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Phytochrome Action during Prechilling Induced Germination of *Betula papyrifera* Marsh[1]

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ABSTRACT

Seeds of paper birch (*Betula papyrifera* Marsh.) were induced to germinate by prechilling at 3 C or by red light. The light requirement was mediated by phytochrome and the action of phytochrome during prechilling was investigated. Red irradiation (R) prior to prechilling markedly enhanced the effectiveness of the prechilling treatment in inducing subsequent germination at 18 C. Reversal of this enhancement by far-red irradiation (FR) was more effective when FR was supplied after a 1-week prechill treatment than after a 2-week treatment. The R enhancement effect exhibited a sharp drop as prechilling temperature was increased from 5 to 7 C. This decline is consistent with a membrane phase transition at about 7 C where Pfr action is diminished by a loss in sensitivity of its receptor sites. Although phytochrome action was observed during prechilling treatments, the seeds failed to germinate at prechilling temperatures. Therefore, it was concluded that while potentiation of germination by Pfr occurred during prechilling, some other reaction(s) leading to radicle protrusion requires higher temperatures. In one seed source loss of germination potential was observed with protracted storage at 3 C. This was prevented by R supplied during the prechilling treatment. Taken collectively the data suggest that action of phytochrome during prechilling is accentuated in these seeds by two factors: (*a*) an increase in the sensitivity (or number) of Pfr receptor sites; and (*b*) preservation of Pfr by deferment of thermal reversion.

The seeds of many plants require light to germinate. The light requirement for germination has been studied extensively and in many species is known to be mediated by phytochrome (3, 4, 18). Moreover, some light-requiring seeds can be induced to germinate by a period of prechilling at low temperature (stratification). Hence, a low temperature treatment may substitute for the light requirement. Nikolaeva (11) has termed such seeds nondeeply dormant to distinguish them from seeds with an obligate chilling requirement. Seeds of the genus *Betula* exhibit this type of response (1, 7, 8, 20). In *Betula pubescens* the light requirement of unchilled seeds is imposed by the pericarp (1) and this has been attributed to an inhibitory substance in the pericarp and to reduced O_2 diffusion to the embryo (2). Excised embryos do not require light or prechilling to germinate (2, 14).

The purpose of this study was to examine the relationship between the role of low temperature and phytochrome in controlling seed dormancy in paper birch. In particular, it was of interest to determine if phytochrome might act during prechilling treatments and how temperature might affect this action. The results suggest interactions between low temperature and Pfr which would explain how prechilling promotes germination in this species. Furthermore, since prechilling promotes germination in other light-requiring species, the results may be helpful in understanding the action of prechilling in other nondeeply dormant seeds.

MATERIALS AND METHODS

Catkins of paper birch (*Betula papyrifera* Marsh.) were collected in the autumn of 1977 and air dried on newspapers at room temperature. Samples from individual trees from New Hampshire, Ontario, and Alaska were used. A New Hampshire seed source (71-C) was collected in the White Mountains near Albany (43°58' N, 71°11' W) and an Ontario seed source (90) came from Ramsey Lake near Sudbury (46°30' N, 81°00' W). Samples from Alaska were collected at three locations. Seed source 67-A was from a coastal population of *B. papyrifera* var. *commutata* at Skagway (59°12' N, 135°19' W). Sources 61-A, 61-B, 61-C, and 62-A were from Fairbanks (64°52' N, 147°46' W) while sources 65-E and 65-F were collected at Canyon Village along the Porcupine River (67°09' N, 142°06' W). The samples from Fairbanks and Canyon Village were *B. papyrifera* var. humilis. Achenes, referred to here as "seeds," were separated from catkin bracts by sieving, and the samples were stored dry in sealed plastic bags at 4 C until used. Samples were removed from storage only for the time required to set up experiments.

