NATURE AND GENETIC CONTROL OF THE VERNALIZATION RESPONSE IN *PHALARIS TUBEROSA* L.

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Summary

Vernalization of seed promoted flowering in *P. tuberosa*, particularly in ecotypes with an obligate cold requirement. The rate of thermoinduction was slower in seed than in seedlings, but all ecotypes responded to seed vernalization by flowering earlier and at a smaller leaf number. Also, combinations of vernalization of seed and seedling were additive in their promotion of flowering.

Attempts to replace the inductive effect of low temperature with either short days or gibberellin treatment were not successful. Short days had a slight inductive effect, but only at intermediate temperatures close to the vernalizing range.

Selection for vernalization requirement indicates that there is considerable additive genetic variation for this charater in the Australian cultivar and that it is under polygenic control. Response to selection in both directions was rapid, and the difference in vernalization requirement between the high and low lines after four generations of selection was greater than the total range found among naturally occurring ecotypes. The vernalization requirement appears to be relatively independent of seedling growth characters, but is correlated with flowering time both in controlled environments and in the field.

In view of the considerable genetic variation stored in populations of *P. tuberosa*, and the high heritability ($0 \cdot 63 \pm 0 \cdot 06$) found for the vernalization response, it should be possible to select for plants which will remain vegetative indefinitely when grown in a mild winter environment.

I. Introduction

Many of the temperate perennial grasses belonging to the subfamily Festucoideae will not flower under favourable day lengths without previous exposure to a period of low temperature (vernalization). Low temperature under these conditions has an inductive role, as inflorescence initiation occurs subsequently when plants are exposed to relatively higher temperatures and long days. Vernalization together with photoperiod represent the two major environmental factors controlling flowering in this group. A detailed account of vernalization and related phenomena is available in a comprehensive review by Lang (1965).

The nature of the vernalization response in grasses varies. Some species have an obligate cold requirement such that without exposure to low temperature plants can remain vegetative indefinitely. Others show a quantitative response with low temperature causing only an acceleration in the rate of flowering. The optimum temperature for thermoinduction lies in the range from 1 to 7°C, although vernal-

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ization can occur over a much wider range, from about −5 to 12–14°C. At the temperature extremes, however, the range, rate, and effectiveness is often greatly reduced (Purvis 1948; Hänsel 1953; Evans 1960*a*).

In *Phalaris tuberosa* L., a typical Mediterranean perennial, all gradations from a quantitative response to an obligate cold requirement occur. Ecotypes from Morocco and Israel have little or no vernalization requirement, but are accelerated in their flowering by cold, whereas populations from more northern latitudes in Turkey and Greece require up to 8 weeks vernalization to induce complete flowering (Cooper and McWilliam 1966). Also, the extent of the cold requirement in the various ecotypes is closely related to the winter temperature of the place of origin (Ketellapper 1960; Cooper and McWilliam 1966).

Because of the outbreeding nature of the species, populations from any one locality are not genetically uniform with respect to their cold requirement. However, the correlation between the proportion of plants that fail to flower without cold treatment, and the duration of the cold requirement to induce flowering of the whole population is high (Ketellapper 1960).

The actual role of vernalization in the control of flowering in temperate perennial grasses may operate more to prevent inflorescence initiation in the autumn than to permit it in the spring (Evans 1964), because in most of the native environments of these species vernalization requirements are probably satisfied early in the winter period. Although there are exceptions to this generalization, as with many of the arctic and subarctic grasses which initiate in the autumn (Hodgson 1966), it appears to be true of the Mediterranean ecotypes of *P. tuberosa*.

In addition to this indirect role of vernalization in flowering, there is also a positive correlation in *P. tuberosa* between the duration of the cold requirement, and the time to ear emergence following vernalization in the field. This suggests that the rate of floral development in the spring is in some way associated with the cold requirement, and is largely responsible for the flowering time of these ecotypes. A similar conclusion has been reached by Silsbury (1964) in the case of *Lolium perenne*, and also by Pugsley (1965) in the flowering of spring wheat.

The general features of the vernalization response in *P. tuberosa*, and in particular the extent of the climatic variation, have been described in previous studies (Ketellapper 1960; Cooper and McWilliam 1966). This paper describes the results of a more detailed study of particular aspects of this response, including the existence of a juvenile phase, and the extent to which certain photoperiodic and chemical treatments are capable of substituting for the cold requirement. Selection experiments were also undertaken to investigate the genetic control of the vernalization response, and the nature of the association between vernalization and flowering time in this species.

