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Photoinduced Seed Germination of *Oenothera biennis* L.

III. ANALYSIS OF THE POSTINDUCTION PERIOD BY MEANS OF TEMPERATURE

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ABSTRACT

The postinduction period of *Oenothera biennis* L. seed germination was examined by temperature treatments. For all experiments, seeds received a standard 24 hour/24°C preinduction period and 12 hour/32°C photoinduction period. Germination is inhibited by postinduction temperatures above 32°C. When seeds are briefly incubated at 44°C and then transferred to 28°C, they germinate at a much lower percentage than 28°C controls. When thermally inhibited seeds are placed in the dark at 28°C for 20 hours, they can be promoted to germinate by a single pulse of red light. Seeds incubated at 12°C or below immediately after photoinduction enter a lag period in which they germinate slowly or not at all for a long time and then resume germination. The length of the lag period is exponentially related to the postinduction temperature. When seeds are incubated at a low temperature and then transferred to a warm temperature, they germinate much more rapidly than seeds not incubated at a low temperature. A model is proposed which is consistent with these and additional results. In the model, a germination promoter is irreversibly formed from a precursor and the synthesis of the precursor is favored at low temperatures and its degradation is favored at high temperatures.

Oenothera seeds are photosensitive and phytochrome is the photoreceptor (12, 13). Maximal or near maximal germination results from (a) prolonged (36–48 h) exposure to red or white light at 24°C (12, 13), (b) two light periods (12 and 2 h) separated by a dark period of 10 h to 16 h (12), (c) a red light pulse applied immediately before or after 2 h of dark incubation at 35°C (12), and (d) a series of red light pulses (13). The percentage of germination is higher when the photoinduction period is at a high temperature (*e.g.* 32°C) than at a low temperature (*e.g.* 16°C), thus indicating the involvement of nonphotochemical processes in the photoinduction period (13). The seeds of many other species also require prolonged or periodic light exposures for germination (1, 2, 4, 10, 11, 15, 21) and these seeds may share with *Oenothera* certain mechanistic similarities in their response to light.

Despite the large number of seeds known to be photosensitive (1, 2, 7, 15, 19) and the extensive study of photoinduced germination (15, 28), very little is known about the postinduction processes which lead to germination. The purpose of this paper is to examine the temperature sensitivity of the postinduction period of *Oenothera* seeds in order to better understand the postinduction process.

MATERIALS AND METHODS

The seeds used were of the same genetic strain and the same harvest as used previously (12, 13), with dark germination below 5% and maximal germination of about 80% at 24°C. They were stored in the dark in a desiccator at 4°C and the germination characteristics remained essentially the same during the experimental period of May to November 1985.

All germination studies were conducted as previously described (12, 13), using 50 seeds per Petri dish and 4 Petri dishes per experimental treatment. The average of the four replicates is reported. All experiments were performed at least twice and typical results are reported. The standard errors of the mean were calculated for each data point and were uniformly below 5%. As before, germination of a seed is defined as the emergence of the embryonic axis from the seed coat and the germination half-time of the seed population is defined as the time from the initiation of photoinduction until 50% germination is achieved. The term germination lag period is used in this paper as the time from the transfer of seeds to a cold temperature until the germination of seeds in the population proceeds uninterruptedly.

For all experiments, seeds were soaked in the dark for 24 h in a 24°C incubator and then transferred in a dark box to a growth chamber maintained at 32°C where they were irradiated with red light for 12 h. This treatment causes near maximal percent germination when seeds are transferred back to a 24°C incubator (13). After irradiation, Petri dishes were placed in a dark box and then transferred to 1 of 11 incubators, each of which was maintained at a constant temperature of 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, or 44°C. Measurements with a thermocouple indicated that the temperature of the seeds equilibrated with the incubator within 5 min after transfer. The temperature of the growth chamber and of each of the incubators was at all times maintained to within 1°C of the designated value. During dark incubation, Petri dishes were wrapped in aluminum foil to prevent evaporative water loss. The red light source and the green safelight were the same as used previously (12, 13).

RESULTS

Effect of Constant Temperature. In initial experiments, seeds were exposed to a single constant postinduction temperature in the range of 4 to 44°C. The results shown in Figure 1, A and B, can be summarized as follows: (a) at all postinduction temperatures, some seeds germinate within 4 h after transfer to the dark incubator; (b) seeds germinate most rapidly at 28 and 32°C, with a germination half-time of about 24 h at each temperature; (c) seeds germinate more slowly at postinduction temperatures of 24, 20, and 16°C than at 28°C, with germination half-times of 29, 35, and 40 h, respectively; (d) at postinduction temperatures of 4, 8, and 12°C seeds enter a lag period in which they germinate to only 4, 6, and 8%, respectively at 8 h after the transfer and then resume germination at 92, 42, and 24 h, respectively after the start of postinduction; and (e) for postinduction temperatures above 32°C, seeds germinate more slowly than at 28°C.