Samples were incubated in Petri dishes (9 × 2 cm) containing one circle of Whatman No. 1 filter paper, two circles of Whatman No. 3 filter paper, and 10 ml of distilled-deionized H_2O. Samples maintained under continuous light (Fig. 1) were placed in reach-in growth chambers illuminated with fluorescent and incandescent light (2,000 ft-c at seed level). Germination was recorded daily for 3 weeks. For other experiments, dishes were placed in the dark in cardboard boxes covered with aluminum foil and incubated in growth chambers. For chilling experiments the dishes were wrapped in two layers of aluminum foil and placed in a walk-in cold room at 3 ± 2 C. There was no germination in these seed sources at 3 C. After chilling, the dishes were moved into a dark room, transferred to the cardboard boxes, and placed in growth chambers at either 18 or 25 C for 2 weeks. Preliminary experiments had shown that dark germination was essentially complete after

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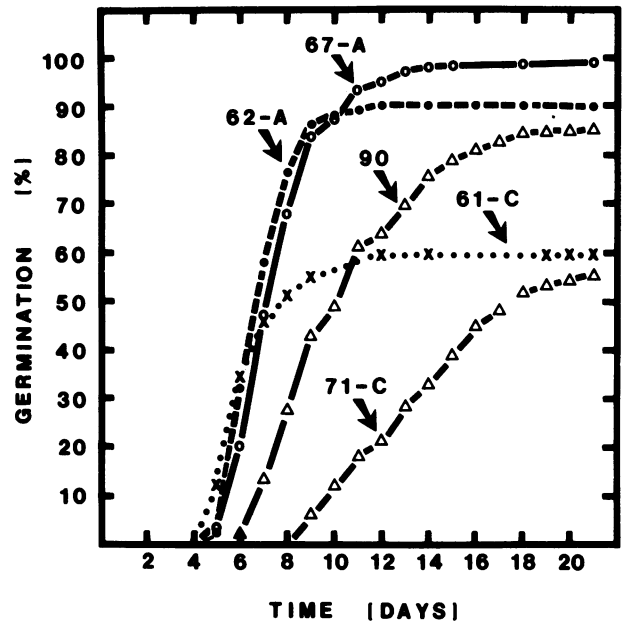

FIG. 1. Germination of unchilled seeds at 18 C under continuous light. Samples were maintained in a growth chamber for 3 weeks under fluorescent and incandescent light (2,000 ft-c at seed level). Germinated seeds were counted daily and removed from plates.

2 weeks. Chilling experiments were scheduled so that all treatments were transferred to growth chambers at the same time. Boxes were removed from the growth chambers and filter papers moistened with water as necessary in the darkroom. All manipulations in the dark were done with a dim green safelight prepared by covering two cool white 15 w fluorescent lamps with 18 layers of No. 623164 green cellophane (Bienfang Paper Co., Metuchen, NJ). This did not induce germination. Temperatures in growth chambers were set with a thermocouple placed inside of a Petri dish and are ±1 C.

R[3] and FR light sources were constructed with modifications after those described by Poff and Norris (13). Plastic filters (30 × 30 cm) obtained from Carolina Biological Supply Co. (CBS), Burlington, North Carolina, were placed over openings (27 × 27 cm) in the tops of light-tight plywood boxes (43.5 × 43.5 × 10 cm). For most experiments R was obtained by passing light from two 40 w cool white fluorescent lamps through a CBS 650 filter. The seeds were 28 cm below the lamps and the irradiance at seed level was about 2.0 μw cm^{-2} nm^{-1} at 665 nm as measured by an ISCO model SR spectroradiometer calibrated against a standard lamp. FR was obtained by passing light from a 500 w incandescent flood lamp through 10 cm of distilled H_2O in a glass tank supported on a wood frame and then through a CBS 750 filter. Seeds were 64 cm below the lamp and irradiance at seed level was about 12.6 μw cm^{-2} nm^{-1} at 750 nm. Petri dish covers were removed prior to irradiation and R and FR were supplied for 10 min and 15 min, respectively, unless indicated otherwise. Spectral energy distributions for the light sources were comparable to those reported by others for these filters (9, 13). For the experiments reported in Figure 4, R was obtained by filtering the fluorescent light through two layers of No. 626162 red cellophane (Bienfang Paper Co.).

