CONTENTS

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Photothermal Time for Flowering in Lentils (*Lens culinaris*) and the Analysis of Potential Vernalization Responses

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ABSTRACT

Two cultivars of lentils, Laird and Precoz, were subjected to 18 potentially vernalizing treatments, comprising constant temperatures of 1, 5 or 9 °C in factorial combination with photoperiods of 8 or 16 h for 10, 30 or 60 d. These seeds or seedlings, together with non-vernalized seeds (as controls), were then transferred to four different growing regimes ('day'/'night' temperatures of 18/5 °C or 24/13 °C, factorially combined with photoperiods of 11 or 16 h). Variation in the number of days from sowing to first flower (f) in the growing regimes for the controls conformed to the equation $1/f = a+b\bar{T}+cP$, where $\bar{T}$ is mean temperature (°C), P is photoperiod (h) and a, b and c are genotype-specific constants. Accordingly, when the environment varies during development, the photothermal time required to flower in day-degrees (°C d) is given by $1/b$ above a base temperature defined as $-(a+cP)/b$. Most variation in time to flower could be accounted for by the photothermal time accumulated in the two successive environments. Therefore, there was no evidence of a specific low-temperature vernalization response in either cultivar. Neither was there evidence of 'short-day' vernalization, i.e. advancement of flowering resulting from preliminary short-day treatments. A potential error inherent in the predictive model described arises because it ignores the presence of a pre-inductive, photoperiod-insensitive phase; but agro-ecological considerations suggest that this error may not be important in practice.

Key words: *Lens culinaris*, lentil, flowering, photoperiodism, vernalization, photothermal time, screening germplasm.

INTRODUCTION

Although there are exceptions, most crops which originate from latitudes greater than 30° show long-day photoperiodic responses, and some of their cultivars also respond to vernalization, i.e. their time to flower is less in long days and, in some cases, is especially early after the plants have experienced a period of chilling (Roberts and Summerfield, 1987). The lentil crop (*Lens culinaris* Medic.) is typical since it is thought to have originated in the east Mediterranean or in west Iran or in Turkey, i.e. between about 30° and 40° N (Muehlbauer, Cubero and Summerfield, 1985). It is generally considered to be a quantitative long-day species and it is reported that many genotypes also show a quantitative vernalization response (Summerfield, Muehlbauer and Roberts, 1985*a*).

In controlled environments it has been shown that six diverse genotypes of lentil – including the two cultivars, Laird (from Canada) and Precoz (from Argentina) chosen for further study here – are indeed quantitative long-day plants; but in all photoperiods they are also sensitive to mean temperature (Summerfield *et al.*, 1985*b*). When time from sowing to first flower, f, is transformed into rate of progress towards flowering by taking the reciprocal, $1/f$, then there is no interaction between photoperiod and mean temperature and the response to each factor is positive and linear as described by the equation:

$$1/f = a+b\bar{T}+cP, \qquad (1)$$

where f is time from sowing to first flowering (d), $\bar{T}$ is mean diurnal temperature (°C), P is photo-

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period (h), and a, b and c are constants specific for each genotype (Summerfield *et al.*, 1985*b*).

This equation, however, is now known to be an oversimplification since it suggests that there is no change in photoperiod sensitivity with time, whereas during the period before flowering there is a pre-inductive phase and a post-inductive phase, both of which are insensitive to photoperiod (Roberts *et al.*, 1986). Sandwiched between these is an inductive phase which is sensitive to, and the length of which is dependent on, photoperiod, being shorter in longer days. The durations of all three phases are probably sensitive to temperature but in this respect they have not yet been separately investigated. At a mean temperature of 21–22 °C, however, the pre-inductive phase continues from sowing for about 10 d in Precoz and 22 d in Laird (Roberts *et al.*, 1986).

Ideally, the durations of these three phases should be separately investigated at different temperatures; but to do so would require considerable work, involving a series of reciprocal-transfer treatments between long and short photoperiods carried out at several different temperatures. This is not a practical proposition when the aim is to devise simple techniques for screening germplasm collections for adaptation to different agricultural environments. For this purpose there is much to be said for using eqn (1) as an overall approximation; it has the advantage that it can be used to devise photothermal units which can be used to predict time to flower under natural conditions where there is a daily change in both temperature and photoperiod; and in theory, observations from only three environments are needed to determine the values of the regression constants in eqn (1) which are required to define the response surface of each genotype (although at least four environments would probably be used in practice) (Summerfield *et al.*, 1985*b*). The approach is based on the thermal-time concept for predicting the timing of developmental processes which, in its original form (e.g. Monteith, 1977), is appropriate only where photoperiodism does not interfere. The original thermal-time concept depends on there being a linear relation between mean temperature and the rate of progress toward flowering, i.e.