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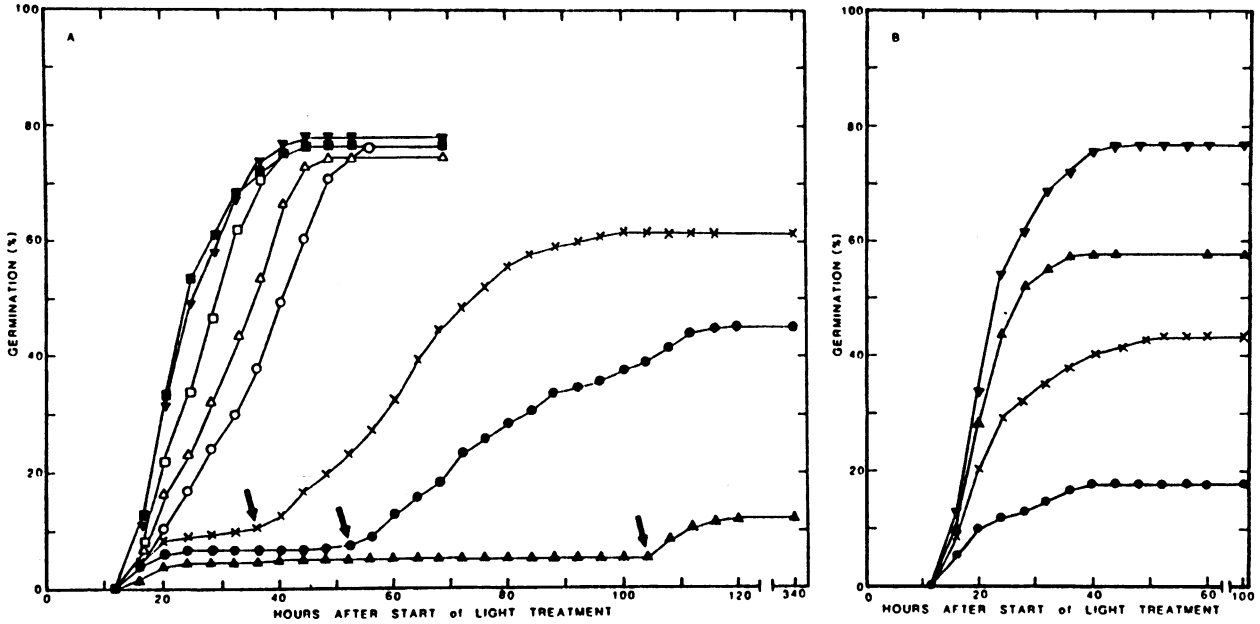

FIG. 1. Effect of postinduction temperature on the time course of germination. A, 4 to 32°C; B, 32 to 44°C. Immediately after photoinduction, seeds were incubated at 4°C (▲), 8°C (●), 12°C (×), 16°C (○), 20°C (△), 24°C (□), 28°C (■), 32°C (▼), 36°C (▲), 40°C (×), or 44°C (●). In this and all subsequent figures, seeds received a standard dark preinduction (24 h at 24°C) and red light photinduction (12 h at 32°C). All figure captions describe postinduction treatments.

Figure 2, A and B, results from an analysis of the data in Figure 1, A and B. In Figure 2A, final percent germination and germination half-times are plotted against postinduction temperature. This figure emphasizes that maximal germination of about 80% occurs from 16 to 32°C with higher and lower temperatures significantly reducing the percentage of final germination. Analogously, the germination half-time is lowest at 28 and 32°C with higher and lower temperatures increasing the germination half-time. Figure 2B shows that for the 4, 8, and 12°C treatments there is an approximately inverse linear relationship between the logarithm of the length of the lag period and the postinduction temperature.

Effect of High Temperature Treatments. Recovery from the inhibition of germination caused by high temperatures was next investigated. Immediately after photoinduction, seeds were incubated at 44°C for 0.5 to 4 h and were then transferred to 28°C. Controls were incubated at 28 or at 44°C throughout the postinduction period. The results (Fig. 3A) show that: (a) for all treatments, germination is slower than the 28°C control but is faster than the 44°C control; (b) with respect to the 28°C control, final germination is only slightly reduced for seeds incubated at 44°C for 0.5 h but is reduced to 49, 37, and 28% for seeds incubated at 44°C for 1, 2, and 3 h, respectively; (c) for all 44°C treatments, germination proceeds slowly upon initial transfer to 28°C but later more rapidly; and (d) the time from transfer to 28°C until the onset of the rapid phase of germination is dependent upon the length of the 44°C treatment (Fig. 3A, inset). In addition, seeds incubated at 44°C for 4 h germinated very similarly to those incubated for 3 h (data not shown). Figure 3B shows that there is an approximately inverse linear relationship (r^2 = 0.95) between the logarithm of the percentage of final germination and the incubation time at 44°C.

