Plant Physiol. (1987) 85, 879–884
0032-0889/87/85/0879/06/$01.00/0

Photoinduced Seed Germination of *Oenothera biennis* L.

I. GENERAL CHARACTERISTICS

Received for publication November 3, 1986 and in revised form August 21, 1987

PETER A. ENSMINGER*[1] AND HIROSHI IKUMA
Department of Biology, University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT

General characteristics of light-induced germination of *Oenothera biennis* L. seeds were investigated at 24°C. During dark imbibition, seeds reached maximal respiration in 7 hours and maximal water content and photosensitivity in 24 hours. After dark imbibition of 24 hours, seeds required a long exposure (>36 hours) to red or white light for maximal germination. Two photoperiods (12 and 2 hours) separated by a period of darkness of 10 to 16 hours gave near maximal germination. For the two photoperiod regime, the first light potentiates a reversible phytochrome response by the second light. A 35°C treatment for 2 to 3 hours in the dark immediately prior or subsequent to 8 hours of light caused a higher percentage of germination. A 2 hour treatment at 35°C also potentiates a reversible phytochrome response. Halved seeds germinated at 100% in light or darkness indicating that the light requirement of the seeds is lost in the halving procedure. After-ripened seeds required less light and germinated more rapidly and at higher percentages than seeds tested shortly after maturation.

Seed germination of *Oenothera biennis* L. (evening primrose) has been shown to be stimulated by light, alternating temperatures, and nitrate (1, 7–10, 18). Short-day light regimes were reported to be more stimulatory than continuous light (11) and photoinduced germination is stimulated by acid scarification (7), but not by gibberellic acid, kinetin, or coumarin (11). *Oenothera* germination is promoted by after-ripening (11) and the germination responses of different genetic strains have been shown to depend on the storage conditions of the harvested seeds (20). The seeds can remain viable for up to 80 years (16).

The light requirement of *Oenothera* seeds is more complex than that of highly photosensitive seeds, such as Grand Rapids lettuce, because *Oenothera* seeds require long or periodic exposure to light. The physiological nature of the stimulation of germination by such light treatments is not yet clearly understood (2, 5). The series of experiments described in this paper were initiated to investigate the general characteristics of photoinduced germination of *Oenothera*.

MATERIALS AND METHODS

The seeds of a single genetic strain of *Oenothera biennis* L. were supplied by Dr. Erich Steiner of the University of Michigan, who originally collected the plant in Litchfield, MI. Plants were grown in the Matthaei Botanical Gardens of the University of Michigan and seeds were propagated by controlled self-pollination. The seeds were collected in the first week of October 1983 and stored at room temperature and humidity. In initial experiments, it was found that the light-response of seeds was not affected by position within individual fruit capsules nor by capsule position on the plant. Thus, all seeds were removed from their capsules and stored together. The majority of experiments reported in this paper were performed shortly after harvest (late November–early December) and 5 to 6 months after harvest (March–April); some experiments were also conducted with seeds 8 to 9 months after harvest (June and July). The seeds used shortly after harvest are designated in this chapter as early winter seeds, those used 5 to 6 months after harvest as spring seeds, and those used 8 to 9 months after harvest as summer seeds.

For germination studies, 50 seeds were evenly spread on two sheets of filter paper in a 5.5 cm plastic Petri dish, wetted in the dark with 2.0 ml of distilled water, irradiated for various periods with two Sylvania cool-white fluorescent lamps placed 15 cm above the Petri dish (energy fluence rate = $(4.5 \pm 0.6) \times 10^5$ ergs·cm^{-2}·s^{-1}), and then returned to darkness for germination. Using the terminology of Ikuma and Thimann (13), the dark imbibition period before exposure to light is referred to as the preinduction period, the period of exposure to light as the photoinduction period, and the dark incubation period after photoinduction as the postinduction period. As indicated in the text of this paper, the length of each period was varied in initial experiments, but was fixed after the length for maximal germination was determined.

For experiments in which seeds were cut in half, they were halved perpendicular to the axis of later growth. This procedure separated the embryonic axis plus a small portion of the cotyledons from the majority of the cotyledons. Because of the small size of the seeds, it was difficult to identify the half seed which contained the embryonic axis. Thus, both halves from 50 seeds were soaked in water. Only the half which contained the axis germinated.

Germination is defined as the emergence of the embryonic axis through the seed coat. When the cotyledonary end emerged before the axis, it was termed atypical germination (12). The standard incubation temperature used in these experiments was 24°C. When seeds were temporarily incubated at 35°C, they were transferred in a dark box to a 35°C incubator. Measurements with a thermocouple indicated that the temperature of the seeds equilibrated with the incubator within 5 min after transfer.

For measurement of water uptake, 100 seeds were weighed (39.7 mg, SE = 1.7 mg) and incubated for a given period on two layers of filter paper in a 5.5 cm Petri dish wetted with 2.0 ml of distilled water. The seeds were removed from the Petri dish, blotted between two sheets of dry filter paper to remove external water, and then weighed. The increase in weight due to water uptake was expressed as percent of the initial weight of the dry seeds.

