EFFECTS OF VERNALIZATION, PHOTOPERIOD, AND THE COTYLEDON ON FLOWER INITIATION IN GREENFEAST PEAS

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Summary

Estimates have been made of the quantitative contribution of each of the determinant factors, photoperiod, vernalization, and colysanthin (a presumed inhibitor of flower initiation formed in the cotyledon), in regulating flower initiation in the late-flowering pea cultivar Greenfeast.

Photoperiod appears to be quantitatively related to the production of an inductive stimulus. This stimulus reaches its threshhold level at about node 12 under 18-hr photoperiods, but not until node 18 under an 8-hr photoperiod.

Colysanthin delays events between photoperiodic induction and flower initiation (evocation), and causes a slightly greater delay to flower initiation in short than in long days (3 and 2 nodes respectively).

Vernalization appears to have two separate effects, both of which promote flower initiation at an earlier node. The smaller effect is manifest on the cotyledonary inhibitor system, and probably results from a reduction of the effective level of colysanthin. The major effect does not appear to involve colysanthin, but is manifest on the young embryo and is effective before photoperiodic induction is completed. The embryo response to vernalization results in advanced flower initiation of some 4 nodes in long days and nearly 6 nodes in short days. This effect may be partially obscured by colysanthin, unless the cotyledons are excised soon after vernalization is completed.

The evidence favours the view that the three determinant factors act in a complementary manner, rather than competitively, to regulate flower initiation in Greenfeast.

I. Introduction

Greenfeast, a late-flowering cultivar of *Pisum sativum* L., behaves as a quantitative long-day plant [node to first flower (NF) = 17 under an 18-hr photoperiod (P_{18}); NF = 24 under an 8-hr photoperiod (P_8)]. Significant advancement of NF can be brought about by grafting Greenfeast scions onto stocks of early-flowering varieties [e.g. Massey (Paton 1956)], by vernalization (Barber *et al.* 1958), or by cotyledon excision during early stages of germination (Johnston and Crowden 1967).

Paton and Barber (1955) proposed a mechanism based on a mobile inhibitor produced in the cotyledons of late-flowering varieties, to account for the grafting behaviour, and this idea is well supported by cotyledon-removal experiments. Barber (1959) introduced the name "colysanthin" for this inhibitor, and suggested that flowering in late varieties occurred when colysanthin was destroyed. Moore (1964) has proposed that vernalization and cotyledon excision in peas may have a common basis, and Paton (1956) concluded from grafting experiments that vernalized stocks of Greenfeast contained less inhibitor than unvernalized stocks.

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An alternative proposal is due to Haupt (1952), who suggested that cotyledons of at least one early-flowering variety, Kleine-Rhinelanderin, produce a flower-stimulatory substance which is graft transmissible. This florigenic substance is thought to be absent from late-flowering varieties, or alternatively its formation is blocked by an inhibitor (possibly a colysanthin) which is produced in the cotyledons of these plants (Haupt 1969).

To date unambiguous experimental verification of either hypothesis has not been made, and all attempts at definitive isolation of a florigenic substance or of a colysanthin have been unsuccessful.

In these present experiments, an attempt has been made to determine more precisely the relationship between the cotyledon system, photoperiod, and vernalization in regulating NF in Greenfeast peas, and in particular to show possible independent or interacting effects.

II. Materials and Methods

Seeds used in these experiments were obtained commercially in Hobart, in a single batch. Before any treatments were commenced, seeds were selected so that their testae were free from cracks or obvious infections, and were surface-sterilized by dusting with Thiram-80. Seeds were planted in a mixture of moist vermiculite–small dolerite chips (1 : 1), contained in 5-lb fruit pulp tins, five seeds per tin. The plants were grown in a glasshouse, under controlled photoperiods of either 8 or 18 hr. Illumination in both photoperiods was provided by natural daylight, supplemented and extended as required by mixed banks of fluorescent and incandescent lamps. Plants were supplied twice weekly with Hoagland's complete nutrient solution (one-quarter strength) and watered as required.