Germination percentages were based on viable, well-filled seeds. Since production of viable seed in paper birch is markedly influenced by climate, site location, and mother tree, not all seeds in a sample contain viable embryos. For this reason unpollinated seeds, poorly filled seeds, and those with aborted embryos were discarded at the end of each experiment by examining each Petri dish with a dissecting microscope. By directing light through the seeds from below the microscope stage it is possible to distinguish well filled seeds from others which would not be expected to germinate (12). Thus, all samples did not contain the same number of viable seeds upon which to determine percentage germination. Generally, there were 70 to 180 viable seeds per Petri dish, but in no case were there less than 50 viable seeds per dish. In some seeds two embryos were observed; these were excluded. The frequency of double embryos was 4% of viable seed or less in the sources used in these experiments. Germination was considered to have occurred when radicle protrusion was 2 mm or greater. Except where noted, data are means of two separate experiments each of which utilized two Petri dishes per treatment. Standard deviations are given for tabular data and presented as vertical bars on figures.

[3] Abbreviations: R, red irradiation; FR, far-red irradiation.

RESULTS

Response of Unchilled Seeds to Light and Temperature. Unchilled seed samples maintained in the dark showed poor germination at temperatures of 25 C or lower (Table I). Data presented in Table II show that the New Hampshire seed source had a light requirement mediated by phytochrome. Similar results were obtained using Alaskan seed sources (results not shown). The promotion of germination by R was greater when the seeds were incubated at 25 C than at 18 C (Table III). Ten min of R induced high germination in seven of the eight seed sources tested at 25 C. However, only two seed sources showed appreciable R-induced germination at 18 C. Germination at 18 C was improved in unchilled samples of three seed sources by continuous light (Fig. 1, compare Tables I and III). The increase in germination at 18 C by continuous light was also observed in other seed sources not used in this study.

Effect of Prechilling on Dark Germination. The New Hampshire seed source and an Alaskan seed source (62-A) were selected to examine the response to prechilling at 3 C. Germination in the absence of light was markedly promoted in both seed sources after

Table I. *The Influence of Temperature on Dark Germination of Unchilled Seeds*

Samples were maintained in the dark for 14 days after which germination was recorded. Average two experiments except where noted.

Seed Source	Germination at			
	18 C	23 C	25 C	30 C
	%			
New Hampshire (71-C)	1 ± 1	3[a]	10 ± 3	31 ± 0
Alaska (62-A)		4[a]	6 ± 2	14 ± 2
Alaska (67-A)	3 ± 4	7 ± 5	10 ± 5	50 ± 0

[a] Values from a single experiment.

Table II. *Phytochrome Control of Germination in Unchilled Seeds*

Samples of the New Hampshire seed source were imbibed for 4 h in the dark at 25 C, irradiated in the sequence shown, and maintained in the dark at 25 C for 14 days. R and FR were given for 10 min and 15 min, respectively.

Treatment	Germination
	%
Dark control	9 ± 1
R	97 ± 1
FR	3 ± 2
R, FR	1 ± 1
R, FR, R	96 ± 2
R, FR, R, FR	2 ± 2

Table III. *Effect of R on Germination of Unchilled Seeds from Sources Maintained at 18 or 25 C*

Unchilled samples were imbibed for 4 h in the dark at 25 C, irradiated for 10 min with R, and incubated for 2 weeks at either 18 or 25 C. Data are means from two to four experiments except as noted.

