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Thermoperiodism in Cocklebur Seed Germination

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ABSTRACT

Germination potential in nondormant, upper cocklebur (*Xanthium pensylvanicum* Wallr.) seeds, which were incapable of germinating under constant temperatures below 25 C in air, was increased by exposure to diurnally alternating temperatures. The cocklebur seeds failed to respond to the temperature fluctuations in the beginning of water imbibition, and their responsiveness appeared only after aerobic presoaking for a limited period or after anaerobic pretreatment for 1 to 3 days.

Maximal germination was obtained after exposure to a thermoperiodic regime of 8 hours at 23 C and 16 hours at 8 C. A process occurring during the high temperature phase was aerobic and had to precede the inductive low temperature phase, its effect increasing with temperature. Critical minimum length of the inductive low temperature phase changed with the duration of a preceding anaerobiosis, for instance about 4 hours after 1 day anaerobiosis, but about 2 hours after 2 days. Percentage of subsequent germination was in proportion to the number of thermoperiodic cycles. A process of the inductive low temperature phase was not perturbed by inserting a brief higher temperature period into its phase; indeed, such insertion rather increased germination potential when performed in the earlier parts of the inductive low temperature phase. The effect of the low temperature survived for 13 to 17 hours during the higher temperature period.

A large literature has accumulated regarding the favorable effect of diurnal temperature fluctuations on seed germination, often called thermoperiodism (see 3, 10, 12–14). While this phenomenon is not necessarily ubiquitous, many kinds of seed germinate well with temperature fluctuations. To our knowledge, there is no report describing that cocklebur seed response to temperature fluctuations for germination. Recently, however, it has become obvious that this seed exhibits bimodal germination responses to temperature (9, 11). Nondormant, upper cocklebur seeds are incapable of germinating at constant temperatures below 25 C, giving progressively higher germination counts as the temperature increases (11). On the contrary, a preexposure of the seed to chilling at 5 to 10 C increases the germination potential, if the seed has been hydrated fully in air over a certain time period, and consequently allowed to germinate at constant 23 C (9). Cocklebur seeds were potentiated to germinate by a chilling treatment which was inserted into an imbibition period at 23 C in which their germination did not occur; this led to a possibility that the cocklebur seeds may also exhibit a thermoperiodic behavior on germination. If it could be defined, an analysis of conditions for inducing a thermoperiodism in the cocklebur seeds would contribute to elucidate its mechanism.

MATERIALS AND METHODS

Fully after-ripened, nondormant upper cocklebur (*Xanthium pensylvanicum* Wallr.) seeds, selected for uniformity, were used in this study. The seeds were sown 60 to 70 in Petri dishes which contained two discs of filter paper and 9 ml of distilled H_2O. Except for an experiment shown in Figure 1, the seeds were aerobically soaked at 23 C for indicated periods prior to alternative temperature treatments, since it is known that the responsiveness of cocklebur seeds to chilling appears after aerobic presoaking (9). Then the dishes with seeds were enclosed in light-tight black bags and manually transferred from one environmental chamber which was controlled at indicated temperatures to another in order to expose to alternative temperatures in darkness. After the temperature treatments, the dishes were returned to 23 C to examine the germinability of treated seeds. Germination, as assessed by emergence of the radicle tip, was determined after 2 or 3 days. Each experiment consisted of two dishes, repeated at least two times, and the data reported herein were representative.

For anaerobic treatment, the dishes were put in a jar, the atmosphere within which was substituted with N_2 after three repetitions of evacuation by vacuum and of subsequent refilling with N_2. Experiments changing O_2 tension in ambient atmospheres were carried out using flasks in which presoaked cocklebur seeds were placed in two discs of moistened filter paper, and the O_2 tensions within the flasks were prepared as described previously (8).

RESULTS

Occurrence of Thermoperiodism. As shown in Figure 1, three cycles of an alternating temperature of 8 hr at 23 C and 16 hr at 8 C or 3 days of continuous chilling at 8 C were inserted into the treatment at various times after the start of soaking at 23 C, and 18 days later the germinated seeds were counted. The response of cocklebur seeds to chilling became much more pronounced with increased duration of a presoaking period, which was in accordance with the result obtained in a previous paper (9). Similarly, the cocklebur seeds were capable of responding to a temperature fluctuation; alternating temperatures were ineffective when given at the start of water imbibition, but caused about 85% germination when given 12 days later. Whenever the alternating temperatures were interposed, they were more effective in potentiating the seeds to germinate than the continuous chilling.

