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Photoinduced Seed Germination of *Oenothera biennis* L.

II. ANALYSIS OF THE PHOTOINDUCTION PERIOD

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ABSTRACT

The photoinduction period of *Oenothera biennis* L. seed germination was analyzed by varying the photoinduction temperature and by substituting red light pulses for continuous red light. At 24°C, seeds require 36 hours of continuous red light for maximal percent germination. The optimal photoinduction temperature is 32°C, with higher and lower temperatures being strongly inhibitory. A 30 minute exposure to far-red light, given immediately after a red light period of 1 to 36 hours, reduces germination by about 25%. Seeds escape from far-red inhibition with a half-time of 5 to 10 hours, depending on the length of the red exposure that precedes the far-red light. Periodic 15 minute pulses of red light can substitute for continuous red light in stimulating germination. Ted red light pulses, with 6 hours of darkness between successive pulses, cause maximal germination. The response to periodic red light is fully reversible by far-red light. Probit analysis of the periodic light response shows that as the length of the dark periods between successive pulses increases, less incident light is needed to induce germination but the population variance in light sensitivity remains constant. Probit analysis of the temperature response shows that as the photoinduction temperature increases from 16 to 32°C, less incident light is needed to induce germination and the population variance in light sensitivity also increases.

Oenothera seeds require a long exposure to red or white light for maximal percent germination (9). The fluence-response curve is biphasic, with a small percentage of highly photosensitive seeds and the majority of the seeds requiring longer exposures to light (9). Two light periods, separated by a dark period of up to 16 h, cause about the same percent germination as a single continuous light period which is as long as both light periods and the intervening dark period combined (9). Twelve h of white light followed by 10 h of darkness potentiates a red/far-red reversible germination response in about 30% of the seeds (9).

A single exposure to light of long duration or to periodic light has also been reported to stimulate the germination of *Betula pubescens* (2), *Hypericum erectum* (24), *Eragrostis ferrugina* (13), *Paulownia tomentosa* (3), *Kalanchöe blossfeldiana* (4, 8), *Chenopodium botrys* (6), and other species (16, 17, 23). Based on the responses to periodic light treatments, Isikawa (15) and Black and Wareing (2) independently classified some species as having either LD seeds or SD seeds. It seems likely that the similar effects of light on the germination of *Oenothera* and other species reflect an underlying similarity in mechanism.

The experiments reported in this paper extend those of the preceding paper. They were designed to further investigate the photoinduction period and to improve understanding of the role of phytochrome in *Oenothera* seed germination. Red light was used to stimulate germination and was applied in a single long exposure or in short pulses. The effect of different temperatures during the photoinduction period was also studied.

MATERIALS AND METHODS

The seeds used were the same genetic strain and same harvest of *Oenothera* as used previously (9). They were collected in the first week of October 1983 and were stored at room temperature and humidity until the last week of June 1984. These afterripened seeds were then placed in a desiccator in the dark at 4°C. The germination characteristics of the seeds did not change appreciably during the experimental period of summer and autumn of 1984. They showed the same germination characteristics as the spring seeds used previously (9), with dark germination typically below 5% and maximal germination of about 80%.

Germination studies were conducted as previously described (9) with 50 seeds per Petri dish, and the average percent germination of four Petri dishes reported. All experiments were performed at least twice. Standard errors were uniformly below 5%. In experiments which were conducted over many days, additional water was added to the Petri dishes to replace the water lost by evaporation. A constant temperature of 24°C was used for all studies except those in which the photoinduction temperature was varied. As before, 'germination' is defined as the emergence of the embryonic axis through the seed coat. For all experiments, the dark preinduction period was set at 24 h, which allowed the seeds to develop maximal photosensitivity (9).

For experiment in which the photoinduction temperature was varied, seeds were first dark soaked for 24 h in a 24°C incubator and were then transferred in a dark box to a temperature-controlled growth chamber where they were irradiated. Then, seeds were transferred in a dark box back to the 24°C incubator. Measurements with a thermocouple indicated that the temperature of the seeds equilibrated with the incubator within 5 min after transfer. Final germination was scored 3 d after the completion of the irradiation period.

The same green safe-light (9) was used as before for all necessary experimental manipulations. Red and far-red light sources and energy fluence rates were the same as previously (9).

For two experiments, germination percentages were transformed to probits. Probit transformation gives a log-linear fluence response curve in which the slope of the linear regression varies inversely with the standard deviation of the distribution of light sensitivity in the seed population. Probit linear regressions were calculated by using the regression equation of Finney (9): $Y = a + bX$, where Y = probit percent germination, $X = \log_{10}$ μmol·m^{-2} of red light, b = slope of regression line, and $a = Y$-intercept. The slope of regressions were calculated according to Finney (10): first, a simple linear regression was determined and then three cycles of successive approximations were performed

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in order to account for the differently weighted probit values. Slopes of regression lines were compared by a one-factor analysis of variance.

