Photoperiodism: An Effect of Darkness during the Light Period on Critical Night Length

Abstract. *The critical night length of the short-day plant* Lemna perpusilla, *grown with sucrose, increases roughly 3 hours under cycles with "light periods" composed of darkness preceded and ended by brief exposures to light. Although plants so grown are white, the effect is due neither to the absence of photosynthesis nor to insufficient total energy. It is inconsistent with current ideas on photoperiodic timing but may be explained by a hypothesis based on reported properties of phytochrome.*

An understanding of the role of phytochrome, the photoperiodic pigment (*1*) in flowering, will require in vivo assays of its state and concentration at various times during photoperiodic cycles. Since such assays are as yet impossible in green tissue (*1, 2*), a plant with little chlorophyll, in which flowering is under precise photoperiodic control, would be valuable. *Lemna perpusilla* 6746, in axenic culture, flowers as a short-day plant when grown in appropriate media; vegetative growth and flowering occur readily under exposure to a few minutes of red light per day if sucrose is supplied; under these conditions the plants are white (*3*). Thus the only element apparently lacking in this system is confirmation of the critical "day" or "night" lengths that are found under photosynthetic conditions (*3, 4*). The experiments described here were expected to provide such confirmation but led instead to a hitherto unreported photoperiodic phenomenon which may have general significance for any hypothesis of photoperiodic timing.

Each culture was started with one vegetative 3-frond colony raised under long-day conditions and grown for 7 days in 50-ml erlenmeyer flasks with about 25 ml of Hutner's medium (half strength), *p*H 6.3, and 1 percent sucrose at 27° to 28.5°C. Light was supplied by three 15-watt red fluorescent tubes behind a single layer of red cellophane and at a distance of 55 cm above the medium. At the end of the 7-day experimental period, the percentage flowering (FL%) was evaluated by dissection. Details of this and all other methods and materials have been published (*3*).

Preliminary experiments showed that a schedule of 1¼ hours of light and 22¾ hours of darkness, hereafter designated 1¼(22¾), gave values for percentage flowering of 25 or above; all new fronds produced remained white. Thus, to determine critical night length under nonphotosynthetic conditions, one might use 24-hour cycles with interrupted light periods, for example 1 hour of light followed by an undetermined period *S* followed by ¼ hour of light, followed by an undetermined period *N*, where *S* and *N* are dark and *N*, to be called night length, is greater than *S*. The cycle may be designated 1(*S*)¼(*N*). However, it seemed unwise simply to assume that the critical value of night length so determined would agree with that obtained with uninterrupted light periods, that is when *S* is light. A series of experiments for testing such agreement was performed (Fig. 1). Since only three light cabinets were available, and since absolute values for percentage flowering vary from experiment to experiment, each experiment compared two cycles of the form 1(*S*)¼(*N*), at a given value of *N*, but with *S* either light or dark, with the standard cycle 1¼(22¾), that is $S = 0$. The results are presented by taking the value for percentage flowering of the standard cycle in each experiment as 100.

The curve for S = light (uninterrupted light periods) essentially confirms previous work (*3, 4*). The slight shift toward longer night lengths, if real, may be due to the use of red instead of white light. However the curve for interrupted light periods—S = dark—is shifted roughly 3 hours in the direction of longer night lengths.

This phenomenon may be explained by the fact that, as intended, new growth under the interrupted light periods was white while it was green under the uninterrupted light periods. The longer critical night might thus be associated with an unsatisfied "high intensity light requirement," presumably photosynthetic (*5*). This can be tested by determining whether the low flowering obtained, for example, under the cycle 1(9¾)¼(13), is raised by interrupting the dark interruption itself with light while at the same time keeping night length and total light energy constant—for example, by testing the cycle ¼(2½)¼(2½)¼(2½)¼(2¼)¼(13). An experiment of this sort is shown in Fig. 2 and indicates that the dark interruption is ineffective when it is itself interrupted by light at frequent intervals. Growth under all cycles shown in Fig. 2 was white. Hence the phenomenon described here is not related to either photosynthesis or total light energy but depends on the time relations of dark and light exposures. This poses a problem for the current hypotheses of photoperiodic timing.

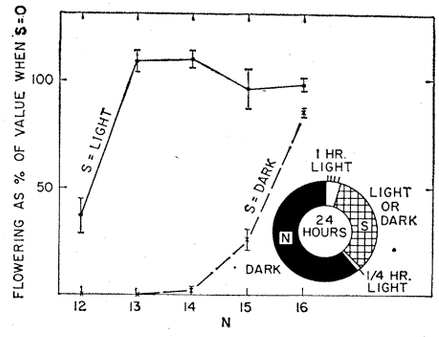

Fig. 1. Flowering of *Lemna perpusilla* 6746 as a function of night length (*N*) after seven 24-hour cycles of the kind indicated in the inset. Both points for each value of *N* are given as percentages of the percentage flowering (FL%) produced under the standard cycle ($S = 0$) in the same experiment. Each point shows the means of five cultures, with brackets equal to twice the standard error.

An interpretation under Bünning's concept of endogenous circadian rhythm (*6*) would suggest that the interrupted light periods inhibit flowering by exposing the plant to darkness during its photophile, or light-requiring, phase. It would not explain, however, why flowering occurs under the schedule 1¼ (22¾), or why the "inhibition" disappears so rapidly with increasing night length.