The rate of O_2 uptake was determined with an oxygen electrode

[1] Present address: Department of Physics, 201 Physics Building, Syracuse University, Syracuse, NY 13244-1130.

(No. 4004, Yellow Springs Instruments Co., Yellow Springs, OH) which was connected to a home-made circuit box (4) and a Heath-Schlumberger recorder (model SR-255B). After 100 dry seeds were weighed, they were incubated in a Petri dish as above for a given period of dark preinduction, dried between two sheets of filter paper, and then placed in a lucite cuvette of 6.0 ml capacity. The cuvette was filled with distilled water containing a drop of Tween 20 to help wet the seed coat. The suspension was stirred and maintained at 25°C by passing thermostatted water through the jacket of the cuvette. The O_2 concentration in the air-saturated water was considered to be 240 μM.

Light transmission through the seed coat was measured with a Bausch and Lomb spectrophotometer connected to a Keithly Instrument microvolt meter. First, a single seed was cut in half and the embryo was removed. The coat of half the seed was then placed over a 0.5 mm hole which was drilled in a 5 cm square, 1 mm thick piece of aluminum which was painted with several coats of flat black paint. A drop of water was placed on top of the seed coat so that it adhered to the aluminum. The aluminum square, with the seed coat attached, was then attached directly to the surface of a Zeis photomultiplier and light transmission was determined. The seed coat was kept moist throughout the measurement period. The results reported are an average of three different seed coats.

All experiments reported herein were repeated at least twice. Unless otherwise stated, all measurements were made in duplicate and average values are reported. Standard errors were uniformly below 5%. All necessary manipulations were performed under dim green light (<80 ergs·cm^{-2}·s^{-1}). Seeds were normally exposed to this light for less than 1 min, but 36 h of exposure to it did not stimulate germination above the dark controls. Red light was obtained by passing Sylvania cool-white fluorescent light through one sheet each of red and amber plexiglas (<0.1% transmission below 580 nm, 50% transmission at 610 nm; energy fluence rate = (6.8 ± 0.4) × 10^4 ergs·cm^{-2}·s^{-1}). Far-red light was obtained by filtering General Electric incandescent tungsten light through 5 cm of water and a red Plexiglas and two layers of dark green acetate sheets (<0.1% transmission below 680 nm; energy fluence rate = (2.2 ± 0.3) × 10^4 ergs·cm^{-2}·s^{-1}). The energy fluence rate was measured with a Yellow Springs Radiometer (model 65).

RESULTS

General Characteristics of Photoinduction. During the preinduction period, photosensitive seeds take up water, become metabolically active, and develop sensitivity to light (2, 13). Thus, the patterns of water uptake, increase in respiration, and development of photosensitivity in *Oenothera* seeds during the preinduction period were examined (Fig. 1). Water uptake reaches a maximal value of 24% of the initial dry seed weight after about 24 h of soaking, with half-maximal uptake in about 5 h. The time-course of water uptake is the same in the dark as in fluorescent light (Fig. 1A). The respiration rate of early winter seeds increases during imbibition and reaches a maximal value of 0.38 nmol O_2·min^{-1}·mg^{-1} dry seed in 7 h; this fate is maintained for more than 36 h in the dark (Fig. 1B). The respiratory rate reaches a maximum when the seeds are half-imbibed. *Oenothera* seeds germinate to low percentages (about 10% for early winter seeds and about 30% for spring seeds) in response to 12 h of white light. Grand Rapids lettuce seeds germinate to 100% with less than 1 min of exposure to the fluorescent light used in these studies (data not shown). Both types of *Oenothera* seeds reach maximal photosensitivity after about 24 h of dark soaking (Fig. 1C). Also, for both types of seeds a long period of dark soaking acts to reduce photosensitivity. For subsequent experiments, the standard preinduction period was set at 24 h, the time at which water content, respiration, and photosensitivity are maximal.

