PHOTOPERIODIC INDUCTION OF FLOWERING IN THE LATE PEA CULTIVAR GREENFEAST: THE ROLE OF EXPOSED COTYLEDONS AND LEAVES

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Abstract

Masking of leaf, stem, and cotyledon tissue below and including the first foliage leaf at node 3 delayed flowering. These masking treatments did not change the rate of node formation and the delayed flowering of masked plants involved equivalent increases in node of first flower and time of initiation of the first flower primordium. Masking of the cotyledons and masking of the first foliage leaf appeared equally effective in delaying flowering. Exposed cotyledons were competent to respond to continuous light before the epicotyl hook opened. It appears that the cotyledons are thus involved in photoperiodic induction in continuous light in addition to the foliage leaves at nodes 3, 4, and 5.

Masking delayed commencement and completion of induction but the delay was not sustained throughout the interval between completion of induction and initiation of the first flower. Reduction of this interval in masked plants probably involves autonomous determination of some evocation events in flowering. Similar relationships may occur in vernalized plants. Juvenility and age cannot be regarded as important factors determining flower initiation.

I. Introduction

Various proposals for the role of the leaf in the flowering of late pea cultivars reveal three conflicting opinions. Haupt (1969) agrees with Köhler (1965) and concludes that flower initiation under normal conditions is promoted by long days and vernalization but hardly influenced by the leaves. Sprent (1966) similarly concludes that leaves apparently have little effect on flowering and suggests that foliage leaves are involved in the photoperiodic response in a qualitative rather than a quantitative fashion. In contrast, Paton (1967) considers the first-formed foliage leaves play an important, though temporary, quantitative part in flowering behaviour. The last concept has led to a new interpretation of the flowering behaviour of cv. Greenfeast (Paton 1968, 1969) and explains, in part, the complex relationships observed (Paton 1960). Not unexpectedly, Paton's interpretation differs in many respects from those favoured by Haupt (1969). Unequivocal evidence either way for the role of leaves in flowering may help resolve some of these differences.

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Since the seed, cv. Greenfeast, was the same in the experiments of Sprent and Paton, their different interpretations are not associated with genotypic differences in experimental material. Haupt refers to Köhler's experiments with cv. Aldermann and thus genotypic differences cannot be excluded as a possible reason for the difference between the Haupt–Köhler concept and that of Paton. But a more likely reason in both cases is in the reduced vegetative growth associated with the defoliation treatments of Köhler and Sprent. Köhler, for example, retained only sufficient leaf material for growth to continue at a greatly reduced rate. On the other hand, Paton (1967) specifically excludes such severe defoliation treatments from the results used in evaluating the effects of defoliation on flowering. There seems ample justification for the latter procedure. Complete defoliation at either node 3 or nodes 3 and 4 clearly delayed flower initiation but the increases in flowering node following these defoliation treatments were reduced and even abolished if further defoliation was sufficiently severe to reduce the rate of node formation. Differences in interpretation seem unavoidable in such defoliation experiments involving concomitant changes in flowering behaviour and growth rate. One solution to the problem is to reduce the severity of the treatment and ensure that changes in growth rate do not occur. This is the approach used in the present paper.

Preliminary treatments have established that masking of pea cotyledons with aluminium foil at an early stage in germination has no observable effect on any aspect of growth rate. In marked contrast, prolonged retardation of the rate of node formation follows early cotyledon excision in peas (cf. Amos and Crowden 1969). For this reason, cotyledon masking appears a more appropriate experimental technique than cotyledon excision for study of the role of exposed cotyledons in flowering. Masking of foliage leaves, however, suffers from disadvantages similar to defoliation. For example, masking of more than one foliage leaf in cv. Greenfeast reduces the rate of node formation and marked compensatory growth of exposed leaves (cf. Thrower 1964) also occurs in extensively masked plants.

The terminology followed is that of Evans (1969) who suggests the events in the leaf (induction) are likely to be so different from the events at the apex (evocation) that different terms should be used to distinguish them. In unvernalized plants of cv. Greenfeast the events related to induction can be clearly separated from those evocation events which occur in the interval between completion of induction and initiation of the first flower (Paton 1969). Frequent reference is made to this interval.

