VERNALIZATION, PHOTOPERIODIC INDUCTION, AND FLOWER INITIATION IN THE LATE PEA CULTIVAR GREENFEAST

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Summary

The events related to the leaf requirement (induction) in the late pea cultivar Greenfeast are separate from events (evocation) between induction and initiation of the first flower. Photoperiod influences induction, whilst vernalization influences evocation. Vernalization represses synthesis of the graft-transmissible floral inhibitor present in unvernalized plants where it delays rapid evocation. Nothing is known of the biochemistry of the floral inhibitor although there is some preliminary histochemical evidence which suggests an interesting relationship between presence of inhibitor and RNA content in the apex.

The effect of seed vernalization is probably additive to the effects of reduced growing temperature and increased photoperiod, which both influence induction by reducing the leaf requirement. The relationship between floral inhibitor and the photoperiodic response is not clear.

I. INTRODUCTION

Some features of the events leading to flower initiation have been described for unvernalized plants of the late pea cultivar Greenfeast (Paton 1967). A quantitative leaf requirement is associated with completion of the photoperiodic events in the leaf and passage of the inductive stimulus out of the leaf. Subsequent events are not dependent on photoperiod or presence of leaves, and thus appear located in the stem, presumably at or near the apex. Initiation of the first flower primordium at the apex occurs about three plastochron intervals after completion of photoperiodic induction in the leaves. Such clear separation of these events both in space and time supports the view (Evans 1969) that the events in the leaf and those at the apex are likely to be so different that different terms should be used to distinguish them. Evans suggests induction and evocation respectively. This terminology will be used in the present paper, which considers vernalization and photoperiodic responses of cv. Greenfeast with respect to events (induction) related to the leaf requirement, and events (evocation) between induction and initiation.

There is good evidence for a graft-transmissible floral inhibitor in late pea cultivars (Paton and Barber 1955). Preliminary reports (Paton 1958; Barber 1959) of grafting experiments between unvernalized and vernalized plants have indicated that the inhibitory effect of a late stock on the flowering of an early scion is not observed after seed vernalization of the late stock. Since this result is particularly

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relevant to the role of vernalization in induction and evocation, data for a typical grafting experiment involving seed vernalization are given for the first time.

Throughout this paper, the effect of low temperature during seed vernalization is interpreted as repression of synthesis of the floral inhibitor which is detectable during germination of unvernalized seed of late cultivars. An alternative interpretation involves low-temperature destruction of the inhibitor (cf. Barber 1959). It has not yet been possible to distinguish experimentally between these two alternatives. However, it is usual to invoke two reactions with different cardinal points and temperature coefficients to explain how low temperatures are promotory, as in "vernalin" production (Lang 1965) and "colysanthin" destruction (Barber 1959). On the other hand, only a single high-temperature-specific step in inhibitor synthesis need be assumed with repression of inhibitor synthesis at low temperature. Apart from its relative simplicity, consideration of the unvernalized condition as an inhibitory effect of increased temperature during germination has the added advantage of allowing direct comparison with the inhibitory effects of increased growing temperature (Paton 1968).

The floral inhibitor has proved just as elusive as florigen. It has some of the characteristics of abscisic acid (Paton, unpublished data) and, in common with this type of compound, it probably has multiple effects. This possibility, considered together with the existence of more than one type of endogenous inhibition in the flowering behaviour of late pea cultivars (Paton 1968), is the main reason why the descriptive but non-committal terms inhibition and inhibitor are retained.

II. Materials and Methods

The late flowering dwarf pea cultivar Greenfeast was used in all experiments. The source of seed and methods of sterilization, germination, and planting of uniform seedling material were as standardized in previous work (Paton 1968). Preliminary experiments on photoperiodic induction in cv. Greenfeast demonstrated appreciably more variable responses when grown in perlite watered with modified Hoagland solution than in standard potting soil. Accordingly, in all the photoperiodic induction experiments described in this paper, the plants were grown in standard potting soil in L.B. growth cabinets located in the Botany Department, A.N.U., Canberra. The grafting experiments were carried out in the Earhart Laboratory and the grafting methods (Paton and Barber 1955) and growth conditions (Paton 1968) have been described previously.