II. Materials and Methods

Four cultivars of *P. tuberosa* were used, representing contrasting ecotypes with a range of vernalization requirements. These are measured as the number of weeks of low temperature (9/4°C) necessary for complete flowering of the population in long days at 24/19°C (Ketellapper 1960; Cooper and McWilliam 1966). Details of the four ecotypes are given in the following tabulation:

Code	C.P.I. Number	Origin	Environment	Vernalization Requirement (weeks)
A	14498	Algeria (Tadmit)	High altitude	>4
B	Australian	Mediterranean (Italy?)	—	4
C	19299	Algeria (Jemmapes)	Coastal	2
D	19305	Morocco (Amizmiz)	Foothills	0

Unless otherwise stated, all experiments were carried out in the Controlled Environment Research Laboratory (CERES) at Canberra, the design and facilities of which have been described by Morse and Evans (1962). With the exception of one experiment which was run in an artificially lit growth cabinet, all plants were grown in natural daylight in cabinets or controlled glasshouses under conditions of fluctuating day/night temperatures. The day temperatures, representing the high light period, extended from 8·30 a.m. to 4·30 p.m. and were 5 degC higher than the night (dark) temperatures. For photoperiods in excess of 8 hr supplementary incandescent lighting was used which provided an intensity of 40 f.c. at plant height.

All seeds were germinated at 25°C, and single seedlings were grown in 4-in. diameter pots, containing an equal mixture of perlite and vermiculite, at 21/16°C in short days (8 hr) until the third leaf stage, when the various treatments were applied. Pots were irrigated twice daily, once with a modified Hoagland nutrient solution and once with water. Following the various treatments, plants were exposed to uniform long day (16 hr) inductive conditions at either 21/16°C or 24/19°C to measure the flowering response. In two experiments this was measured by dissection of the apices on the primary shoot after 1 month in inductive conditions. For this purpose the following scoring system was adopted: 0 = vegetative; 1 = initiated (double ridge); 2 = florets differentiating; 3 = inflorescence fully differentiated; 4 = inflorescence "boot" stage; 5 = inflorescence emerged. In all other experiments the flowering response was measured by recording the percentage of plants with an inflorescence (ear) emerged, together with the number of long days required for emergence, and the leaf number at emergence.

(*a*) *Seed and Seedling Vernalization*

Seed was vernalized in the dark on moist filter paper at 2°C for 0, 4, 8, and 10 weeks, and then germinated and grown to the third leaf stage in short days (8 hr) at 21/16°C. Eighty seedlings of each ecotype, representing the four seed treatments, were then vernalized in natural daylight under short days at 9/4°C for 0, 1, 2, 3, 4, and 6 weeks. Following seedling vernalization, all plants were exposed to long days at 21/16°C, and the flowering response recorded. All response times were measured from the date of exit from the cold, as there is no evidence for initiation and development during short-day vernalization. A lower temperature (21/16°C) was used during the long-day induction to increase the frequency of flowering plants in those ecotypes with a vernalization requirement. The experiment was terminated after 12 weeks of long days.

(*b*) *Short-day Induction*

Plants were exposed at the third leaf stage to two non-vernalizing temperatures 25/20°C and 19/14°C, and at three photoperiods (8, 12, and 16 hr) in each. Controls at the same stage were vernalized for 4 weeks under the same photoperiods. Fifteen plants of each ecotype were subjected to each treatment, and progress towards flowering was measured by dissection after 1 month in long days at 24/19°C. In a separate experiment plants were vernalized as before, and after transfer to inductive long days, flowering data were recorded at the time of ear emergence.

(*c*) *Gibberellin Treatment*

The gibberellin used was the potassium salt of gibberellin A_3 (GA_3) and was applied both to seed during imbibition, and to young seedlings. Seeds were soaked for 6 hr at 25°C in a 3×10^{-5}M solution of GA_3. Following the treatment half of the treated and control seedlings were

exposed to 4 weeks of vernalization. The seedling treatment was carried out on a separate population of plants half of which had previously received a similar vernalization exposure. The plants were sprayed on each of 10 consecutive days with a $1 \cdot 5 \times 10^{-4}$M solution of GA_3 plus 0·1% of Tween 20. The flowering response of the treated and control plants in both groups, based on 10 plants of each ecotype per treatment, was determined by dissection after 1 month in long days at 24/19°C.

