PHOTOPERIODIC AND TEMPERATURE CONTROL OF FLOWER INITIATION IN THE LATE PEA CULTIVAR GREENFEAST

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Summary

The number of green foliage leaves at initiation is related to a quantitative leaf requirement for flowering in the late pea cultivar Greenfeast. When grown in various temperature and photoperiod regimes the leaf requirement is least in continuous light and low temperatures. The additive nature of the photoperiodic and temperature responses suggests that photoperiod and temperature probably act independently.

The different response types with increased temperature point to three temperature-dependent reactions. A high-temperature promotory reaction (type 1) is indicated by the reduced leaf requirement at high temperatures in continuous light. At normal growing temperatures, the leaf requirement increases with either a rise in temperature (type 2) or reduction in photoperiod. Increased flowering node and delayed initiation time above the temperature optima for early initiation (type 3) are not associated with equivalent changes in the leaf requirement. High temperatures in short days appear to affect the flowering process indirectly.

It is suggested that high-temperature acceleration of some early step in leaf senescence delays attainment of the leaf requirement in short days but has no effect when initiation precedes the onset of senescence as in continuous light or low temperatures. These results further our understanding of the temperature dependence of flowering in a quantitative long-day plant.

I. Introduction

Late varieties of several legume species behave as quantitative long-day plants. One feature of their flowering behaviour is the marked temperature response of unvernalized plants grown in short days. The delay in initiation at low temperature in short days is slight relative to plants grown in long days and Barber (1959) suggests that a flower-delaying substance or colysanthin is preferentially destroyed at low temperatures and in long days in late varieties of *Pisum sativum*. Initiation is greatly delayed at high temperatures in short days and in addition to low-temperature promotion Evans suggests a high-temperature dark inhibitory process in late varieties of *Trifolium subterraneum* (Evans 1958) and a late variety of *Vicia faba* (Evans 1959). The present paper suggests a common link between low-temperature promotion and high-temperature inhibition in the flowering of the late pea cultivar Greenfeast. The leaf status at photoperiodic induction in this variety is known (Paton 1967) and indicates the general nature of the mechanism involved.

The work was carried out in the Earhart Laboratory and, when seasonal effects are taken into account, the main results have been confirmed in glasshouse studies at Hobart and using controlled-environment cabinets at Canberra.

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II. Materials and Methods

An almost complete range of constant temperatures from 4 to 30°C at intervals of about 5 degC was combined factorially with three levels of photoperiod of 8 (P_8), 16 (P_{16}), and 24 hr (P_{24}) of artificial light of at least 1000 f.c. in a normal 24-hr cycle. Details of the cultural conditions used are given in Went (1957).

Greenfeast, a late flowering dwarf cultivar of *Pisum sativum*, was used in all treatments. The commercial source of fresh seed was the same as that used in previous experiments, in which procedures for sterilization of dry seeds, germination, and selection of uniform seedling material was standardized (Paton and Barber 1955). An additional fumigation of the dry seed with methyl bromide was involved in the present work. After soaking for 6 hr at 17°C in water which was changed frequently lots of about 200 imbibed seeds were covered with about 1 cm of vermiculite and all further growth occurred at the temperature and photoperiod for the particular treatment.

Single seedlings were planted in plastic cups of vermiculate–gravel mixture when the first scale leaf was just separated from the apical bud. At this stage the epicotyl (0·5–1·0 cm) and radicle (5–6 cm) were sufficiently well developed to select the 36 uniform seedlings required for each treatment. In planting, the cotyledons were left partially exposed to reduce bacterial rotting. Subsequent growth was remarkably uniform within each treatment. Axillary and cotyledonary buds were removed as soon as they were visible. This check was particularly important in short photoperiods where growth of lateral shoots was most vigorous and apical dominance was easily lost.