II. Materials and Methods

The late-flowering dwarf pea cv. Greenfeast was used in all experiments. The source of the seed and methods of sterilization, germination, and planting of uniform seedling material were as standardized previously (Paton 1968). Plants used in the masking and transfer experiments were grown and transferred between growth cabinets as in previous transfer experiments (Paton 1969).

Strips of aluminium foil were used for masking. Appropriate overlapping and crimping ensured a light-proof but loose cover for cotyledons, foliage leaves, and the part of the stem bearing the two scale leaves. The foliage leaves were usually remasked once to allow for complete cover without restricting the developing stipules and leaflets. Remasking involved exposure of previously light-deprived tissue to dim light for about 1 min. The cotyledons were positioned at the surface of the soil when transplanting at day 4. Germination usually occurred in the dark and unmasked cotyledons were subsequently exposed to normal light at transplanting. The testa did not appear to influence the photoperiodic responses of the unmasked cotyledons, but to ensure uniformity the testa was removed at transplanting in most experiments.

A standard temperature of 20°C was chosen for sterilization, soaking, germination, and growth. The plants were grown in normal potting soil in growth cabinets. At predetermined times, replicates of each masking treatment were transferred from a cabinet having continuous light to a cabinet having 8 hr light (8 a.m. to 4 p.m.). The same light intensity (3500 f.c.) was used for both photoperiods. Periodic dissections confirmed the earlier observation (Paton 1969) that the increases with time of total number of nodes (N) and the number of unfolded leaves (NL) were unaffected by day length. Similarly, the rates of increase of N and NL values were always determined in masking experiments to establish any possible effects of the experimental treatments on these rates. The mean value of the node of the first flower (NF) was determined from dissection of from 10 to 20 plants after dissection of some test plants indicated that all plants of the treatment had initiated at least one flower primordium. The standard errors of the means for NF values are usually too small to show graphically but it was not possible to obtain standard errors for estimated values derived by transposition (cf. Paton 1968). The total number of plants in any one experiment was limited by the size of the cabinet which holds 200 plant containers of suitable size for unrestricted growth of a single pea plant. Treatments were reduced in number in preference to reduction in replicates below 10. This becomes an important point when the number of transfer treatments in some experiments was reduced to three and accurate determination of the time of completion of photoperiodic induction was not possible. In such cases, the 50% response level in the linear reduction of NF values with increasing days in continuous light was taken as 50% induction (cf. Paton 1969).

III. Results

The masking treatments which did not affect the rate of node formation were:

Treatment UM—unmasked control plants;

Treatment MC—masked cotyledons;

Treatment M2—masked cotyledons and masked scale leaves at nodes 1 and 2;

Treatment M3—masked cotyledons, masked scale leaves, and masked leaflets and stipules of the foliage leaf at node 3.

Data for a typical experiment are given in Figure 1. The flowering behaviour of unmasked plants (treatment UM) confirmed the main results of previous transfer experiments conducted at 20°C (Paton 1967, 1969). Masking of all leaf, stem, and cotyledon tissue below and including the first foliage leaf at node 3 (treatment M3) increased the NF values for all transfer times. Less extensive masking involving cotyledons and scale leaves (M2) or cotyledons alone (MC) gave intermediate NF values. None of the masking treatments affected the increase with time of either N or NL values. Changes in NF values were not associated with changes in the rate of node formation and the increased NF values of masked plants involved equivalent delays in time of initiation of the first flower primordium.

The magnitude of the delayed flowering of masked plants depended on both the extent of masking and the transfer time. The increase in NF values with the most extensive masking treatments (M3) was greater at the intermediate transfer times at day 15 (3·8 nodes) and day 10 (3·3 nodes) than for the long-day controls transferred after flower initiation (day 25, 0·9 nodes; day 20, 1·0 nodes) and the short-day controls (day 5, 1·8 nodes). A maximum delay with transfers at day 15 also occurred with treatments MC and M2. The delays associated with the M2 treatment were often not significantly different from those associated with the MC treatments, and it is doubtful whether masking of the scale leaves and associated stem tissue affected flowering to the same extent as masking of either the cotyledons or the first foliage leaf.

The hitherto unrecognized delay in flowering with masking of cotyledons in the MC treatment suggests that exposed cotyledons play an active part in events of induction and should be included in the number of leaves involved in induction. Thus exposed cotyledons now appear additional to the three foliage leaves at nodes 3, 4, and 5, proposed previously (Paton 1967). The magnitude of this cotyledon component was surprisingly large, all experiments (cf. Figs. 1–3) involving delays in *NF* values between 0·5 and 0·8 nodes.

