibit flowering depending upon the time in the cycle that the light is given, the duration of the perturbation, and the intensity of the light. Figure 6 depicts diagrammatically the postulated response of Biloxi soybean to light perturbations given during a 48-hour cycle. This curve postulates that the 8-hour photoperiod which initiates each

![Diagram](https://example.com/diagram.png)

**Fig. 6.** Diagrammatic representation of the basic endogenous rhythm in Biloxi soybean. Figure A shows the basic rhythm divided into alternating 12-hour periods. The empty rectangles correspond to the 8-hour photoperiod. Each graph contains 2 photoperiods in order to complete the curve. Any light given during the photophile phase is inhibitory and light given during the photophobe phase may be stimulatory. Figures B, C, and D represent the effect of 30-minute, 2-hour and 4-hour perturbations on the rhythmic flowering response of Biloxi soybean respectively. The horizontal line in the last 3 figures corresponds to the control level of flowering. (See text for explanation.)
cycle induces a basic endogenous rhythm (fig 6A). During such a cycle, there are presumably two 12-hour phases during which light may be stimulatory. These have been called photophil phases. These photophil phases alternate with two 12-hour phases during which light is inhibitory, the photophobe (or scotophil) phases (1).

During the photophil phases, the duration of the perturbation has a pronounced effect on the flowering response of Biloxi soybean as shown in figure 6B, C, D. Thirty minutes of light during the second photophil phase may be slightly inhibitory or nonstimulatory, while 2- or 4-hour perturbations produce a marked stimulation in the flowering response. Any light given during the photophobe phases is inhibitory. Thus, at the 16-hour point in the cycle, any light results in total inhibition regardless of the duration, and light at the 20-hour point in the cycle is partially inhibitory with any duration of light. Light at the 16-hour point is also totally inhibitory to flowering regardless of the total cycle length (6).

The effect of 2 perturbations, in an otherwise long dark period, presents a more complex problem. In all cases where 2 perturbations were used, if either perturbation fell in the photophobe phases, inhibition of the flowering response occurred. When two 30-minute perturbations are given in the middle of the second photophil phase, no significant stimulation occurs (fig 1). On the other hand, 2 perturbations, each of 2-hours duration, given in the second photophil phase of the rhythm raises the level of flowering to more than twice that of the control plants (fig 3). When the first perturbation is 3 minutes and the second is 4 hours, the total light given to the plants is longer by 3 minutes than in the previous experiment. Yet the response of the plants to such treatments is very different. When both perturbations are given in the second photophil phase of the rhythm, some stimulation of flowering is obtained (fig 5). However, this stimulation of flowering is much less than that observed when both perturbations are 2-hours in duration.

When 2 perturbations are given, one in a photophobe phase and one in a photophil phase, the inhibition caused by the photophobe interruption may be only partially overcome by prior or subsequent illumination in a photophil phase. This partial recovery only takes place when the perturbation given during the photophil phase is 2 hours or more in duration. Even 3 minutes of light given during a photophobe phase causes an inhibition that may not be overcome by 4 hours of light in the photophil phase. In all cases, it is clear that even a 4-hour interruption does not destroy the basic rhythm induced by the 8-hour photoperiod.

When the cycle length is extended from 48 to 72 hours, one is provided with a third photophil and photophobe phase. The flowering response of plants subjected to such a cycle and with the dark period interrupted with two 2-hour perturbations is shown in figure 4. When the first perturbation is given in the photophobe phases, a decrease in the number of flowers produced below the control level is observed. When such a perturbation is followed by a second 2-hour perturbation in a subsequent photophil phase, the level of flowering is increased. When the first perturbation is given in a photophobe phase, some stimulation in the flowering response is produced. When this is followed by a second perturbation in a photophobe phase the flowering response of the plants is inhibited below the control level. In addition, when the first perturbation is given between the 24- and 40-hour points in the cycle a second perturbation 12 hours later is markedly inhibitory. This indicates that there is a strong interaction between the 2 perturbations when both are given near the middle of the long dark period.