The plants were scored at regular intervals for number of foliage leaves (NL), including the number of dead or senescent leaves (ND). Of the 36 plants in each treatment, about half were used for dissections of the apical bud to determine the increase in total number of nodes (N) with time. The plan of dissections was to dissect a group of eight plants both before and after flower initiation, leaving two plants available to determine approximately when flower initiation had occurred. In a few treatments with greatly delayed flowering, the number of plants dissected at one time was reduced to four and sometimes two so that dissection times were evenly distributed over the long vegetative period. The increase in total number of nodes above the initial six nodes in the embryo of the dry seed was linear with time except during germination when an initial slow rate of node formation before plumule emergence was balanced by a temporary high rate when plumule commenced to elongate. However, the overall rate during this fluctuation was the same as the rate finally established.

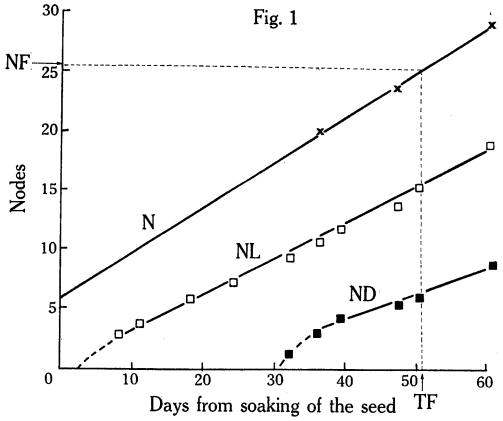
Determination of the mean value for the node of first flower (NF) was made from the remaining 20 plants in each treatment when the first flower was at the stage of anthesis and the plants were harvested. The cotyledonary node was counted as zero. Some treatments caused various degrees of flower abortion but no example of reversion of a flower structure to vegetative growth was found. In all cases the remaining rudiments of an aborted flower were recognizable. An accurate estimate of the time of initiation of the mean flowering node (TF) was obtained from the time graph of total node number (Fig. 1). Since flower primordium initials are evident at about the same time as the subtending leaf is morphologically distinct at the apex (Paton 1967), times of initiation of the first flower primordium and the flowering node were equated. The leaf status of the plant at the time of initiation of the first flower was determined from the time graphs for the number of foliate leaves and the number of dead leaves. These relationships, together with all necessary notation, are given in graphical form in Figure 1 for a typical treatment (P_8, 14°C) in which the onset of leaf senescence occurred before flower initiation.

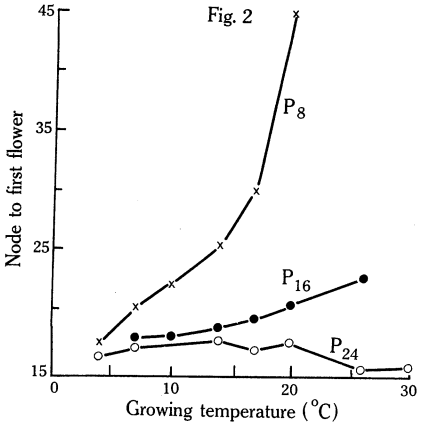
The standard errors of the mean NF values were generally small but tended to increase in short days at high temperatures. Transposition between the node and time axes involved compounded variances and statistical treatment of derived values was restricted to covariance analysis of the number of green foliage leaves at initiation (NL − ND) on temperature. A routine analysis of variance of NF values was made. It was not possible to obtain the standard error of means for the derived values TF (Fig. 3) and rate of node formation (Fig. 4).

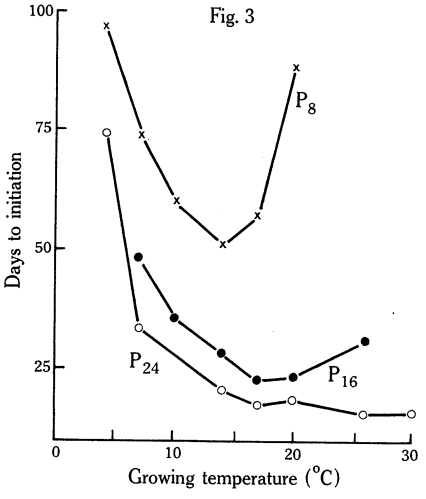
III. Results

(a) *Node and Time of Initiation of the First Flower*

Treatment means for node and time of initiation of the first flower are shown in Figures 2 and 3 respectively. Blanks in the factorial design resulted from death of plants before flower initiation (P_8, 26 and 30°C) and because three of the photoperiod–temperature combinations (P_{16}, 4 and 30°C; P_{24}, 10°C) were not available during the