$$1/f = a + b\bar{T}. \quad (2)$$

Equation (2) can be considered as a special case of eqn (1) in which the photoperiodic constant, c, is set to zero. Where eqn (2) applies, e.g. in photoperiod-insensitive genotypes of many species, it can be shown that flowering occurs when the number of day-degrees (°C d) above the appropriate base temperature, T_b, accumulate to a given value, i.e. the thermal time, θ, appropriate for the cultivar. In this case the base temperature is given by

$$T_b = -a/b, \quad (3)$$

and the thermal time by

$$\theta = 1/b, \quad (4)$$

(Monteith, 1981; Roberts and Summerfield, 1987). However, when eqn (1) applies, although thermal time can still be calculated in the same way, i.e. according to eqn (4), the base temperature which has to be subtracted from the daily mean temperature, varies with photoperiod and is given by:

$$T_b = -(a + cP)/b \quad (5)$$

(Summerfield *et al.*, 1985*b*; Roberts and Summerfield, 1987). In this case although the daily contribution to thermal time is expressed in similar units (°C d), it is preferable to describe them now as photothermal units as a measure of photothermal time. This important distinction serves to remind that the base temperature used in their calculation varies with photoperiod because of the extra photoperiodic term in eqn (1) as compared with eqn (2).

The term vernalization, it has been suggested (Vince-Prue, 1975), is best restricted to the specific promotion of flower initiation by a previous cold treatment given to the imbibed seed or young plant; and, unless otherwise qualified, that is how the term is used here. It may occur at temperatures between −5 and 16 °C, but with maximum effect typically between 1 and 7 °C (Whyte, 1960; Vince-Prue, 1975). But it does not seem to have been realized that even if a preliminary cool temperature shortens the time taken to flower after transfer to a warmer environment, it does not automatically follow that a specific vernalization effect has been demonstrated. Clearly, if eqn (1) or (2) applies, then any preliminary cool-temperature (potentially vernalizing) treatment which is above the base temperature will allow thermal or photothermal time to accumulate. Thus, the time taken to flower after transfer from the supposedly 'vernalizing' treatment to another environment will be reduced accordingly, even when vernalization has not occurred.

The demonstration of a true vernalization effect, then, requires that any reduction in time to flower resulting from a vernalization treatment is greater than that which would be expected from the accumulation of thermal or photothermal time alone. It is already known that exposing the imbibed seeds of a diverse range of six lentil genotypes for 30 d in the dark at 1·5 ± 0·5 °C decreases the time taken to flower after transfer to warmer environments (Summerfield *et al.*, 1985*b*). But what is less clear, is whether this is a specific vernalization effect only achieved by chilling temperatures, or whether it is merely the effect of a more general developmental process which responds positively to temperature as reflected in eqn (1).

When the term vernalization is used without qualification, as we have discussed, it is generally understood to mean a specific response to chilling. But in some temperate species, including some of the autumn-sown cultivars of temperate cereals, it is known that short-days may substitute for, enhance, or be required instead of cool temperatures if the maximum response – or in some cases if any response – to subsequent long days is to be achieved. This effect is sometimes known as short-day vernalization and, where it exists, gives rise to the category of photoperiodic response sometimes referred to as SLDP (short-long-day plants), implying that earliest flowering occurs when short days are followed by long (Vince-Prue, 1975; Roberts and Summerfield, 1987). We are not aware that such a response has been observed in lentil, but the investigation described here was designed to expose either low-temperature or short-day vernalization responses, should either or both exist.

MATERIALS AND METHODS

Plant material

Two cultivars which had been included in previous experiments (Summerfield *et al.*, 1985*b*; Roberts *et al.*, 1986) were chosen for further study here: Precoz (ILL 4605), originating from Argentina and said to be early to mature and slightly sensitive to photoperiod, and Laird (ILL 4349), a cultivar released in Canada but which originated from USSR, matures later and is reputedly more sensitive to photoperiod. Designations in parenthesis are the accession numbers of the International Centre for Agricultural Research in Dry Areas (ICARDA) at Aleppo in Syria from where the seed stocks were obtained.

