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Effects of Low Temperature and Age of Plant on Flowering in *Lolium perenne* L.[1]

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EXPOSURE to winter conditions of low temperatures and short days greatly hastens the emergence of the inflorescence of many temperate grasses when they are subsequently exposed to long days and warm temperatures. Species and strains vary from those with no cold or short-day requirement for flowering to those in which such are obligatory. Between these extremes, the flowering response is proportional to length of exposure to these pre-induction conditions. Research on "seed chilling" has been conducted for more than a century, but the importance of the short-day component of winter conditions has been realized only in recent years. Although efforts to elucidate the mechanisms of vernalization have not been very rewarding, charting the effects of various pre-induction treatments has enabled the plant scientist to control life cycles of many species (2).

The authors encountered difficulties in obtaining expected results by chilling germinated seeds in preliminary experiments with those strains of perennial ryegrass known to have high pre-induction cold requirements as mature plants. The present experiments were conducted to determine if the stage of seedling development or age of plant influenced subsequent floral response to low temperatures. A further objective was to compare effects of natural vernalizing conditions with those of constant low temperature and artificial lights.

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MATERIALS AND METHODS

All experiments were conducted with a single strain of perennial ryegrass originating from a certified seed lot produced in Oregon. One hundred plants were selected and screened by the root fluorescence test to eliminate any showing annual characteristics. After vegetatively subdividing each plant into 9 equal segments, the resulting 900 plants were established in an isolated nursery in the field to permit random pollination. Natural photoperiods were extended to 18 hours by artificial light to facilitate intercrossing of plants differing in natural time of anthesis. Seed used in these experiments was a composite from this planting.

The initial experiment was designed to compare *(1)* germinating seeds vernalized in complete darkness at 35° F. ± 3°, *(2)* seedlings grown at greenhouse temperatures for 4 weeks and 10-hour photoperiods before transferring to 35° F. ± 3°, and 10 hours light daily, and *(3)* seedlings grown as in *(2)* above but transferred out-of-doors after 4 weeks for exposure to natural winter conditions. The 4-week-old seedlings were in the 3-to 4-leaf stage when exposed to the natural or artificial pre-induction conditions.

Seed vernalization treatments were conducted in petri dishes by permitting 48 hours of germination in the dark at room temperature before transferring to low temperature. Dishes were wrapped in aluminum foil to exclude light, but were opened briefly each week in diffuse light to permit air exchange. Water was added as needed to keep the filter paper saturated.

Seedlings were started in a soil-sand-peat mixture in the greenhouse. Temperatures in the greenhouse were 75° to 80° F. during daytime and 65° to 70° F. at night throughout these experiments. Upon emergence, theseedlings were exposed to 10 hours of natural light daily until they were 4 weeks old. At this time half of the plants were placed in the cold chamber at 35° F. ± 3° and provided with 10 hours artificial light daily from a fluorescent source at an intensity of 500 to 600 foot-candles. The remaining half were transferred out-of-doors to natural winter conditions.

In each of the treatment conditions described above, lots of 48 seeds or 4-week-old seedlings were maintained for 0-, 2-, 4-, 8-, 12-, 16-, and 20-week periods, except that there was no 2-week period in natural winter conditions. Treatments were begun on November 11, 1961, and concluded on March 31, 1962. The monthly mean minimum and maximum temperatures and mean photoperiods for this period are shown in Table 1. The photoperiods are those at the mid-month period including civil twilight (6° below horizon), and may be slightly in excess of effective light during winter periods with cloudy days.

Upon completion of the natural or artificial cold treatments, the plants were transferred to a greenhouse and placed under continuous light in a randomized complete block design with eight replications. Natural daylengths were extended by incandescent lights providing about 100 f.-c. at plant height.

A second experiment concerned the effect of plant age on heading. Plants were grown to ages of 4, 8, 12, 16, and 20 weeks in the greenhouse with photoperiods restricted to 10 hours. Half of the plants were then transferred to 35° F. temperature with 8-hour photoperiods and 900 f.-c. fluorescent light for 4 weeks. The other half remained in the greenhouse conditions for this period. All plants were then transferred to continuous light in the greenhouse. Each treatment consisted of 3 individual plants in each of 6 replications.

In both experiments responses to all treatments were measured by the number of days from transfer to continuous light until emergence of the tip of the inflorescence from the sheath. Data are recorded as "days to heading." In the first experiment, the percentage of plants heading was a further measurement.