Seeds to be vernalized were planted in tins as above, and placed in a room at 3°C for periods of up to 4 weeks. Excision and culture of embryos was carried out as described by Johnston and Crowden (1967). When embryos were to be vernalized, they were planted onto sloped agar in tubes to afford good illumination during the vernalization period. In these cases photoperiod was provided by artificial light only.

The technique used for grafting was as described previously by Paton and Barber (1955). For grafting vernalized plants, seeds were planted 3 in. deep in moist vermiculite and given 4 weeks vernalization at 3°C. This deep planting encouraged extension of the epicotyl and facilitated the grafting procedure. When mixed grafts were performed, i.e. vernalized with unvernalized partners, seeds for the unvernalized material were planted 4 days before the due completion of the vernalization treatment. This ensured that both graft partners were at a comparable stage of development as determined by apical dissection. The grafts were made at the stage of opening of the plumular hook, when the epicotyl was approximately 1 in. long (about 6–8 days for unvernalized plants).

To allow for statistical treatment of the data, the experiments were planned as randomized-block experiments with four replications in each treatment. A minimum of 20 plants was involved in each treatment. For the scoring of NF, all plants were grown to anthesis, and the node at which the first flower (or aborted rudiment) appeared was recorded, taking the cotyledonary node as zero. Values for means, standard errors, and numbers of plants scored for the various treatments are quoted in the tables. Average rates of node formation for plants in various treatments were determined by dissection of groups of 10 plants at intervals throughout the growing period. Experiments were conducted throughout the year under controlled photoperiod conditions, yet there is evidence of variations in NF due to seasonal (but not photoperiodic) differences. These variations are possibly related to seasonal variations of the night temperatures but this point has not been investigated thoroughly. Control of temperature in our glasshouse is not absolute, and whereas reasonably uniform day temperatures can be maintained, it is not uncommon for the night temperatures, particularly in winter, to fall to about 12°C. Since all plants in any one experiment were grown under comparable conditions with adequate randomization, it is assumed

that there is no significant effect of this phenomenon within individual experiments. All results are recorded showing the season in which the plants were grown.

III. Results

Factors affecting the determination of NF in Greenfeast were investigated in a series of experiments involving cotyledon removal, grafting, vernalization, and photoperiod in various combinations of treatments.

Table 1 shows the effects of cotyledon removal and vernalization treatments on flower initiation under 18-hr and 8-hr photoperiods. It can be seen that vernalization and cotyledon excision led to advancement of NF in both photoperiods, and that the two treatments supplemented one another in effect, the maximum advancement of

Table 1

Effect of cotyledon excision, vernalization, and photoperiod on node to first flower in Greenfeast (winter crop)

Photo-period	Treatment	Node to First Flower (no vernalization)	No. of Plants	Node to First Flower (4 weeks vernalization)	No. of Plants
18 hr	Cotyledons intact	16·90±0·21	20	14·42±0·16	19
	Cotyledons removed	14·94±0·15	18	12·50±0·22	6
8 hr	Cotyledons intact	24·35±0·21	20	20·11±0·21	18
	Cotyledons removed	21·43±0·25	14	18·57±0·20	7
18 hr*	Both cotyledons intact	18·97±0·14	29	13·73±0·12	26
	Right cotyledon removed	14·85±0·13	26	13·04±0·04	26
	Left cotyledon removed	14·81±0·12	27	12·97±0·12	29
	Both cotyledons removed	13·80±0·17	10	11·90±0·10	10

* Summer crop.

NF being achieved when both treatments were given. Under long days, removal of both cotyledons advanced flowering by 2 nodes for vernalized as well as unvernalized plants, whilst the vernalization effect was to advance NF by 2·5 nodes for both intact and decotyledonized plants. In contrast, when plants were grown in short days cotyledon removal had a much greater effect in unvernalized than in vernalized plants (2·9 and 1·6 nodes respectively), and the vernalization treatment was more effective in intact than in decotyledonized plants (4·2 and 2·9 nodes respectively). Removal of one cotyledon gave an intermediate level of effect in both vernalized and unvernalized plants.