The limitations imposed by size restrictions in this type of experiment are evident with only three transfer times at days 10, 15, and 20 in Figure 1. One important consequence is that the lack of data between these times does not allow accurate determination of the time when photoperiodic induction was completed. It is possible, however, to derive approximate values of the leaf number at commencement of induction and at 50% induction from the curves in Figure 1. Transposition

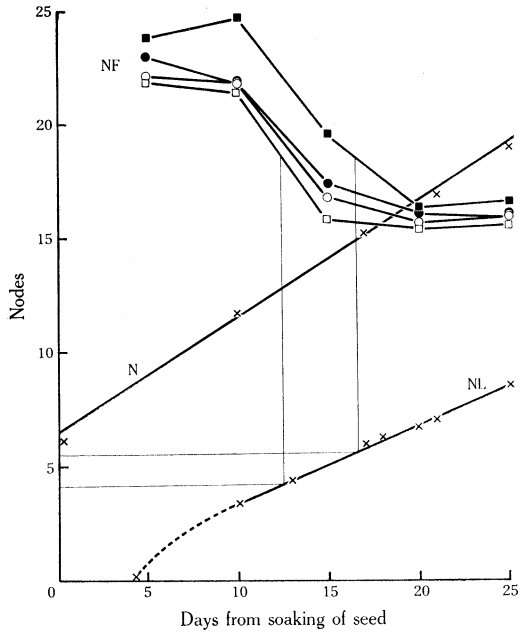

Fig. 1.—Relationship in unmasked and masked plants between node of first flower (*NF*) and number of days in continuous light before transfer to 8-hr days. Exposure to continuous light commenced at 4 p.m. on day 4 and terminated at the times indicated. □ Unmasked plants (UM). Masking involved either the cotyledons (MC, ●) or cotyledons and two scale leaves (M2, ○) or cotyledons, two scale leaves, and all the first foliage leaf at node 3 (M3, ■). Changes in the total number of nodes (*N*, ×) and unfolded leaves (*NL*, ×) are shown for the same time scale.

lines are given for 50% induction in the UM controls and at this level of induction the *NL* values increased from 4·1 (UM) to 4·5 (MC) and 5·6 (M3) with increased severity of masking. Commencement of induction in M3 was not evident at day 10 and was delayed presumably until day 11 or 12 when the first unmasked leaf at node 4 was unfolded. These approximate values are included in Table 2.

The data for Figure 2 were obtained from a further experiment in which daily transfers of MC and M3 masking treatments were made during the critical period between day 12 and day 21. The errors involved in derivation of the time of completion of induction and time of initiation of the first flower from these curves are unlikely to be more than 12 hr. The derived values given in Table 1 show that masking treatment MC (and M3) delayed completion of induction by 2·0 days (3·0)

and delayed initiation of the first flower by 1·0 days (1·5). Thus the delay in completion of induction in masked plants was not sustained throughout the interval between completion of induction and initiation. The two masking treatments each reduced the interval by about 30% (1·0 and 1·5 days). The magnitude of this reduction of the interval was not large but two full-scale check experiments have