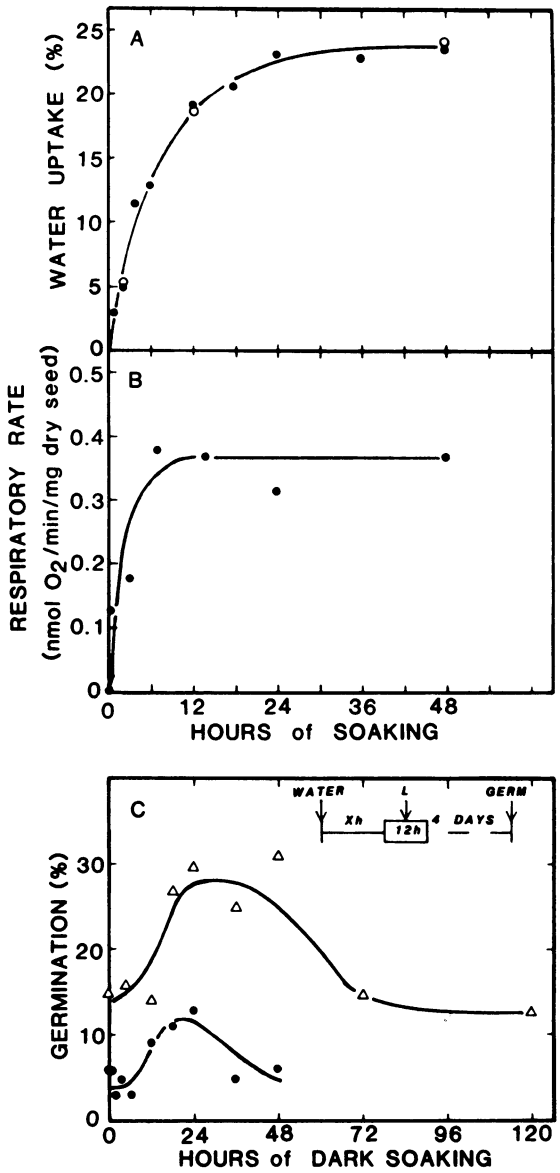


FIG. 1. Characteristic changes during preinduction. A, Increase in fresh weight; B, increase in respiration; C, change in photosensitivity. Early winter seeds were soaked in darkness (●) or in white fluorescent light (○). Spring seeds (△) were soaked in darkness. In this and subsequent figures of this paper, inset diagrams show experimental protocols and the × in each diagram is plotted on the abscissa.

Absorbance and reflectance of light by the seed coat affect the amount of light which reaches the embryonic axis. Thus, the light transmission spectrum through a seed coat was determined. The results (Fig. 2) show that light transmission is very low in the blue, green, and orange part of the spectrum with a gradual increase in transmission in the red and maximal transmission in the far-red region. Although the amount of light which reaches the embryonic axis cannot be quantitatively determined from these results alone, this transmission spectrum suggests that the amount of red light which reaches the embryonic axis is significantly reduced by the seed coat. Absorbance of light by the seed coat also makes spectrophotometric determination of phytochrome extremely difficult in these seeds.

The fluence-response relationship of *Oenothera* was next studied by dark soaking seeds for 24 h and then exposing them to white light for varying times. The experiments were performed

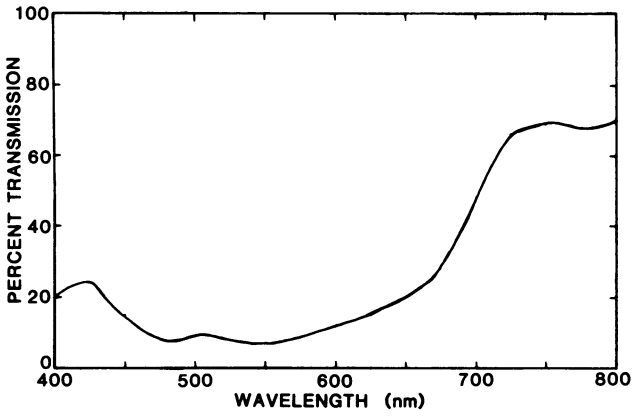


FIG. 2. Light transmission spectrum through a seed coat.

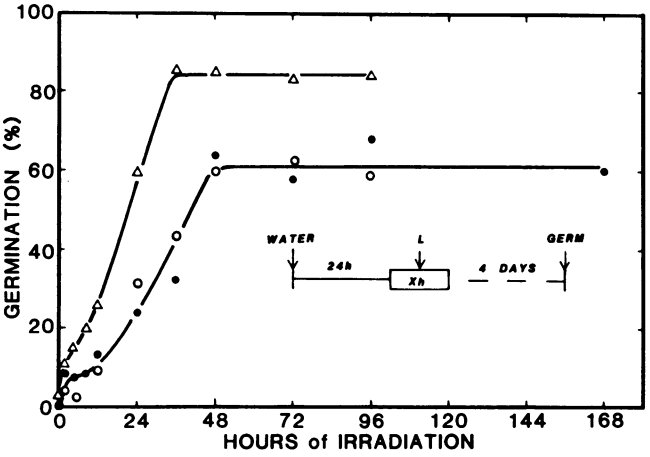


FIG. 3. Fluence response curves for white fluorescent light and red light. Spring seeds (△) or early winter seeds (●) were exposed to white light. Early winter seeds (○) were exposed to red light.

with early winter and spring seeds. The results in Figure 3 indicate that: (a) early winter seeds require 48 h of light to germinate to a maximum of about 63%, whereas spring seeds germinate to a maximum of 85% after 36 h of light; (b) the response of early winter seeds to red light is the same as to white fluorescent light, even though the energy fluence rates are vastly different (see "Materials and Methods"); and (c) the fluence-response curves appear to be biphasic for both types of seeds, with a small stimulation of germination caused by up to 12 h of light and a second stimulation of germination caused by longer exposures to light. Both the first and the second phases of the fluence curve are influenced by after-ripening. Summer seeds showed a fluence-response curve nearly identical to that of spring seeds (data not shown).