In a recent paper (9) it was found that the responsiveness of cocklebur seeds to the chilling falls gradually as the duration of the aerobic presoaking is increased beyond about 15 days. Similarly, we could not detect a responsiveness to alternating temperatures in the cocklebur seeds after a prolonged presoaking (data not shown).

Chilling Responsiveness after Anaerobiosis. We have also found that the decreased chilling responsiveness in aged cocklebur seeds is again increased by a preceding anaerobic treatment (9). However, it is not known how the responsiveness to the chilling changes with duration following anaerobiosis. Therefore, presoaked seeds were subjected to 8 C for 2 days after 3-day anaerobiosis according to the schedules illustrated in Figure 2.

Cocklebur seeds which received neither anaerobiosis nor chilling failed to germinate. Chilling applied immediately after the anaerobiosis led to about 12% germination. A short period at 23 C

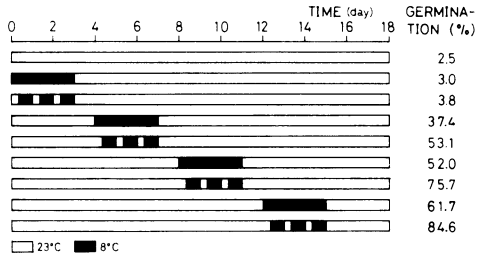

FIG. 1. Occurrence of thermoperiodism for germination during a soaking period. At 0 day, cocklebur seeds were imbibed to water and 18 days later germination was recorded.

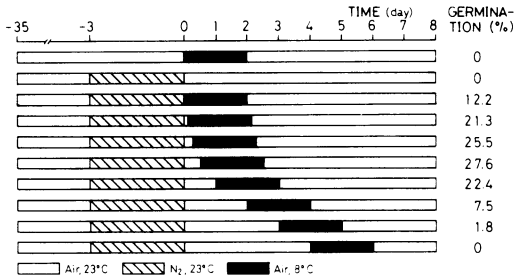

FIG. 2. Enhancement of the chilling effect by an intervening higher temperature period. Seeds presoaked for 35 days were incubated in anaerobiosis at 23 C for 3 days and exposed to chilling at 8 C for 2 days after various durations at 23 C in air.

intervening between the anaerobiosis and the chilling caused significantly more germination, the maximum germination being achieved at 12 hr. A prolonged exposure to 23 C decreased germination below that obtained with no intervening period.

Thermoperiodic Germination. In Figure 2, it was brought out that a period of moderate temperature after an anaerobiosis enhances the chilling responsiveness of aged seeds. This suggests also that the excessively presoaked seeds, which have lost the chilling responsiveness, are potentiated to germinate by the diurnal temperature fluctuations, if they were previously subjected to anaerobiosis. Seeds presoaked aerobically and kept in anaerobiosis for 3 days were subjected to the varied durations of continuous chilling at 8 C, n(OH + 24L), or to the diurnal regime of alternating temperatures of 23 and 8 C, n(8H + 16L)[1] (Fig. 3).

No germination occurred in the seeds that had not received chilling. Concurrently with the result of a preceding paper (9), germination increased in proportion to the number of cycles, the duration of the chilling, in n(OH + 24L) series, the effect of the chilling being cumulative only within a limited period.

In a series of n(8H + 16L), on the other hand, cocklebur seeds invariably gave much higher germination counts, although the total lengths of the chilling period were short compared to those in the n(OH + 24L) series. The germination increased to about 96% with four cycles of temperature fluctuations.

Necessity of O_2 for High Temperature Phase. A process proceeding at lower temperature is known to be aerobic (9). An experiment shown in Table I was designed to examine the necessity of O_2 for the high temperature phase in the 2(8H + 16L) regime. The low temperature phase at 8 C was aerobic.

There was little or no thermoperiodic germination in the absence of O_2. Germination was permitted only at the O_2 tensions of 3% or higher.

Temperature Dependence of High Temperature Phase. An experiment given in Table II shows how temperatures in the high temperature phase influence the thermoperiodic germination. For comparison, half of pretreated seeds were incubated at constant temperatures, and the other half were exposed to a regime of 3(10H + 14L).