Vernalization treatments were commenced after sterilization and soaking. The imbibed seeds were positioned on about 6 cm of perlite in a well drained container, covered with 1 cm of perlite, and placed in a cold room at 1–3°C for the 4 weeks required for maximum vernalization. The watering schedule was minimal to prevent drying out. Germination proceeded slowly over the duration of the cold treatment, and at the end of 4 weeks the radicles were about 1–2 cm in length and the epicotyl was just about to break through the testa. The unvernalized seeds were soaked and grown at 20°C 2 days prior to termination of the cold treatment. Soaking of the unvernalized seed was considered the beginning of day 0. The vernalized seedlings were grown at 20°C during the night of day 2. This procedure provided similar-sized vernalized and unvernalized plants (epicotyl 0·5–1 cm and radicle 5–6 cm) for planting on day 3 when the photoperiodic treatments were commenced.

The photoperiodic treatments consisted of daily transfers to 8-hr days of vernalized and unvernalized plants grown in continuous light from day 3. The same light intensity (3500 f.c.) was used for both photoperiods. The temperature control in the artificially lit controlled-environment cabinets allowed transfer from the cabinet giving continuous light to one giving an 8-hr day without changes in temperature, rate of vegetative development, light quality, or light intensity.

Daily dissections of 15 plants from each of the vernalized and unvernalized treatments in continuous light gave data on the increase with time in total number of nodes (N), and the number of foliage leaves (NL). Periodic dissections of vernalized and unvernalized plants grown in 8-hr days from day 3 confirmed the earlier observation (Paton 1967) that N and NL values were unaffected by day length. The daily dissections involved removal of the outer leaves of the apical bud and exposure of the meristematic dome. It was not necessary to remove the youngest three to four leaf primordia for determination of N values and the meristem and youngest primordia were preserved for later histochemical study (Knox and Paton, unpublished data). The mean value for the node of first flower (NF) was determined from dissection of 20 plants after dissection of five test plants indicated that all plants of the treatment had initiated at least one flower primordium. The standard error of the means for N, NL, and NF were generally too small to be shown graphically with the node scale used in Figure 1. Statistical analysis of values derived by transposition between the node and time axes in Figure 1 was not attempted.

III. Results

(*a*) *Transfer Experiments*

Unvernalized plants required only 12 days of growth in continuous light before transfer for flower initiation to occur at the same node as plants kept continuously in the light until flower primordia were present [Fig. 1(*a*)]. Since flower initiation