The results shown in Figure 3A indicate that when seeds are incubated at 44°C immediately after photoinduction, their germination is significantly inhibited but that they can partially recover from this inhibition in the dark at 28°C. Since the 44°C treatment was given immediately after the photoinduction period, the Pfr formed by the red irradiation may have reverted to Pr in the heat-inhibited seeds (3, 20). Such seeds should be stimulated to germinate by reexposure to red light. In order to investigate light-promoted recovery from thermal inhibition, seeds were given the standard preinduction and photoinduction treatment, incubated at 44°C for 3 h, and then incubated in the dark at 28°C for varying times before reexposure to red light for 15 min or 12 h. The results (Fig. 4) show that: (a) in response to 15 min of red light, germination reaches a maximum of about 60% after 20 h of dark incubation at 28°C, then slowly declines for longer 28°C incubation periods; and (b) in response to 12 h of red light, germination reaches a value of 65% after 10 h of dark incubation at 28°C and increases to 70% for a 45 h 28°C incubation period. In addition, the stimulation of germination caused by 15 min of red llight is fully reversible by far-red light and the seedlings which develop from these seeds grow normally (data not shown). These results cannot be interpreted exclusively in terms of phytochrome thermal reversion and indicate that an additional process is involved in the thermal inhibition of germination (see "Discussion").

Effect of Low Temperature Treatments. One notable feature of Fig. 1A is that at postinduction temperatures of 12°C and below, the seed population enters a lag period for a prolonged time before resuming germination. A series of experiments were performed in order to further examine the physiological nature of this lag period.

First, in order to see if a seed population that has started germination also shows the arrest and resumption of germination when incubated at a low temperature, photoinduced seeds were transferred to 8°C after 8 h of postinduction incubation at 28°C. Controls were held continuously at 28°C or at 8°C throughout postinduction. The results (Fig. 5) indicate that: (a) seeds incubated at 8°C after 8 h at 28°C also enter a lag period which is slightly shorter (28 h) than that of seeds treated at 8°C throughout postinduction (32 h), and (b) seeds transferred to 8°C after 8 h at 28°C germinate maximally whereas seeds exposed to 8°C throughout postinduction germinate only to 46%.

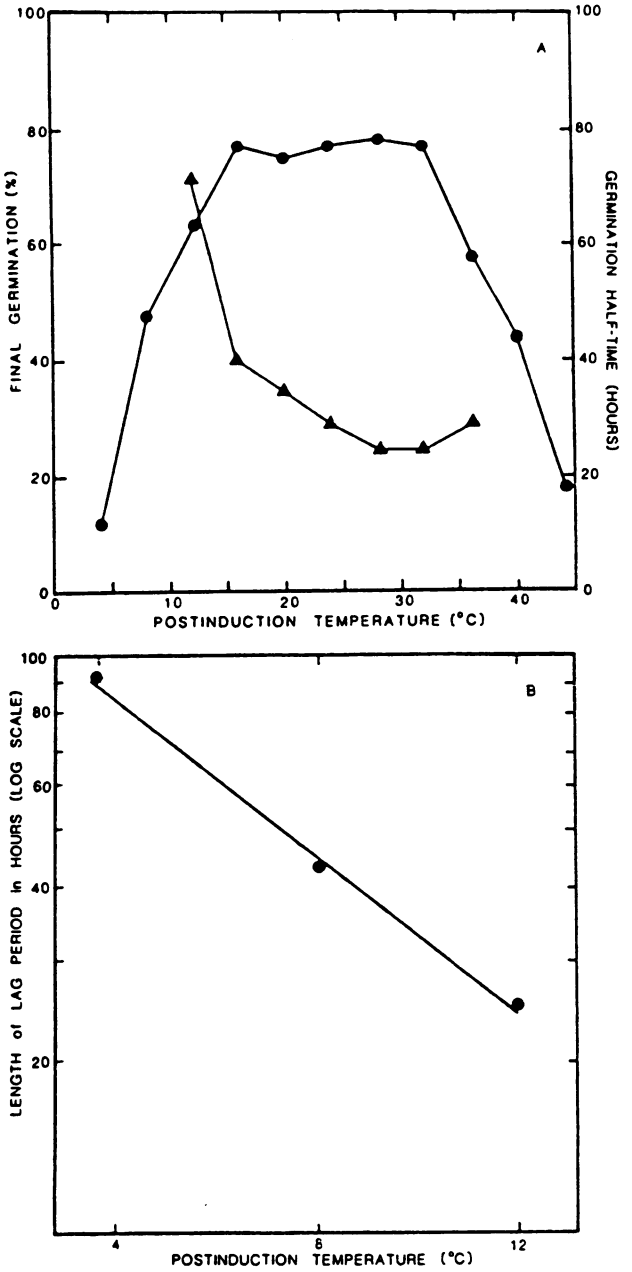

FIG. 2. A, Effect of postinduction temperature on the percentage of final germination (●) and on the germination half-time (▲); B, effect of postinduction temperature (4–12°C) on the length of the lag period (see arrows in Fig. 1A).