Table 1. Monthly temperatures and daylengths during the experimental period at Davis, Calif., 1961–62.

Month	Temperatures (° F.)			Photoperiod, hours*
	Mean	Min.	Max.	
Nov.	52	40	64	11.1
Dec.	44	37	52	10.6
Jan.	42	32	53	10.8
Feb.	48	40	55	11.7
Mar.	50	39	62	12.8
Mean	47	38	57	11.4

* Based on the 15th day of each month and includes civil twilight (6° below horizon).

RESULTS

Cold treatment of seedling plants resulted in a greater percentage of plants heading and more rapid heading than treatment of germinating seeds. As shown in Figure 1, all seedling plants headed after exposure to 4 or more weeks of either natural environment or constant 35° F. In contrast, the germinating seeds exposed to 4 weeks at 35° F. headed only 50%. As the length of cold treatment was extended, the percentage of heading increased in those treated as seeds but even with 20 weeks of cold, nearly 10% of the plants remained vegetative. It appears that individuals with very high cold requirements cannot be induced to flower by cold treatment of the germinating seeds unless treatment is continued for very long periods. There was no difference in heading percentage between seedling plants exposed to the natural environment of winter and those exposed to constant 35° F. temperatures for 4 or more weeks.

Seedling plants exposed to the natural environment, and those maintained in 35° F. also responded similarly as measured by days to heading. Days to heading decreased in a curvilinear manner as length of cold periods increased. The curve leveled out at about 18 days suggesting this as a minimum time for the developing inflorescence to emerge. Plants exposed to constant 35° F. for 4 weeks headed about 9 days earlier than plants exposed to the natural environment in December 1961. The mean maximum and minimum temperatures for December were 52° F. and 37° F., respectively. Although 54% of the plants receiving no cold treatment had headed after 120 days in continuous light, the percentage of such plants heading in natural photoperiods, which reach a maximum of 16 hours at 38° N latitude, would be considerably less than this. Under greenhouse conditions it was found that continuous light resulted in a much higher percentage of plants heading than 18-hour photoperiods.

The nature of the response curve for days to heading of the cold-treated seeds was similar to that of cold treated seedling plants. However, individuals exposed to cold as seeds required from 23 to 53 days longer to reach the heading stage. The largest difference between the response of seeds and seedling plants occurred with the 4-week cold treatment. The interval between 2 and 4 weeks of cold caused the greatest reduction in days to heading for treated seedling plants while the cold-treated seeds responded most to the 4- to 8-week cold interval. In considering these responses, it must be remembered that the seedling plants were 28 days older than the germinating seeds at the time of cold treatment and therefore might be expected to progress towards heading more rapidly. In the 0-, 8-, 12-, 16-, and 20-week cold treatments, differences between responses of plants grown from cold-treated seeds and those treated as seedling plants approximated 28 days. In the 2- and 4-week treatments, even after subtraction of the 28 days, there remains an 8- to 25-day advantage. A more detailed comparison of the response of germinating seeds and seedlings to cold is possible by analyzing the rapidity of heading.

The cumulative percentage of plants heading for each cold treatment at 35° F. over a period of 120 days is shown in Figure 2 as days to heading on a logarithmic scale. When germinating seeds received no cold treatment but were placed immediately into 24-hour photoperiods, only 33% of the resulting plants headed in 120 days. Two and 4 weeks of chilling greatly reduced the time required for the earliest plants to head, but in neither case did more than 50% of the plants head. Seed treatment in 35° F. for 8, 12, 16, and 20 weeks produced plants which began heading after about 30 days in continuous light. Figure 2 shows that approximately 10% of this population has cold

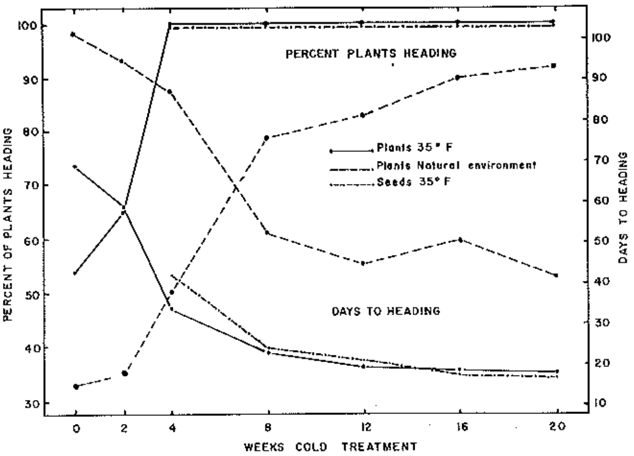

Figure 1. Effect of cold treatment of germinating seeds and 4-week-old plants on percentage of plants heading and mean days to heading.