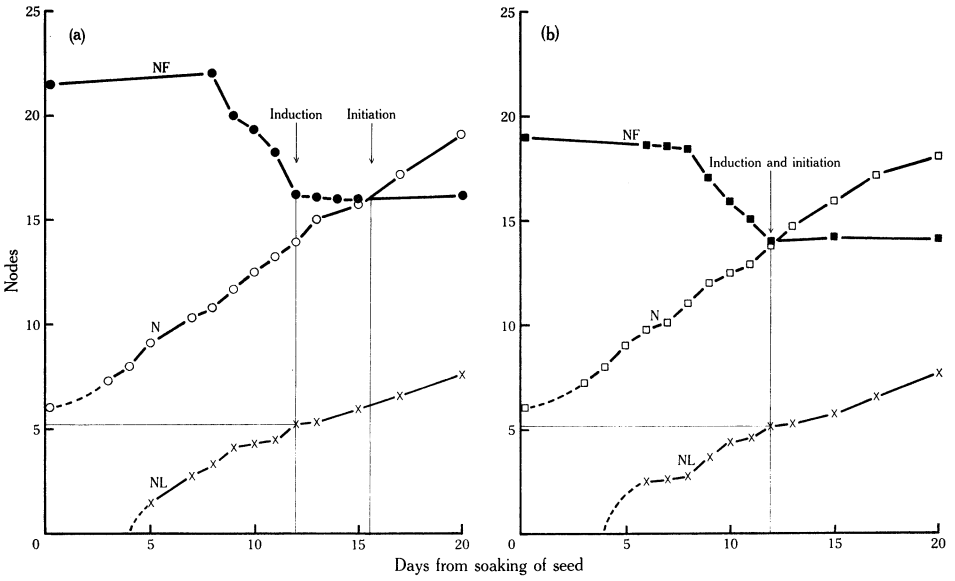

Fig. 1.—Relationship in unvernalized (*a*) and vernalized plants (*b*) between node of first flower (NF; ●, ■) and number of days in continuous light before transfer to 8-hr days. Changes in leaf status for the same time scale are shown for total number of nodes (N; ○, □) and unfolded leaves (NL, ×).

occurred between days 15 and 16, the node of first flower was predetermined and became independent of the photoperiodic regime at least 3 days before the first appearance of flower primordia. This irreversible commitment to flower at a predetermined node is good evidence for the existence of photoperiodic induction as distinct from evocation in cv. Greenfeast (cf. Paton 1967).

Figure 1(*b*) shows that this is not the case in vernalized plants. Completion of photoperiodic induction and initiation of the first flower coincide at day 12 [Fig. 1(*b*)]. Figure 2, which combines on a larger scale pertinent data from Figures 1(*a*) and 1(*b*), shows in detail the different responses of vernalized and unvernalized plants. Vernalization abolished the additional time required for evocation in unvernalized plants. Inspection of N values in Figure 2 shows that absence of the delay between induction and initiation was not associated with any change in the rate of node formation, which remained constant (0·67 nodes per day) before and after induction for both treatments. At this rate of node formation the difference of 3 days between induction and initiation in the unvernalized plants accounts exactly for the two-node vernalization response in NF values for plants grown in continuous light in this experiment.

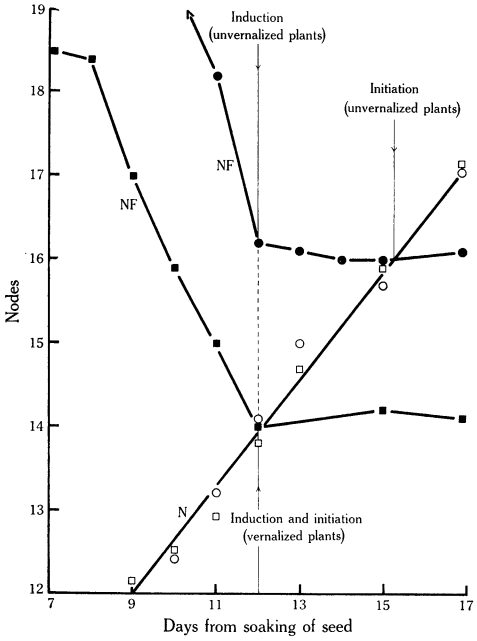

Fig. 2.—Comparison of induction and initiation of first flower in vernalized and unvernalized plants. Symbols as in Figure 1.

Some features of the reduction in flowering node [NF values, Figs. 1(*a*) and 1(*b*)] with increased days in continuous light were very similar for unvernalized and vernalized plants. The reduction commenced about day 7, 50% response occurred at about day 10, and maximum reduction was achieved after 12 days. The standard error for the means of the NF values with 50% response was only slightly larger (±0·2) than the standard error values for the long-day control (±0·15). Thus, there was no evidence of a bimodal distribution of NF values. Progress towards completion of photoperiodic induction and subsequent evocation was quantitative and not qualitative. The vernalization response was only slightly larger in the short-day controls (2·5 nodes) than in the long-day controls (2·0 nodes). The NF values were not affected by any large photoperiod × vernalization interaction.