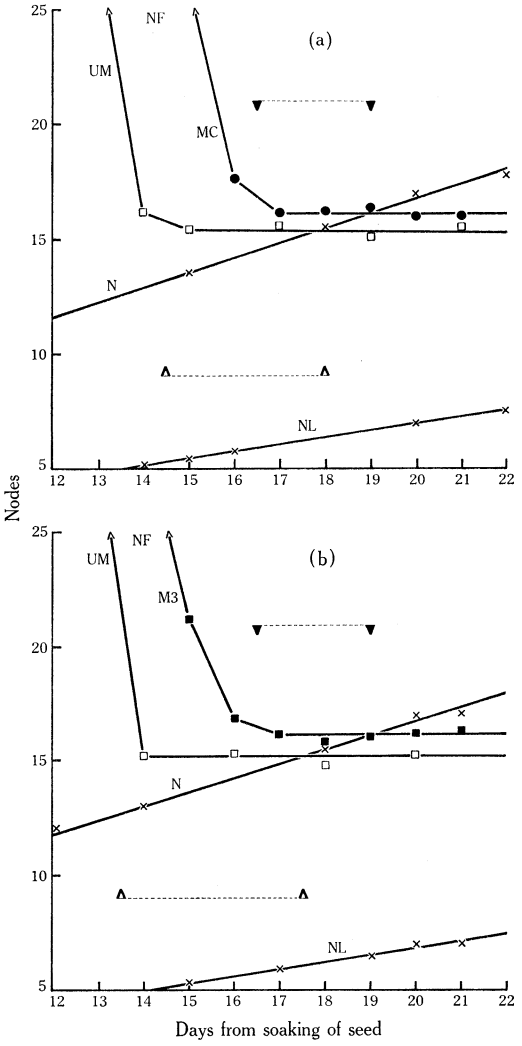

Fig. 2.—Comparison of MC (*a*) and M3 (*b*) masking treatments. Symbols as in Figure 1. The interval between completion of induction and initiation of the first flower is shown for unmasked plants (△.....△) and masked plants (▼.....▼).

confirmed the result. Despite the small magnitude, the reduction in the interval in masked plants is of physiological interest since at least some events in evocation apparently progressed independently of the delayed completion of induction in masked plants. This conclusion raises an interesting point for discussion.

The delayed completion of induction in masked plants was associated with increased *NL* values at all stages of induction (Table 2). As expected from previous experiments (Paton 1967, 1969), the *NL* values of the unmasked controls at

TABLE 1

EFFECT OF MASKING OF COTYLEDONS AND LEAVES ON PHOTOPERIOD INDUCTION AND FLOWER INITIATION

Time after soaking of the seed for completion of induction and initiation of the first flower and the interval between them. Masking treatments as in Figure 1. Times for induction and initiation estimated from Figure 2

Treatment	Induction Period (days)	Flower Initiation Time (days)	Interval (days)	Figure Reference
UM	14·5	18·0	3·5	2(*a*)
MC	16·5	19·0	2·5	
Delay (days):	2·0	1·0		
UM	13·5	17·5	4·0	2(*b*)
M3	16·5	19·0	2·5	
Delay (days):	3·0	1·5		

completion of induction varied only slightly between 5·3 (MC control) and 5·0 (M3 control). Masking increased the *NL* values at completion of induction by 0·6 and

TABLE 2

NUMBER OF UNFOLDED LEAVES (*NL* VALUES) AT THREE STAGES OF INDUCTION IN MASKED AND UNMASKED PLANTS

Estimates of the NL values were obtained by transposition from the NF values in Figure 1, and at the indicated time for completion of induction in Figure 2. Masking treatment as in Figure 1

Treatment	*NL* Values			
	Commencement of Induction (Fig. 1)	Induction 50% Complete (Fig. 1)	Induction Complete [Fig. 2(*a*)]	Induction Complete [Fig. 2(*b*)]
UM	0–2	4·1	5·3*	5·0*
MC	2–3	4·5	5·9	—
M3	3–4	5·6	—	6·0

* Differences between UM controls commonly observed in concurrent transfer experiments using two apparently similar pairs of L.B. cabinets.

1·0 respectively, and the magnitude of each response was confirmed generally in check experiments. The remaining values in Table 2 suggest that the increased *NL*

values of masked plants were already evident at commencement of induction and at 50% induction. This type of overall delay of induction in masked plants supports the proposal (Paton 1967) that the first-formed foliage leaves play a quantitative part in flowering.

The MC treatment was again surprisingly effective compared with the M3 treatment. If it is assumed that increases of 0·5–0·6 in *NL* values were associated with masking of the cotyledons in M3 as well as the MC treatments, then the further masking of the stipules and leaflets of the first foliage leaf, together with the two scale leaves and adjoining stem tissue, increased the *NL* values by only 0·4–0·5. Even allowing for complete ineffectiveness of the scale leaves, the cotyledons and the first foliage leaves appeared equally effective organs for perception of light during photoperiodic induction. There is no indication that ontogenetic rank (age) was involved in the effectiveness of the cotyledons (node 0) and the first foliage leaf (node 3). Similarly, there was no clear indication that ontogenetic rank was a major factor in the effectiveness of exposed leaves between nodes 3 and 6. Although the leaf at node 6 in M3 plants, was sufficient substitution for the exposed cotyledons and first foliage leaf of unmasked plants, masking treatments of a single leaf between nodes 3 and 5 in preliminary experiments have not disclosed any large changes in leaf efficiency with ontogenetic rank. Presumably the small reduction in the number of exposed leaves involved in induction of masked plants followed either from a summation of small increases in leaf efficiency of individual leaves or, as is more likely, from the unavoidable exposure of leaves whilst they were unfolding from the apical bud and before they could be masked.