(*d*) *Selection Procedures*

The response to four generations of selection for both an increased (high) and decreased (low) vernalization requirement was measured in a population of the Australian cultivar B. In the first two generations selection was carried out by planting unvernalized seedlings in the field at 14-day intervals during the late winter. The high selection line was made up of plants remaining vegetative following the longest cold exposure, and the low line comprised plants flowering after the shortest exposure. The same procedure was followed for the third and fourth generations of selection, but the cold exposure was given under controlled-environment conditions at 9/4°C and the flowering response measured under inductive long days at 24/19°C. The selection intensity was relatively low, averaging 10% during the first two generations but was increased to an average of 3·6% under controlled conditions. Selected genotypes (15–20) were induced to flower and recombined after each selection cycle, and the vernalization requirement of the progeny measured. After four generations of selection, the cold requirement in the high, low, and control lines was analysed by measuring the flowering response in groups of 30 plants per line, after exposure to a wide range of vernalization treatments from 0 to 90 days.

From these results estimates of the phenotypic standard deviation of the cold requirement in days were obtained for the high and control lines, and as these were not significantly different, the realized heritability was calculated from the expression:

$$\Delta G = (\Sigma\tau)h^2\sigma_p ,$$

where ΔG is the total response in the high selection line based on the increase in the number of days to 50% flowering over the four generations of selection; $\Sigma\tau$ is the accumulated selection differential in standard units (7·95); h^2 is the realized heritability; and σ_p is the mean phenotypic standard deviation assumed to be constant throughout the period of selection.

The behaviour of the high, low, and control lines after four generations of selection, when exposed to different durations of winter temperatures in the field, was examined by establishing lots of 100 unvernalized seedlings at the third leaf stage in the field at Canberra at 14-day intervals for a period of 12 weeks commencing on July 1st. The percentage of plants flowering in each line was recorded for each planting date.

(*e*) *Estimation of Correlated Responses*

The high, low, and control lines were subjected to a growth analysis after four cycles of selection, to detect the existence of any correlated growth responses as a consequence of selection for vernalization requirement. Plants were grown at two constant temperatures (10 and 15°C) in growth cabinets in which the light intensity was 3000 f.c. for an 8-hr photoperiod, and the relative humidity was maintained at 70–80%. The relative growth rate (R_g) of the shoots, and the rate of leaf area expansion (mm^2/day) were calculated for plants between the appearance of the fourth and seventh leaf on the main shoot. Total tiller numbers were also recorded at the final harvest. Data on flowering time and yield under field conditions were obtained for all three lines from the serial plantings described in the previous section.

III. Results

(*a*) *Seed and Seedling Vernalization*

The effect of vernalizing imbibed seed on the subsequent flowering of four *Phalaris* ecotypes is illustrated in Figure 1(*a*). Seed vernalization alone caused a marked reduction in the leaf number at ear emergence (flowering), and also an increase

in the percentage of flowering plants in ecotypes A and B which have an obligatory cold requirement for complete flowering in long days at 21/16°C. In the case of the

