The Flowering Response of Coleus in Relation to Photoperiod and the Circadian Rhythm of Leaf Movement

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Abstract. The flowering response of Coleus frederici and Coleus blumei × C. frederici is dependent on the photoperiod; both plants have a critical day length of about 12 hr. The inductive phase, defined as the period when light signals inhibit floral development, started 10 hr after the onset of darkness under 4 and 8-hr photoperiods, and 8 hr after the onset of darkness under a 12-hr photoperiod. However, a fixed temporal relationship between the inductive phase and the minimum leaf position was observed for Coleus frederici. The inductive phase always started 5 hr after the minimum leaf position. This evidence supports the theory that a circadian clock participates in the time measurement process of photoperiodic floral induction.

It is generally agreed that photoperiodism in both plants and animals is a phenomenon which involves a process of time measurement (see, for example, 16, 23). However, the mechanism of the time measurement process is still disputed. To explain photoperiodic induction of flowering in higher plants, the 2 principal theories that have been advanced are the hour glass and the coincidence theory. The first one advocates a process that is initiated at the beginning of each dark period, mainly considered to be a pigment conversion process (i.e., Phytochrome FR to Phytochrome R), which measures the length of time required to attain the critical day length (1, 16).

The coincidence theory hypothesizes that a circadian rhythm is used by the organism as a time scale (2, 3, 6, 7, 10, 13, 19, 20, 21). In nature light entrains the rhythm and controls its phase. The coincidence of an inductive phase of the rhythm with the main light period controls the future development of the organism.

One difficulty in providing evidence for the theory that the time measurement process in photoperiodism involves circadian rhythms stems from the fact that in very few instances have both phenomena been studied with the same organism. In higher plants, a circadian pattern in floral induction has been demonstrated in Xanthium (22), Pharbitis (14), Soybea (10), Chenopodium (6, 7), and Lemma (12, 13); however, there is no report in the literature of an overt circadian rhythm in these plants. On the other hand, most of the literature dealing with the nature of the circadian rhythm in plants is based on the day neutral plant, Phaseolus multiflorus (4). The only plant in which both phenomena have been studied is a short day plant Kalanchee blossfeldiana, that exhibits an overt circadian rhythm of petal movement (5, 8). The experiments with Kalanchee have neither proven nor disproven that the time measurement process of the photoperiodic response involves the clock which controls the circadian rhythm.

It is the purpose of this paper to present experimental evidence to support the model suggested by Pittendrigh and Minis (21) in which the inductive phase (the time of the cycle when light has a photoperiodically inductive effect) is correlated with the phase of the circadian rhythm (leaf movement). The results cannot be explained by a simple hour glass mechanism. Furthermore, they agree with Pittendrigh and Minis’s model (21) in that the inductive phase lies at the end of the main dark period rather than at its beginning as was suggested by Bunning (2, 3).

Materials and Methods

C. frederici and C. blumei × C. frederici (later designated as C. hyb.) plants were propagated by cuttings and grown in the greenhouse under continuous light as described before (9).

Most of the experiments were conducted in growth chambers at 21 ± 1° (9). The light signals, of 200 ft-c intensity, were provided by a 40 watt cool-white fluorescent tube and a 150 watt incandescent bulb. For the experiments in which C. frederici was grown under 4- and 8-hr daylengths with night interruption by light signals, special cabinets were used. For these experiments the temperature was 28 ± 2° and a 40 watt cool-white fluorescent bulb (13'36 T12 CW GE) provided about 200 ft-c of illumination.

At the end of the experiments, the apical meristems of the plants were examined under a dissecting}

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microscope for the determination of floral induction. Vegetative apices of Coleus exhibit the typical appearance of a flat meristem. Development of the reproductive phase was first indicated by a broadening of the apex, which later develops successive pairs of bracts. When 2 bracts are fully formed and another 2 are just starting to emerge, the length of the transformed apex is about 0.2 mm. When 6 bracts can be detected, the length is about 0.3 mm. In a 0.4 mm long inflorescence there are 8 to 10 bracts; later on the bracts are all tightly folded one above the other and it is difficult to count them precisely any more. Most of the experiments were duplicated and provided similar results.

Recording of leaf movement was done as described before (9).

Results

**Flowering and Leaf Movement Rhythm Under Various Photoperiods.** The purpose of the following experiments was to determine the critical daylength for floral induction of both *C. frederici* and *C. blumei* x *C. frederici* and in parallel to follow the pattern of their leaf movement rhythm under different daylengths. Kribben (15) reported that both species are short-day-plants with a critical day length of 12 to 13 hr. Since his experiments were done in the greenhouse under natural light conditions, I repeated them under controlled environmental conditions.