Next, the time-course of germination of early winter and spring seeds were examined by using the standard dark preinduction period of 24 h and a photoinduction period of 48 h. Germination was scored periodically after the start of irradiation. The results show that spring seeds germinate more rapidly and to a greater extent than early winter seeds (Fig. 4). The time from the start of photoinduction to 50% germination is about 70 h for early winter seeds and about 40 h for spring seeds.

When spring seeds were cut in half and soaked in light or in darkness, all half seeds germinated atypically within 2 d, whereas the germination of intact seeds was all typical and less than 100% (data not shown). This indicates that the embryonic axes of *Oenothera* seeds are capable of growing without irradiation, and that the light requirement of the seeds is lost in the halving procedure.

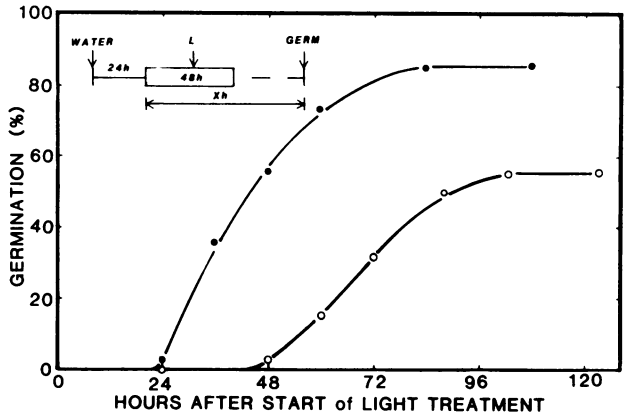


FIG. 4. Time course of germination for early winter seeds (○) and for spring seeds (●).

The results thus far presented for *Oenothera* indicate a general similarity of its preinduction and postinduction periods to those of other photosensitive seeds (2, 5, 13) in that (a) respiration, water content, and photosensitivity increase during dark preinduction and (b) seeds only require light for germination if the seed coat is intact. However, *Oenothera* seeds are unlike lettuce and many other highly photosensitive seeds in that they require prolonged exposure to light for maximal percentage of germination.

Effect of Periodic Light and Temperature. The requirement for a long exposure to light for maximal percent germination raised the possibility that *Oenothera* seeds may be stimulated by periodic exposure to light (2, 5, 11). To examine this possibility, seeds were exposed to two light periods (12 and 2 h) which were separated by an intervening dark period, the length of which was varied. Figure 5A shows the results for spring seeds. With no intervening darkness, *i.e.* with a single photoperiod of 14 h, germination is about 25%, but it increases to a maximum of 60 to 63% with an intervening dark period of 10 to 16 h, then declines slowly and linearly for longer intervening dark periods. The same data are plotted in Figure 5B, together with the data for continuous illumination in Figure 3. In this figure, the abscissa shows the hours between the start and the end of the light treatment for the single and the two light regimes. When the intervening dark period is 0 to 10 h for the two-light regime, the percentage of germination is practically identical with that of seeds given continuous light for 12 to 24 h.

When the duration of the second irradiation was varied, with the first photoperiod and the intervening dark period fixed at 12 and 10 h, respectively, germination increased dramatically during the first 2 h of the second exposure to light (Fig. 6A). This dramatic increase was followed by a slow and linear increase in germination as the intervening dark period increased to 20 h. The same data are plotted in Figure 6B with the data for continuous irradiation shown in Figure 3. As in Figure 5A, the abscissa shows the time between the start and the end of irradiation for each treatment. The portion of the curve in Figure 6A in which there is a slow linear rise agrees quite well with the response to continuous irradiation of 24 h and longer.

Photoinduced seed germination of many species is often stimulated by short treatments at an elevated temperature (21, 23). Thus, spring seeds were treated at 35°C for varying lengths of time immediately before or after 8 h of light. The preinduction period was fixed at 27 h in these experiments. The results (Fig. 7) show that 2 to 3 h of incubation at 35°C, given either before or after light exposure, stimulates germination but the stimulatory effect declines as the length of the incubation period exceeds 3 h. In the next experiment, seeds were incubated at 35°C for 3 h immediately before or after varying times of exposure to light