The effect of alternating temperatures was positively correlated with the temperature of the high temperature phase. Thus, the greater the temperature differential, the more pronounced was the thermoperiodic germination. It is already known that under constant temperatures, the percentage of germination of cocklebur seeds increased with increasing temperature (11). Also in this case, a few seeds were capable of germinating at 26 C.

Critical Lengths of the Inductive Low Temperature Phase. The critical minimum and optimal lengths of the inductive low temperature phase in the thermoperiodic regimes were determined in relation to the duration of the anaerobic pretreatment. The anaerobic pretreatment is known to raise by itself the germination potential of this seed (6, 8).

Seeds presoaked aerobically for 25 days were incubated in anaerobiosis for the various periods indicated in Figure 4, and thereafter subjected to the diurnal regimes represented by 3[(24-X)H + XL], in which the duration of the low temperature phase

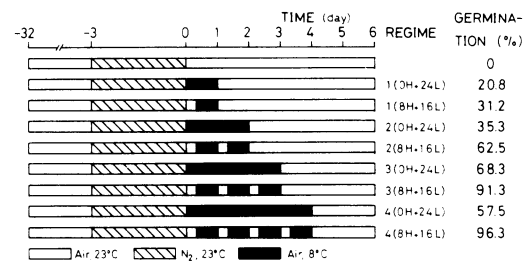

FIG. 3. Thermoperiodic germination of cocklebur seeds aged by excessive presoaking. Seeds presoaked for 32 days were treated according to illustrated schedules.

Table I. Effect on thermoperiodic germination of O_2 tension in the higher temperature phase

Cocklebur seeds which were pre-soaked for 30 days and then incubated in anaerobiosis for 2 days were subjected to a 2(8H + 16L) regime, in which the high and low temperature phases were 23 and 8 C, respectively.

O_2 Tension	Germination
0 %	2.4 %
3	29.5
10	42.8
air	40.2

Table II. Effects on thermoperiodic germination of temperature during the high temperature phase

Cocklebur seeds which were pre-soaked for 40 days and then incubated in anaerobiosis for 3 days were subjected to either 3(24H + 0L) or 3(10H + 14L) regime, in which the low temperature phase was 8 C.

Temperature	Germination	
	3(24H + 0L)	3(10H + 14L)
17 C	0 %	4.6 %
20	0	19.3
23	0	44.8
26	3.2	72.0

[1] Abbreviations: n(XH + YL): diurnal alternations of n cycles between a high temperature period for X hr and a low temperature period for Y hr; n[(24-X)H + XL]: diurnal alternations of n cycles between low temperature periods for X hr and high temperature periods for the remainder of a day.

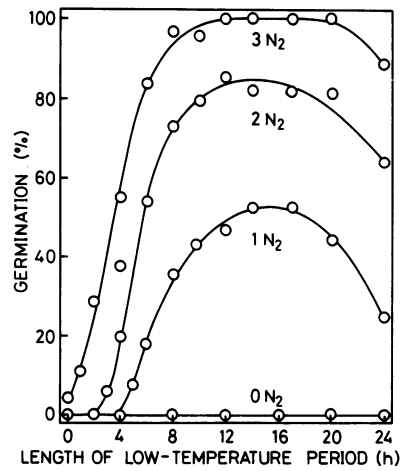

FIG. 4. Critical low temperature periods of thermoperiodic germination of aged cocklebur seeds. Twenty-five days presoaked cocklebur seeds were incubated in anaerobiosis for different days indicated in the figure, and then subjected to three cycles of the diurnal regimes of temperature fluctuations. 3[(24-X)H + XL].

was altered. The seeds which received no anaerobiosis failed to germinate under any regime. In anaerobically pretreated seeds, the optimal length of the low temperature phase at 8 C was about 16 hr, regardless of the duration of the anaerobiosis. The extent of subsequent germination potentiated by a continuous low temperature, 3(OH + 24L), as well as that of thermoperiodic germination, increased as the duration of the anaerobiosis was extended. Similarly, the critical minimum length of the inductive low temperature phase was decreased with the duration of the anaerobiosis. The critical length was about 4 hr after 1 day of the anaerobiosis, 2 hr after 2 days, and disappeared after 3 days. The anaerobic pretreatment of 3 days led to slight germination without exposure to chilling.