Seed Source	Germination at			
	18 C		25 C	
	Control	R	Control	R
	%			
New Hampshire (71-C)	2[a]	3[a]	13 ± 7	96 ± 2
Ontario (90)	0	85 ± 4	3 ± 0	100
Alaska (61-B)	0	1 ± 1	2 ± 1	39 ± 2
Alaska (61-C)	0	0	0	63 ± 5
Alaska (62-A)	0	4 ± 0	7 ± 1	74 ± 4
Alaska (65-E)	0	0	0	17 ± 1
Alaska (65-F)	0	2 ± 1	1 ± 1	77 ± 1
Alaska (67-A)	2 ± 2	88[a]	11 ± 2	99 ± 1

[a] Means from a single experiment.

only 1 week at 3 C when samples were transferred to 25 C after prechilling (Fig. 2). The Alaskan sample achieved and maintained a higher level of germination after 2 weeks of prechilling than did the New Hampshire sample. There was a pronounced difference in the effectiveness of the prechilling treatment when samples were transferred to 18 C. The Alaskan seed source showed high germination after chill times of 2 weeks or longer. The New Hampshire seed source showed a gradual increase in germination potential with chill times up to 3 weeks; thereafter the prechilling stimulus was progressively lost. After 10 weeks of prechilling, the New Hampshire seed source reacted like unchilled seeds and showed low germination when transferred to 18 C. Prechilling the Alaskan seeds for 9 and 10 weeks caused a downward trend in germination potential at 18 C similar to the New Hampshire seed source. Loss of the prechilling stimulus by the New Hampshire seed source suggested that some factor(s) which accumulates during prechilling is lost unless the seed is transferred to a temperature warmer than 18 C. Loss of the prechilling stimulus with transfer to 18 C has been observed in other seed sources from Alaska, Ontario, and New Hampshire.

Action of R and FR on Loss of the Prechilling Stimulus. Because the light requirement of this seed is mediated by phytochrome, the effects of R and FR on loss of germination potential during prolonged prechilling of the New Hampshire seed source were examined. Samples were prechilled, given R during prechilling, and returned to 3 C for the remainder of the prechilling period. Samples were then transferred to 18 C to permit germination (Fig. 3). In this experiment, the loss of prechilling stimulus was more rapid than in Figure 2. This may have been due to small temperature fluctuations during the prechilling treatments. Nonetheless, R prevented the decline in germination potential when supplied throughout the prechilling treatment and caused essentially complete germination. The enhancement of germination by R given at the 3-week point of a 7-week prechill period was reversed by FR (Table IV). Samples of the New Hampshire seed source which had been prechilled for 7 weeks showed low germination at 18 C when maintained in the dark. The enhancement of germination potential obtained by R given at 3 weeks was removed by FR and reinstated by subsequent R. In other experiments, samples of the New Hampshire seed source were prechilled for 23 weeks and irradiated after 20 weeks as described in Table IV. When transferred to 18 C for 2 weeks, the prechilled samples which were not irradiated showed only 3% germination. R supplied after 20 weeks of prechilling induced 89% germination, and as before, FR removed the R enhancement. Therefore, in this seed source photoreversible control of germination persists through a storage period of at least 20 weeks at 3 C. Moreover, the data suggested that Pfr action may occur at 3 C.