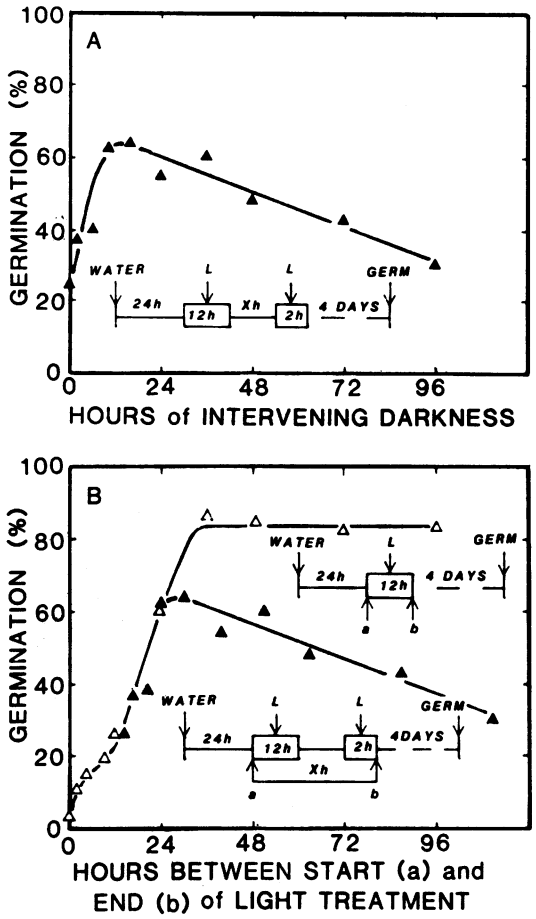


FIG. 5. Effect on percent germination of the length of an intervening dark period between two photoperiods of 12 and 2 h, using spring seeds. A, Percent germination as a function of the length of the intervening dark period; B, comparison of the data for two photoperiods (▲) with that for a single photoperiod (△) shown in Figure 2. The time between the beginning and the end of light treatment is plotted on the abscissa.

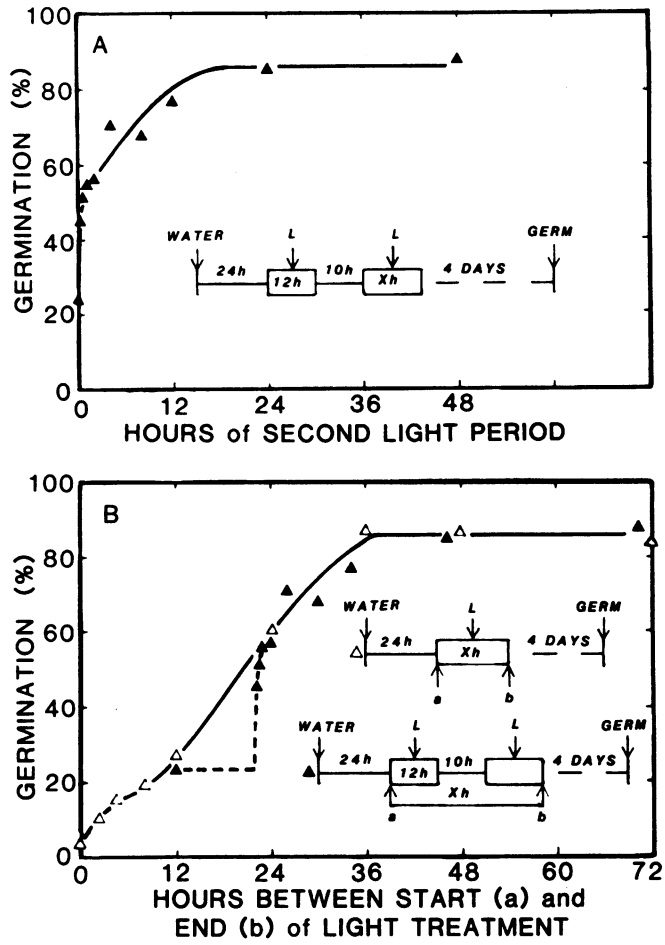


FIG. 6. Effect on percent germination of the length of the second irradiation period of the two-light regime when the length of the first irradiation period is set at 12 h and the intervening dark period at 10 h. A, Percent germination as a function of the length of the second light period; B, comparison of the data for two photoperiods (▲) with that for a single photoperiod (△) shown in Figure 2. The time between the beginning and the end of the light treatment is plotted on the abscissa. All data are for spring seeds.

and the results were compared with 24°C controls (Fig. 8). The maximal germination obtained after 36 h of light is about 85% for all three treatments. However, when seeds are exposed to light for less than 12 h, incubation at 35°C stimulates germination markedly above the control, particularly when seeds are incubated immediately before light.