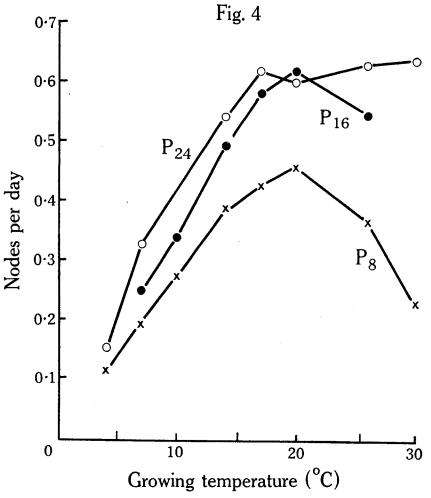


Fig. 1.—Leaf status from soaking of the seed (time = 0) to initiation of the first flower for a typical treatment (P_8, 14°C) in which the onset of leaf senescence preceded initiation. At the time of initiation (TF) the node of first flower (NF) is equivalent to the total number of nodes (N). The leaf status at initiation consists of the number of nodes bearing unfolded leaves (NL) and dead shrivelled leaves (ND). The number of green foliage leaves at initiation is the difference between NL and ND.

Figs. 2–4.—Effect of temperature on the node of first flower (Fig. 2), on time from soaking of the seed to initiation of the first flower (Fig. 3), and on the rate of node formation at the apical meristem (Fig. 4) of plants grown in photoperiods of 8 (P_8), 16 (P_{16}), and 24 hr (P_{24}). In Figure 2 the standard error of means is 0·69.

course of these experiments. Variance analysis of NF values for complete combinations demonstrates very significant effects ($P < 0{\cdot}001$) due to photoperiod and temperature and to the photoperiod × temperature interaction. The values for the incomplete combinations are included in Figure 2 and they further emphasize the magnitude of these effects. The pattern of values in Figure 3 suggest similar effects for TF values.

Increased photoperiod lowered the node and shortened the time of initiation as expected for a quantitative long-day plant. Particularly at medium to high temperatures, the response to a change of 8 hr in day length was larger with P_8 and P_{16} than P_{16} and P_{24} (Figs. 2 and 3). Thus the magnitude of the photoperiodic responses varied with different photoperiod and temperature regimes and this provided a large component of the photoperiod × temperature interaction effects.

TABLE 1

DIFFERENT RESPONSES TO INCREASED GROWING TEMPERATURES

NF, node of first flower; TF, time of initiation of mean flowering node

Response Type	NF	TF	Temp. Range and Photoperiod Involved
1	Decreased	Decreased	Above 20°C in P_{24}*
2	Increased	Decreased	Below 14°C in P_8†
			Below 17°C in P_{16}†
			Below 20°C in P_{24}*
3	Increased	Increased	Above 14°C in P_8†
			Above 17°C in P_{16}†
4	Decreased	Increased	Not observed

* Optimum temperature for maximum NF values in P_{24}.

† Optimum temperature for low TF values in P_8 and P_{16}.

Three different types of temperature responses with *increase* in temperature were detected by the direction of change of NF and TF values. These are listed in Table 1. The type 1 response was observed in P_{24} above 20°C where increased temperature promoted flowering with a reduction in NF as well as TF values. Such high-temperature promotion of flowering suggests the existence of a high-temperature promotory reaction similar to that proposed by Evans (1958, 1959). Type 2 and type 3 responses were separated by the optimum temperatures for low TF values which occurred at 14°C in P_8 and 17°C in P_{16} (Fig. 3). The corresponding NF values (Fig. 2) in P_8 and P_{16} show no sign of these temperature optima. This combined with the rarity with which node and time of initiation are considered together probably explains why these two response types have not been clearly separated in previous work.