RESULTS

A reversible phytochrome response was previously detected in about 30% of *Oenothera* seeds that were given 12 h of white light followed by 10 h of darkness (9). This finding posed a question as to whether partial far-red reversibility throughout the 36 h photoinduction period is a characteristic of *Oenothera* seeds. In order to examine this question, seeds were given red light for varying periods of time and irradiated with far-red light for 30 min immediately following the red light period. As a control, seeds were given red light alone for varying periods. The results (Fig. 1) for red light alone are very similar to those reported previously for white light (Fig. 3 in Ref. 9): maximal germination is attained with 36 h of light and the fluence-response curve appears to be biphasic. For red irradiation periods of less than 4 h, far-red light completely reverses the stimulatory effect of red light, reducing germination below the dark control level. As the exposure time to red light is increased from 4 to 36 h, far-red light only partially reverses the stimulatory effect of red light. Seeds given far-red light after red irradiation typically germinated about 15 to 25% less than those given red light alone. Far-red reversal was not detected in seeds given more than 48 h of red irradiation.

In the experiment above, the amount of far-red light which was applied did not completely inhibit the red light stimulation of germination. Thus, far-red saturation was examined by irradiating seeds with far-red light of varying duration immediately after red irradiation periods of 2, 18, or 36 h. The results (Fig. 2A) show that for all three red light treatments, far-red irradiation of 2 min or longer saturates the reversal response. Half-maximal inhibition was attained by 1 min of irradiation (about 1×10^6 ergs·cm^{-2}). Red light applied immediately after far-red light is expected to reverse the far-red inhibition. Thus, the red light saturation was examined as follows: seeds were given an initial red irradiation of 2, 18, or 36 h, then 15 min of far-red light, and finally a second red irradiation for varying times. The results (Fig. 2B) show that, for all three red light treatments, red irradiation of 10 min or longer saturates the reversal of the far-red effect. Half-maximal effect was achieved by about 2 min of red light (about 8×10^6 ergs·cm^{-2}).

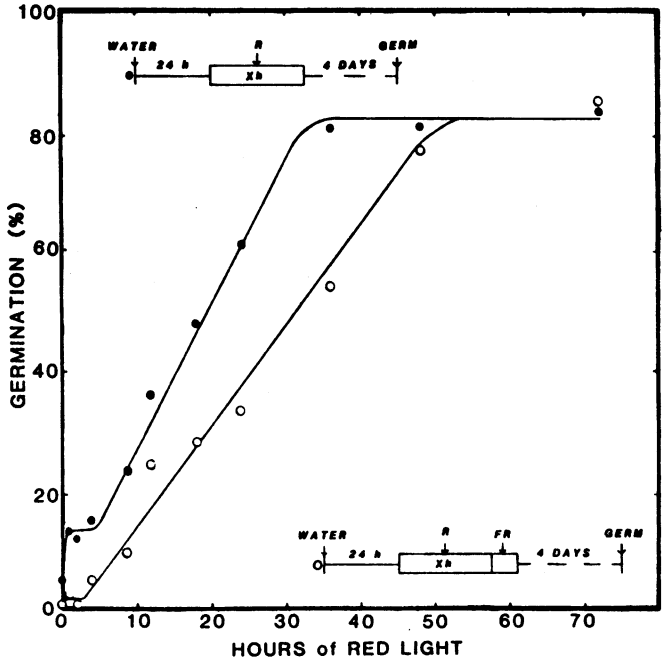

FIG. 1. Effect of far-red light on the reversal of germination induction produced by different times of exposure to red light. Seeds were given red light alone (●) or were given red light and then 30 min of far-red light (○). In this and subsequent figures, the inset diagrams or the diagrams above the figures shown experimental regimes and the × in each diagram is plotted on the abscissa.