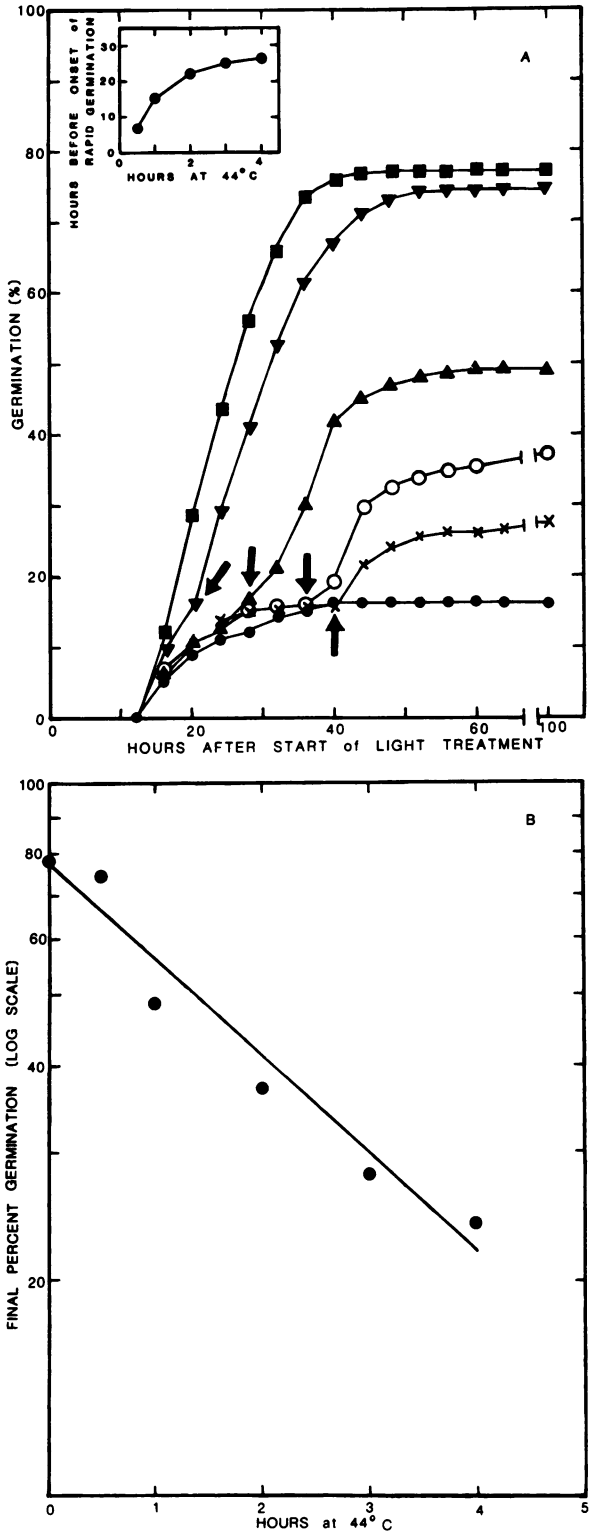

FIG. 3. A, Effect of the length of postinduction incubation at 44°C on subsequent germination at 28°C. Immediately after photoinduction, seeds were incubated at 44°C for 0.5 h (▼), 1 h (▲), 2 h (○), or 3 h (×) and then transferred to 28°C. Controls were held at 28°C (■) or at 44°C (●) throughout postinduction. Arrows indicate the time of onset of rapid germination, as determined by the point at which the curve changed from a decreasing slope to an increasing slope. Inset shows the length of the 44°C incubation as a function of the time before the onset of rapid germination at 28°C. B, Effect of the length of postinduction incubation at 44°C on final percentage of germination.

Next, the change in the physiological state of the seeds as they progress through the 8°C lag period was investigated. Immediately after photoinduction, seeds were placed at 8°C for 12 h (beginning of lag period), 24 h (middle of lag period), or 36 h (end of lag period) and were then transferred to 28°C. Controls were maintained at 8°C or at 28°C throughout the entire postinduction period. The results (Fig. 6A) show that germination proceeds very rapidly after seeds are transferred from 8°C to 28°C, with the slope of the germination time-course curve being much steeper for 8°C treated seeds than for untreated seeds. The time from transfer to 28°C until 50% germination is attained dramatically decreases as the time of 8°C incubation increases (Fig. 6B).

The difference in the germination response at the beginning

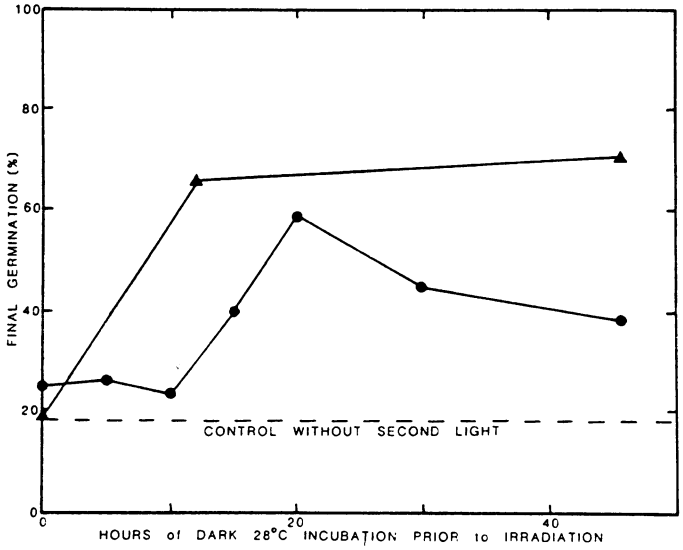

FIG. 4. Effect of the length of a dark incubation period at 28°C and subsequent exposure to red light on the germination of seeds initially incubated at 44°C. Immediately after photoinduction, seeds were incubated for 3 h at 44°C and then for a varying time at 28°C before they were given 15 min (●) or 12 h (▲) of red light. Final germination is plotted against dark incubation time at 28°C prior to irradiation.