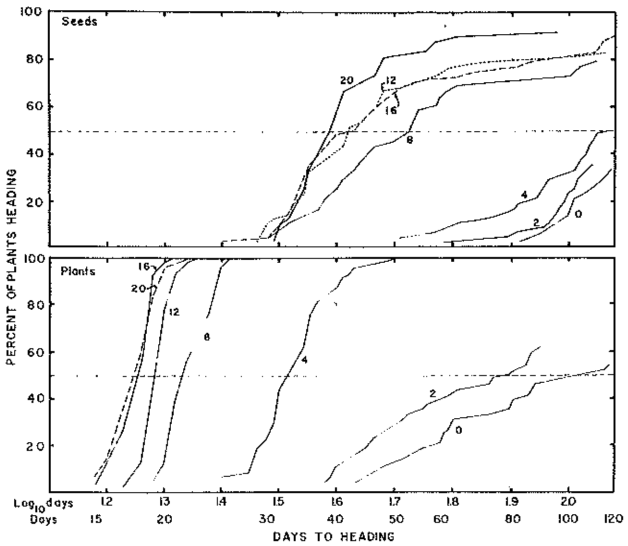

Figure 2. Effect of number of weeks of cold treatment of germinated seeds and 4-week-old plants on percentage of plants heading, cumulated over 120 days. Numbers on curves denote weeks of treatment. Broken lines are merely for ease of discernment.

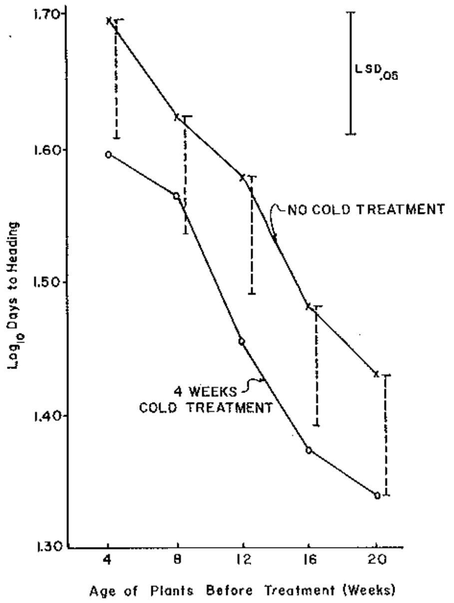

Figure 3. Effect of age of plant on the response to 4 weeks of low temperature as measured by days to heading.

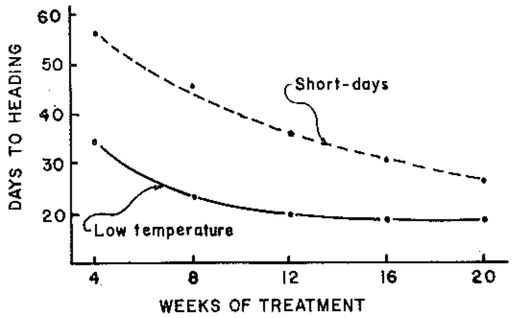

Figure 4. Comparison of the effects of short-day pre-induction conditions (10-hour daylength + warm temperature) with those of low temperature (10-hour daylength + 35° F.).

requirements that are fully satisfied by 8 weeks of seed chilling. Cold periods longer than this did not hasten heading in this segment of the population. The remainder of the population showed a further decrease in days to heading with 12- and 16-week treatments. Only in the last 50 to 60% of the population did days to heading decrease further with 20 weeks of cold. About 8% of the population failed to head even with the longest cold treatment as germinating seeds.

Four weeks of cold treatment of seedling plants was more effective than the 20-week treatment of germinating seeds as measured by both days to heading and percentage of plants heading. Increasing lengths of cold treatments reduced not only the mean days to heading but also the variation within each treatment. The coefficients of variation for the 4-, 8-, 12-, and 16-week treatments were 22.6, 10.4, 6.0, and 6.8%, respectively. The reliability of the data for the 20-week treatment may be questionable due to inadvertent nitrogen burning of the plants in this treatment early in growth.