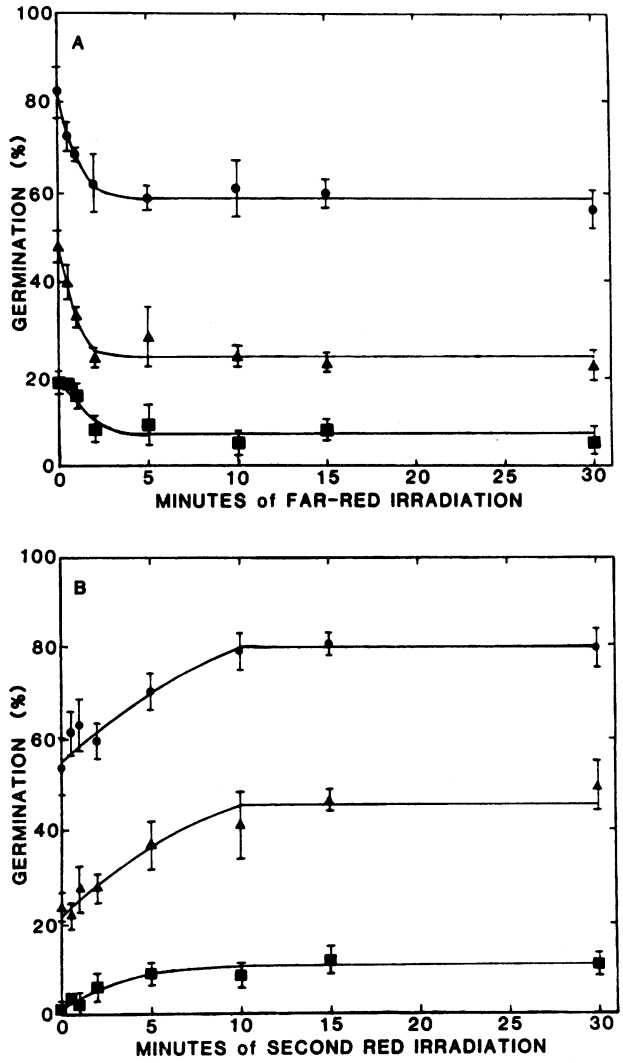

FIG. 2. Fluence response curves for far-red light (A) and red light (B) to reverse red and far-red effects, respectively. In (A), seeds were photoinduced by 2 h red light (■), 18 h red light (▲), or 36 h red light (●) then exposed to far-red light for various times. In (B), seeds were initially given the same red light treatments as in (A), were exposed to 15 min of far-red light, and were then exposed to red light for various times. Vertical bars show standard deviations.

The biphasic fluence response curve that is repeatedly observed (Fig. 1 and Ref. 9) indicates that a small percentage of seeds germinate in response to a brief irradiation of red or white light, and the majority of the seeds germinate only in response to longer irradiation periods. The seeds that germinate in response to a brief irradiation period behave like highly photosensitive seeds (*e.g.* Grand Rapids lettuce) in that the red effect is fully reversible by far-red light. In contrast, the majority of the seeds require long light exposures and the red light induction is only partially reversed by far-red light (Figs. 1 and 2).

The time course of escape from far-red inhibition was next examined. Seeds were first given an initial red light period of 2,

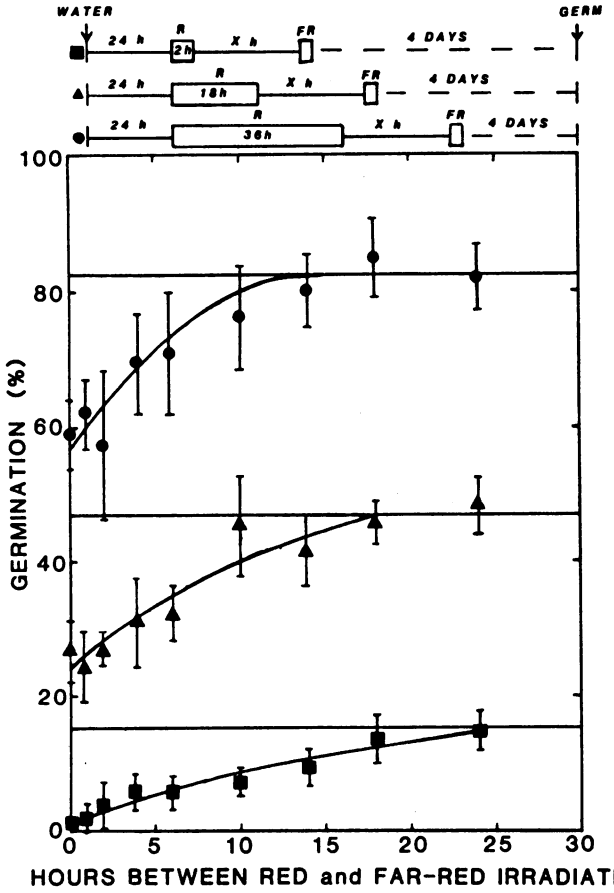

FIG. 3. Escape from far-red inhibition. Seeds were photoinduced by 2 h of red light (■), 18 h of red light (▲), or 36 h of red light (●) and then were given 15 min of far-red light after various periods of darkness. Horizontal lines in the figure are red light controls without far-red light. 2 h red light control: 15.0%; 18 h red light control: 45.5%; 36 h red light control: 82.5%. Vertical bars show standard deviations.