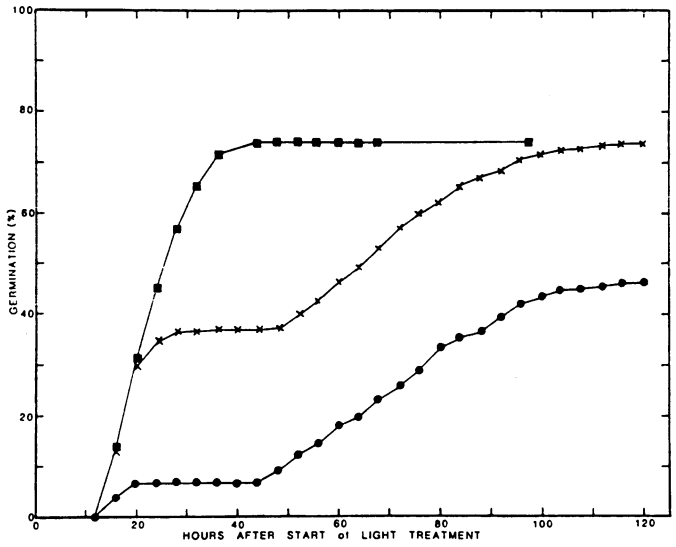

FIG. 5. Effect of incubation at 8°C after 8 h of postinduction at 28°C on the time-course of germination. Immediately after photoinduction, seeds were incubated at 8°C (●), incubated at 28°C for 8 h and then transferred to 8°C (×), or were held at 28°C throughout postinduction (■).

and the end of the lag period (Fig. 6B) indicates that *Oenothera* seeds apparently pass through certain physiological processes at 8°C which make them germinate more rapidly at 28°C. This phenomenon was further examined by placing seeds which had been incubated at 8°C and then at 28°C back into 8°C. The length of the original 8°C period was set at 12 h (beginning of lag period) or 36 h (end of lag period). The results (Fig. 7) show that seeds which were initially incubated at 8°C for 12 h pass through a second lag period of about 12 h in length. For this treatment, the total length of the first plus second lag periods (24 h) is shorter than the single lag period of control seeds (32 h) which were incubated at 8°C throughout postinduction. However, the total length of time from the start of the first lag period to the end of the second lag period (32 h) is the same as that of the single lag period of the 8°C control seeds. In contrast to these results, seeds incubated at 8°C until the end of the lag period (36 h) do not exhibit a second lag period during the second 8°C incubation (Fig. 7). All treatments in this experiment, except for the one of incubation at 8°C throughout postinduction, result in maximal germination of about 80%.

The results shown in Figures 6 and 7 indicate that after 36 h of postinduction incubation at 8°C, *Oenothera* seeds have completely passed through the lag period. Next, the temperature sensitivity of these seeds was reinvestigated by incubating them at 8°C for 36 h and then transferring them to temperatures in the range of 16 to 44°C. A control was kept at 8°C throughout postinduction. The results (Fig. 8A) show that: (a) seeds incubated at 28°C exhibit the most rapid germination, with 50% germination achieved 3 h after transfer to 28°C; (b) seeds incubated at 16, 20, or 36°C germinate somewhat slower than those held at 28°C; and (c) seeds incubated at 44°C germinate very rapidly at first and quickly reach a maximal value of 43% (cf Fig. 1B). Below 36°C, the percentages of final germination attained from this experiment are virtually identical to those shown in Figure 2A of the seeds which did not receive the 8°C incubation (Fig. 8B). However, final germination is higher at 36 and 44°C for seeds which were incubated at 8°C (Fig. 8B). Figure 8B also emphasizes that the germination half-times of the different experimental treatments shown in Figure 8A are nearly identical to one another, whereas the germination half-times of seeds not incubated at 8°C show a much greater temperature dependence.

DISCUSSION

High Temperature Effect. Short treatments at high temperatures stimulate photoinduced germination of *Oenothera* (Table 1 in Ref. 12; Fig. 5B in Ref. 13) and many other photosensitive seeds (2, 15, 30). In contrast, prolonged treatments at high temperatures are highly inhibitory to *Oenothera* (Fig. 7 in Ref. 12; Fig. 5B in Ref. 13; Fig. 3B in this paper) and many other photosensitive seeds (30). Postinduction temperatures above 32°C lead to slower germination and prevent maximal germination in *Oenothera* (Fig. 1B). As the length of postinduction incubation at 44°C increases, seeds become less capable of recovery when they are transferred to 28°C (Fig. 3A). It appears that the heat treatment has completed its inhibitory action in about 3 or 4 h (Fig. 3A, inset).