Interruption of the Inductive Low Temperature Phase by Higher Temperature. In an experiment shown in Figure 5, the inductive low temperature phase on a 2(16H + 8L) regime was interrupted by various durations of a higher temperature period at 23 C. Prior to exposure to fluctuating temperatures, seeds were aerobically presoaked for 18 days and thereafter kept in anaerobiosis for 2 days, and the perturbative higher temperature periods were interposed in the middle of a low temperature phase of 8 hr at 8 C.

Contrary to expectations, an intervening higher temperature for periods as short as 30 to 90 min increased germination potential, consequently giving much higher germination counts under a subsequent condition for germination test. The perturbation of the inductive low temperature phase was brought about only by the intervention of higher temperature periods longer than 2 hr. If the duration of the intervening higher temperature period was 3 hr, the duration of actually continuing low temperature phase was no more than 2.5 hr, which suggests that the reduction of germination percentages by the higher temperature interruption may be due to the shortening of the inductive low temperature phase itself. Thus, the duration of the low temperature phase became insufficient for inducing the effect of the low temperature.

Time Sensitivity to Perturbative Higher Temperature during the Inductive Low Temperature Phase. The intervention of a higher temperature was thought to nullify the effect of the low temperature period depending upon its intervening time. Therefore, seeds pretreated as in Figure 5 were subjected to a similar thermoperiodic regime, 2(16H + 8L), in which a higher temperature of 3 hr at 23 C was interposed into the different times of the inductive low temperature phase of 8 hr at 8 C (Fig. 6).

Germination inhibition by the interposed higher temperature occurred near the end of the low temperature phase. The extent of reduced germination was almost similar to that obtained by a 2(21H + 3L) regime. Accordingly, the reduction of germination percentage is probably due to the shortening of an actual low temperature phase by its higher temperature interruption. However, the earlier parts of a low temperature phase did not result in the reduction of germination, despite the fact that it split the low temperature phase into the same partition as did the interruption at the later parts. This may result from the existence of the promotive effect of a higher temperature, which probably occurs only at the earlier part of the low temperature phase. Therefore, the time of occurrence of the promotive effect of a higher temperature was tested in the next experiment.

Time Sensitivity to Promotive Higher Temperature during the Inductive Low Temperature Phase. Seeds which received 18 days of aerobic presoaking and 2 days of anaerobic pretreatment were subjected to 2(12H + 12L) of 23 and 8 C. One hr of 23 C temperature was applied to interrupt a 12-hr low temperature phase at various times (Fig. 7).

The stimulative effect of higher temperature of 1 hr was most effective when inserted into the earlier parts of a low temperature phase, causing the maximum increase in final germination percentage at 3 hr after the start of the low temperature phase. No reduction of germination occurred at any part of the low temper-

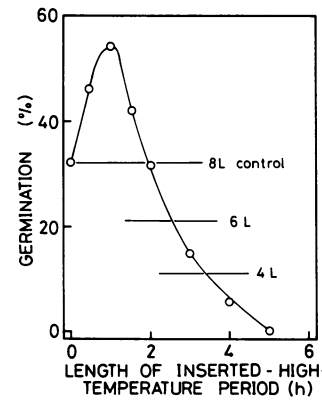

FIG. 5. Interruption of the inductive low temperature (8 C) phase by a higher temperature (23 C) of various durations. Cocklebur seeds which were presoaked for 18 days and pretreated in anaerobiosis for 2 days were subjected to a 2(16H + 8L) regime, into the middle of which 23 C periods were inserted. Horizontal lines with the numbers in figure show the germination percentages in different regimes in which the low temperature phases were 8, 6, and 4 hr.

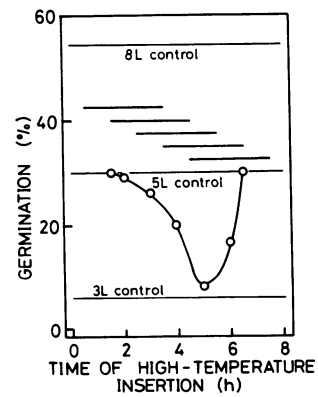

FIG. 6. Time sensitivity to the perturbative higher temperature during the inductive low temperature phase. Cocklebur seeds which were presoaked for 18 days and pretreated in anaerobiosis for 2 days were subjected to a 2(16H + 8L) regime, in which the higher temperature at 23 C for 3 hr was inserted into the various parts of an 8-hr low temperature phase of 8 C. Horizontal lines in figure show the germination percentages at 2(21H + 3L), 2(19H + 5L), and 2(16H + 8L) regimes, and horizontal bars in figure show the times of the higher temperature insertion.

ature phase by the short insertion of higher temperatures, unlike the result of the long insertion in Figure 6.