Rates of node formation for control, vernalized, and decotyledonized plants under long photoperiod are shown in Figure 1. For unvernalized plants the average rates of node formation to the time of flower initiation were 0·47 nodes/day (cotyledons intact), and 0·26 nodes/day (cotyledons removed). For vernalized plants the average rates of node formation in the post-vernalization interval were 0·51 and 0·27 nodes/day respectively. The apparently slower rate for non-vernalized plants reflects the lag of 2–3 days following imbibition before any new node formation

becomes evident. In contrast, vernalized plants show no such lag in the immediate post-vernalization period, and, in fact, have laid down one additional node during the 4-week period of the vernalization treatment. If the average rate for control plants is estimated from day 2 onwards, a rate equivalent to that for vernalized plants is obtained (0·51 nodes/day). Similar results to those shown in Figure 1, obtained in a separate experiment, are summarized in Table 3.

An equivalent rate of node formation for both vernalized and unvernalized plants is also reported by Paton (1969). The slightly higher rate in Paton's experiments (0·67 nodes/day) is probably related to a higher and constant ambient temperature during the main growing period. That the plastochron interval should also be similar for vernalized and unvernalized plants after cotyledon removal indicates that the effect of seed vernalization on the flowering response of this plant is not manifest

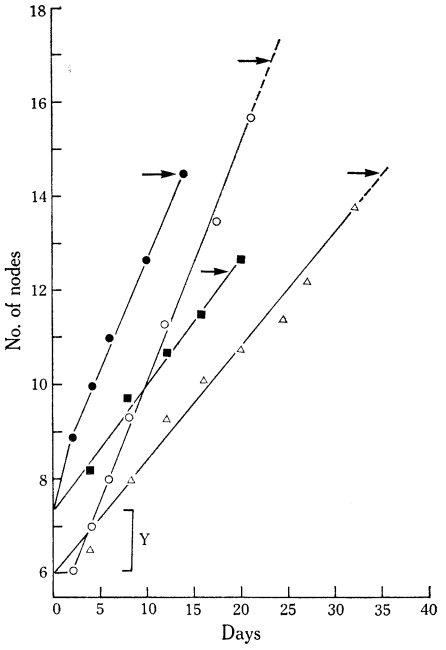

Fig. 1.—Rates of node formation in control, vernalized, and decotyledonized Greenfeast plants under an 18-hr photoperiod (summer crop). The data were obtained in an experiment parallel to that recorded in Table 1. Arrows indicate the node of initiation of the first flower. Y = nodes formed during the vernalization treatment.
○ Cotyledons intact, no vernalization.
△ Cotyledons removed, no vernalization.
● Cotyledons intact, 4 weeks vernalization.
■ Cotyledons removed, 4 weeks vernalization.

through any alteration in the rate of leaf formation. On the other hand, there is a clear correlation between the retarded rate of node formation in decotyledonized plants and the initiation of flower primordia at an earlier node.

To investigate the effect of the time of vernalization and cotyledon-removal treatments on NF, plants were germinated for varying intervals before vernalization treatments were begun, and cotyledons were excised from different groups of plants immediately before or after vernalization. Treatments were staggered to allow all plants to commence post-vernalization growth concurrently. Because of limited facilities in the vernalization room, this experiment was conducted under long-day conditions only. The results are given in Table 2. It can be seen that, irrespective of whether the cotyledons were present or not, the response to vernalization decreased as germination and growth of the plants progressed. In fact vernalization had no

TABLE 2

EFFECT OF TIME OF COTYLEDON-REMOVAL TREATMENTS AND VERNALIZATION ON NODE TO FIRST FLOWER IN GREENFEAST (WINTER CROP)