Figure 1 indicates that the critical day length for *C. frederici* was above 12 hr and just below 13 hr; *C. hyb.* reacted similarly. *C. frederici* plants grown under a 13-hr photoperiod showed a very early stage in the formation of a reproductive apex, i.e., broad-

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**Fig. 1.** Flowering response of *Coleus frederici* under 24-hr light-dark cycles of different daylengths. The number in parentheses near each closed circle indicates the number of cycles the plants experienced under that photoperiod. Each point is the average of about 15 plants.

**Fig. 2.** (top) The leaf movement rhythm of *Coleus blumei* x *C. frederici* and *C. frederici* under 24-hr light-dark cycles of different photoperiods. The onset of light occurs at hr 0800 eastern standard time. Vertical guide lines indicate hr 2400. Numbers on the left side indicate the daylengths in hr.

**Fig. 3.** (bottom) Plot of the time of mean minimum leaf position of *C. frederici* and *C. hyb.* under different daylengths (ordinate). Diagonal line designates the onset of darkness. Each point is an average of 3 to 5 plants for 4 cycles, starting on the second day in the light-dark conditions. The standard error of each point is within the range of ± 0.00 to ± 0.35.
ening of the apex, but did not progress beyond this stage even after 29 cycles. When plants at that stage were transferred to the greenhouse, they stayed vegetative for a period of 2 months.

The phase of the circadian rhythm as measured by the time of minimum leaf position changed slightly relative to the onset of the dark period. The time between the onset of dark and the minimum leaf position was about 5 hr under 2 to 8-hr photoperiods and about 3 hr under 12 to 16-hr photoperiods in the case of C. frederici.

A similar trend was observed for C. hyb. as shown in figure 3.

The phase relationship between the minimum leaf position and the light cycle persisted for 4 cycles in constant dim light. This supports the assumption that the phase of the overt rhythm indicates the phase of the light sensitive oscillator.

Flowering and Leaf Movement Rhythm Under Various Daylengths With Night Interruptions by Light Signals. The following experiments were designed to locate the inductive phase, i.e., the clock time when illumination inhibits flowering, under 4, 8, and 12-hr daylengths. It was expected that the inductive phase would be determined by the circadian rhythm of leaf movement. Since C. frederici is faster to flower than C. hyb. (19 cycles compared to 40), experiments were restricted to C. frederici.

Figures 4 to 6 show the shift of the inductive phase in different photoperiods. While inhibition of floral induction by light signals started 10 hr after the onset of dark under 4 and 8-hr daylengths, it started 8 hr after the onset of dark for a 12-hr daylength. However, the onset of floral inhibition always occurs 5 hr after the time of the minimum leaf position under each photoperiod.

In addition, there was a lengthening of the inductive phase under increasing photoperiods. One can estimate that the duration of the inductive phase under a 4-hr photoperiod is no more than 1 hr, while under a 8-hr photoperiod it is probably over 1.5 hr, and for a 12-hr photoperiod it is clearly over 2.5 hr. Estimates are based on those portions of the cycles covered by the light signals which inhibited flowering maximally.

The interruption of the dark period with a light signal (table I) had a very slight effect on the phase of the circadian rhythm when the light signal was given after the time of the minimum leaf position.

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**Fig. 4.** (top) Flowering response of C. frederici under a 4-hr photoperiod with 60 min light signals interrupting the night at different clock time (indicated by the bottom scale as EST). Empty and dark bars indicate the light and dark periods of the cycle, respectively.

**Fig. 5.** (middle) Flowering response of C. frederici under 8-hr photoperiod with 30 min light signals interrupting the night at different clock times.

**Fig. 6.** (bottom) Flowering response of C. frederici under 12-hr photoperiod with 30 min light signals interrupting the night at different clock times.
On the other hand, when the light signal was given before the minimum position of the leaves, the phase of the rhythm was delayed in such a way that the termination of the light signal simulated the comparable full photoperiod. For example, *C. frederici* plants under a 4-hr daylength with a light signal at hr 1500 to 1600 eastern standard time showed a mean minimum leaf position at hr 20;31 ± 0.19 simulating a full photoperiod of 8:16.

An advance of the phase occurred in only 1 case, when the light signal started about the time of minimum leaf position. In this case an advance of about 4 hr was observed.

All the light signals that inhibit flowering fall after the minimum leaf position, so they do not affect the phase of the circadian rhythm; when light signals do shift the rhythm, they do not effect a photoperiodic response since the direction and the amount of phase shift is such that the inductive phase remains in the dark.