Husbandry

Techniques were based on those developed for tropical grain legumes in general (Summerfield, Huxley and Minchin, 1977) and subsequently modified for lentils (Summerfield and Muehlbauer, 1982). Seeds from the median weight range of each stock were immsersed in 50% ethanol for 30 s, surface sterilized for 2 min in 0·2% mercuric chloride in 0·01 M HCl, and washed three times in sterile distilled water. These seeds were then inoculated with strain of *Rhizobium* LE19 and sown, five to each 18 cm diam. pot containing a mixture of vermiculite, sand, gravel and loam-less peat compost without fertilizer (4:2:4:1 by volume) soaked in deionized H_2O and allowed to drain for 24 h. After sowing, the seeds were covered with a 2 cm layer of rooting medium, all components of which had been steam-sterilized before use. Pots were not irrigated until 10 d after sowing. Thereafter, sufficient nutrient solution, complete except for containing only 20 mg l^{-1} inorganic nitrogen (Summerfield *et al.*, 1977), was delivered automatically four times each day so that drainage through the pots occurred at every application. Seedlings were later thinned to leave three uniform plants ('replicates') in each pot.

Experimental design

Seeds of the two cultivars were sown in 18 preliminary environments, viz. three constant temperatures (1, 5 and 9 °C) × two photoperiods (8 and 16 h), for three durations (10, 30 or 60 d). These preliminary environments were selected to vernalize, to a greater or lesser extent, any plant capable of responding to chilling. Furthermore, the 8 and 16 h photoperiodic treatments were included to expose any short-day vernalization response.

After the preliminary treatments the seeds or seedlings, together with untreated seeds which served as unvernalized controls, were transferred to each of four different photothermal environments in which the plants would complete their development to the point when the first open flowers (corolla colour visible) were recorded. The final environments comprised four combinations of two photoperiods (11 and 16 h), with two mean diurnal temperatures (12 and 19 °C). In the case of the 16 h photoperiod the 'day' temperature started 3 h after the lights came on and ended at lights-off; while in the 11 h photoperiod the day temperature started 2 h before lights-on and ended at lights-off. And so mean diurnal temperature of 12 °C was achieved by using a 'day' temperature of 18 °C for 13 h each day and a 'night' temperature of 5 °C for the remaining 11 h of the 24 h cycle. The mean temperature of 19 °C was achieved by using a 'day' temperature of 24 °C for 13 h and a 'night' temperature of 13 °C for 11 h. In all, there were 152 treatment combinations.

The experiment was carried out in 12 modified Saxil controlled-environment cabinets in which temperatures were controlled to within 0·2 °C of the respective nominal values (except immediately after temperature change-over), carbon dioxide concentrations were controlled at 340 ± 15 mg l^{-1} and the vapour pressure deficit was maintained at