At the completion of induction at day 12 both vernalized and unvernalized plants had unfolded leaves at nodes 3, 4, and 5 (cf. Fig. 1). The leaves at node 6 were

just commencing to grow free of the ensheathing stipules. This number of foliage leaves at completion of induction has been interpreted as leaf requirement for completion of the photoperiodic reactions involved in induction (Paton 1967). Seed vernalization did not affect this leaf requirement and thus did not affect induction.

The value of three foliage leaves exclusive of the two scale leaves at nodes 1 and 2 agrees well with the values for the leaf requirement in continuous light in other experiments conducted at 20°C where values of 3·0–3·2 foliage leaves have been obtained repeatedly. Such reproducibility was not shown by other parameters. For example, unvernalized plants varied in NF values from 15·2 to 18·5, and the times between induction and initiation varied between 3 and 8 days and 2–5 plastochrons. A long-term experiment using a wide range of seed storage conditions has been commenced in an attempt to identify the factor causing this apparent seasonal variability.

(*b*) *Grafting Experiment*

As in other experiments involving grafted pea cultivars, substantial and prolonged reduction in scion growth occurred in some grafted plants. These weak scions remained stunted apparently indefinitely. Such permanence of reduced growth in graft combinations of closely related genotypes suggests some incompatability more characteristic of an immunological reaction than temporary injury associated with grafting. Whatever type of reaction is involved in weak scion growth, it is associated with a marked delay in NF values (cf. Table 1). This reciprocal relationship between reduced growth and increased NF values is less often observed than the direct and possible causal relationships (Paton, unpublished data) between growth and NF values; usually either reduction in rate of node formation is associated with reduced NF values (e.g. in cuttings and treatments with growth retardants) or accelerated node formation is associated with increased NF values (e.g. treatment with gibberellic acid). Only in the absence of concomitant changes in growth rate can changes in NF values be attributed wholly to factors directly influencing the processes of flowering. It is for this reason that NF values of the normal scions and not the weak scions in Table 1 will be considered in this paper. Those scions which established normal growth rate within about 10 days after grafting gained in fresh weight between grafting and flowering at an overall rate greater than 100 mg/day. The rate for weak scions was usually less than 50 mg/day. In Table 1, a rate of 75 mg/day was chosen to separate normal and weak scion growth.

Compared with the control grafts, unvernalized stocks of cv. Greenfeast caused significant delays in flowering of cv. Massey scions of 2·9 nodes in short days ($P < 0{\cdot}001$) and 1·1 nodes ($0{\cdot}05 > P > 0{\cdot}01$) in long days. Delayed flowering or inhibition of cv. Massey scions did not occur with seed vernalization of the stock. Intact plants of early cultivars, such as Massey, are unaffected by vernalization and photoperiod, and thus provide a useful assay for inhibition effects. These results have been interpreted (Paton 1958; Barber 1959) as the transfer of a floral inhibitor from the unvernalized late stock to the early scion. This graft-transmissible inhibitor was not present following seed vernalization of the late stock cultivar.

Equivalent confirmation has not been possible in the case of the photoperiodic responses which occurred in early scions on unvernalized stocks in Table 1. In five

separate experiments three showed no photoperiodic effects, whilst the significant responses in two were associated with either the absence of inhibition in long days or extremely large inhibition in short days. Thus, these grafting experiments provide no firm evidence either way to link the presence of inhibitor with photoperiodic responses. Other opinions are similarly equally divided. Barber (1959) suggests that the photoperiodic responses of late pea cultivars involves destruction of a floral inhibitor or colysanthin in long days. On the other hand, Johnston and Crowden (1967) suggest that photoperiod and colysanthin have independent effects on flowering of peas.