Surviving Duration of Low Temperature Effect. All of the thermoperiodic regimes used so far in this study were daily alternations of temperatures. The fact that the germination potential of cocklebur seeds was increased also by a single temperature shift, as shown in Figures 1, 2, and 3, would suggest that alternating temperatures are not necessarily implicated in a diurnal fluctuation. Nevertheless, it is an unquestionable fact that the effect of low temperature is cumulative when temperature alternations were applied. The last experiment was designed to examine a condition for the accumulation of the low temperature effect, that is, as to observe how the effect declines during a period of higher temperature at 23 C. Seeds which received 24 days of presoaking and 2 days of subsequent anaerobiosis were subjected to a schedule illustrated in Figure 8. Thirty hr of 23 C temperature was taken in order to remove the promotive effect of a higher temperature inserted after the anaerobiosis and before the first low temperature period (Fig. 2), and 3 hr of 8 C temperature was used as the unit of a low temperature period near its minimum critical length (Fig. 4). How long the effect of the preceding low temperature period survives during the higher temperature period at 23 C was examined by adding the effect of a second low temperature period for 3 hr to that of the first one.

As noted in Figures 5 and 7, the inserted higher temperature period greatly stimulated subsequent germination when relatively short, lasting 2 to 8 hr. When it was extended to about 13 hr, however, germination percentage was almost equal to that obtained by a continuous low temperature period for 6 hr. When it reached about 17 hr, the effect of the preceding low temperature was completely lost, and when it was prolonged beyond 20 hr, even the effect of the following chilling period did not appear. These results suggest that the effect of the low temperature survives for 13 to 17 hr.

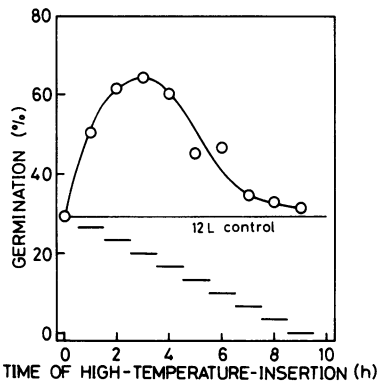

FIG. 7. Time sensitivity to the promotive higher temperature during the inductive low temperature phase. Cocklebur seeds which were presoaked for 18 days and pretreated in anaerobiosis for 2 days were subjected to a 2(14H + 10L) regime, in which the higher temperature at 23 C for 1 hr was inserted into the various parts of a 10-hr low temperature phase at 8 C. Horizontal bars in figure show the times of the higher temperature insertion.

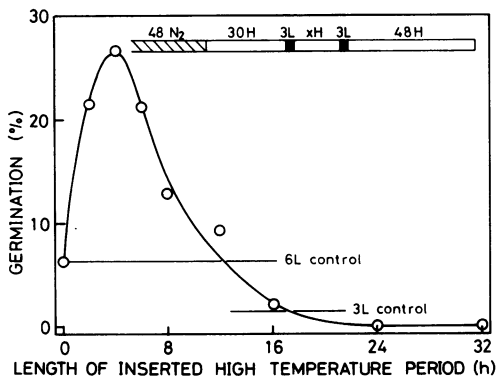

FIG. 8. Survival times of the effect of a low temperature period during a higher temperature period. Cocklebur seeds which were presoaked for 24 days were treated according to a schedule illustrated in figure, in which the duration at 23 C between two low temperature periods at 8 C for 3 hr was changed. Horizontal lines in figure show the germination percentages at 2(2H + 3L) and 2(18H + 6L) regimes.

DISCUSSION

In a previous paper (9), it was found that cocklebur seeds which imbibe water and are held under aerobic conditions for several days are potentiated to germinate by a single exposure to chilling, a phenomenon which was termed "chilling induction" of seed germination. The experiments described here have shown, however, that regimes of diurnal alternations between a higher and a lower temperature are more effective for their potentiation than a single chilling (Figs. 1, 3, and 4). This shows clearly that like seeds of many plant species which germinate better in a regime of diurnal temperature fluctuations than at constant temperatures, cocklebur can show a thermoperiodic behavior on germination.