Further experiments have established the time after soaking of the seed when exposed cotyledons were competent to respond to continuous light by reducing the *NF* value. Using an intermittent mist spray it was possible to expose the cotyledons to continuous light from imbibition of the dry seed and during the first 4 days of germination. Such early exposure of the cotyledons did not reduce *NF* values compared with dark-germinated plants and there seems no evidence suggesting that cotyledons develop competence to respond to light before the fourth day after soaking. Full competence, however, developed between day 4 and day 7. This is shown by two curves in Figure 3 which gives the data of a large-scale experiment combining three times for transfer from continuous light and a range of times after soaking of the seed for masking of the cotyledons. The *NF* values of plants transferred after 11 days (T11) and 14 days (T14) were reduced 2·0 and 1·6 nodes respectively ($P < 0·001$) with the additional exposure of the cotyledons to continuous light when masking of the cotyledons was delayed from day 4 to day 7. The magnitude of these reductions in *NF* values associated with exposure of the cotyledons to continuous light for 3 additional days appears remarkably large. It is unlikely that the plumule was involved since for most of the time between day 4 and day 7 the epicotyl was still in the hook stage and barely emerging from between the cotyledons. Even when the first foliage leaf was unfolding at about day 10, additional exposure of the cotyledons effected a reduction in the *NF* values of T14 plants equivalent to that found for a similar period of exposure before day 10. The same equivalence was found in preliminary experiments combining masking and demasking treatments of the cotyledons of plants

transferred from continuous light at about 50% induction (cf. T14, Fig. 3). Exposure to continuous light for 3 days reduced *NF* values by similar amounts irrespective of the time between day 4 and day 12 when the cotyledons were exposed. Thus the magnitude of the response elicited from exposure of active cotyledons to a period of continuous light appears independent of cotyledon age. The only restriction involved exposure of the cotyledons to continuous light at least 3 days before day 15. This restriction may be related either to completion of induction in continuous light or to inception of cotyledon senescence both of which occurred about day 15 in the growth conditions used.

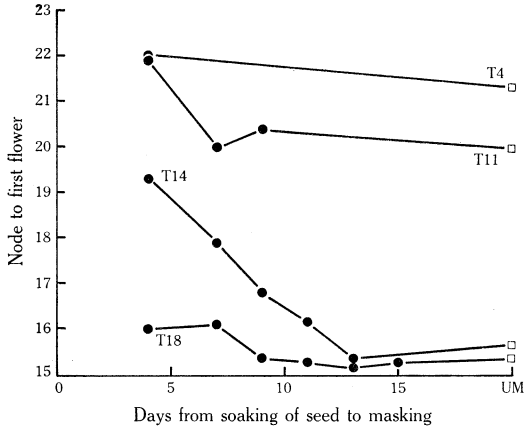

Fig. 3.—Effect of time of cotyledon masking on the flowering node of plants transferred from continuous light at the times (days) indicated after soaking of the seed (T4, T11, T14, and T18). UM, unmasked control plants.

The large masking responses of T14 plants in Figure 3 commonly occurred with intermediate transfer times (cf. Figs. 1–3). Presumably the large photoperiodic response has supplemented the small masking responses of the short-day and long-day control plants. This supplementary effect is perhaps best illustrated in the situation where commencement of induction in masked plants was delayed until induction was almost completed in unmasked plants. Transfer from continuous light at this stage would give *NF* values for unmasked plants close to the continuous-light controls whereas the masked plants could be close to the short-day controls. Such a situation has yet to be observed, but the large masking responses of plants transferred at day 14 (see Figs. 2 and 3) suggest that it is a distinct possibility.