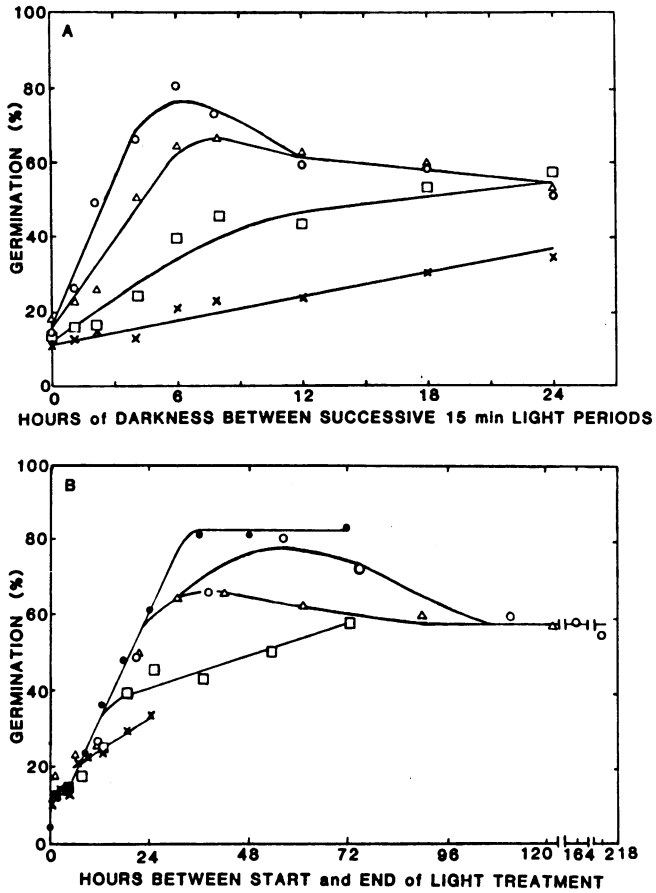

FIG. 4. Effect of 2, 4, 6, and 10 periodic 15 min red light pulses on final germination. A, percent germination plotted as a function of the length of the dark period between successive red light pulses; B, percent germination plotted as a function of the time between the start and end of light treatment and compared with the data for the continuous red light treatment of Figure 1 (●). Seeds were given 2 red light pulses (×), 4 red light pulses (□), 6 red light pulses (△), or 10 red light pulses (○).

18, or 36 h, then were placed in darkness for varying periods before they were given 15 min of far-red light. The final percentage of germination was determined 4 d later. The results (Fig. 3) show that the seeds in all three treatments have fully escaped from far-red inhibition after about 20 h and that those which have completed the escape reaction germinate to the same percentage as the respective controls without far-red light. The half-time of escape for seeds given 2 h of red light is about 10 h. Seeds given 18 and 36 h of red light escape more rapidly and have escape half-times of about 7 and 5 h, respectively. Thus, the small number of highly photosensitive seeds escape from far-red inhibition more slowly than seeds which require a longer exposure to red light for germination.

Two periods of white light, separated by a dark period, stimulate germination more than a single exposure which equals the sum of two light periods (9). Since red light gives the same response as white light (9), it seemed likely that red light given in short pulses would stimulate germination to the same extent as a single continuous exposure to red or white light. Thus, seeds were exposed to 2, 4, 6, or 10 red light pulses, each of 15 min duration. The dark period between successive light pulses was varied from 0 to 24 h. The data are presented in Figure 4A with percent germination plotted as a function of the length of the dark period between successive light pulses.

The results in Figure 4A indicate that: (a) for seeds that received 2, 4, 6, or 10 light pulses without intervening dark periods (*i.e.* for continuous light periods of 0.5–2.5 h), germination is about the same (15%); (b) for seeds that received 2 and 4 light pulses, germination increases gradually as the intervening dark periods increase to 24 h, with maximal germination of 35% for 2 light pulses and of 59% for 4 light pulses; (c) for seeds given 6 light pulses, germination reaches a maximum of 66% with intervening dark periods of 6 and 8 h, and then declines gradually to 57% as the intervening dark periods lengthen to 24 h; (d) for seeds given 10 light pulses, germination reaches a maximum of 80% with intervening dark periods of 6 h and declines slowly to 60% as the intervening dark periods increase to 24 h; and (e) for seeds given 4, 6, and 10 light pulses with intervening dark periods of 24 h, the germination is about the same (55%).

The data in Figure 4A are replotted in Figure 4B together with the fluence response curve of Figure 1 for continuous red light alone. The percent germination is plotted as a function of the total time between the start and end of irradiation, *i.e.* the total length of the photoinduction period. For each of the four periodic light regimes, the results indicate that when the intervening dark periods are short, the percent germination is similar to that of the continuous light treatment. However, when the intervening dark periods are long, the fluence response curves for the periodic light regimes deviate from the fluence response curve for continuous light. After the points of deviation, germination increases linearly for the 2 and 4 light regimes, whereas it slowly reaches a maximum and is followed by a decline for the 6 and 10 light regimes.

One noteworthy point in Figure 4B is that the periodic light regimes induce germination with far less incident light energy than a single exposure to continuous light. Thus, a light pulse apparently stimulates certain processes which sensitize the seeds to subsequent exposure to light. These processes occur in continuous light and in the dark periods between successive light pulses. If metabolic (*i.e.* nonphotochemical) processes are responsible for the sensitization process, then incubating the seeds at different temperatures during the photoinduction period should have an effect on germination.

In order to examine this possibility, seeds were photoinduced by continuous red light periods of varying lengths at a temperature from 16 to 40°C. Preinduction and postinduction temperatures were held constant at 24°C. The results (Fig. 5A) indicate the following: (a) as the photoinduction temperature increases from 16 to 32°C, seeds become more sensitive to light; (b) maximal germination is approximately 80% for photoinduction temperatures from 24 to 32°C and is 75% and 67% at 20°C and 16°C, respectively; (c) at 36 and 40°C, maximal germination of 56 and 47%, respectively, is attained with 1 h of red light, but for periods longer than 1 h, germination progressively declines to about 30% for the 36°C treatment and to 0% for the 40°C treatment; (d) at temperatures of 28°C and higher, the seeds no longer exhibit a biphasic response; and (e) the biphasic response becomes more pronounced as the temperature is progressively lowered from 28 to 16°C with the percent germination of the first phase relatively unaffected (15–25%) from 16 to 24°C.