The previous paper (9) also indicated that cocklebur seeds increased their responsiveness to chilling with aerobic soaking, followed by a decrease beyond 15 days. Also, the responsiveness to a thermoperiodic regime changes similarly in this respect; hence, the cocklebur seeds could not respond to temperature fluctuations in either the earlier periods (Fig. 1) or the later periods (data not shown) of soaking. As in our previous paper (9), the responsiveness of aged cocklebur seeds to the thermoperiodic regime which was lost during an excessively prolonged presoaking, was recovered by subsequent exposure to anaerobiosis for a few days (Figs. 3 and 4). These facts suggest that many seeds which have been considered to lack thermoperiodic behavior on germination may become responsive to alternating temperatures after some preconditioning. If this is true, the response of seeds to temperature fluctuations may be a very widespread phenomenon among plants, apart from whether their responsiveness is actual or latent.

The physiological mechanism of thermoperiodic germination is still obscure, although diverse explanations for this have been tried (2). In fact, more of the foregoing explanations is sufficient to account for the results presented here. Specific conditions as a prerequisite for the occurrence of thermoperiodic germination in cocklebur seeds may give us some clue for understanding its mechanism.

Anaerobic pretreatment during a soaking period is known t potentiate cocklebur seeds to germinate, termed "anaerobic induction" of seed germination (6, 8). Parallel to the change in responsiveness to a single shift to a low temperature or fluctuating temperatures during a soaking period, the responsiveness of cocklebur seeds to the anaerobic induction increased and then decreased with increasing imbibition time (7). After such prolonged water imbibition, which has not permitted their germination, the cocklebur seeds have been considered to be in a state of secondary dormancy. For inducing secondary dormancy at room temperatures, the upper seed of cocklebur did not require any specific treatment besides water imbibition, whereas the lower one had to be exposed to atmospheres very poor in O_2 (4). In previous papers (7, 9) involving the occurrence of anaerobic and chilling inductions during the soaking period, secondary dormancy was supposed to be a condition in which a balance between the germination-inhibiting system and the germination-stimulating system was maintained in favor of the former. Increase in the germination ability due to chilling was considered to be a result of a balance shift to the suppression of the former's activity and the accumulation of the latter's potential (9). Similarly, anaerobic induction was speculated to shift the balance by inactivating the germina-

tion-inhibiting system, and as a result, probably, to lead to a loss of the secondary dormancy. In Figures 2 and 3, however, the insufficient duration of the anaerobic induction led to bringing back the sensitivity to alternating temperatures which had been lost during aging, although an anaerobic induction of 3 days allowed germination without exposure to alternating temperatures (Fig. 4). These data imply that thermoperiodism on germination may be a phenomenon which is realized only when two antagonistic systems coexist and their balance in action is restricted within a certain range. In this sense, the condition for thermoperiodism seems essentially common to that for photoperiodism. From studies on photoperiodic tuberization and tuber sprouting in *Begonia* (5), we have presented evidence that the photoperiodic response may be determined by a balance between the photodependent and -independent reaction systems, which are antagonistic in their effects and compete for the product of the main light period common to the two systems.

Could we speculate on the existence of some product (precursor) or situation common to the two antagonistic systems in thermoperiodism and in photoperiodism? In preceding papers (7, 9), the existence of certain metabolite(s) produced during aerobic soaking and involved in the process of germination has been assumed. In Figure 2, higher temperature periods for several hr applied after anaerobiosis markedly increased the effectiveness of the subsequent chilling. This indicates that the higher temperature is more effective when given before rather than after the low temperature, and thus that the higher temperature to be effective must precede the low temperature. A similar relation was suggested from data in Figure 8, in which a brief insertion of a higher temperature period further increased the germination potential accumulated during the inductive low temperature period. This increase occurred, moreover, only when the brief higher temperature was followed by a low temperature period greater than a critical length.

In Figure 2, on the other hand, sensitivity to chilling became less as the inserted higher temperature period was extended, and finally disappeared. This suggests that the germination-inhibiting system which would be inactivated by anaerobic pretreatment is probably reactivated and exerts its action during a prolonged higher temperature period. The necessity of long term higher temperature for the manifestation of action of the germination-inhibiting system is suggested also from the results of Figure 5, in which shorter exposure to higher temperature failed to perturb the process proceeding at the low temperature. This necessity would thus permit a postulation that also the germination-inhibiting system requires the accumulation of certain metabolite(s) produced during the higher temperature period, in order to exert its inhibitory action. Based on these premises, we could define thermoperiodism, as well as photoperiodism, in seed germination as a phenomenon which occurs only when two counteracting systems, differing in temperature dependency, coexist for use of a common precursor.