18-hr photoperiod only was used. n, number of plants scored; N_1, number of nodes at commencement of vernalization treatment; N_2, number of nodes at conclusion of vernalization treatment

Days from Sowing to Commencement of Vernalization	Length of Vernalization Treatment (weeks)	Cotyledons Intact				Cotyledons Removed before Vernalization				Cotyledons Removed after Vernalization			
		n	NF	N_1	N_2	n	NF	N_1	N_2	n	NF	N_1	N_2
0	0	18	16·78±0·21	6·0	—	23	14·84±0·14	6·0	—				
	4	18	14·39±0·12	6·0	7·0	20	12·28±0·12	6·0	—	10	12·80±0·20	6·0	7·0
6	0					17	15·76±0·25	8·7	—				
	4	20	15·60±0·15	8·8	11·0	13	13·92±0·08	8·8	9·0	18	14·39±0·12	9·5	11·4
10	0					19	15·37±0·14	11·2	—				
	4	19	15·47±0·16	11·2	12·5	18	14·22±0·10	11·2	11·8	18	15·44±0·16	11·6	14·5
14	0					19	16·37±0·19	13·2	—				
	4	20	16·50±0·15	13·1	15·4	20	15·90±0·16	13·1	14·7	18	15·94±0·19	13·2	15·5

TABLE 3

EFFECTS OF VERNALIZATION OF EXCISED EMBRYOS ON NODE TO FIRST FLOWER OF GREENFEAST (SUMMER CROP)

18-hr photoperiod only was used. *n*, number of plants scored; N/D, rate of node formation (nodes/day) from end of vernalization treatment to time of initiation of first visible flower primordium

Length of Vernalization Treatment* (weeks)	Cotyledon Status	Length of Vernalization Treatments after Cotyledons Removed									
		0 Weeks			1 Week		2 Weeks		3 Weeks		4 Weeks	
		n	NF	N/D	*n*	NF	*n*	NF	*n*	NF	*n*	NF
0	Intact	20	15·40±0·15	0·46								
	Excised	23	13·48±0·14	0·24	24	12·67±0·12	27	11·76±0·09	7	12·14±0·14	16	12·06±0·11
1	Intact	19	15·50±0·12	0·45								
	Excised	29	12·48±0·15	0·23	30	12·33±0·12	27	12·22±0·11	27	12·44±0·12		
2	Intact	20	14·60±0·15	0·51								
	Excised	24	12·17±0·10	0·27	17	11·53±0·24	26	11·92±0·13				
3	Intact	20	13·50±0·11	0·51								
	Excised	24	12·33±0·12	0·25	26	12·58±0·11						
4	Intact	19	13·00±0·09	0·50								
	Excised	24	12·33±0·10	0·26								

* Preceding cotyledon excision.

significant effect on the NF of plants which had already reached the node-12–13 stage of development (between 10 and 14 days after germination). Cotyledon removal at 14 days was without effect on unvernalized plants, but an effect of marginal significance ($P = 0{\cdot}05$) was still apparent with vernalized plants. Removal of cotyledons from growing plants before giving the vernalization treatment resulted in greater advancement of NF than did post-vernalization excision.

The decreasing effect of vernalization on NF does not appear to be correlated to the change in rate of node formation of the plants during the vernalization period. For intact plants, the rates of node formation during vernalization at 6, 10, and 14 days after germination were nearly the same in all cases (approximately 0·5 nodes/week). When cotyledons were removed before vernalization, the number of nodes formed per week in the embryos during vernalization increased sharply from 0·05 at 6 days to 0·15 at 10 days and 0·5 at 14 days, whereas the effect of vernalization on NF in these groups of plants showed an almost linear decline.