About 60 to 65% germination occurs when seeds are given a short period of light after 12 h of light plus 10 h of darkness (Fig. 6) or a short period of light immediately after a brief 35°C incubation (Fig. 8). About 35% germination occurs when seeds are given a short period of light immediately before a brief 35°C incubation (Fig. 8). Since phytochrome is often implicated in plant responses to brief light exposures (2, 3, 5), the possible involvement of phytochrome in *Oenothera* germination was directly tested with red and far-red light for these potentiating treatments. Table I shows the results of these experiments for summer seeds. Although the difference between the red and far-red light treatments is not as great as that of lettuce or other highly photosensitive seeds, the data clearly show the stimulation of germination by red light and the reversal of the red light effect by far-red light. For each treatment, far-red light alone or red light followed by far-red light results in a percentage of germination about equal to that of the dark controls. The difference in percent germination between red and far-red treatments is about 35% for treatment A (12 h light followed by 10 h darkness) and treatment B (2 h dark incubation at 35°C immediately before

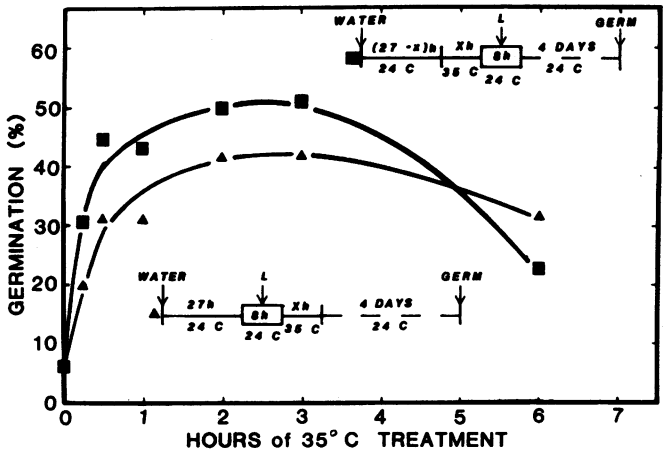


FIG. 7. Effect on percent germination of the length of a 35°C dark incubation period given immediately before (■) or after (▲) 8 h of white light. Experiments were performed with spring seeds.

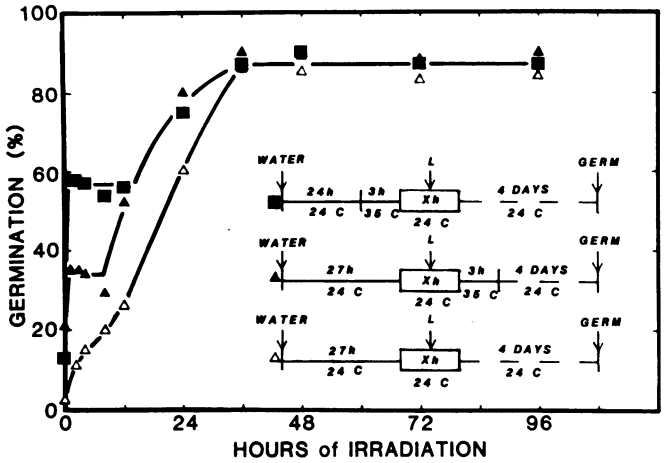
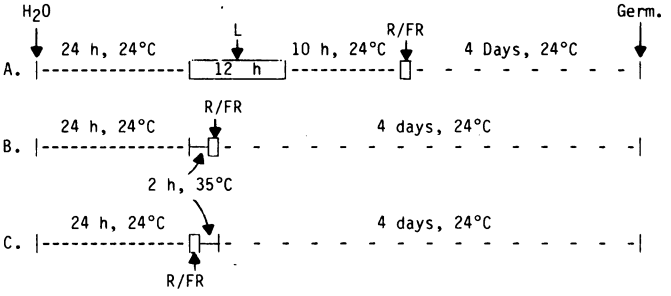


FIG. 8. Comparison of the fluence response curves for spring seeds which were incubated for 3 h at 35°C immediately before (■) or after (▲) photoinduction with that of seeds not incubated at 35°C (△). The preinduction period was set at 27 h.

Table I. *Effects of 12 h of Light and of Incubation at 35°C on the Potentiation of a Reversible Phytochrome Response in Oenothera Seeds*

Experimental protocols for the three potentiating treatments are shown in the diagrams below, with germination percentages resulting from irradiation with red and far-red light (R/FR in the diagrams) given in the table. Each entry shows the mean and standard error of four replicates. The percent germination of seeds which received a potentiation treatment alone without being irradiated with red or far-red light is presented under the treatment 'Darkness.'



Light Treatment	Germination		
	Treatment A	Treatment B	Treatment C
		%	
Darkness	35.5 ± 2.9	17.5 ± 1.8	23.5 ± 2.0
Red	67.0 ± 2.6	55.5 ± 3.8	43.0 ± 2.1
Far-red	29.5 ± 2.5	22.5 ± 1.5	17.0 ± 2.1
Red → far-red	31.5 ± 4.0	17.5 ± 3.7	22.0 ± 3.2

red or far-red irradiation). The difference is about 20% when seeds are incubated at 35°C immediately after irradiation (treatment C). All differences between the red and far-red light treatments are statistically significant ($P < 0.01$). In summary, 12 h of light followed by 10 h of darkness or a brief incubation at 35°C can elicit a reversible phytochrome response in 20 to 35% of the seeds.