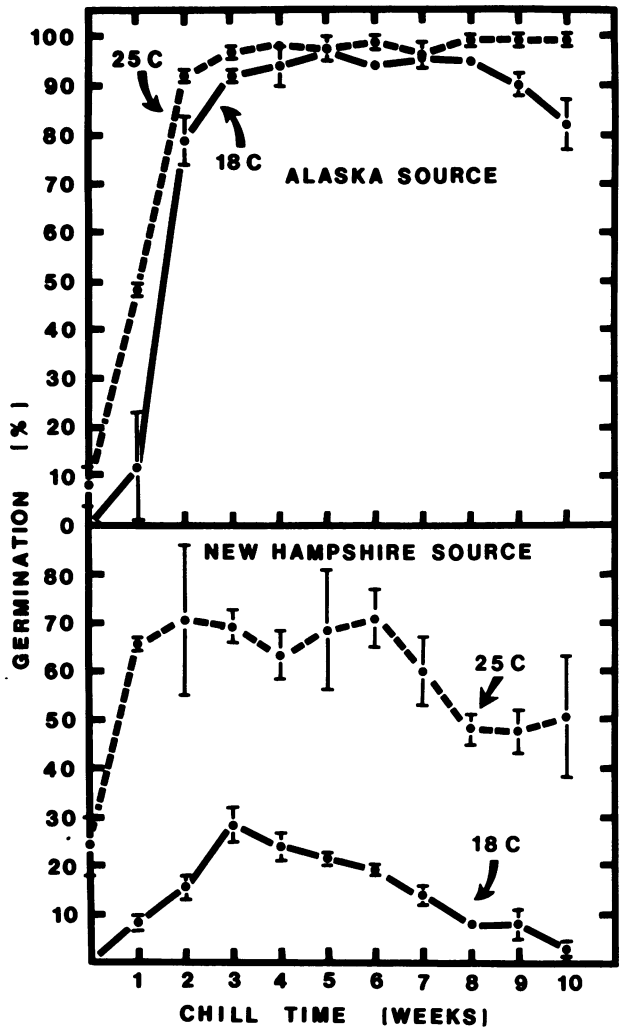

FIG. 2. Effect of prechilling on dark germination in the New Hampshire seed source and an Alaskan seed source (62-A). Samples were sprinkled onto moist filter paper in Petri dishes, wrapped immediately in aluminum foil, and placed at 3 C. Treatments were scheduled so that all prechill times were completed on the same day. Samples were then incubated for 2 weeks in the dark at 18 C or 25 C after which germination was recorded.

Phytochrome Action During Prechilling. If phytochrome action takes place during the prechilling period, the effectiveness of prechilling should be increased as the level of Pfr is increased in the seed. To examine this possibility, samples were given increasing doses of R prior to prechilling to establish different levels of Pfr and then prechilled for 1 week at 3 C. The samples were then transferred to 18 C for 2 weeks after which germination was counted. Because the supply of Alaskan seed source 62-A had been consumed, two other Alaskan seed sources (61-B, 61-C) were selected. The effectiveness of the prechilling treatment increased as the dose of R was increased (Fig. 4). Although 15 min of R failed to induce germination in unchilled samples of either seed source at 18 C, as little as 20 s of R prior to prechilling caused significant germination. Apparently prechilling sensitizes these seeds to low levels of Pfr. Similar results were obtained with other seed sources from Alaska and from New Hampshire.

To demonstrate phytochrome action during the prechilling

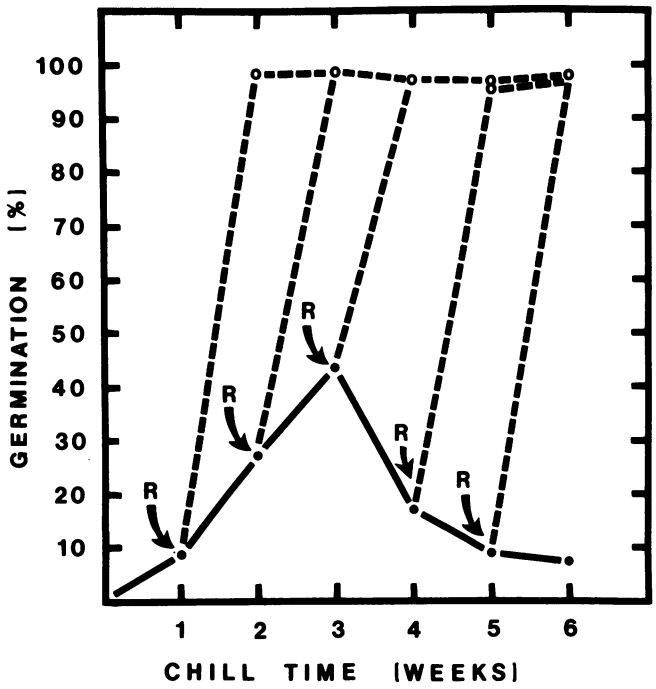

FIG. 3. Effect of R supplied during the prechilling treatment on loss of prechilling-induced germination potential. Samples of the New Hampshire seed source were prechilled in the dark at 3 C and then transferred to 18 C for 2 weeks after which germination was recorded. Dark controls (——) received no irradiations. Other samples (-----) received 10 min of R during the prechilling period at the times indicated and were then returned to the cold.