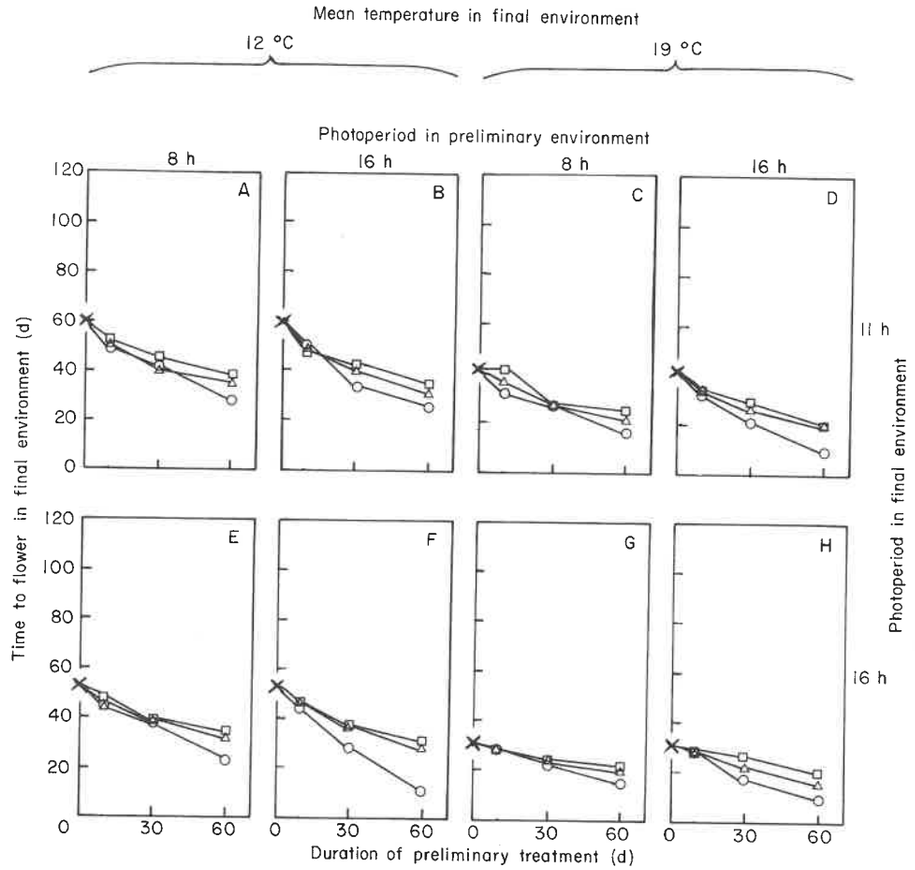

FIG. 1. The effects in cv. Precoz of preliminary treatments for various periods (0, 10, 30 or 60 d) at cool constant temperatures (1, 5 or 9 °C) in two contrasting photoperiods (8 or 16 h) on the time taken to first flower from the time of transfer to warmer mean temperatures (12 or 19 °C) in each of two photoperiods (11 or 16 h). Each point represents the mean of three plants. Temperature of preliminary treatment: □, 1 °C; △, 5 °C; ○, 9 °C. No preliminary treatment (controls): ×. Variation in time to flower amongst the individual plants in any given treatment can be seen by examining Fig. 4 or 5.

0·265 ± 0·020 kPa. 'Daylight' fluorescent tubes supplemented with tungsten incandescent lamps providing 10% of the total rated wattage per cabinet gave an irradiance of 170 J m^{-2} s^{-1} at pot level. Photoperiods were controlled by switching the total lighting systems rather than using any supplementary lighting scheme, for reasons discussed elsewhere (Hadley *et al.*, 1983).

RESULTS AND DISCUSSION

The pre-inductive phase and the possibility of short-day vernalization

The mean times taken for plants to flower after they had been transferred from the various treatments which had potential vernalization characteristics are shown in Figs 1 and 2. A comparison of the results of all those treatments in which the plants had experienced 8 h photoperiods during the initial exposure to cooler environments with those which had experienced 16 h days (cf. consecutive pairs of graphs, A with B, C with D, etc. in Figs 1 and 2) show that neither photoperiod (nor total radiation, which was deliberately confounded with photoperiod) during the early phase of development had little or, in most cases, no effect on the time taken to flower. There is, therefore, no evidence of 'short-day' vernalization for, had there been, then earlier flowering would have been consistently expected following preliminary treatment with 8 h as compared with 16 h photoperiods. One small anomaly, however, is apparent within this otherwise consistent array of data: a comparison between Fig. 2C and D shows that most preliminary treatments in 8 h photoperiods resulted in earlier flowering when the plants were subsequently transferred into an 11 h photoperiod at a mean temperature of 19 °C, as compared with the corresponding treatments where 16 h photoperiods had been imposed during the first phase of development. If this had been a common observation for similar comparisons (viz. A with B, E with F, and G with H in Fig. 2) then it would be indicative of a short-day vernalization response in Laird but, in isolation, it remains an unexplained anomaly.

It is concluded that there is no short-day vernalization response, nor indeed any effect of daylength during the early period of development. In the absence of short-day vernalization it is possible that some advancement of flowering resulting from exposure to the 16 h (as compared with the 8 h) photoperiod during the preliminary period might have been expected in this long-day species. However, it has been shown previously that in lentil there is a pre-inductive phase during which the plants are insensitive to photoperiod: at a mean temperature of 21 °C it lasts from sowing for about 10 d in Precoz and about 22 d in Laird (Roberts *et al.*, 1986). Accordingly, we would expect no effect of photoperiod in any of those environments which lasted for 10 d. Although it seems likely that the duration of the pre-inductive phase will increase with a decrease in temperature, we have no information as to the extent of such variation. Nevertheless, it seems reasonable to