DISCUSSION

Oenothera seeds become more photosensitive (Figs. 1C and 3) and germinate more rapidly (Fig. 4) after a brief storage period at room temperature and humidity. This after-ripening appears to be nearly complete by 5 months after seed harvest (11). In comparison with Grand Rapids lettuce seeds, after-ripened *Oenothera* seeds are still weakly photosensitive in that they require about 36 h of irradiation for maximal germination.

The initial dark preinduction period is a preparatory phase of germination in *Oenothera* as in the seeds of other species (2, 13). During preinduction, seeds take up water, increase in respiration, and develop photosensitivity (Fig. 1). As with lettuce (13) and other seeds (2), the photosensitivity of *Oenothera* seeds declines as the preinduction period becomes long (Fig. 1C), although the time-course is much slower in *Oenothera*. In *Oenothera*, there is also an increase and subsequent decline of photosensitivity during the intervening dark period when seeds are given two light exposures (Fig. 5A). Thus, *Oenothera* seeds increase in photosensitivity during the dark preinduction period and as a consequence of exposure to light. In contrast, lettuce and other highly photosensitive seeds take up water, increase in respiration, and fully develop photosensitivity all during the preinduction period.

The photoinduction period is also complex in *Oenothera* seeds. First, much longer exposure to light is necessary for maximal germination of these seeds (Fig. 3) in comparison to highly photosensitive seeds. Second, the fluence-response curve of *Oenothera* appears to be biphasic (Fig. 3); a small percentage of seeds germinate with less than 12 h of light, while the majority of seeds require a much longer exposure to light. The biphasic nature of the fluence-response curve is more pronounced in seeds shortly after collection than in after-ripened seeds (Fig. 3). It is also more pronounced in seeds briefly incubated at 35°C immediately before or after irradiation than in those maintained at 24°C throughout (Fig. 8). Biphasic fluence-response curves have been reported for several other species (5). Third, the amount of light needed to induce a given percentage of germination decreases if the photoperiod is interrupted by an intervening dark period (Fig. 6B; also Ref. 11). Fourth, photosensitivity increases and then decreases as the length of the intervening dark period is extended (Fig. 5A). Fifth, while phytochrome is involved in the photoinduced germination of *Oenothera* seeds, the treatments used here brought about a reversible phytochrome response in only 20 to 35% of the seeds (Table I).

Interestingly, photosensitivity increases during the first 10 h of the dark period that separates two periods of irradiation (Fig. 5A) and the increase is similar to what occurs during continuous exposure to light (Fig. 5B). This indicates that the physiological processes which occur during those 10 h of darkness are not limited by the availability of light. The red/far-red reversibility of the second light period indicates that phytochrome is involved in *Oenothera* germination (Table I). However, since only 20 to 35% of the seeds exhibit a reversible phytochrome response (Table I), it appears that in the treatments used here, many of the seeds have escaped from photoreversibility.

While phytochrome is obviously involved in *Oenothera* germination, the light response is clearly much different from that of Grand Rapids lettuce. Although the seed coat of *Oenothera* would be expected to significantly diminish the amount of red light which reaches the embryonic axis (Fig. 2), the requirement of these seeds for prolonged light exposures cannot be interpreted simply in terms of the optical properties of the seed coat. Experimental treatments which potentiate a reversible phytochrome response (Table I) are likely to be closely associated with the phytochrome system or with the reactions in which phytochrome participates.

Responses to light as observed in *Oenothera* seeds have also been reported in the seeds of other species. For example, *Chenopodium album* seeds increase in photosensitivity during a dark period which intervenes between two light periods (15) and the seeds of many other species require several exposures to light for germination (2, 14, 17, 24).

In *Oenothera* (Fig. 8; Table I) and in many other species (21–23), a brief incubation at an elevated temperature dramatically

increases photosensitivity. Unlike other species which are stimulated by heat treatments of a few minutes (2, 21), *Oenothera* seeds require 2 to 3 h of high temperature incubation for maximal increase in photosensitivity (Fig. 7). Prolonged high temperature incubation inhibits germination of *Oenothera* (Fig. 7) and other species (2, 6, 13, 21). When *Oenothera* seeds are irradiated before a high temperature incubation they germinate to a lower percentage than when irradiated after a high temperature incubation (Fig. 8; Table I). The lower percentage of germination of seeds irradiated before 35°C incubation may be caused by some of the phytochrome in the far-red absorbing form which is formed in these seeds undergoing thermal reversion to phytochrome in the red absorbing form when they are subsequently incubated at 35°C.

The postinduction period is terminated by protrusion of the embryonic axis through the seed coat. In *Oenothera* seeds which were cut in half, the axes grow in light and in darkness. The axis outgrowth of half-seeds is 100% within 2 d, whereas intact seeds germinate to 81% by 3 to 4 d after the beginning of photoinduction (Fig. 4). The inhibition of axis elongation in the presence of the seed coat may be overcome by weakening the mechanical restriction of the coat (12) or by the axis gaining a germination potential (19). How such an effect is induced by light and the mechanism of treatments that enhance photosensitivity in *Oenothera* remain to be explained. The following paper examines the photoinduction period of *Oenothera* germination.