The question arose as to whether further growth or older plants would result in still greater response to cold. A second experiment compared heading responses of plants exposed to 4 weeks of constant 35° F. temperature after they had been grown in short-day conditions in warm temperatures for 4, 8, 12, 16 and 20 weeks. A check treatment remained in warm temperatures (65° F. at night, 75° F. during day) and 10-hour daylengths during the 4-week treatment. Statistical analysis of flowering response is frequently hindered by the failure of some plants to flower during the course of the experiment. Only 5 plants of the 180 used in this experiment failed to head in 120 days—one in every 4- and 8-week age group, and one in the 12-week group in the check treatment. For the purpose of analysis, the value *120* was used as days to heading for these plants. An analysis of variance was performed on logarithmically transformed data. Results are presented in Figure 3 as a comparison of cold-treated and noncold-treated plants for each age group.

The 4-week cold treatment reduced the number of days to heading the same amount in all age groups. Thus, increase in age of plants beyond 4 weeks did not increase the response to cold, but both cold treatment and increasing age of plants resulted in more rapid heading when plants were subsequently subjected to continuous light and warm temperatures.

In this experiment plants were grown for various periods in short days and warm temperatures before treatment. Consequently, what has been referred to as "age of plant" can be considered as duration of exposure to short days. Several workers (3, 4) have reported that some long-day grasses, including perennial ryegrass, show a response similar to that produced by low temperatures when exposed to short days and warm temperatures. This has been referred to as "short-day vernalization." By comparing the heading responses of the plants grown for various periods in short days plus warm temperatures to those of plants in the first experiments treated for equivalent periods at 35° F., the relative efficiencies of these two pre-induction treatments can be determined (Figure 4). In both the 4- and 8-week treatments, plants exposed to low temperature headed about 22 days earlier than those kept in short days and nonvernalizing temperatures. However, this difference decreases in treatments of longer duration as a minimum limit is reached in the low temperature treatment.

DISCUSSION

Cooper proposed and developed the method of seed chilling of ryegrass to analyze local populations as to their flowering behavior (1). The method affords the advantages of economy of time and space but has been found inadequate in many cases where vernalization of all individuals of a population of *Lolium perenne* was desired. Studies by Evans (4) pointed to the fact that 4-week-old plants resulted in more rapid vernalization than treatment of seeds at 10° C. He exposed seeds as well as plants to 8-hour photoperiods. Our studies, as well as those of others (3), indicate that heading responses are the same whether seeds are treated in the dark or in 8-hour photoperiods. Peterson et al. (5) found that in a strain of darnel which responded to short periods of seed chilling, cold treatment in the advanced seedling stage was seldom more effective than seed treatment. In the present experiment the increased effectiveness of treatment in the 4-week old stage is seen only in that portion of the population having the higher cold requirements (Figure 2). If only the earliest 50% of the population is considered, treatment of seeds resulted in a shorter time from germination to heading in cold treatments of 8 weeks or longer. However, in the 30% of the population having the highest cold requirements, treatment of germinating seeds was ineffective as compared with treatment of 4-week old plants. *Lolium* individuals with relatively low cold requirements can be fully vernalized in the germinating seed stage. Adequate vernalization of individuals having a high cold requirement require treatment in a more advanced growth stage.

The precise stage at which a plant is most responsive to cold treatment has been only partially determined. Evans (4) found that plants treated at the 8-week stage showed no higher response to cold than plants at 4 weeks. In this experiment 4-week-old plants were as responsive as those 20 weeks old. Optimum response in our material is acquired at some stage between the germinating seed and the 4-week-old plant (3 to 4 leaves).

Studies of *Lolium perenne* dealing with the effects of the short-day component of winter conditions have produced results varying from no vernalizing effect (4) to complete vernalization (3). Cooper (3) has attributed differences in results to genetic differences between strains used and to differences in temperatures during the short-day treatment. Our results show that in this strain plants growing in short days and warm temperatures gradually increase in rapidity of heading in subsequent long days and that, given enough time a response equal to that of the maximum low temperature response might be achieved.

SUMMARY

Four-week-old plants of a relatively early flowering strain of perennial ryegrass were found to be vernalized equally well in artificial conditions of 8-hour photoperiods and constant 35° F. as in natural winter conditions.

When germinating seeds and 4-week-old plants were given equivalent low temperature treatments and then transferred to continuous light and warm temperatures, the percentage of plants heading was greater and days to heading were fewer with treatment at the 4-week-old stage.

Increase in age of plants beyond 4 weeks did not result in increased response to cold treatment.

Plants grown in short days and warm temperatures gradually progressed toward a vernalized condition.

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