IV. Discussion

Early competence of exposed cotyledons to respond to photoperiod is well known (cf. Ballard and Grant Lipp 1964) in plants having an epigeal type of germination. During the hypogeal germination of peas, the cotyledons are not normally exposed and it is perhaps surprising that they develop clear competence between the fourth and seventh day after soaking of the seed. Freshly cut hand sections of cotyledons under a light microscope did not reveal the presence of green chloroplasts until after the cotyledons were exposed for 8 days and thus development of photosynthetic ability does not seem associated with the early competence of pea cotyledons to respond to photoperiod. Exposure of cotyledons to low light intensities will

probably elucidate this point. Similarly, light-induced changes in permeability affecting transport of physiologically active substances out of the cotyledons should be tested. The most simple interpretation in cv. Greenfeast, however, is that exposed cotyledons and exposed foliage leaves are equally effective organs for perceiving long days. The main differences between the cotyledons and foliage leaves are probably quantitative and related to inception of senescence which, in the conditions used, was appreciably earlier for the cotyledons (about day 15) than for the first foliage leaf (day 35). There is a clear indication in this study that duration of exposure of the cotyledons regulates their contribution to induction but it is difficult to imagine those conditions in which inception of senescence of the cotyledons was delayed sufficiently to enable them alone to cause induction. In this respect cv. Greenfeast differs from *Anagallis arvensis* in which Ballard and Grant Lipp (1964) consider it is probably true that cotyledons on their own are competent in induction. Other features of induction in *A. arvensis* suggest further differences (e.g. rapid vegetative reversion in non-inductive short days and a decline in efficiency of percipient leaves with increase in ontogenetic rank) but one interesting similarity is that both *A. arvensis* and cv. Greenfeast initiate the first flower in the axil of a leaf which was not present at the time of induction.

Although one of the results to be discussed is explained by the autonomous determination of flowering favoured by Haupt (1969), other results suggesting the quantitative role of exposed leaves and cotyledons in the flowering of cv. Greenfeast are difficult to reconcile with the concepts of critical age and juvenility also favoured by Haupt. He assumed that juvenility has adverse effects with regard to the importance of age for flower initiation in peas and proposes that one of the most important factors determining flower initiation is age. In contrast, early competence of the cotyledons to respond to long days, a constant level of efficiency of the cotyledons between day 4 and day 12 after soaking of the seed, and an approximate equivalence of the cotyledons and first foliage leaf for perception of long days now demonstrated for cv. Greenfeast suggest that juvenility and age are of little importance in this cultivar. The apparently unusual situation with regard to RNA content of the cells at and near the apex of cv. Greenfeast (Paton 1969), in which there is no marked central zone even in 1-day-old seedlings, suggests the interesting possibility that absence of a juvenile phase in exposed leaves and cotyledons during induction may be associated with absence of a vegetative phase in the apex. Histochemical studies are in progress to elucidate this point. The significance of Haupt's concepts of critical age and juvenility in the flowering of cv. Greenfeast is probably related to the concomitant growth necessary to develop a sufficient number of exposed leaves for induction.

The 30% reduction in the interval between completion of induction and initiation of the first flower in masked plants implies at least partial independence of evocation events on completion of induction. Such independence can be interpreted as an example of autonomous determination of flowering in peas as proposed by Haupt (1969). It is not clear from the present study, however, whether the level of autonomous determination, as indicated by the degree of independence, is related to the magnitude of the induction delay. One possibility is that autonomous determination may be involved in the vernalization responses of peas. The zero interval between completion of induction and initiation of the first flower which occurs in vernalized

plants of cv. Greenfeast (Paton 1969), presumably represents the maximum degree of independence that can be achieved. On this basis, it is tempting to consider some causal relationship between maximum independence in vernalized plants and a substantial delay of induction relative to commencement of evocation if it is assumed that some autonomous events in evocation occur during the vernalization treatment of 30–40 days.

Heslop-Harrison and Heslop-Harrison (1970) view the general problem of photoperiodic induction in a similar way to Haupt's autonomous determination. They favour the hypothesis that the march of developmental events is governed by the endogenous controls whilst the specific leaf-generated hormone functions as a rate modulator of autonomous processes. But if the delayed flowering of cv. Greenfeast in short days involves an association of delayed induction and autonomous determination, there is no evidence suggesting that the level of autonomous determination increases greatly in short days. In contrast, the relative constancy of the number of green foliage leaves involved in induction of cv. Greenfeast (Paton 1968) suggests that the amount of the leaf-generated stimulus affecting flower initiation remains relatively constant, even when the flowering node is delayed until the 45th node or more in short days and high growing temperatures.

V. Acknowledgments

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