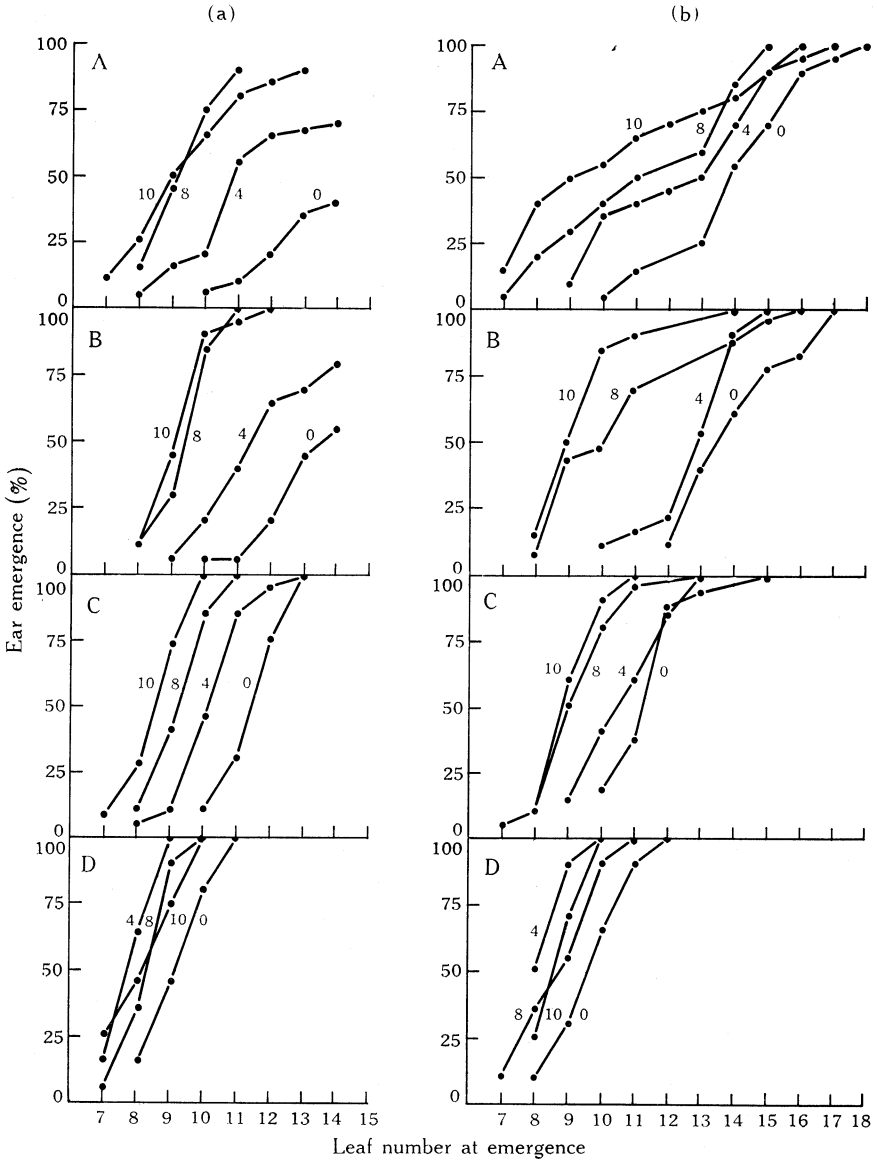

Fig. 1.—Effect of seed vernalization on the flowering response in *P. tuberosa*. (*a*) Influence of seed vernalization (0, 4, 8, 10 weeks) on the cumulative percent of ear emergence at successive leaf numbers in four ecotypes (A, B, C, and D) which differ in their cold requirements. (*b*) As for (*a*) plus a subsequent seedling vernalization sufficient to satisfy the cold requirement. Ecotypes A and B received 6 weeks vernalization as seedlings, and C and D 2 weeks.

Australian cultivar B, 8 weeks of seed vernalization satisfied the cold requirement of all plants in the population.

Where seed vernalization was combined with a range of seedling vernalization treatments, it was still effective in reducing leaf number at emergence. This effect of seed vernalization in combination with one of the seedling treatments is shown in Figure 1(*b*). Over all these seedling treatments the effect of seed vernalization increased with the duration of the cold treatment except in D (no cold requirement) in which about 4 weeks of seed vernalization gave the maximum response.

Vernalizing seed also had a marked effect on the number of inductive long days required for ear emergence. This effect was measured over a range of seedling vernalization treatments some of which are illustrated in Figure 2. In all ecotypes, seed vernalization enhanced the effect of seedling vernalization, but in those ecotypes

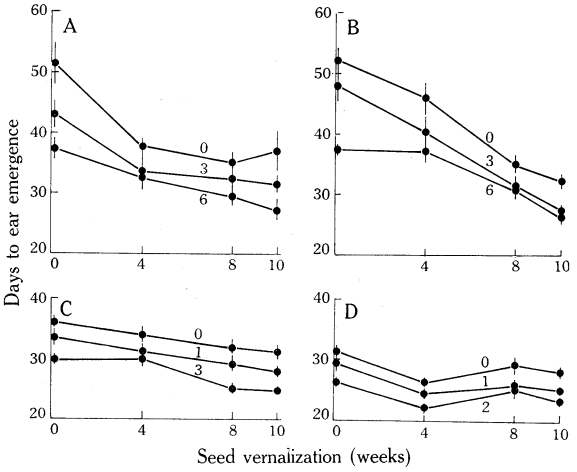

Fig. 2.—Complementary action of seed plus seedling vernalization treatments on the time to ear emergence of four *P. tuberosa* ecotypes (A, B, C, and D) in long days. Curves represent various times (in weeks) of seedling vernalization.

which responded most to cold treatment (A, B, and C) there was no significant difference in the time to emergence between the 8- and 10-week seed vernalization treatments. However, at this level of seed vernalization all populations showed a significant response to further seedling vernalization. This suggests that seed and seedling vernalization may not be equivalent, and that these ecotypes were not capable of responding to more than about 8 weeks of seed vernalization. The same pattern is evident in ecotype D, which has no cold requirement and in which seed vernalization for 4 weeks gave the maximum response.