The hypothesis of photoperiodic timing so far developed from work on phytochrome (P) relates the critical night length to the time required for the far-red absorbing and presumably

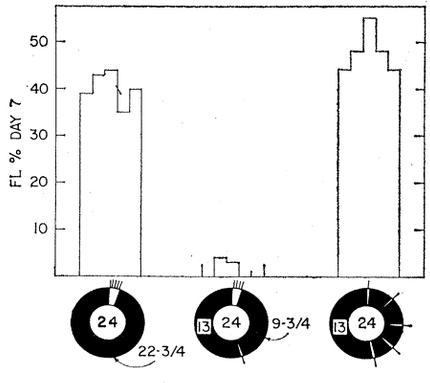

Fig. 2. Flowering under the light (dark) schedules 1¼(22¾), 1(9¾)¼(13), and ¼(2½)¼(2½)¼(2½)¼(2¼)¼(13). Divisions of bars represent replicate cultures. Range of frond numbers per culture in each treatment: 61 to 73, 68 to 80, 56 to 68. Each projecting line on the cycles represents ¼ hour of red light.

active form of the pigment, P_{FR}, to revert to the red-absorbing form, P_R, in darkness (*1, 7*). The assumption is that in short-day plants P_{FR} must remain below a certain critical level for a given time for flowering to occur; light acts only to maintain or restore a high level of P_{FR}. On this hypothesis, once photosynthetic interactions are eliminated, there is no reason why flowering should occur readily under the schedules 11(13) or ¼(2½)¼(2½)¼(2½)¼(2¼)¼(13) but not under 1(9¾)¼(13). An explanation of this phenomenon in terms of phytochrome may nevertheless be at hand.

The Beltsville group has reported (*2, 7, 8*) that in some intact etiolated tissues the presence of P_{FR}, brought about by red light, causes an apparent decrease in the total level of phytochrome, at least as assayed by in vivo reversibility. While the actual fate of the phytochrome is completely unknown, these observations can be taken at face value as representing a decrease of phytochrome level after treatment with light; on this admittedly speculative basis, the present results with *Lemna* become explicable. Those plants on schedules in which the "light period" consists largely of uninterrupted darkness would be expected to have more phytochrome than plants on schedules with the usual light periods, since, during the latter, the phytochrome level should decrease. Thus the plants on the first type of schedule would start each night with a higher total phytochrome level, all of it in the P_{FR} state because of the final light exposure. It would then require a longer night to allow the P_{FR} level to fall below the critical level, by reversion to P_R; hence there is a shift in critical night length (Fig. 1).

Whether or not this is a valid hypothesis will only be determined by direct in vivo assays and extended kinetic studies of the phenomenon itself. A general hypothesis of photoperiodic timing based on the interaction of phytochrome state (P_R or P_{FR}) and total photochrome level, the type proposed here, has obvious advantages in analyzing many complex light-dark interactions now inexplicable and possibly also certain anomalies in the red, far-red responses of plants such as *Pharbitis* seedlings (*9*) or *Lemna perpusilla* itself (*4*). However, the relevant data are at present too scanty to justify an extended discussion.

There are few reports in the literature comparable with the present observations. Krumwiede (*10*), using the short-day plant *Kalanchoe* grown photosynthetically, found a ¼-hour increase in the critical night length when part of the light period was of reduced intensity.

More relevant are the results of Könitz (*11*) who observed a marked inhibition of flowering in the short-day plant *Chenopodium* when the main (13-hour) light period was interrupted by treatments with far-red light for varying periods. In the controls a light period was interrupted with dark for up to 4 hours; such interruptions were ineffective or else produced only slightly increased flowering. Longer interruptions might have given results similar to those reported here, though always with the difficulties and ambiguities encountered when plants are grown under photosynthetic conditions. Könitz concluded that the photophile phase was highly sensitive to far-red light, but his results are equally consistent with the more concrete hypothesis suggested here.

Confirmation of these observations on many plants may prove difficult unless interactions with photosynthesis are eliminated. For this reason, *Lemna* and other small plants that can be grown under axenic, nonphotosynthetic conditions should be further exploited for analyses of the mechanism of photoperiodic timing, especially in relation to the phytochrome system (*12*).

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Rhodium-102 Fallout: Variations in Deposition and Concentrations in Precipitation

Abstract. *Rhodium-102 was produced as a tracer for U.S. high-altitude detonations in August 1958 and has been detected and monitored in precipitation since October 1960. Between January and September 1961, when atmospheric tests were resumed, the contribution of this high-altitude debris in fallout increased.*

Rhodium-102 was produced as a tracer in the Orange shot of the U.S. "Hardtack" nuclear test series 11 August 1958 (*1*). This detonation was a high-altitude air burst at about 43 km above Johnston Island (lat 16°N, long 170°W) (*1*). It was hoped that this nuclide could be used as a unique tracer for debris injected into the higher stratosphere. In a similar manner W^{185} was used to trace lower stratospheric equatorial debris (*2*). The debris was in all probability placed at an altitude above 100 km (*1*).

The total amount of the Rh^{102} injected was generally estimated to be about 3 megacuries (Mc) (*1, 5*). In addition about 0.3 Mc of Rh^{102} from other U.S. tests and some from Soviet sources (*1*) could have contributed to material deposited from the stratosphere in 1958–59.

Scientists have reported (*1, 3–11*) on the Rh^{102} high-altitude tracer experiment and the distribution of the radioactivity in the atmosphere; thus far little information has been presented on the occurrence of Rh^{102} in precipitation. Under the auspices of the U.S. Atomic Energy Commission, Rh^{102} has been monitored in rainwater at Westwood, New Jersey, since July 1960. Data from two other stations (Pittsburgh, Pa., and Richmond, Calif.) are available for a shorter period (*12*).

Volumes of precipitation (obtained with a large rainfall collector) (*13*) were processed because of the expected low concentrations of Rh^{102}. The minimum volume of water processed in any month was 75 liters. The procedures have been described (*14*).

Two known isomers of Rh^{102} were believed to have been produced by the Orange shot, one with a half-life of 210 days and another with