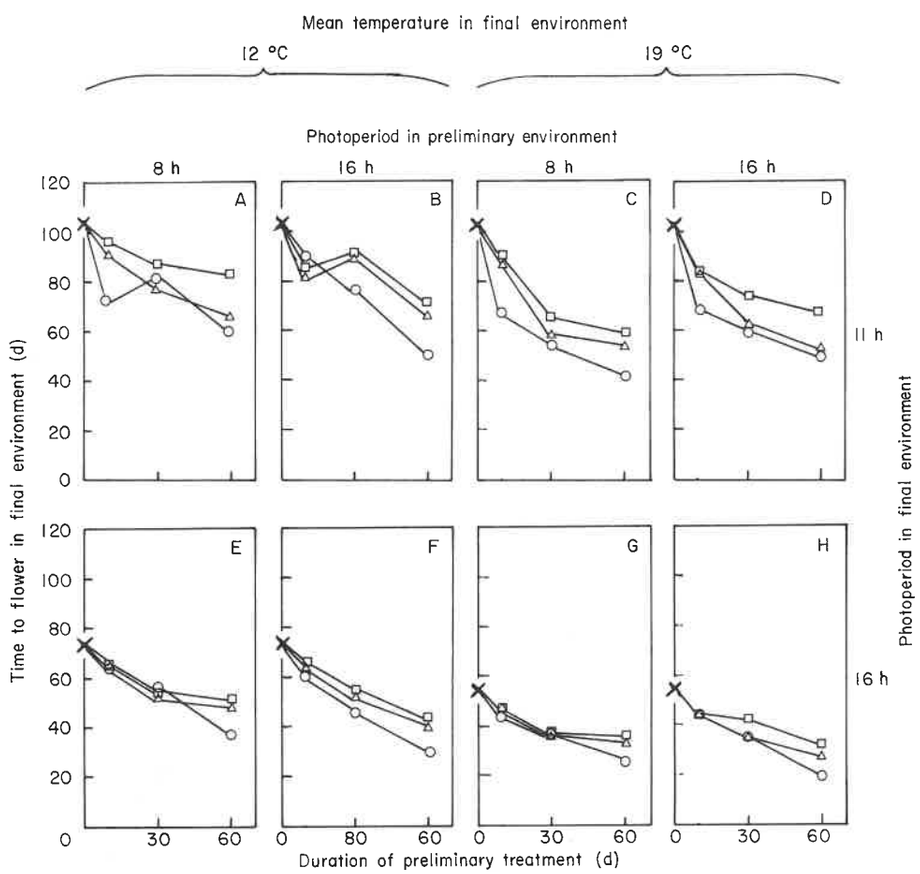

FIG. 2. The effects in cv. Laird of the same treatments described in the caption to Fig. 1. Variation in time to flower amongst the individual plants in any given treatment can be seen by examining Fig. 6 or 7.

assume that the pre-inductive phase might be considerably extended in the temperatures used here for the preliminary treatments, and especially at 1 °C where it may well have continued throughout most of the 60-d treatment. In summary, then, it is certainly expected that there would be no effect of photoperiod in any of the 10 d preliminary treatments and probably little effect in those which lasted 30 d. But it might be possible that, where the preliminary treatment was extended for 60 d at 9 °C, long days would begin to have some effect towards the end of that period as the plants progressed into their photoperiodically sensitive inductive phase. Examination of Figs 1 and 2 show that these expectations are generally met.

The general photothermal response and the possibility of low-temperature vernalization

It is clear that all preliminary treatments shortened the time required for flowering in all the environments to which the plants were subsequently transferred. But, as explained in the Introduction this is to be expected, irrespective of whether or not the plants have a specific low-temperature vernalization response; this is because they will, in any case, accumulate photothermal units during the preliminary treatments. Before allowing for this fact in testing for a specific vernalization response, it is necessary to consider the values of the constants to be used in the determination of photothermal time.

One of the problems of experimenting with lentil is the germplasm accessions, and even so-called cultivars, are far more heterogeneous than those of other grain legume species (e.g. soyabean) which have been more intensively selected by plant breeders. And so times to flower in similar environments on different occasions using small samples or different seed lots, vary rather more than if genetically pure seed stocks were used.

Table 1 shows the constants applicable in eqn (1) as calculated by multiple-regression analysis on the four control treatments (i.e. on the twelve plants which were not subjected to 'vernalization' treatments and which, therefore, were maintained under unchanging regimes throughout development). For comparison, it also shows those values calculated from 12 different photothermal combinations over a similar range of photoperiod and temperature on a previous occasion (Summerfield *et al.*, 1985*b*). It is difficult to appreciate the combined implications of simultaneous variations in the values of a, b and c in eqn (1). Figure 3, therefore, graphically displays the overall consequences of these variations between experiments for both cultivars over a wide range of temperature and photoperiod. It also shows some results from another investigation at a single mean temperature (Roberts *et al.*, 1986).

Although the differences illustrated are not entirely unexpected in view of the heterogeneous nature of lentil cultivars, it is reassuring, at least, that the principal characteristics of each cultivar remain clearly evident on different occasions: for example, Precoz is consistently more temperature-sensitive and earlier than Laird, and both cultivars show considerable photoperiod-sensitivity under all conditions. Furthermore, when common seed stocks are used, then predictions based on eqn (1) and derived from work in controlled environments are reasonably accurate when extrapolated to fluctuating field environments (Summerfield *et al.*, 1985*b*). Nevertheless, it is obvious that any estimate of the values of the constants is subject to error which will affect the accuracy of prediction across experiments and between seed lots; but this problem should not affect analysis within experiments. Accordingly, in spite of the fact that they were based on fewer treatments than in the previous investigation, we have used the constant values estimated in the present investigation in the next stage of the analysis.