Table IV. *Effect of R and FR Supplied after 3 Weeks of Prechilling on Germination of New Hampshire Seeds Chilled for 7 Weeks and Transferred to 18 C*

Samples were prechilled at 3 C in Petri dishes wrapped with aluminum foil, given R (10 min) and/or FR (15 min) in the sequence indicated at the 3-week point of the 7-week prechill period, and then returned to 3 C for 4 weeks. After prechilling, samples were placed at 18 C in the dark for 2 weeks after which germination was recorded. Germination of nonirradiated seeds was 39% after 3 weeks of chilling.

Treatment	Germination
	%
Dark (unchilled)	0
Prechilled 7 weeks	6 ± 1
Prechilled 7 weeks + R after 3 weeks	97 ± 1
Prechilled 7 weeks ± FR after 3 weeks	8 ± 8
Prechilled 7 weeks + R, FR after 3 weeks	10 ± 11
Prechilled 7 weeks + R, FR, R after 3 weeks	98 ± 3

period, samples of two seed sources were irradiated before and after chill periods of 1 week and 2 weeks. After prechilling, samples were incubated for 2 weeks in the dark at 18 C and germination was recorded (Table V). Germination was promoted to a greater extent by prechilling in the Alaska seed source than in the New Hampshire seed source. In both seed sources R given before prechilling enhanced the effectiveness of prechilling and this response was antagonized by FR given immediately after R. When supplied after 1 week of prechilling, FR was highly effective at antagonizing the promotive action of R given prior to prechilling. However, when the prechilling period was lengthened to 2 weeks, the inhibitory effect of FR after prechilling declined in both seed sources. These data suggest that Pfr can act during the prechilling treatments as well as later when the seeds are transferred to a warmer temperature.

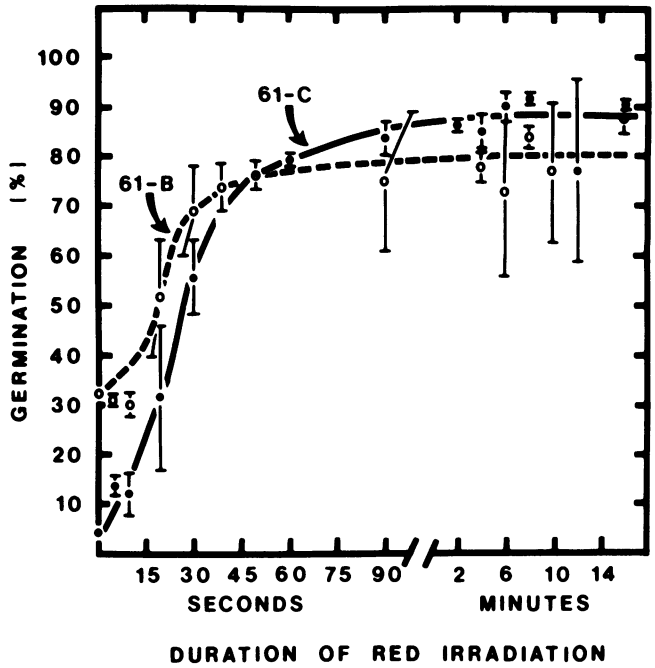

FIG. 4. Effect of R supplied prior to prechilling on the effectiveness of the chilling period. Samples of two Alaskan seed sources were imbibed for 6 h at 25 C, given various doses of R, and chilled in the dark for 1 week at 3 C. Samples were then incubated for 2 weeks at 18 C and germination was recorded. After 2 weeks at 18 C unchilled dark controls failed to germinate, and a 15 min dose of R failed to induce germination in unchilled samples of either seed source.