Would the thermoperiodism in this case, like photoperiodism, be implicated in an endogenous diurnal rhythm? All of the present experiments, except for Figures 2 and 8, were carried out in diurnal regimes of temperature alternations. In fact, the maximal germination percentage was obtained in a regime of n(8H + 16L) (Fig. 4), regardless of the anaerobic pretreatment, and the thermoperiodic germination response was in proportion to the number of cycles (Fig. 3). These findings imply the participation of endogenous diurnal rhythm in the germination of cocklebur seeds. Similar suggestions with other seeds have been offered by others (12–14).

However, an inductive low temperature phase in thermoperiodism was not perturbed by a short duration of an inserted higher temperature (Figs. 5 and 6), although the dark period in photoperiodism was perturbed by a brief irradiation given at its middle. In Figures 5 and 7, a short higher temperature insertion even promoted germination. These data suggest that the process of the inductive low temperature phase, unlike that of the inductive dark period in photoperiodism, does not involve any time-measuring system, such as the phytochrome system, a conclusion which is supported also by the data in Figure 8. Thereupon, a nondiurnal regime of temperature fluctuations, such as shown roughly by 2(5H + 3L), gave higher germination counts. Similar results were obtained by Butler (2), who observed that the seed germination of *Stylosanthes humilis* occurs most rapidly when the cut pods were subjected to an alternating temperature regime of 10 and 35 C based on short length cycles of 1.5 and 4.5 hr. Similarly, vigorous germination under nondiurnal temperature fluctuations is also observed with *Capsella bursa-pastoris* (1). These findings seem to rule out the participation of circadian rhythm in thermoperiodic germination.

LITERATURE CITED

1. ABIDIN ZU 1956 Die Beeinflussung der Samenkeimung durch den Temperaturwechsel. Z Bot 44: 207–220
2. BUTLER JE 1957 Germination of *Stylosanthes humilis* (Townsville stylo) in short cycles of alternating temperature. Seed Sci Technol 3: 323–328
3. CROCKER W, LV BARTON 1953 Physiology of Seeds. Chronica Botanica Co., Waltham Mass
4. DAVIS WE 1930 Development of dormancy in seeds of cocklebur (*Xanthium*). Am J Bot 17: 77–87
5. ESASHI Y 1964 Studies on the formation and sprouting of aerial tubers in *Begonia evansiana* Andr. X. Tuberization under long-days and in darkness. Plant Cell Physiol 5: 101–117
6. ESASHI Y, K KOTAKI, Y OHHARA 1976 Induction of cocklebur seed germination by anaerobiosis: a question about the "inhibitor hypothesis" of seed dormancy. Planta 129: 109–112
7. ESASHI Y, Y OHHARA 1977 Enhancement by low temperatures of the anaerobic induction of cocklebur seed germination. Aust J Plant Physiol. In press
8. ESASHI Y, M OKAZAKI, K WATANABE 1976 The role of C_2H_4 in anaerobic induction of cocklebur seed germination. Plant Cell Physiol 17: 1151–1158
9. ESASHI Y, Y TSUKADA, Y OHHARA 1977 Interrelation between low temperature and anaerobiosis in the induction of cocklebur seed germination. Aust J Plant Physiol. In press
10. HARRINGTON GT 1923 Use of alternating temperatures in the germination of seeds. J Agric Res 23: 295–332
11. KATOH H, Y ESASHI 1975 Dormancy and impotency of cocklebur seeds. I. CO_2, C_2H_4, O_2 and high temperature. Plant Cell Physiol 16: 687–696
12. KOLLER D 1972 Environmental control of seed germination. *In* TT Kozlowski, ed, Seed Biology. Academic Press, New York, pp 1–101
13. LANG A 1965 Effects of some internal and external conditions on seed germination. Encycl Plant Physiol 15-2: 843–893
14. MAYER AM, A POLJAKOFF-MAYBER 1975 The Germination of Seeds. Pergamon Press, Oxford