TABLE 1. *Values of the parameters in eqn (1) estimated from the four control photothermal regimes compared with those estimated from 12 environments in a previous investigation* (*Summerfield* et al., *1985b*)

Investigation and cultivar	Parameter values*		
	a	*b*	*c*
Current			
Precoz	−0·011 200 (±0·00 330)	0·001 427 (±0·000 141)	0·000 871 (±0·000 197)
Laird	−0·008 172 (±0·00 276)	0·000 309 (±0·000 112)	0·001 187 (±0·000 156)
Previous			
Precoz	−0·005 223	0·000 936	0·000 751
Laird	0·001 469	0·000 306	0·000 446

* With standard errors (where available) in parentheses.

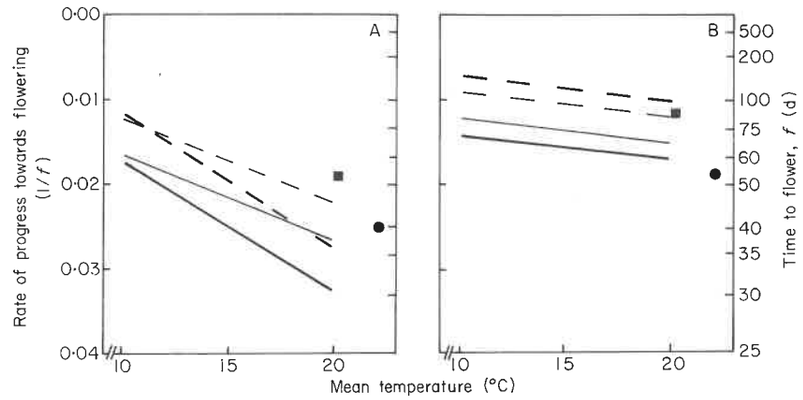

FIG. 3. The relation between mean temperature and rate of progress towards flowering $(1/f)$ for cv. Precoz (A) and cv. Laird (B) calculated according to eqn (1) for photoperiods of 11 h (– – –) and 16 h (——) using values of constants estimated from previous experimental results (Summerfield *et al.*, 1985*b*) (thin lines) and from the control treatments in the present experiment (thick lines), both using the same suite of controlled-environment cabinets. The points show experimental results obtained at the mean temperatures indicated in photoperiods of 11 h (■), and 16 h (●) in another investigation in normal daylight (Roberts *et al.*, 1986).

The next stage was to test the hypothesis that both cultivars are responsive to low-temperature vernalizations, i.e. that flowering is accelerated more by cool preliminary environments than would be accounted for on the basis of accumulated photothermal time during exposure to these conditions. In order to do this, the predicted times to flower were calculated for every combination of preliminary and final environment, on the assumption that there was no specific vernalization response. Thus, the actual number of days from sowing to first flower recorded for every plant could be plotted against the calculated (predicted) value.

The principles of calculating predicted times have been described (Roberts *et al.*, 1986) and subsequently discussed in greater detail (Roberts and Summerfield, 1987). Briefly, the procedure was as follows. First, the base temperature, T_b, was calculated for all the preliminary and final environments in the various treatments according to eqn (5). It was then possible to calculate the daily contribution to the photothermal time necessary for flowering θ, by subtracting the base temperature from the actual daily mean temperature. This daily contribution $(\bar{T}-T_b)$ was then accumulated throughout the cool preliminary environments and then in the final warmer environments for each treatment until the photothermal time θ, necessary for flowering and predicted by eqn (4) had been reached. The number of days which it had taken to achieve this value in the combined environments was recorded as the predicted time to flower.

The results of these predictions are compared with the actual time taken to flower in Figs 4–7. In Precoz (Figs 4 and 5) it is clear that when plants are grown for various periods in potentially vernalizing temperatures before transfer to warmer temperatures in either long or short days, most of the variation in the time taken to flower can be accounted for by the general response to temperature and photoperiod which is described by eqn (1). If there were a true low-temperature vernalization response in this cultivar, it would have been exposed by consistently shorter observed times to flower than expected in all the regimes which had included a preliminary cool-temperature treatment. Furthermore, based on reported optimum vernalization temperatures in other species (Roberts and Summerfield, 1987), such deviations might be expected to have been greater in those treatment combinations which included preliminary chilling environments at 1° or 5 °C rather than at 9 °C. However, no consistent deviations of these types were observed in either Precoz (Figs 4 and 5) or Laird (Figs 6 and 7). It must be concluded that if there is a low-temperature vernalization response in these cultivars, then it is too small to be detected.