These results also illustrate the association between the cold requirement and the number of long days required for ear emergence following vernalization. Populations of A and B for which the seedlings require a minimum of 4 weeks of low temperature for complete vernalization, flowered considerably later than C and D which have little or no cold requirement.

(*b*) *Short-day Induction*

The influence of short days on floral induction and their capacity to replace the vernalization requirement in *P. tuberosa* is summarized in Table 1. At high temperature (25/20°C), short days had no apparent effect on the flowering response

of any of the ecotypes, and provided no substitute for low temperature (9/4°C) in satisfying the vernalization requirement. At a lower temperature (19/14°C), slightly above the temperature range for vernalization, short days caused a small increase in the number of plants initiating in long days in those ecotypes with an obligatory cold requirement. This effect was enhanced by doubling the duration of the short-day exposure. Although more plants initiated following a short-day pretreatment, the development of the inflorescence tended to be more advanced following the long-day pretreatment. The effect of an intermediate day length (12 hr) is not shown as there was no detectable enhancement in either initiation or development.

TABLE 1

ROLE OF SHORT DAYS IN THE FLOWERING RESPONSE OF *P. TUBEROSA* ECOTYPES WHEN GIVEN IN CONJUNCTION WITH DIFFERENT TEMPERATURES PRIOR TO EXPOSURE TO INDUCTIVE LONG DAYS FOR 4 WEEKS

Ecotype	Temperature (°C)	Photoperiod 8 hr		Photoperiod 16 hr	
		% Initiated	Apex Score†	% Initiated	Apex Score†
A	25/20°C (4 weeks)	9	0·0	7	5·0
B		13	1·5	0	0·0
C		60	3·0	67	4·0
D		100	3·3	93	4·2*
A	19/14°C (4 weeks)	13	1·0	0	0·0
B		13	2·0	13	3·0
C		93	3·5	87	4·8**
D		100	4·6	100	5·0*
A	19/14°C (8 weeks)	25	2·1	0*	0·0
B		33	3·0	0**	0·0
C		96	2·8	66*	4·6**
D		100	3·8	100	5·0**
A	9/4°C (4 weeks)	100	3·5	93	3·2
B		100	2·7	100	2·5
C		100	3·5	100	3·7
D		100	4·2	100	4·4

* Difference between 8 and 16 hr photoperiod significant at $P<0·05$.
** Difference between 8 and 16 hr photoperiod significant at $P<0·01$.
† Mean apex score for initiated plants only.

Exposure of plants to different photoperiods during vernalization at 9/4°C, had no effect on the subsequent flowering response in long days. All ecotypes initiated flowers and there was no significant difference between photoperiods in the time of ear emergence or in the leaf number at emergence as shown in the following tabulation:

Photoperiod (hr)	8	12	16
Average number of long days to ear emergence	37·3	38·2	37·6
Average numer of leaves at emergence	11·4	11·3	11·2

(c) *Effect of Gibberellin*

The application of GA_3 to either seeds or seedlings was not effective in promoting initiation or flower development in any of the four ecotypes tested. Also GA_3 was not able to substitute for vernalization in those ecotypes with an obligatory cold requirement (Table 2). Seeds imbibed in the presence of GA_3 developed normally and showed no effects of the treatment. However, where young plants were sprayed with GA_3 during induction, excessive elongation of culms occurred accompanied by leaf chlorosis and extreme epinasty of stems. Elongation under these conditions was quite independent of the reproductive condition of the apex.