Conclusions and Discussion

The literature on plant photoperiodism has shown that flowering may follow a circadian pattern, and gives an indication that the control of flowering might depend on the circadian rhythm, but it does not show direct evidence for a correlation between the 2 phenomena. It is demonstrated here, for the first time, that the inductive phase of the short day plant *C. frederici* has a fixed phase relationship to the phase of the circadian rhythm of the plant. Under different photoperiods maximum inhibition of floral induction by light signals always started 5 hr after the minimum leaf position (but 10 hr from onset of darkness under 4 and 8-hr photoperiods and 8 hr under a 12-hr photoperiod). If an hour glass mechanism were operating here as the time measurement process, the inductive phase would be timed from the onset of darkness and would thus show a fixed relation to it under different photoperiods [as was suggested, for example, by Bonner (1)].

However, it should be noted that such a correlation has been shown in insect photoperiodism, mainly with *Pectinophora gossypiella* (18, 21) and bird photoperiodism (11, 17). These results follow the main idea expressed before, namely that the position of the inductive phase relates to the phase of the overt circadian rhythm.

The results shown in figures 4 to 6 also suggest that the duration of the inductive phase lengthens under increasing photoperiods. Considering these 2 effects of prolonged photoperiods on the inductive phase, i.e. delaying the phase and increasing its duration, one could predict that under a 13-hr photoperiod, part of the inductive phase will be already illuminated by the beginning of the main photoperiod, and therefore inhibition of floral development takes place.

The Precision of the Clock. Although photoperiodic induction of flowering is considered to be an all-or-none phenomenon, the developmental rate of the reproductive stage appears to be a quantitative one (1). Quantitative reduction in floral development was observed under photoperiods only slightly longer than the critical daylength as for example a 13-hr photoperiod in the case of *Coleus* plants (fig 1), or as a result of experiments with light signals falling near the inductive phase (fig 4). The idea generally proposed to explain this phenomenon was that, under such circumstances, less "florigen" is produced during each cycle (1).

Another explanation is suggested here, in which the precision of the biological clock is considered.

| Table I. Time of Minimum Leaf Position in a 24-hr Light-Dark Cycle When the Night is Interrupted With a Light Signal |
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| Photoperiod | Time at which light signal is commenced | Mean time of minima EST ± S.E. |
| **a. Coleus blumei × C. frederici** | | |
| 8-hr | 2000 | 22.26 ± 0.38 |
| 8-hr | 2400 | 23.67 ± 0.18 |
| 4-hr | 2200 | 22.50 ± 0.37 |
| 4-hr | 2200 | 21.00 ± 0.36 |
| **b. Coleus Frederici** | | |
| 8-hr | 1830 | 18.38 ± 0.19 |
| 8-hr | 1500 | 17.12 ± 0.19 |
| 4-hr | 2200 | 16.25 ± 0.29 |

Fig. 7. Frequency distribution illustrating variation in time of minimum leaf position in light-dark cycles relative to the time of most frequent occurrences of the minima (Hr zero). Empty bars: *Coleus Frederici*; Dark bars: *Coleus blumei × C. Frederici*. 

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Examination of the circadian rhythm of leaf movement of individual plants for successive cycles under full photoperiod shows that the time of the minimum leaf position is not precisely the same in each cycle. Analysis of variance has shown that variability in time of minimum leaf position for individual plants on successive days is the same as the variability between plants on the same day (over 99% level of confidence). This justifies pooling the data for minimum leaf position recorded under different photoperiods, designating as time 0 the time where the peak in number of minima occurred. The plot of these data (fig 7) shows that they have a bell shaped distribution around the mean. Assuming that the inductive phase is determined by the phase of the circadian rhythm, the existence of small errors or fluctuations in the timing of the inductive phase may play a role under photoperiods which are close to the critical daylength. Induction of flowering under such circumstances will occur only when the circadian rhythm is slightly advanced; in all the slightly delayed cycles the terminal part of the inductive phase will be illuminated by the succeeding main light period. For example, with a 13-hr photoperiod the observed developmental stage after 29 cycles is a broad apex for Coleus fridrici plants. On the other hand, this developmental stage may occur with as few as 15 or 16 cycles of maximally inductive photoperiods. Consequently, it is suggested that only 15 to 16 cycles of the 29 given with a 13-hr photoperiod were fully inductive and the rest were entirely non-inductive. This reasoning may also explain why complete inhibition of floral induction did not occur in the night-interruption experiments with a 4-hr photoperiod. In these cases, the 1 hr duration of the inductive phase might be so short that the light signal “missed” the inductive phase occasionally, thus resulting in partially inductive cycles.

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Literature Cited