In a further experiment, embryos were dissected from imbibed seeds which had been vernalized for varying periods up to 4 weeks. Some of these embryos were then given extended vernalization treatments, up to a total of 4 weeks, in isolation from the cotyledon influence. This experiment also was conducted under long days only. The results are shown in Table 3. The data show that 1 week of vernalization was sufficient to obtain nearly maximum advancement of NF, provided the cotyledons were excised before the plants were allowed to grow under normal temperatures. It did not matter whether cotyledons were present or not during the vernalization interval. On the other hand, when cotyledons remained intact during the post-vernalization period of growth, there was only progressive advancement of NF as the vernalization treatment was extended to the full 4 weeks.

Two grafting experiments were conducted. In the first experiment, vernalized and unvernalized scions of Greenfeast (GV and GU respectively) were grafted to both vernalized and unvernalized Greenfeast stocks. The results are shown in Table 4. For plants grown in a long photoperiod it is seen that grafting vernalized scions, either GV/GU or GV/GV (scion/stock), promoted flowering at an earlier node than did GV controls. The NF of these vernalized scions is in fact comparable with that resulting from the dual treatments of cotyledon removal plus seed vernalization shown in Tables 1, 2, and 3. This is in sharp contrast to the performance of vernalized scions in short days, where no effect of grafting was evident. Thus plants from each of the treatments GV, GV/GU, and GV/GV have almost identical NF values. Moreover, this value of NF (approximately 20·5) is some 2 nodes higher than that for vernalized, decotyledonized plants grown under short days (Table 1). Similarly, with unvernalized scions (GU, GU/GU, and GU/GV), a significant effect of grafting is evident only when plants are grown under long-day conditions. Thus it would seem that the effect of grafting in Greenfeast is nullified during the prolonged interval of vegetative growth which precedes flower initiation in short days.

In the second grafting experiment, an early flowering variety, Massey, was used as stock. Grafts on Massey stocks grown under short days were not always successful, and scion mortality was high. However, survival was satisfactory under long-day conditions. In all cases grafting to Massey stocks promoted flowering at an earlier node than did comparable grafts to Greenfeast stocks. More significant

perhaps is the observation that vernalized Greenfeast scions grafted to Massey stocks flowered out of the same nodes as vernalized, decotyledonized plants (Table 1) in both photoperiods, in marked contrast to the behaviour of GV/GU and GV/GV grafts.

TABLE 4

EFFECT OF VERNALIZATION, GRAFTING, AND PHOTOPERIOD ON NODE TO FIRST FLOWER IN GREENFEAST (WINTER CROP)

Graft Type (scion/stock)*	8-hr Photoperiod		18-hr Photoperiod	
	NF	n†	NF	n†
GU (control)	23·35±0·30	20	16·90±0·18	20
GV (control)	20·50±0·21	20	14·53±0·12	19
GU/GU	23·13±0·23	8	15·60±0·21	18
GU/GV	22·71±0·29	14	14·17±0·19	18
GV/GU	20·67±0·20	18	12·79±0·14	19
GV/GV	20·42±0·20	24	12·90±0·17	20
GU/MU	19·86±0·54	7	13·88±0·09	8
GU/MV	20·71±0·47	7	13·37±0·22	19
GV/MU	18·10±0·52	10	12·67±0·14	12
GV/MV	19·00±0·68	7	12·67±0·14	18

* G = Greenfeast; M = Massey; U = unvernalized; V = vernalized.
† Number of plants scored.

IV. DISCUSSION

Most plants undergo a period of vegetative development before reaching the ripeness-to-flower condition, whereupon they may produce reproductive structures. The ripeness-to-flower condition is probably an absolute expression of a plant's genetic constitution, but the subsequent realization of this genetic potential may require an appropriate combination of environmental conditions. There is good evidence in these present experiments that in Greenfeast peas the minimum node at which the initiation of flower primordia may occur is about node 12. (We have scored only a very small number of NF-11 plants, less than 5% of the total, following treatments which promote the maximum advancement of NF.) Since there are usually 6 nodes already present in the dormant embryo, then the attainment of the minimum node number for flower initiation (which may well coincide with ripeness-to-flower for this plant) involves vegetative development of a further 6 nodes after the commencement of germination. However, under normal growing conditions the observed NF for this plant is delayed beyond node 12. Quantitative reduction of this delay may be brought about by treatments such as cotyledon removal, vernalization, or long photoperiod given independently or in combination.