Acknowledgments—Professor Erich Steiner of the Department of Biology, University of Michigan kindly supplied all seeds used in the present investigation. The transmission spectrum of *Oenothera* seed coats were taken by an instrument assembled by Professor Conrad S. Yocum. We thank Professors Harry A. Douthit, Alfred S. Sussman, and Conrad S. Yocum for discussion and critical reading of the manuscript.

LITERATURE CITED

1. AXENTJEV BN 1930 Über die Rolle der Schalen von Samen und Früchten, die bei der Keimung auf Licht reagieren. Bot Zentralbl 46: 119–212
2. BEWLEY JD, M BLACK 1982 Physiology and Biochemistry of Seeds, Vol 2. Springer-Verlag, New York, pp 60–120
3. BORTHWICK HA, SB HENDRICKS, EH TOOLE, VK TOOLE 1952 A reversible photoreaction controlling seed germination. Proc Natl Acad Sci USA 38: 662–666
4. ESTABROOK RW 1967 Mitochondrial respiratory control and polarographic measurement of ADP:O ratios. Methods Enzymol 10: 41–47
5. FRANKLAND B, R TAYLORSON 1983 Light control of seed germination. *In* W Shropshire Jr, H Mohr, eds, Encyclopedia of Plant Physiology, Vol 16A. Springer-Verlag, New York, pp 428–456
6. FUJII T, S ISIKAWA 1961 The effects of temperature after light-exposure on the germination of *Oenothera* seeds. Bot Mag 74: 414–418
7. GARDNER WA 1921 Effect of light on germination of light-sensitive seeds. Bot Gaz 71: 249–288
8. GASSNER G 1915 Über die keimungsauslösende Wirkung der Stickstoffsalze auf lichtempfindliche Samen. Jahrb Wiss Bot 55: 259–342
9. GASSNER G 1915 Altes und Neues zur Frage des Zusamenwirkens von Licht und Temperatur bei der Keimung lichtempfindlicher Samen. Ber deut Bot Ges 33: 203–217
10. GROSS KL 1985 Effects of irradiance and spectral quality on the germination of *Verbascum thapsus* L. and *Oenothera biennis* L. seeds. New Phytol 101: 531–541
11. HARTMAN W 1970 Untersuchungen zur Lichtabhängigen Samenkeimung von *Oenothera biennis* L. Biochem Physiol Pflanzen 161: 368–376
12. IKUMA H, KV THIMANN 1963 The role of the seed-coats in germination of photosensitive lettuce seeds. Plant Cell Physiol 4: 169–185
13. IKUMA H, KV THIMANN 1964 Analysis of germination processes of lettuce seed by means of temperature and anaerobiosis. Plant Physiol 39: 756–767
14. ISIKAWA S, Y YOKOHAMA 1962 Effect of "intermittent irradiations" on the germination of *Epilobium* and *Hypericum* seeds. Bot Mag 75: 127–132
15. KARSSEN CM 1970 The light promoted germination of the seeds of *Chenopodium album* L. IV. P_{fr} requirement during different stages of the germination process. Acta Bot Neerl 19: 297–312
16. KIVILAAN A, RS BANDURSKI 1981 The 100 year period for Dr. Beal's seed viability experiment. Am J Bot 68: 1290–1292
17. NAGAO M, Y ESASHI, T TANAKA, T KUMAGA, S FUKUMOTO 1959 Effects of photoperiod and gibberellin on germination of seeds of *Begonia euansiana* Andr. Plant Cell Physiol 1: 39–47
18. OTTENWALDER A 1914 Lichtintensität und Substrat bei der Lichtkeimung. Z Bot 6: 785–848
19. SCHEIBE J, A LANG 1965 Lettuce seed germination: evidence for a reversible light induced increase in growth potential and for phytochrome mediation of the low temperature effect. Plant Physiol 40: 485–492
20. STEINER E 1968 Dormant seed environment in relation to natural selection in *Oenothera*. Bull Torr Bot Club 95: 140–155
21. TAYLORSON RB, SB HENDRICKS 1972 Interactions of light and a temperature shift on seed germination. Plant Physiol 49: 127–130
22. TAYLORSON RB, SB HENDRICKS 1972 Phytochrome control of *Rumex crispus* L. seeds induced by temperature shifts. Plant Physiol 50: 645–648
23. TOOLE EH, VK TOOLE, SB HENDRICKS, HA BORTHWICK 1957 Effect of temperature on germination of light-sensitive seeds. Proc Int Seed Test Assoc 22: 1–9
24. WAREING PF 1959 Photoperiodism in seeds and buds. *In* RB Withrow, ed, Photoperiodism and Related Phenomena in Plants and Animals, American Association for the Advancement of Science, Washington, DC, pp 73–87