Interestingly, the relationship between the time of incubation at 44°C and the logarithm of the percentage of germination is approximately linear (Fig. 3B). This response would be considered a one-hit response in terms of target theory (8). According to target theory, such a result occurs when each seed in the population is thermally inhibited by the hitting of a single target in the seed.

When postinduction seeds are initially incubated at 44°C and then allowed to recover for 15 to 20 h in the dark at 28°C, they are markedly stimulated by a pulse of red light (Fig. 4). For such seeds, 12 h of red light is slightly more stimulatory than 15 min of red light. The sensitivity to 15 min of red light declines for prolonged recovery periods, although the sensitivity to 12 h of light does not decline for recovery periods of up to 45 h (Fig. 4). *Oenothera* photosensitivity also increases and then declines: (a) during the preinduction period at 24°C (Fig. 1 in Ref. 12), (b) during the intervening dark period when seeds are given two light periods (Fig. 5 in Ref. 12), and (c) in response to periodic light pulses as the time between successive pulses increases (Fig. 4A in Ref. 13). It seems likely that similar metabolic processes are involved in causing the increase and subsequent decline in photosensitivity in these experimental treatments.

Grand Rapids lettuce seeds are somewhat similar to *Oenothera*

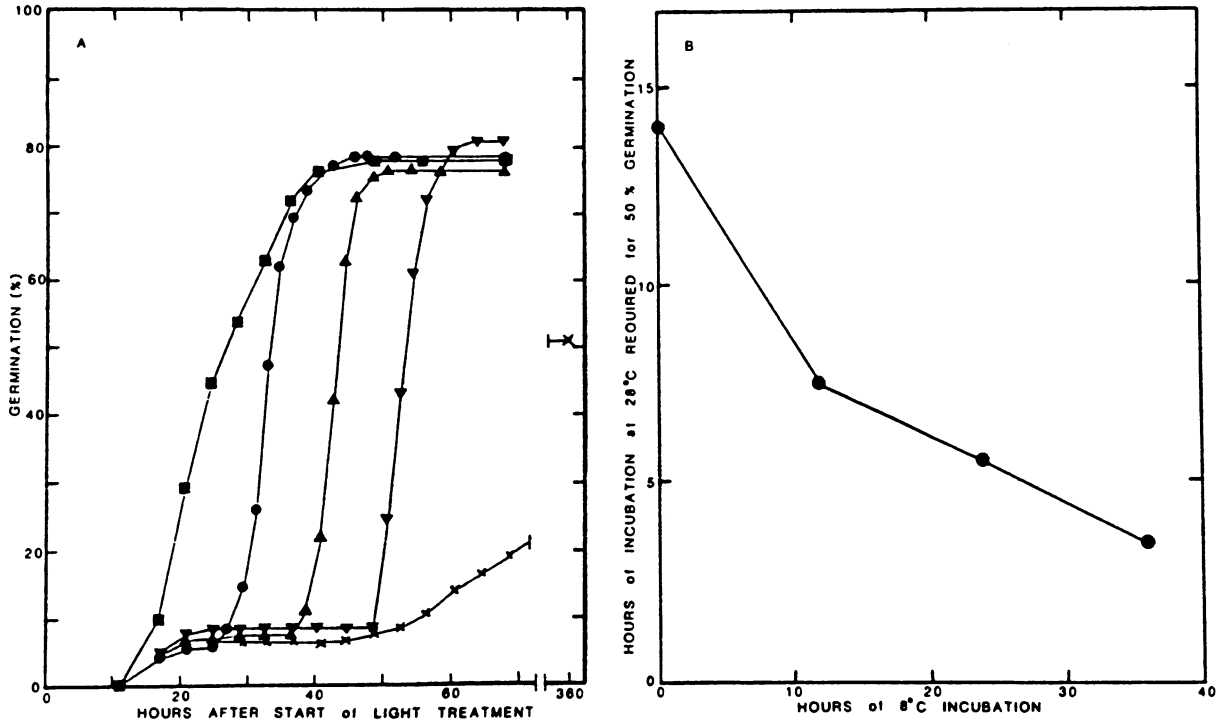

FIG. 6. A, Effect of the length of an 8°C incubation period on subsequent germination at 28°C. Immediately after photoinduction, seeds were incubated at 8°C for 12 h (●), 24 h (▲), or 36 h (▼) and then transferred to 28°C. Controls were held at 8°C (×) or at 28°C (■) throughout postinduction. B, Effect of the length of an 8°C incubation period on the length of a 28°C incubation period required for germination.