Reduction of TF values in the type 2 response was most likely associated with the increased rate of node formation with increased temperature over the low to medium range (cf. Figs. 3 and 4). Such direct dependence of initiation time on rate of vegetative development occurred only with this response type. Delayed initiation occurred in the type 3 response despite the tendency for increased vegetative development to be associated with reduced TF values. This is clearly demonstrated

for temperature increases between the optimum temperature for low TF values and the temperatures for maximum rate of node formation in P_8 and P_{16} (cf. Figs. 3 and 4). Some high-temperature process inhibitory to flowering seems involved in the type 3 response. The results on leaf status suggest that a large component of this high-temperature inhibition of flowering in cv. Greenfeast in short days may be due to indirect effects of high temperature.

(*b*) *Leaf Status at Flower Initiation*

The presence of dead leaves at the lower nodes gave a characteristic appearance to plants grown at high temperatures. While young leaves unfolded from the apical bud, leaf senescence caused death of the older leaves at successive nodes from the base (Fig. 1). The foliage leaves below the apical bud formed a block of green leaves which moved upwards with growth of the plant. If, as observed with growing temperatures greater than 25°C, leaf senescence progressed at a faster rate than leaf unfolding, the block of green leaves was gradually reduced in size until the plant finally died. The two rates were about equal between 17 and 20°C and a block of 6–10 green foliage leaves was maintained at the top of the plant often when more than 50 extended internodes were present. It was during dissections of such plants grown in P_8 that flower initiation was observed not to occur until more than eight green foliage leaves had developed. The presence of 10 or more green leaves was a good indication that flower initiation had already occurred. Flower initiation occurred rarely with eight green leaves and seemed delayed indefinitely with only six green leaves or less. These early observations indicated some link between leaf status and flowering and, accordingly, leaf status data was obtained at flower initiation in all treatments.

Since the number of nodes to first flower is made up of two scale leaves (constant), the number of dead foliage leaves [0·0–21·6 (ND values, Table 2)], the number of green foliage leaves [4·4–10·1 (NL — ND values, Table 2)], and the number of nodes in the apical bud (7·0–10·5), the large variation in NF values (14·9–44·2) was associated mainly with variation in ND values. This was particularly noticeable with increases in NF values in the type 3 response which were very similar to the increases in ND values in corresponding conditions. Both occurred only in short days at high temperatures and both showed the same trends. This similarity suggests that a large component of the photoperiod × temperature interaction affecting NF values may be related to the photoperiod × temperature interaction which, as can be seen by inspection of Table 2, affected ND values.

Increased temperature and, to a less extent, increased photoperiod accelerated the onset of leaf senescence (Table 3). The interval between soaking of the seed and shrivelling of the first foliage leaf was large at low temperatures and showed a smooth asymptotic reduction to 27 days in P_8 and 17 days in P_{24} at 30°C. The onset of leaf senescence occurred after flower initiation in the type 2 response but preceded it in the type 3 response, thus appearing related in some way to the temperature optima for early flowering which separated the two response types. The acceleration of the onset of leaf senescence observed in long days has been confirmed in later experiments and

TABLE 2

EFFECT OF TEMPERATURE AND PHOTOPERIOD ON LEAF STATUS AT FLOWER INITIATION

The number of nodes, exclusive of the two scale leaves at nodes 1 and 2, bearing shrivelled dead leaves (ND) and green foliate leaves (NL−ND) are given. Horizontal bars separate the type 2 (above), type 3 (P_8 and P_{16}), and type 1 (P_{24}) responses to increased temperature

Temp. (°C)	P_8		P_{16}		P_{24}	
	ND	NL−ND	ND	NL−ND	ND	NL−ND
4	0	5·4	—	—	0	4·4
7	0	7·7	0	5·9	0	5·5
10	1·5	7·9	0	5·8	—	—
14	4·7	9·0	0	6·8	0	5·8
17	8·1	9·9	0	7·4	0	6·1
20	21·6	10·1	1·2	8·0	0	6·8
26	—	—	7·1	8·2	0	6·3
30	—	—	—	—	0	6·2