TABLE 2

EFFECT OF GIBBERELLIN (GA_3) ON THE FLOWERING OF *P. TUBEROSA* ECOTYPES

I(%), percentage initiated; A.S., mean apex score for initiated plants only

Ecotypes	Treatment	No cold, $-GA_3$		No cold, $+GA_3$		Cold, $-GA_3$		Cold, $+GA_3$	
		I(%)	A.S.	I(%)	A.S.	I(%)	A.S.	I(%)	A.S.
A	Seed	0	0·0	10	2·0	100	3·2	60	3·3
	Seedling	0	0·0	10	1·0	90	3·4	100	3·3
B	Seed	20	1·5	0	0·0	90	2·2	90	2·4
	Seedling	0	0·0	10	1·0	90	2·9	100	2·5
C	Seed	90	2·9	80	3·3	100	3·5	100	3·2
	Seedling	70	2·4	90	2·8	100	3·4	100	3·2
D	Seed	100	3·6	100	3·3	100	4·2	100	4·5
	Seedling	100	3·1	100	2·8	100	4·3	100	3·0
Mean		46	1·7	50	2·0	96	3·4	94	3·2

(d) *Response to Selection*

There was a marked response to selection for both high and low vernalization requirement as illustrated in Figure 3. The mean cold requirement of the unselected population averaged 16 days with a phenotypic standard deviation (σ_p) of 9·4 days. This was increased to 70 days ($\sigma_p = 12·2$) in the high line after four generations of selection. In terms of the vernalization requirement for flowering of all plants in the population, this represented an increase of from 28 to 90 days. In the low line the cold requirement was entirely eliminated after only two generations of selection, which was equivalent to the rate of response over the same period in the high line.

The realized heritability for the vernalization requirement (based on the response of the high line over four generations of selection) was high, with a value of $0·63 \pm 0·06$ and an average phenotypic standard deviation of 10·8 days.

The flowering responses of the high, low, and control lines when grown during the late winter in the field are illustrated in Figure 4. In the high line almost half the population flowered following the first planting on July 1st (1750 hr of vernalization), but virtually all plants established after July (less than 1000 hr vernalization) received insufficient cold and failed to flower. In contrast, practically all of the plants in the low line flowered when established during July and August, and even 30% of

the plants flowered from the latest September planting. These later values would have been substantially higher but for the onset of high summer temperatures and moisture stress, which prevented further flowering. The control line plotted over the same period gave an intermediate response.

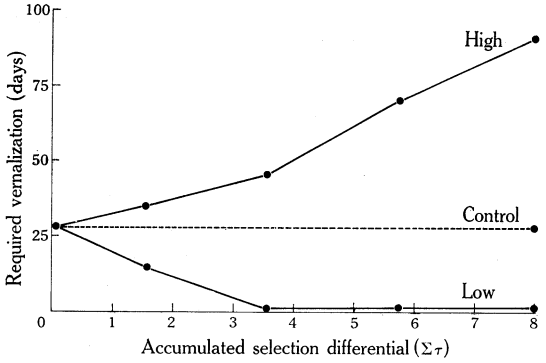

Fig. 3.—Response to selection for both high and low vernalization requirement in *P. tuberosa* ecotype B. The vernalization requirement for flowering of all plants in the population (days at 9/4°C) is plotted against the accumulated selection differential ($\Sigma\tau$) in standard units. Results represent progress from selection both under field and phytotron conditions.

Flowering under field conditions showed good correlation with the results obtained over a similar range of vernalizing treatments in controlled environments, especially for the high line in which the flowering expression was not disturbed by high summer temperatures and drought. This indicates that the cold requirement, as measured in controlled environments, has good predictive value for flowering under field conditions.

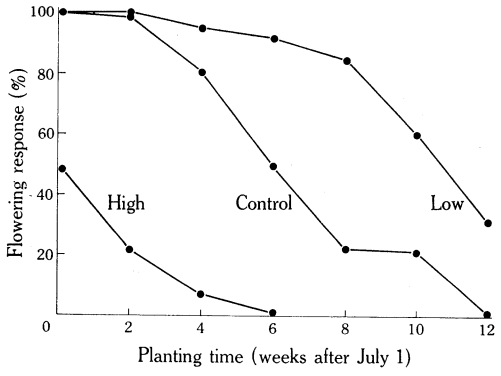

Fig. 4.—Flowering response (% flowering) in *P. tuberosa* lines selected for a high and low vernalization requirement, together with the unselected control, following exposure to a decreasing period of winter cold. Plants were established as described in Section II(*d*), and flowering recorded in the following summer. The vernalization received (hr below 10°C) was 1750, 1410, 1060, 770, 450, 290, and 100 hr for planting times of 0, 2, 4, 6, 8, 10, and 12 weeks respectively.