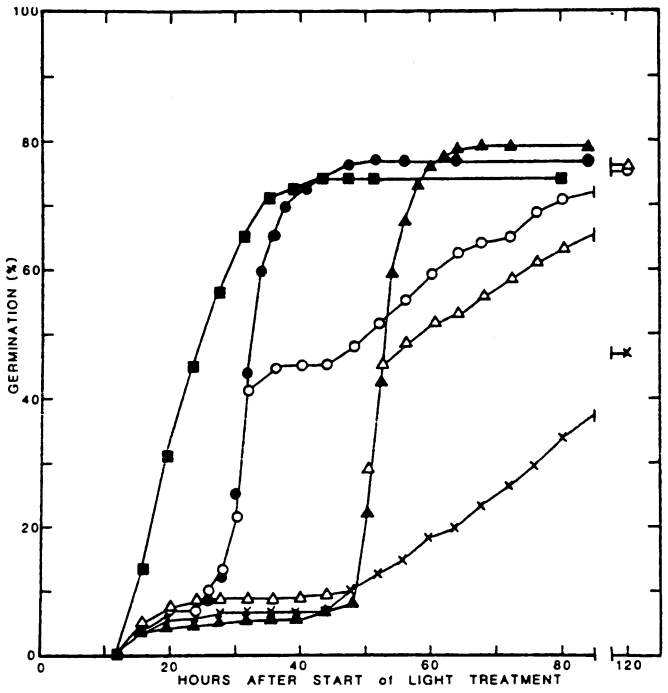

FIG. 7. Effect of the length of an initial 8°C incubation period on the germination of seeds subsequently incubated at 28°C and then at 8°C. Immediately after photoinduction, seeds were incubated at 8°C for 12 h, transferred to 28°C for 8 h, and then returned to 8°C (○) or were incubated at 8°C for 36 h, transferred to 28°C for 4 h, and then returned to 8°C (△). Controls were held at 8°C for 12 h (●), or 36 h (▲) before transfer to 28°C or were held at 8°C (×) or at 28°C (■) throughout postinduction.

seeds in their response to high temperature inhibition. When lettuce seeds are given a pulse of red light and then incubated in the dark at 30 or 35°C for 12 to 24 h, germination is inhibited (3, 20). Since lettuce seeds can be restimulated by a red light pulse given immediately after a high temperature incubation, this inhibition has been interpreted as simply being caused by thermal reversion of Pfr to Pr (3), although it is quite likely that another process is involved (20). For example, it has been suggested that the thermal inhibition of lettuce germination may be related to the heat-mediated inhibition of the capacity of seeds to synthesize ethylene (5). In the case of *Oenothera* seeds, red light has no effect when given immediately after high temperature incubation (Fig. 4) and thus the results presented here also cannot be interpreted exclusively in terms of the thermal reversion of phytochrome. Nevertheless, the fact that photoinduced seeds of *Oenothera* (Fig. 4), lettuce (3, 20), and other species (22, 30), as well as photoinduced fern spores (6) can recover from thermal inhibition with the aid of a red light pulse, suggests possible similarities in the mechanism of light-stimulated recovery from high temperature inhibition.

Low Temperature Effect. The seeds of many species, such as those in the Rosaceae (2, 9), require a prolonged incubation period at a low temperature for germination. In *Oenothera*, incubation at a low temperature has practically no effect on seeds which are not given light (data not shown). However, when *Oenothera* seeds are incubated in the cold (4–12°C) immediately after photoinduction, the seed population enters a lag period before germination proceeds uninterruptedly (Fig. 1A). As the temperature increases from 4 to 12°C, the length of the lag period decreases dramatically (Fig. 2B), indicating a very large temperature coefficient (Q_{10} = 5.5) for this phenomenon. Since germination occurs without a noticeable lag period at postinduction temperatures above about 12°C (Fig. 1A), the processes which cause the lag period seem to have a threshold at this temperature and the experiments presented here are unable to