Effect of Prechilling Temperature on Red Light Enhancement of Germination. Since phytochrome action occurred during the prechilling treatment, it was of interest to determine how differences in prechilling temperature would affect potentiation of germination by Pfr. Seeds were given R, prechilled for 3 weeks at different temperatures, and then transferred to 18 C for 2 weeks (Fig. 5). Although the two seed sources responded differently to the prechilling treatment, both showed a dramatic loss of red light enhancement of prechilling at temperatures greater than 5 C. At a prechilling temperature of 12 C the seeds failed to respond to R. The effectiveness of prechilling was lost in a similar fashion in the dark controls. These data suggest that the action of Pfr during prechilling is markedly impaired in some way at temperatures between 7 and 12 C. Seeds which received R and were frozen at −5 C were fully potentiated and germinated when transferred to 18 C.

DISCUSSION

The promotive action of low temperature has been ascribed to deferment of the loss of Pfr by thermal reversion (15, 16). Taylorson and Hendricks (17) suggested that in *Amaranthus* seeds low temperature depresses thermal reversion of Pfr more than the interaction of Pfr with its receptor site. By this interpretation the major role of prechilling would be to preserve Pfr levels. In *B. papyrifera* part of the promotive action of prechilling may be attributed to such preservation; however, the experiments reported here suggest that in some way low temperature may also change the availability or sensitivity of Pfr receptor sites (substrate). In Figure 4 the increased sensitivity to prechilling induced by R could be interpreted as an increase in Pfr receptor sites during the low temperature treatment. Several lines of evidence have linked temperature responses in seeds to membrane integrity (5, 6), and some phytochrome mediated responses appear to involve changes in membrane properties (10). Therefore, it seems reasonable that

Table V. *Effects of R and FR Given Before or After Prechilling on the Germination of a New Hampshire Seed Source (71-C) and an Alaskan Seed Source (67-A)*

Samples were prechilled for 1 or 2 weeks in the dark at 3 C and then maintained in the dark at 18 C for 2 additional weeks after which germination was recorded. Samples which were irradiated prior to prechilling were imbibed in the dark at 25 C for 4 h (71-C) or for 6 h (67-A) before irradiation. For those samples receiving no light or receiving light only after prechilling, the 25 C imbibition was omitted. Controls were not irradiated. Unchilled seeds failed to germinate after 2 weeks at 18 C; however, R induced 11 ± 7% and 86 ± 5% germination, respectively, in unchilled samples of the New Hampshire and Alaskan seed sources maintained at 18 C for 2 weeks.

Treatment	New Hampshire Source (71-C)		Alaska Source (67-A)	
	Prechill period (weeks)		Prechill period (weeks)	
	1	2	1	2
Controls	5 + 1	31 ± 7	28 ± 1	62 ± 7
Controls (imbibed at 25 C before chill)	7 ± 5	19 ± 10	9 ± 6	23 ± 6
R before chill	79 ± 2	98 ± 1	71 ± 11	77 ± 3
R, FR before chill	3 ± 0	16 ± 1	6 ± 5	26 ± 4
FR before chill	2 ± 1	21 ± 5	9 ± 4	27 ± 4
R before chill + FR after chill	9 ± 5	54 ± 4	12 ± 7	40 ± 6
FR before chill + R after chill	91 ± 0	98 ± 0	95 ± 5	99 ± 1
R after chill	92 ± 4	99 ± 1	99 ± 1	99 ± 0
FR after chill	5 ± 1	26 ± 4	18 ± 6	81 ± 2