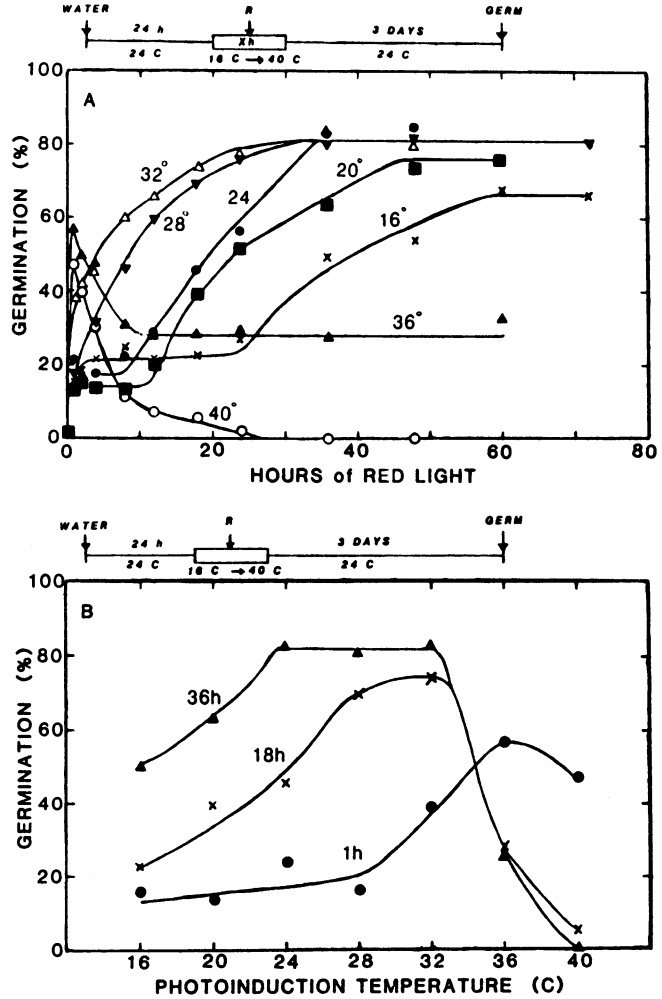

FIG. 5. Effect of photoinduction temperature on the response to red light. A, Seeds were given continuous red light for varying periods shown on the abscissa at 16°C (×), 20°C (■), 24°C (●), 28°C (▼), 32°C (△), 36°C (▲), or 40°C (○). All dark controls germinated below 3%. B, Percent germination is shown for seeds given 1 h (●), 18 h (×), or 36 h (▲) of red light at varying temperatures shown on the abscissa.

Part of the data of Figure 5A are replotted in Figure 5B to clarify the effect of temperature for 1, 18, and 36 h photoinduction periods. This figure indicates that germination is low for seeds given 1 h of red light, at photoinduction temperatures between 16 and 28°C but it is significantly higher for temperatures above 28°C and reaches a maximum at 36°C. Seeds given red light for 1 h at 40°C are somewhat inhibited. For seeds given 18 h of red light, the germination increases steadily from 16 to 28° with a maximum of 76% of 32°C. Higher temperatures are strongly inhibitory. When seeds are given 36 h of red light, germination is 50% at 16°C, increases to a maximum of about 80% at 24, 28, and 32°C and decreases sharply at higher temperatures.

Next, the red/far-red photoreversibility was reexamined by using the 10-light regime with intervening dark periods of 6 or 24 h (Table I). When each light period consists of red light alone or of far-red light followed by red light, germination is high at 60 to 68%. When each light period consists of far-red light alone or of red light followed by far-red light, germination is low at 4% or less. These results conclusively demonstrate that the stimulation of germination which is caused by periodic light is controlled by a series of reversible phytochrome responses.

In order to examine the postinduction processes which occur after a periodic light treatment, the time course of germination was determined for seeds given one, two, three, or four 15 min light pulses with intervening dark periods of 24 h. These results were compared with the germination time-course of seeds given 18 h of continuous red light. The results (Fig. 6A) indicate that, while the four-light regime and 18 h of continuous light result in similar final germination percentages (63 and 55%, respectively), the time-course of germination for seeds given periodic light is considerably slower (half-time = 59 h) than for seeds given 18 h of continuous light (half-time = 37 h). The germination half-times for the one-, two-, and three-light regimes are 41, 48, and 53 h, respectively.

The time-course of germination for periodic light treatments with short (4 h) intervening dark periods was also determined for seeds given two, four, six, or ten 15 min red light pulses. The germination time-courses for these treatments were compared with those of seeds given 2, 12, 18, and 36 h of continous red light. The results (Fig. 6B) show that the time-course for each periodic light treatment is very similar to that of the continuous light treatment which gives a similar final percentage of germination. A comparison of Figure 6A with 6B demonstrates that

Table I. *Reversibility of Periodic Red Light by Far-Red Light in Oenothera Seeds*

Ten light periods were given with intervening dark periods of either 6 h (experiment A) or 24 h (experiment B) between successive light doses. Light periods consisted of: 1) 15 min red light, 2) 15 min far-red light, 3) 15-min red light followed by 15 min far-red light, 4) 15 min far-red light followed by 15 min red light. In all cases the first light period was given after 24 h of dark soaking. Germination percentages are given as the means and standard deviations of four runs.