TABLE 3

EFFECT OF TEMPERATURE AND PHOTOPERIOD ON TIME TO FIRST FLOWER INITIATION AND ONSET OF SENESCENCE

Times given in days from soaking of the seed to initiation of the first flower (TF) and to the onset of senescence indicated by shrivelling and death of the first foliate leaf at the third node (TD_3). The horizontal bars have the same meaning as in Table 2

Temp. (°C)	P_8		P_{16}		P_{24}	
	TF	TD_3	TF	TD_3	TF	TD_3
4	97·0	140·0	—	—	64·5	126·4
7	74·5	95·5	47·8	62·0	33·5	55·0
10	59·8	61·0	35·3	45·0	—	—
14	51·4	34·5	25·6	30·0	20·9	27·5
17	57·0	31·6	22·6	27·0	17·5	23·0
20	88·4	28·0	23·5	23·1	18·8	21·6
26	—	27·0	32·4	22·0	15·8	20·0
30	—	27·0	—	—	16·0	17·5

seems similar to the acceleration of abscission in detached lupin leaves with increased photoperiod between P_8 and P_{24} (Burrows and Carr 1967).

The rate of node formation (Fig. 4) was controlled mainly by temperature but small effects due to photoperiod were observed. Similar relationships existed for the rates at which foliage leaves unfolded from the apical bud. Later experiments under glasshouse and controlled-environment facilities have failed to confirm the promotion of leaf formation in long days.

An analysis of covariance of the number of green foliage leaves at initiation (NL − ND) on temperature was made for the three photoperiods. The effects due to temperature and photoperiod were both highly significant ($P < 0{\cdot}001$). No significant photoperiod × temperature interaction could be demonstrated in this analysis. The values increased with rise in temperature and in shorter photoperiods. These effects were additive at all growing temperatures except that the rate of increase with temperature was slightly lower in P_{24} than P_{16} and P_8 and this was probably associated to some extent with the type 1 response. The number of green foliage leaves at initiation followed the pattern of NF values for the type 1 and type 2 responses and apart from the type 1 response this temperature dependence was similar in all photoperiods. As with NF values the photoperiodic responses were greater with P_8 and P_{16} (*c.* two leaves) than with P_{16} and P_{24} (*c.* one leaf) but in contrast to NF values the magnitude of these photoperiodic responses was not affected to any great extent by growing temperatures. The number of green foliate leaves at initiation was more at 20 °C in P_{24} than at 10 °C or less in P_{16} and 4 °C in P_8. Evidently exposure to low temperatures partially replaced exposure to long day.

IV. Discussion

The temperature dependence of values for flowering node in short days may be interpreted equally well either as high-temperature inhibition (cf. Evans) or as low-temperature promotion (cf. Barber). It is tempting to think that the temperature optima for early initiation may indicate some separation of high- and low-temperature effects if both mechanisms were involved. However, a quantitative leaf requirement is directly concerned in photoperiodic induction of flowering in cv. Greenfeast (Paton 1967) and a flowering model for this variety should reconcile the results for flowering node and leaf status. In particular, it should account for the difference between the small additive responses to photoperiod and temperature shown by the number of green foliate leaves at initiation and the large responses, especially the interaction effects, shown by the values for the flowering node. This is possible if the number of green foliate leaves at initiation is considered a relative measure of the leaf requirement for flowering in all photoperiod and temperature regimes. That no large error is involved in this assumption is supported by current work (Paton, unpublished data) which indicates that vernalization is the only environmental treatment which affects the number of plastochron intervals between induction and initiation to any great extent.

It has already been demonstrated (Paton 1967) that cv. Greenfeast plants are photoperiodically induced in continuous light when very few foliage leaves are present. The actual number depends on temperature as well as photoperiod, and increases in a linear fashion from about 2 at 10 °C to more than 3 at 20 °C. This

increase with temperature is accompanied by equivalent increases in the number of foliage leaves at initiation and also in the total number of nodes both at induction and subsequently at flower initiation. The same relationships hold in the present work. Thus the small increases in foliate leaves at initiation and equivalent increases in flowering node between 4 and 20°C in continuous light, are related to the temperature dependence of the leaf requirement.