Other workers (Paton and Barber 1955; Barber 1959; Paton 1969) have proposed that this delay to flower initiation can be largely explained in terms of a graft-transmissible inhibitor produced in the cotyledons of Greenfeast (and probably other late-flowering varieties as well). The data in these present experiments is consistent with this view that flowering in Greenfeast is regulated, at least in part,

by an inhibitory effect of the cotyledon. Whether the cotyledon effect is due to the presence of an inhibitor (i.e. colysanthin; Barber 1959), or to the absence or retarded formation of a florigenic substance (Haupt 1969) is not unequivocably determined, but on the evidence available we favour the former view.

Perhaps the best evidence that a colysanthin is directly involved comes from the results of grafting experiments, summarized in Table 4. Thus, considering the Greenfeast on Greenfeast grafts, it is significant that a discrete effect of grafting is seen only under a long photoperiod, when the NF of grafted scions is comparable to that of decotyledonized plants. In contrast, the NF of scions in a short photoperiod is the same as for intact plants. This difference in NF between comparable graft treatments in different photoperiods may simply reflect the length of time that is required to establish a functional graft union (presumably a phloem connection), and permit transfer of colysanthin from the cotyledons of the stock to the scion. Plants at grafting already contain 8 or 9 nodes, so that vernalized scions only need to form 4 more before flower initiation occurs. Thus, in long photoperiods events in the vernalized scion leading to flower initiation may well be completed before the graft union is adequate for regular colysanthin transport, and hence colysanthin does not attain its normal inhibitory threshhold. Similarly, with unvernalized scions under long days, NF is always below that for the ungrafted control plants (first reported by Paton and Barber 1955), suggesting that the graft union is still not fully functional after about 6 or 7 nodes of growth, and the quantity of colysanthin reaching the apex is insufficient to delay initiation to the normal extent as in the ungrafted controls. In short days, 12 or more nodes of vegetative growth from the time of grafting precede the formation of the first flower primordium. By this time it is most likely that the graft union is fully established, and normal colysanthin transport has been restored.

In contrast, by using stocks of the early flowering variety, Massey, a grafting effect was apparent in both photoperiods, and the Greenfeast scions behaved in all treatments simply as decotyledonized plants. The Massey stocks contributed no effective inhibitor to the graft partner.

Apart from the physiological property of causing delayed flower initiation there is little additional evidence available concerning the nature of colysanthin. Paton (1969) has commented that it has some properties characteristic of abscissic acid, with possibly a variety of physiological effects. In these present experiments we have shown a significant correlation between removal of colysanthin (by cotyledon excision) and the effects of this treatment on flower initiation and rate of node formation (Fig. 1), but the mechanism of this relationship is not at all clear.

In Barber's hypothesis (1959), vernalization and long photoperiod both act in a competitive fashion to destroy colysanthin. However, Johnston and Crowden (1967) reported that photoperiod and cotyledon removal appeared to be additive in their effect, and Paton (1969) has recently shown physiological separation of the photoperiod and vernalization effects. The degree of interaction of these three factors in regulating NF in Greenfeast is shown in the variance analysis of the data in Table 1. Thus the interaction between cotyledon removal and vernalization is highly significant ($P < 0{\cdot}001$). However, the interaction of photoperiod with both cotyledon removal and vernalization is comparatively weak ($0{\cdot}02 < P < 0{\cdot}05$ in each case), indicating that photoperiod is relatively independent of these other treatments in its effects.