Light Treatment	Germination	
	Experiment A	Experiment B
	%	
1) Red	66.0 ± 5.8	62.0 ± 3.1
2) Far-red	0.0 ± 0.0	3.5 ± 1.6
3) Red → far-red	1.0 ± 0.7	4.0 ± 3.1
4) Far-red → red	68.0 ± 4.0	60.0 ± 5.3

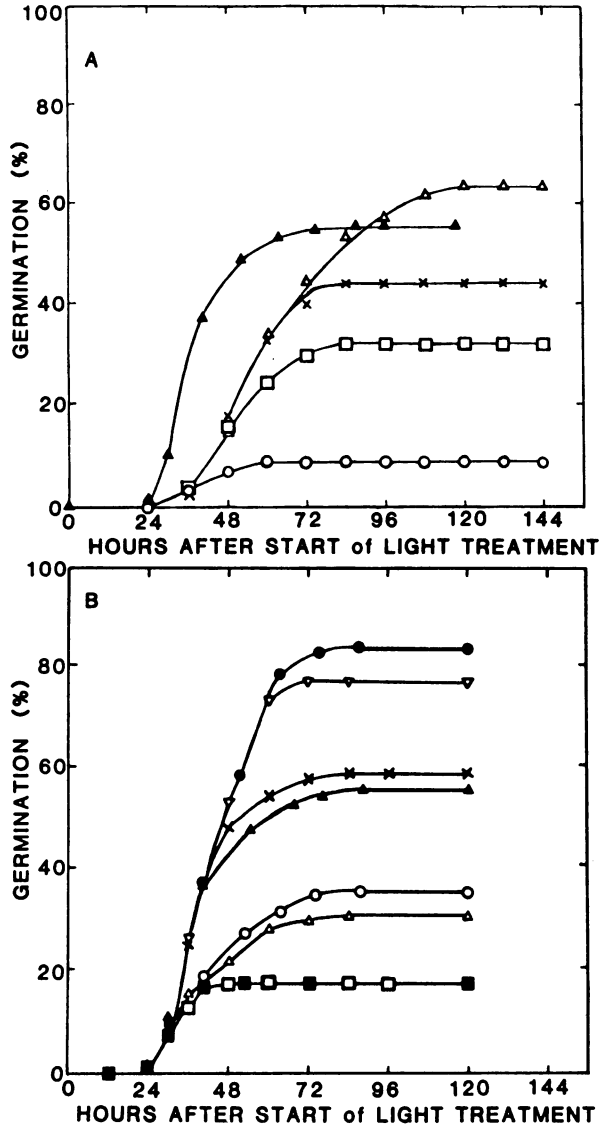

FIG. 6. Comparison of the time-courses of germination of seeds photoinduced by periodic red light with those photoinduced by continuous red light. A, Seeds were photoinduced by 18 h of continuous red light (▲) or by 1 (○), 2 (□), 3 (×), or 4 (△) 15 min pulses of red light with 24 h of darkness between successive light pulses. B, Seeds were photoinduced by 2 (▽), 4 (△), 6 (×), or 10 (□) 15 min pulses of red light with 4 h of darkness between successive light pulses or seeds were photoinduced by 2 h (■), 12 h (○), 18 h (▲), or 36 h (●) of continuous red light.

(a) when the intervening dark periods are short, a periodic light treatment induces germination with a time-course similar to that of continuous light and (b) when the intervening dark periods are long, the time-course of germination is much slower for seeds given a periodic light treatment. The significance of these results is discussed in the next section.

DISCUSSION

Effect of Continuous Red Light. A single long exposure to red or white light causes maximal percent germination in *Oenothera* seeds (Fig. 1; Ref. 9). The fluence-response curve is biphasic if the exposure to red or white light is continuous, with a small percentage of sensitive seeds stimulated by brief light exposures, and a large percentage of insensitive seeds requiring longer light exposures (Figs. 1 and 5A; Ref. 9). Far-red light fully inhibits the sensitive seeds but only partially inhibits the insensitive seeds (Fig. 1 and 2A). The inhibitory effect of far-red light is fully reversed by a subsequent exposure to red light (Fig. 2B), thus indicating the involvement of phytochrome in the germination of both the sensitive and insensitive seeds. The percentage of sensitive seeds is dramatically increased by a brief 35°C dark incubation period applied immediately before or after a light pulse (9) but is unaffected by photoinduction temperatures up to 28°C (Fig. 5A). At the photoinduction temperature of 32°C, it becomes difficult to distinguish between sensitive and insensitive seeds (Fig. 5A).

Although the sensitive seeds are fully inhibited by far-red light, the insensitive seeds are only partially inhibited (Figs. 1 and 2A). Some of these insensitive seeds must have escaped from far-red inhibition before the end of the photoinduction period (Figs. 1, 2A, and 3). The seeds induced to germinate by long photoinduction periods apparently take less time to pass through the escape step (Fig. 3).