It does appear, however, according to the

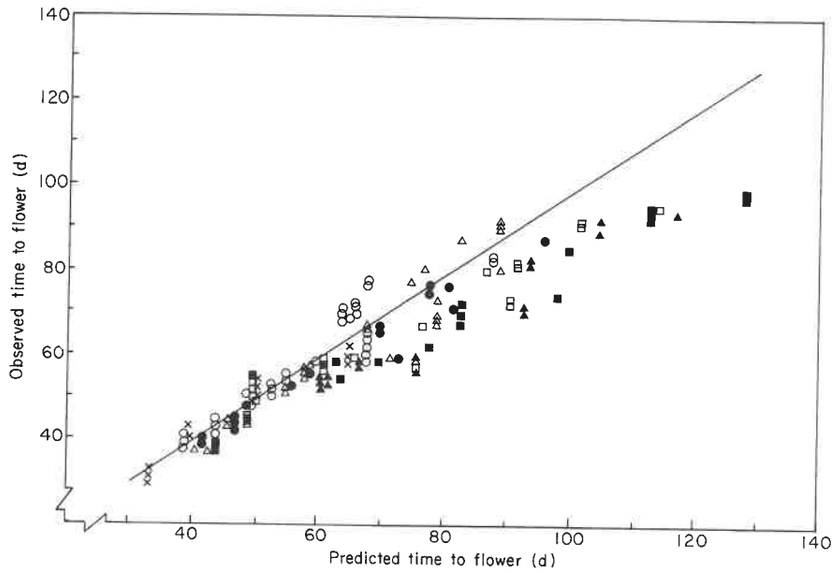

FIG. 4. The relation in cv. Precoz between predicted and observed times from sowing to first flower, including the period spent in preliminary cool environments before transfer to the final warmer environments in which flowering occurred. Each point represents a single plant. There were three replicates in each of the 76 treatment combinations but some points are obscured by coincidence of position. Temperature of preliminary treatments: □, 1 °C; △, 5 °C; ○, 9 °C. Photoperiod of preliminary treatment: closed symbols, 8 h; open symbols, 16 h. No preliminary treatments (controls): ×. The diagonal line ($y = x$) represents the position of perfect agreement between observed times to flower and those predicted from eqn (1).

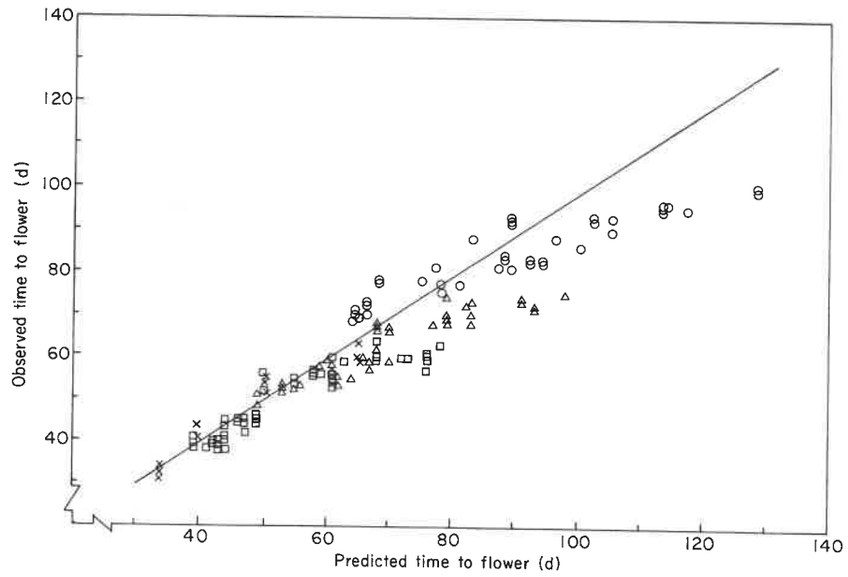

FIG. 5. The same data for cv. Precoz as shown in Fig. 4 but with duration of the preliminary treatments identified as follows: □, 10 d; △, 30 d; ○, 60 d.