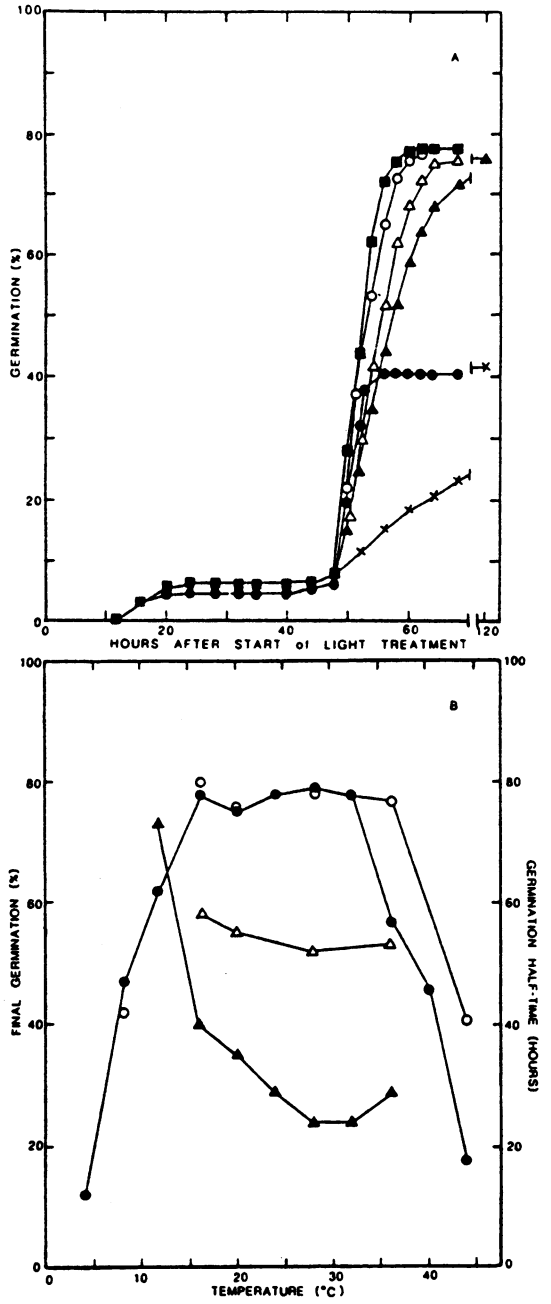

FIG. 8. A, Effect of 36 h incubation at 8°C on subsequent germination at various temperatures. Immediately after photoinduction, seeds were placed at 8°C for 36 h and maintained at 8°C (×) or incubated at 16°C (▲), 20°C (△), 28°C (○), 36°C (■), or 44°C (●). B, Effect of incubation temperature applied after 36 h of incubation at 8°C on the percentage of final germination (○) and on the germination half-time (△) in comparison with seeds which received no 8°C incubation (see Fig. 2A).

detect their occurrence at higher temperatures.

Another effect of incubating *Oenothera* seeds in the cold is that it stimulates them to germinate more rapidly when they are transferred back to 28°C (Fig. 6, A and B). Cold incubation also allows these seeds to germinate more rapidly (Fig. 8A) and to a higher percentage (Fig. 8B) when they are transferred to a very high temperature (36–44°C). A similar response is found in pea seedlings, which become heat resistant after a vernalisation treatment (18). In *Oenothera* seeds, the amount of stimulation caused by cold incubation increases with the length of the cold incubation period (Fig. 6B). There is no stimulation when seeds are incubated at 16°C immediately after photoinduction and then transferred to 28°C (data not shown). Thus, the low temperature mediated stimulation of germination at 28°C and the low temperature mediated germination lag period are quite likely related phenomena because: (a) both have an upper threshold at about 12°C and (b) both appear to have completed their action after about 36 h at 8°C.

When postinduction seeds are briefly incubated at 28°C and then transferred to 8°C, they exhibit a lag period which is slightly shorter than the 8°C control (Fig. 5). When postinduction seeds are incubated at 8°C for a short time, then briefly at 28°C, and then returned to 8°C they show a second lag period before resuming germination (Fig. 7). The total length of both lag periods is less than that of the single lag period of the 8°C control. However, the total time between the start of the first lag period and the end of the second lag period is the same as that of the single lag period of seeds incubated at 8°C throughout. This complex response suggests that the changes in seed metabolism which are initiated by a low temperature treatment are not reversed when the seeds are briefly exposed to a warm temperature.

The stimulation of *Oenothera* germination by a low temperature treatment is somewhat similar to the phenomenon of vernalization in *Hyoscyamus niger* as described in a classical paper by Lang and Melchers (23; see also Ref. 26). Both can be interpreted in terms of a model in which a promoter is irreversibly formed from a precursor at low temperatures and its destruction is favored at high temperatures.

$$\leftrightarrows \text{PRECURSOR} \rightarrow \text{PROMOTOR}$$

Two significant conclusions of a recent mathematical formulation (14) of this model are: (a) incubation below a certain threshold temperature is necessary for forming the promoter (Fig. 1) and (b) a brief period of incubation at a high temperature prior to or in the middle of a cold period increases or has no effect on the rate of formation of the promoter (Figs. 5 and 7). The results for *Oenothera* germination which were discussed in the previous three paragraphs are consistent with this model.

Apple seeds show some similarities to *Oenothera* seeds in their response to low temperature. For germination, apple seeds require several weeks of cold pretreatment and then a return to a warm temperature (9, 25, 29). Apple seeds germinate very slowly after short cold pretreatments, but as the cold period is extended, germination becomes more rapid. This is similar to *Oenothera*, in which incubation at 8°C increases the germination rate at 28°C (Figs. 6 and 7). In apple, cold pretreatments also lead to an increase in the amount of detectable free gibberellins A_4 and A_7 in the embryonic axis (16, 17). The correlation of gibberellin content with germination response in apple seeds (16, 17) is highly suggestive, although a low temperature treatment affects many other metabolic processes and it is difficult to prove that a change in gibberellin content alone causes the change in germination response. Nevertheless, *Oenothera* seeds and apple seeds respond to cold temperature in a similar manner and these germination responses are also similar to the phenomena of vernalization (23, 26), breaking of bud dormancy (24), and induction of tuber growth (27). The similar effect of cold temperature on *Oenothera* seeds and these other phenomena suggests that there is a similar mechanism which regulates these responses.

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