Paton (1969) has proposed that in Greenfeast photoperiod has a quantitative effect, which is directly concerned with the attainment of the minimum leaf requirement for flowering (i.e. induction), and the production within the leaves of an inductive stimulus. This stimulus passes from the leaves to the stem apex where flower initiation takes place. Vernalization, on the other hand, influences those reactions at the stem apex which follow induction and culminate in the initiation of flower primordia (i.e. evocation, Knox and Evans 1968). From Paton's data (1969), the processes of evocation occupy about 3 plastochron intervals, or less following seed vernalization treatment. It can be estimated also that photoinduction is completed in Greenfeast (under continuous light) by about node 13 or 14.

In these present experiments, it can be seen that treatments which promote maximum advancement of NF, i.e. both vernalization and cotyledon removal, allow flower initiation to occur as early as node 12 (mean 12·5) under an 18-hr photoperiod. Thus, under these conditions at least, the inductive stimulus has reached its effective threshhold by about the node-11–12 stage of development. In short photoperiods (8 hr), this threshhold is not reached until about 18 nodes are produced (NF = 18·57 for vernalized, decotyledonized plants; Table 1). This difference of 6 nodes is regarded as an expression of the quantitative difference in photoperiodic induction between 18- and 8-hr photoperiods for this variety. If it may be assumed that photoperiodic induction occurs at the same minimum leaf number in intact as in decotyledonized plants, then the duration of the evocation processes in this plant is extended from 3 plastochron intervals, as suggested by Paton's data (1969), to about 5 (4·40 in P_{18} and 5·78 in P_8).

Since flowering at node 12 has been observed in these present experiments, it appears that, provided seed vernalization has been performed, the induced apex can proceed immediately to floral initiation, in both photoperiods, and this will occur in the absence of cotyledons. However, should cotyledons remain attached to the growing plant after vernalization then flower initiation is delayed. The magnitude of the delay is about 2 nodes in both photoperiods (2 nodes in P_{18}, and slightly less, 1·6 nodes, in P_8). If seed vernalization was not given, flower initiation is delayed still further, by some 2·5 nodes in P_{18} and about 4 nodes in P_8.

Both Barber (1959) and Paton (1969) have implied that the response to vernalization can be interpreted in terms of a direct effect of vernalization on the cotyledon inhibitor, either by destruction (Barber 1959) or by reduced synthesis (Paton 1969). In contrast, the present experiments show that any effect of vernalization on the cotyledon system is significantly less than the maximum vernalization response that can be realized. Thus removal of cotyledons from vernalized plants, e.g. Table 1, advanced NF by 4·5 nodes in P_{18} and nearly 6 nodes in P_8 compared with the unvernalized controls. However, when the cotyledons were left attached to vernalized plants, apparent vernalization responses of 2·5 and 4 nodes in P_{18} and P_8 respectively were obtained. These differences in NF between intact and decotyledonized plants after vernalization could result from colysanthin which had moved into the shoot of the intact plant during the post-vernalization period of growth. The quantitative nature of the colysanthin effect is shown (Table 1) by removal of single cotyledons, when values of NF intermediate between intact and fully decotyledonized plants were obtained. In Table 3, it is seen that the maximum

vernalization effect was achieved only if cotyledons were removed before post-vernalization growth at normal temperature was allowed to take place.

These results suggest that a major effect of vernalization is manifest directly on the embryo itself. Since under normal growing conditions the NF of vernalized plants with intact cotyledons does not regain the original value for unvernalized controls, it seems that the embryo vernalization effect is not readily reversible, but that it may be partially obscured when cotyledons are left attached to the growing plant after vernalization. Table 3 also shows that this effect of vernalization on the embryo requires only a comparatively short-term exposure to low temperature in order to yield maximum response.