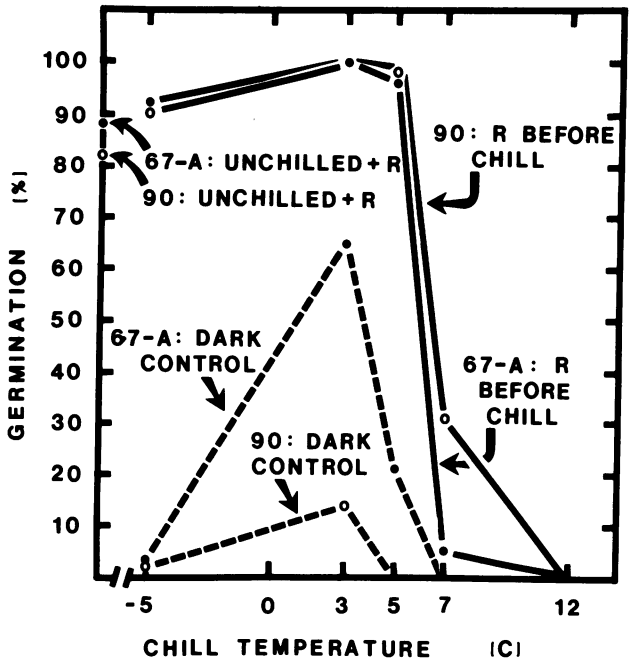

FIG. 5. Effect of prechilling temperature upon R enhancement of germination. Alaskan (67-A) and Ontario (90) seed sources were prechilled for 3 weeks at the temperatures shown and then incubated for 2 weeks at 18 C. Samples which were irradiated before prechilling (——) were imbibed in the dark for 6 h at 25 C, given 10 min of R, wrapped in aluminum foil and prechilled. Dark controls (-----) received no imbibition prior to prechilling. Dark, unchilled controls of both seed sources failed to germinate after 2 weeks at 18 C.

Pfr action may be modified by the physical state of cellular membranes. Moreover, VanDerWoude and Toole (19) recently reported that in lettuce seeds prechilling temperatures below 18 C induce a large increase in sensitivity to low levels of Pfr. They proposed that this effect was associated with a membrane phase transition near 18 C. The sharp increase in red light enhancement of the prechilling effect as temperature decreased from 12 to 5 C is a similar phenomenon (Fig. 5). We feel that this also represents a membrane effect. From this view Pfr receptor sites which remove dormancy become markedly more sensitive to Pfr (or increase in number) as temperature decreases from 12 to 5 C.

In these seeds temperature delineates two responses to R, a low temperature response associated with the prechilling effect and a higher temperature response in unchilled seeds. While R enhanced the promotion of germination by prechilling at temperatures below 7 C, the seeds failed to respond to R at 12 C (Fig. 5). This "dead spot" of R sensitivity disappeared at 18 and 25 C (Table III). We suggest that high sensitivity of Pfr receptor sites induced by prechilling is lost at temperatures above 5 C because of physical changes in some cellular membrane(s). However, not all receptor sites were lost above 5 C since sensitivity to R was apparent at higher temperatures in unchilled seeds (Table III). The failure of seeds to display a response to R at 12 C may have been due to a depression of events following potentiation. That is, the seeds may not have germinated because enzymic steps required for radicle growth did not proceed adequately. The sensitivity to R increased as temperature was increased from 18 to 25 C (Table III) and at 30 C the seeds began to lose the light requirement and germinate in the dark (Table I). This change in behavior with increased temperature could be explained by increases in three components: (*a*) the interaction of Pfr with its receptor sites (*i.e.* the potentiation event); (*b*) enzymic steps which follow potentiation and culminate in radicle protrusion; and (*c*) loss of Pfr by thermal reversion. Increases in the first two components would account for the greater sensitivity to R at 25 C compared to 18 C and for partial loss of the light requirement at 30 C. An increase in the third component would explain why the dark germination at 30 C was less than samples given R at 25 C (compare Tables I and III). That is, while the first two reactions would be accelerated with increased temperature the loss of Pfr would also be enhanced and some seeds would not fulfill their phytochrome requirements. Hence, these seeds do not exhibit thermodormancy at 30 C.

In the New Hampshire seed source loss of germination potential with protracted prechilling suggests the development of secondary dormancy. Because it can be reversed by R it may be due to a slow loss of pre-existent Pfr by thermal reversion. Thus, Pfr interacts with its receptor sites during the first 3 weeks of prechilling and as time increases, more seeds fulfill their phytochrome requirements. However, loss of Pfr by thermal reversion seems to catch up with phytochrome action after 3 weeks and germination potential at 18 C declines as less and less Pfr remains. It may also be that some loss of Pfr receptor sites occurs with protracted storage at 3 C.

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