The energy fluence of red light which is required to reverse the effect of far-red light (Fig. 2B) is much greater than that required by Grand Rapids lettuce seeds, which germinate to 100% with less than 1 min of the red light used in these experiments (data not shown). The reason for this difference is not yet entirely clear, but the very low transmittance of red light by *Oenothera* seed coats (9) may likely be involved.

Effect of Periodic Red Light. In *Oenothera*, periodic exposure to red light can substitute for continuous exposure to light in stimulating germination (Fig. 4). Red/far-red reversibility of this response (Table I) indicates control by phytochrome. When the dark periods between light pulses are short, periodic light and continuous light result in the same response when final percent germination is plotted as a function of the total time between the start and end of irradiation (Fig. 4B). Thus, for a periodic light treatment, less light energy is required to produce a given percentage of germination. In order to further analyze this effect, the data in Figure 4B were replotted in Figure 7 (top) with percent germination on a probit scale as a function of the logarithm of the total amount of incident light. The slope of the regression line was calculated for each data set in which the length of the dark period between successive flashes was held constant and the number of flashes was varied. The results show that the slopes of the regression lines for the continuous light and the periodic light treatments are not significantly different. This indicates that the periodic light treatments increase the light sensitivity of the seed population, but that the variance in light sensitivity in the population remains unaffected (7, 10, 11).

Periodic light cannot cause maximal percentage of germination when the intervening dark periods are very long (Figs. 4A and 7, top). For example, 4 light pulses are as effective as 10 light pulses when the intervening dark periods are 24 h long (Figs. 4 and 7, top). Fluence response curves plotted on a probit scale which are parallel but which have different light saturation points must result from some factor(s) that limits germination only in the portion of the seed population that has a greater light requirement (7, 11).

Interestingly, even though seeds given periodic light with 24 h intervening dark periods can only germinate to about 60% (Figs. 4A and 7, top) the fluence response curve for that 60% is shifted to lower amounts of light relative to treatments with shorter intervening dark periods (Fig. 7, top). Thus, the processes which shift the fluence response curve to lower amounts of light and the processes which cause different light saturation points (see above paragraph) are occurring simultaneously in *Oenothera* seeds.

Changes in the light sensitivity of seeds have been previously interpreted as being caused by changing amounts of phytochrome. For example, Taylorson and Hendricks (21) interpreted

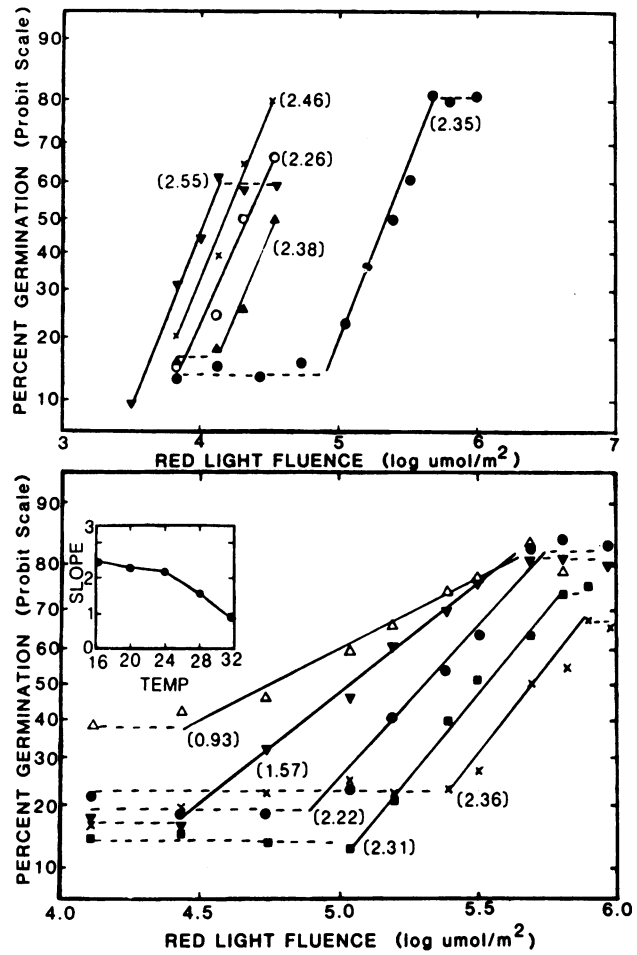

FIG. 7. (Top), Probit analysis of the effect of periodic light on germination photoinduction. Percent germination on a probit scale is plotted against log μmol m^{-2} of red light. Seeds were given continuous red light (●), or 15 min red light pulses with intervening dark periods of 2 h (▲), 4 h (○), 6 h (×), or 24 h (▼). For a one-factor analysis of variance of the slopes (shown in parentheses), $F_{[4,12]} = 0.165$ indicating no significant difference in the slopes. (Bottom), Probit analysis of the effect of photoinduction temperature on germination. Seeds were given continuous red light at 16°C (×), 20°C (■), 24°C (●), 28°C (▼), or 32°C (△). For a one factor analysis of variance of the slopes (shown in parentheses), $F_{[4,12]} = 3.70$ indicating a significant difference ($P < 0.05$) in the slopes. Inset shows the effect of photoinduction temperature on the slope of the probit regression lines.

an increase in the light sensitivity of *Amaranthus retroflexus* seeds as being caused by phytochrome synthesis. Although it is difficult to directly measure changes in the amount of phytochrome in an embryonic axis, most seed physiologists currently believe that phytochrome levels in photosensitive seeds remain constant prior to axis protrusion (1, 12). However, even if an increase in the phytochrome content of an axis can be demonstrated, such an increase may not be responsible for increasing seed photosensitivity since phytochrome concentration in other plant tissues is often not correlated with the amount of light necessary to cause a physiological response (18, 19).