(*e*) *Correlated Responses*

A comparison of the growth and development of the progenies of both selection lines and the control population is given in Table 3. Under controlled environments the seedling performance of the three lines was comparable, although some differences were apparent. At 15°C the low line had a significantly higher relative growth rate, and at the lower temperature (10°C) the high line produced more tillers. These variations, however, did not cause any significant difference in the total weight of

seedlings at the final harvest, and there was no other evidence of any marked change in seedling behaviour as a consequence of selection for vernalization requirement.

With mature plants under field conditions, differences between lines were more obvious. Plants of the high line flowered 16 days later than the control, and the low line 3 days earlier, following a midwinter planting. Also, the average yield of dry matter from the same plants of the high line in the following autumn was significantly greater.

TABLE 3

CORRELATED RESPONSES TO SELECTION FOR VERNALIZATION RESPONSE IN *P. TUBEROSA*

Comparison of the three selection lines (high, low, and control) after four generations of selection

Selection Line	Controlled Environment (seedlings)						Field Environment* (mature plants)	
	R_g (mg/mg/day)		Leaf Area Growth (mm²/day)		No. of Tillers		Days to Ear Emergence	Autumn Yield [log(g/plant)]
	15°C	10°C	15°C	10°C	15°C	10°C		
Unselected (control)	0·125	0·072	253	76	8·2	6·8	149	2·09
High	0·122	0·067	247	73	8·1	7·6	165	2·22
Low	0·134	0·069	239	76	8·2	6·2	146	2·02
L.S.D.(0·05)	0·006		19		0·9		1·5	0·09

* Data from midwinter planting (July 1st): 100 plants per line.

IV. DISCUSSION

Four *P. tuberosa* ecotypes, ranging in vernalization requirement from 0 to 4 weeks, responded to cold treatment when applied either to imbibed seeds or to young seedlings. Seed vernalization alone, or in combination with seedling vernalization, caused an increase in the percentage of plants which flowered, and also caused a reduction in the leaf number at ear emergence and in the time to flower in long days.

The finding that imbibed, but non-growing, seed of *P. tuberosa* can be successfully vernalized is at variance with the results of Ketellapper (1960), who found that some vegetative growth was necessary before plants would respond to low temperature. This may have been due to insufficient cold treatment, as seed vernalization has been demonstrated in other temperate perennial grasses including *Lolium perenne* (Frischknecht 1959; Cooper 1960; Evans 1960*b*; Bommer 1961; Silsbury 1964) and is widespread in winter cereals (Lang 1965).

The response to seed vernalization in *P. tuberosa* was most marked in those ecotypes (A and B) with an obligate cold requirement. With these, short doses of seed or seedling vernalization were about equally effective in reducing the time to ear emergence. However, the duration of seed vernalization required to satisfy completely the cold requirement, was at least twice that required by seedlings to achieve the same result. This decline in the rate and effectiveness of seed vernalization with prolonged exposure to low temperature, suggests that the capacity of *P. tuberosa*

seed to respond to cold treatment is limited, possibly by the size of the shoot apex in the embryo where the vernalization process is localized.

The apparent association between the vernalization requirement of the four ecotypes and the number of days required for ear emergence confirms earlier work with the same species (Ketellapper 1960; Cooper and McWilliam 1966). It suggests that the rate of floral development in long days differs in these ecotypes, but it is not known to what extent these responses are physiologically related, or whether they have simply become associated through the action of natural selection. Under field conditions there is probably also an interaction with temperatures during initiation, as both temperature and photoperiod have been shown to influence the rate of inflorescence development in *Lolium* (Evans 1960*a*, 1960*b*).

In a number of perennial grasses that respond to vernalization e.g. *Bromus inermis*, *Lolium perenne*, and *Hordeum bulbosum*, short days can under certain circumstances substitute for the low temperature requirement (Newell 1958; Cooper 1960; Evans 1960*a*; Koller and Highkin 1960), but in these species short-day induction results in a slower rate of initiation and floral development. By contrast in *Poa pratensis*, short days and low temperature are both necessary for floral induction (Peterson and Loomis 1949).