These results indicate that any light in the photophobe phases of the rhythm is inhibitory and light in the photophil phase may be stimulatory if the duration of the perturbation is sufficient. Three minutes or 30 minutes of light are inhibitory or nonstimulatory at all points in the cycle. The flowering response of Biloxi soybean to 2 perturbations given during the dark period of a 48-hour cycle follows a pattern that would be expected on the basis of alternating photophil and photophobe phases, each of 12-hours duration, as postulated by Bunning (1). The response of the plants to 2 perturbations in a 72-hour cycle also follows closely this curve of alternating photophil and photophobe phases. A 2-hour perturbation may initiate its own rhythm and light during the photophobe phase of this secondary rhythm would tend to be inhibitory. Thus, figure 4 shows that a second 2-hour perturbation given 12 hours after the first in the middle portion of a 72-hour cycle results in a decrease in the flowering response. In other words, the basic endogenous rhythm may be interacting with weaker rhythms initiated by the perturbations. In addition to this sensitivity to light, there may be an interaction between 2 perturbations given near the end of the cycle and the next photoperiod.

One may conclude, therefore, that the 8-hour photoperiod obviously controls the basic or fundamental rhythm. While perturbations given during long dark periods may influence the response of the plants to this endogenous rhythm, they are not able to appreciably alter the basic rhythm initiated by the main light period.

**Summary**

Biloxi soybean plants were subjected to 48- and 72-hour cycles and the dark periods interrupted with 2 perturbations of various durations. In experiments employing a 48-hour cycle with an 8-hour photoperiod the flowering response of the plants follows a basic pattern of 12-hour photophil phases alternating with 12-hour photophobe phases. If either perturbation is given in a photophobe phase of the rhythm, the flowering response is inhibited. When 2 perturbations of 2-hours duration were given in a photophobe phase, the flowering response was stimulated. When 1 perturbation is given in a photophobe phase and
the other in a photophil phase, the inhibition caused by the interruption in the photophobe phase can only partially be overcome by a light interruption in a photophil phase either before or after the photophobe perturbation.

Two-hour perturbations may initiate weak rhythms which interact with one another to some extent, however, they are not capable of altering the basic endogenous rhythm which is initiated by the 8-hour photoperiod.

Literature Cited


Regulation of Photosynthesis in Chlamydomonas reinhardi 1, 2

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Phototrophic growth is possible only in those organisms that possess photoreceptors, photopigments, the photosynthetic electron transport chain, and the reductive pentose-P cycle or other biochemical pathway for carbon reduction. There are many different organisms, however, that are facultative phototrophs, for they can grow as heterotrophs when provided with an appropriate source of reduced carbon as an energy source.

The activity of one enzyme of the reductive pentose-P cycle, ribulose 1,5-diP (RuDP) carboxylase, is a function of growth conditions in several organisms (2, 3, 8). This enzyme catalyzes the reaction of CO₂ with RuDP in photosynthetic organisms (4, 6, 12, 19). Fuller and Gibbs (2) showed that the activity of this enzyme in albino mutants of barley was low compared to that found in green barley. Dark-adapted or streptomycin-bleached Euglena were found to contain lower RuDP carboxylase activity than green, light-grown Euglena. Chlorella variegata contained high levels of RuDP carboxylase activity when grown as a phototroph, but heterotrophic growth, either in the light or in the dark, resulted in both a decreased chlorophyll content and decreased RuDP carboxylase activity. Fuller et al. (3) have also shown that the presence of sodium acetate or malate in the growth medium of Chromatium results in a decrease in RuDP carboxylase activity. Lascelles (8) demonstrated that the synthetic of this enzyme occurs at about the same rate as bacteriochlorophyll synthesis in Rhodopseudomonas spheroides. Growth conditions which resulted in a cessation of bacteriochlorophyll synthesis also resulted in an immediate cessation of RuDP carboxylase synthesis.

These observations suggest that it should be possible to investigate the cellular regulation of photosynthesis by examining chloroplast formation, pigment synthesis, the level of activity of certain enzymes of photosynthesis, and the activity of the photosynthetic electron transport chain under conditions of phototrophic and heterotrophic growth. To this end, we have initiated an investigation concerned with the regulation of photosynthesis in the unicellular green alga Chlamydomonas reinhardi. The purpose of this report is to describe the effect of growth conditions