Judging by the overall trends observed in the number of green foliate leaves at initiation, this temperature dependence is simply additional to and independent of the increased leaf requirement in 16- and 8-hr days. At the temperature separating the type 2 and type 3 responses for node and time of initiation, these temperature-dependent increases in the leaf requirement show no break which might indicate the presence of both low-temperature promotion and high-temperature inhibition. The type 2 response apparently extends to high temperatures in 16- and 8-hr days but is masked in continuous light at high temperatures by the reduced leaf requirement associated with the type 1 response. Thus two separate temperature-dependent reactions appear directly involved in the flowering mechanism. Increased growing temperature is inhibitory in one reaction (type 2) but promotory in the other (type 1).

Since the type 3 response involves no change in the leaf requirement other than increases expected from the type 2 reaction, the delayed flowering at high temperatures in short days indicates the presence of some temperature-dependent reaction not directly involved in the flowering mechanism. The similarity of the photoperiod × temperature interactions affecting flowering node in the type 3 response and the number of dead leaves at initiation suggests that leaf senescence is related to flower initiation in the same way that pre-induction defoliation increases both node and time of initiation but post-induction defoliation is ineffective (Paton 1967). As expected on this basis, no delay in flowering is observed when induction and initiation occur prior to the onset of leaf senescence. However, when the increased leaf requirements in short days (photoperiodic response) and high temperatures (type 2 response) are combined with accelerated leaf senescence at high temperatures, the onset of leaf senescence may occur before initiation and presumably before induction. Under these conditions additional node development (increased flowering node) and additional time (delayed initiation) are required before sufficient green foliage leaves are developed for the leaf requirement. In continuous light, initiation always precedes the onset of senescence and despite the greatly accelerated leaf senescence, the type 3 response does not occur. Thus the greatly delayed flowering in short days at high temperature as well as the large photoperiod × temperature interaction effects for node and time of initiation may be explained.

Comparable leaf status data have not been obtained in previous studies and consideration of the various flowering mechanisms proposed for other quantitative long-day plants is necessarily restricted to one general but critical point. This concerns the interrelationships of the reactions involved in the temperature and photoperiodic responses. The reactions are additive for the leaf requirement but, as emphasized by Barber (1959), they are competitive for flowering node. Additive and competitive effects suggest different flowering mechanisms and it is important to distinguish between them. The competitive relationship for flowering node is now

demonstrated to be associated with the type 3 response; that is, with high-temperature acceleration of leaf senescence and not with the effect of high temperature on processes directly involved in flowering. In the absence of the type 3 response at low to medium temperatures and more especially for the leaf requirement at all temperatures, the photoperiod and temperature reactions are mostly additive. Low temperature partially replaces the necessity for exposure to long days but, except at 4°C, such replacement is not associated with reduction in the photoperiodic response. The exceptionally low leaf requirement in P_8 at 4°C does indicate almost full replacement but this is more likely associated with inductive effects of vernalization during germination at this temperature than with temperature dependence of the leaf requirement evident at 7°C and above.

Partial defoliation does not delay flowering in cv. Greenfeast (Sprent 1966) and the effect of prior leaf senescence on initiation is unlikely to be associated with reduction in leaf area. Presumably leaves become inactive in the inductive processes well before any morphological indications of senescence. Possibly high-temperature acceleration of some early step in leaf senescence is involved. The activity of foliage leaves for photoperiodic induction in cv. Greenfeast may depend on leaf age (cf. Lam 1967), but it seems likely that any inductive stimulus originating from leaves which subsequently shrivel before flower initiation is not accumulated and does not affect the quantitative leaf requirement. This is very similar to the concept that the flowering stimulus is "dissipated" in some plants in about 4–6 days (Salisbury 1961).

V. Acknowledgments

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