In *P. tuberosa* short days had a slight inductive effect after prolonged exposure, but only at temperatures at or slightly above the upper limit for vernalization, and there was no detectable effect at high or at low (vernalizing) temperatures. A similar response has been found in *Lolium perenne* (Wycherley 1952; Cooper 1960). This lack of response to short days at vernalizing temperatures makes it difficult to see how short days can influence flowering in *P. tuberosa* when fully induced under natural winter conditions.

Gibberellin has been one of the most successful substances used in attempts to replace the vernalization requirement in long-day plants, although for every success there have been many negative responses (Lang 1965). In *P. tuberosa*, GA_3 was unable to replace the vernalization requirement, and caused no significant promotion of inflorescence development, irrespective of the duration of the vernalization requirement, or of previous exposure to cold.

These results are in general agreement with those obtained for other grasses and cereals (Koller, Highkin, and Caso 1960; Peterson and Bendixen 1963; Hurd and Purvis 1964; James and Lund 1965; Sugi and Yamada 1965), although the transient stimulation usually found in the development of floral primordia was not observed. The failure to achieve a flowering response with GA_3 may indicate some inadequacy in the treatment, such as the dose rate or nature of the particular gibberellin used. However, it seems more likely, as suggested by Hurd and Purvis (1964), that gibberellins may have no specific association with the vernalization process, but may be associated subsequently in some way with the thermoinduced state by potentiating the apex to respond to the inductive photoperiod.

Considerable variation exists both within and between populations of *P. tuberosa* with respect to the cold requirement, much of which represents adaptive variation in response to local climate. As with most other climatic responses that have been studied in detail (Clausen and Hiesey 1958; Cooper 1959, 1960; Cooper and Edwards

1961) vernalization requirement shows continuous variation and appears to be under polygenic control.

In the ecotypes with an obligate cold requirement, the flowering response under a particular photoperiod can be thought of in terms of a threshold model, with the response to low temperature representing the underlying continuous variable, and the threshold, which is under genetic control, the amount of cold needed by a particular individual to cause induction. A similar model has been proposed by Cooper (1954) for the control of heading responses in *Lolium*. In these outbreeding populations of *P. tuberosa* individual plants are heterozygous, and the position of their threshold on the underlying scale depends on their genetic constitution.

The results of selection for cold requirement confirm the assumption that considerable additive genetic variation is present for this character in the Australian population. The response to directional selection was large, and sufficient to produce highly significant differences between lines selected in opposite directions. The shift towards increased cold requirement was greater, because in the low line no further response could be obtained when the obligate requirement was removed. However, the range in mean vernalization requirement between the high and low lines after only four generations of selection (70 days) was greater than the total range found between the extremes in the naturally occurring ecotypes. This indicates that an outbreeding population, such as the one used in this study, can carry much more potential genetic variation than is expressed phenotypically under natural conditions. A similar genetic structure for the winter requirement and date of ear emergence has been described in *Lolium* by Cooper (1959, 1960).

The high realized heritability ($0 \cdot 63 \pm 0 \cdot 06$) agrees quite well with the heritability for date of ear emergence obtained by Latter (1965) for the same Australian cultivar. His calculated heritability was $0 \cdot 53 \pm 0 \cdot 02$, but after correction for the degree of phenotypic assortative mating, a more realistic estimate based on random mating in a population under artificial selection was $0 \cdot 40$. The higher heritability estimate for cold requirement is to be expected because vernalization is only one component of the flowering response, and is less likely to be affected by environmental influences than the actual date of ear emergence.

There was a good correlation between the inductive requirement of the three selection lines as assessed in controlled environments and their winter requirement as measured by the extent of flowering in the field after serial plantings in the late winter and spring. Correlated responses in other characters after four generations of selection for cold requirement were mainly confined to mature plants under field conditions. The delayed flowering time found in the progeny of the high selection line was to be expected, in view of the positive association found in *P. tuberosa* between the vernalization requirement and flowering date after complete induction under winter conditions in the field (Cooper and McWilliam 1966). Also, the more vigorous growth of this material in the autumn was a direct reflection of the higher tillering capacity of vegetative plants.

In view of the considerable potential genetic variation stored in these populations of *P. tuberosa*, it should be possible to select for plants that remain vegetative indefinitely in a particular region because their requirements for winter cold are never

satisfied (Peterson, Cooper, and Vose 1958). In *P. tuberosa*, since there is no evidence of any undesirable correlated response to selection, as found by Cooper (1961) following selection for extreme early and late flowering in *Lolium*, such a non-flowering variety could well have a useful agronomic role.

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