There is no evidence in these present experiments to endorse Barber's proposal (1959) that vernalization leads to destruction of colysanthin at the plant apex. In all experiments where both vernalization and cotyledon treatments have been investigated simultaneously, it is evident that vernalization treatment was more effective in advancing NF than cotyledon removal alone (e.g. Table 1: 0·5 nodes in P_{18}, $0{\cdot}02 < P < 0{\cdot}05$; 1·5 nodes in P_8, $P < 0{\cdot}001$). Thus if colysanthin is indeed the substrate for the vernalization reaction, then it follows from Barber's hypothesis that the embryo of the imbibed seed already contains a significant quantity of the inhibitor. However, experiments involving sequential cotyledon removal (Johnston and Crowden 1967) and leaching (Sprent and Barber 1957) show that the removal of colysanthin from the cotyledons does not start until about day 4 or 5 after germination.

Further, it is clear that the period of active movement of colysanthin into the shoot (up to about day 14–15) corresponds to the period of decreasing sensitivity of the shoot to vernalization treatment (Table 2), and it may be argued that it is the presence of colysanthin at the apex which decreases or masks the effect of vernalization in young plants. In Table 2 it is also seen that cotyledon excision from growing plants at 6, 10, and 14 days after germination, prior to a vernalization treatment, was more effective in advancing NF than post-vernalization excision. Whilst it can be expected that some colysanthin had already entered the shoot during the period of germination preceding the vernalization treatment, thus providing for the progressive delay to NF in both groups of decotyledonized plants, it is apparent that, in the latter group, inhibitor movement from the cotyledons continued throughout the vernalization treatment, in company with the limited growth which took place during this period. In each of the above cases, it is implied that colysanthin present at the apex survives vernalization treatment and effectively reduces the vernalization response.

Although there is no evidence as to the precise nature of the apical vernalization reaction it appears fairly certain that it does not involve colysanthin. Rather it seems more logical to interpret the embryo response in terms of production of a positive flowering stimulus. Since photoperiodic induction in Greenfeast is completed by about node 12 (under long days), it is apparent that following seed vernalization treatment this stimulus is stable for at least 5–6 plastochron intervals and even longer (12 or more plastochron intervals) under an 8-hr photoperiod. It is apparent from the data in Table 2 that vernalization does not have a significant effect on apices which have passed the node-12 stage of development (between 10 and 14 days after germination). This implies that vernalization has an effect on the embryo only when given to plants before the time of photoperiodic induction. Thus it is possible

that apical vernalization acts in some manner to predispose the young plant to photoinductive processes, rather than be implicated at a later stage in the evocation events, as Paton (1969) has suggested. On the other hand, colysanthin appears to be more concerned with the post-inductive events, and may partially obscure the vernalization response.

There are two lines of evidence which suggest that vernalization has a direct effect on the cotyledon inhibitor system. The magnitude of this effect on the cotyledon is appreciably less than that on the embryo. Firstly, comparison of the values of NF obtained by grafting unvernalized scions to both vernalized and unvernalized stocks (14·17 and 15·60 respectively in P_{18}) shows a significant difference of 1·43 nodes ($P < 0{\cdot}001$). However, NF values for the corresponding grafts under short days, although showing the same trend, are not significantly different. Secondly, there is the observation of a progressive increase in the effect which vernalization has on intact plants with lengthening exposure to cold treatment (Table 3). This effect of vernalization on the cotyledon system may reflect either a steady, low-temperature destruction of colysanthin (cf. Barber 1959), or more probably that there may be a progressive repression of the capacity to synthesize the inhibitor (cf. Paton 1969).

In either case, the net result appears to be that before the inhibitor level can be restored to the effective threshhold, the minimum level of growth is achieved by the shoot for it to become photoinduced, and for flower initiation to be evoked. Unlike the effect of vernalization in dissected embryos, vernalization of the cotyledon system requires a long period of treatment (at least 4 weeks) in order to register the full effect. An apparent reversal of vernalization in peas (devernalization) at high growing temperatures has been reported (Highkin 1956; Barber 1959; Moore and Bonde 1962). The mechanism of this effect is not known but it may well be explained in terms of higher colysanthin synthesis at elevated temperatures.

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