Thermal reversion of phytochrome is likely to be involved in causing *Oenothera* seeds to require long light exposures or periodic pulses of light for germination. Thermal reversion of Pfr[2] to Pr is well known to occur in seeds and fern spores (5, 14, 20). Thermal reversion also apparently occurs in *Oenothera* seeds since 2 h of incubation at 35°C is significantly more effective in promoting germination when given before light than when given after light (see "Discussion" in Ref. 9). The results of the present paper are consistent with the idea that a single flash of light may be insufficient to cause maximal percent germination because Pfr undergoes thermal reversion before all seeds are stimulated to germinate. Therefore, if Pfr must act for a long time in order for a seed to germinate, then continuous or periodic light may be required since both of these treatments repeatedly form Pfr from phytochrome which has undergone thermal reversion to Pr. A similar interpretation was previously proposed to explain the light response of *Paulownia tomentosa* seeds (3).

The germination time-course for a periodic light treatment with short intervening dark periods is the same as for a continuous light treatment which induces the same percentage of germination (Fig. 6B). This indicates that the kinetics of signal transduction (*i.e.* Pfr → germination) are very similar for these two different treatments. In contrast, the germination time-course for a periodic light treatment with long intervening dark periods is much slower than that for a continuous light treatment which produces the same percentage of germination (Fig. 6A). This indicates that the kinetics of signal transduction are different for these two treatments even when the final percentage of germination is the same. If phytochrome thermal reversion is important in regulating *Oenothera* germination (see previous paragraph) then, relative to seeds given pulsed light with short intervening dark periods, seends given pulsed light with long intervening dark periods would be expected to experience larger changes in the Pfr:Pr ratio throughout the course of the light treatment. This difference may be related to the difference in the kinetics of signal transduction for these two treatments.

Effect of Photoinduction Temperature. The light sensitivity of *Oenothera* seeds is increased by a brief dark incubation at 35°C applied either before or after a light pulse (9) and can also be increased by increasing the photoinduction temperature (Fig. 5). Temperature treatments are well known to increase the light sensitivity of the seeds of many other species (22) although the mechanism which causes this response is still not well understood.

In order to analyze the effect of photoinduction temperature on *Oenothera* seeds, the data of Figure 5A were replotted in Figure 7, bottom with probit percent germination as a function of the logarithm of the total amount of light. The data (Fig. 7, bottom) show that as the photoinduction temperature increases from 16 to 32°C, the seeds become more sensitive to light and the population variance in light sensitivity increases. The increase in population variance, as indicated by the decrease in the slope of the regression lines, is especially pronounced between 24 and 32°C (Fig. 7, bottom [inset]). In other words, as the photoinduction temperature increases, the light sensitivity of low fluence-requiring seeds is greatly increased whereas that of high fluence-requiring seeds is less affected. In contrast to the periodic light experiments (Fig. 7, top), this temperature experiment (Fig. 7, bottom) cannot be interpreted in terms of phytochrome thermal reversion since red light is given throughout the duration of the temperature treatment and this would result in a high Pfr:Pr ratio throughout the photoinduction period. It seems likely that high photoinduction temperatures increase seed photosensitivity by stimulating signal transduction processes. Additional results (data not shown) which show that the time course of germination is more rapid for seeds photoinduced at 32°C than for those photoinduced at 24°C support this idea.

The work in the present and preceding paper has described several experimental manipulations which affect the photosensitivity of *Oenothera* seeds: (a) after-ripening enhances seed photosensitivity (9), (b) as the dark preinduction period lengthens,

[2] Abbreviations: Pfr, phytochrome in the far-red absorbing form; Pr, phytochrome in the red absorbing form.

photosensitivity increases for about 24 h and then declines after about 2 d (9), (c) a brief dark incubation period at 35°C before or after a light period increases photosensitivity (9), (d) increasing the photoinduction temperature increases photosensitivity (Figs. 5 and 7, bottom), (e) photosensitivity increases for about 16 h and then declines during the intervening dark period when a light pulse is given after 12 h of light (9), and (f) photosensitivity increases during the intervening dark periods when a series of red light pulses is given (Figs. 4 and 7, top). A better understanding of the mechanism(s) by which experimental treatments affect seed photosensitivity will help seed physiologists to better understand how phytochrome regulates seed germination. The next paper in this series examines postinduction processes in *Oenothera* seeds by means of temperature.

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