TABLE 1

EFFECT OF VERNALIZATION OF SEEDS OF CULTIVAR GREENFEAST USED AS STOCK ON THE FLOWERING NODE OF CULTIVAR MASSEY USED AS SCIONS

Grafts were grown in 8-hr and 16-hr days (P_8 and P_{16} respectively). The flowering behaviour of grafts with normal scion growth (> 75 mg fresh wt/day) differed from grafts with weak scion growth (< 75 mg fresh wt/day). There were 25 grafts per treatment, and about equal numbers of normal and weak scions occurred in each treatment

Graft Type	Normal Scion		Weak Scion	
	P_8	P_{16}	P_8	P_{16}
Massey/Massey	10·4±0·2	10·6±0·2	11·6±0·4	12·1±0·3
Massey/unvernalized Greenfeast	13·3±0·6	11·7±0·5	14·1±0·4	13·0±0·3
Massey/vernalized Greenfeast	10·5±0·4	10·9±0·2	13·4±0·4	13·3±0·4

IV. DISCUSSION

A clear link exists in unvernalized plants of cv. Greenfeast between a delay in evocation and the presence of a graft-transmissible floral inhibitor. Neither delay nor inhibitor can be demonstrated in vernalized plants. Thus, vernalization in cv. Greenfeast leads to the absence of a floral inhibitor which is present in unvernalized plants, where it delays rapid evocation at the apex. In general terms this interpretation agrees with a recent proposal (Chouard and Tran Than Van 1969) that vernalization removes the inhibition exerted by the apical meristem on the flowering of axillary meristems.

Nothing is yet known of the biochemistry of the inhibitor. Attempts to determine differences in apices of vernalized and unvernalized plants have met with little success. Vernalization increases periclinal zonation of the outer layers of cells on the apical dome and youngest leaf primordia (Knox and Paton, unpublished data), but this effect is more likely to be a general effect of reduced growth rate as observed in some grasses (Thielke 1965) than a specific effect of seed vernalization on flowering. Preliminary histochemical investigations (Knox and Paton, unpublished data) suggest an apparently unusual situation with regard to RNA content of cells at and near the apex. Compared with other plants where apices undergo a transition from vegetative to intermediate, prefloral, and finally reproductive phases (cf. Nougarède 1967), apices of cv. Greenfeast appear "intermediate" as early as 1 day after soaking of unvernalized seed or by the time cold treatment is terminated in vernalized seed.

Presumably this has some significance in relation to mode of action of the floral inhibitor.

The concept that vernalization but not photoperiod influences events in evocation at the apex, and photoperiod but not vernalization influences events in induction in the leaves, has several important implications.

The photoperiodic and vernalization reactions may now be separated physiologically. In contrast, Barber (1959) was unable to achieve such separation in late pea cultivars, either physiologically or genetically. Repetition of the present transfer experiments using F2 seed from crosses between early and late pea cultivars may help resolve this question.

The response to low temperature during seed vernalization may be distinguished from the response to low temperature during seedling growth. The decreased leaf requirement with decreased growing temperature in the type 2 response (Paton 1968) most likely extends down to vernalization temperatures. Thus, the exceptionally low flowering node of cv. Greenfeast grown in 8-hr days at 4°C from soaking of the seed (Paton 1968) probably results from the additive effects of this low temperature on induction (reduced type 2 response) and evocation (repression of inhibitor synthesis).

The effect of vernalization may be described as a conditioning of the apex. Comparable conditioning of the apex by vernalization has been tentatively proposed by Wellensiek, Doorenbos, and Zeevaart (1956), but they also considered that vernalization conditions the leaves in the photoperiodic reactions particularly with regard to perception of the photoperiodic stimulus. There is no feature of the flowering behaviour of cv. Greenfeast which favours the latter interpretation. If the vernalized state is transmitted to cells derived from a vernalized apex (cf. Wellensiek, Doorenbos, and Zeevaart 1956), then it is not surprising that seed vernalization does not affect a leaf requirement involving embryonic leaves only. This is the case with induction in continuous light in the present experiments. However, the increased leaf requirement in short days (Paton 1968) may involve non-embryonic foliage leaves above the 6th or 7th node, and the possibility that seed vernalization might then have some effect on the leaf requirement cannot be excluded.

V. Acknowledgments

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