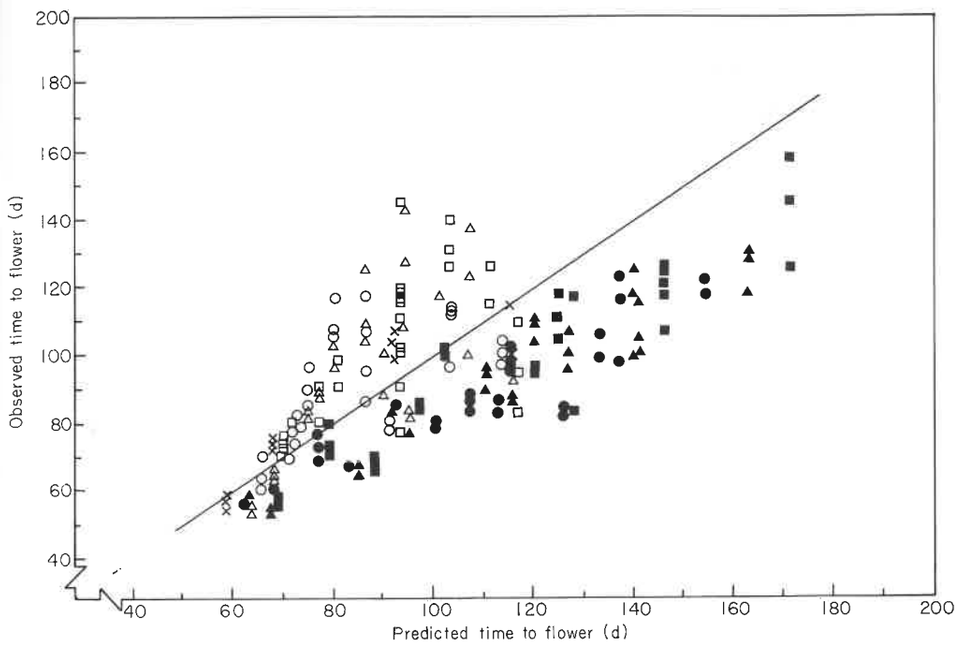

FIG. 6. The relation in cv. Laird between predicted and observed times from sowing to first flower. Temperature of preliminary treatment: □, 1 °C; △, 5 °C; ○, 9 °C. Photoperiod of preliminary treatment: closed symbols, 8 h; open symbols, 16 h. No preliminary treatment (controls): ×. Further explanation as for Fig. 4.

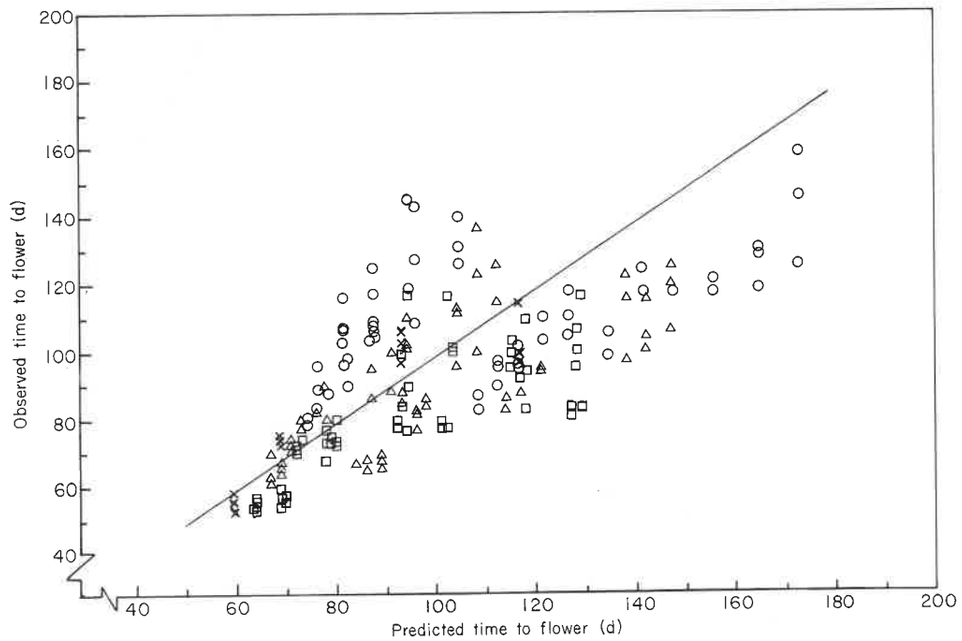

FIG. 7. The same data for cv. Laird as shown in Fig. 6 but with the duration of the preliminary treatments identified as follows: □, 10 d; △, 30 d; ○, 60 d.

assumptions made, that those plants of both cultivars which had experienced short photoperiods (8 h) in the preliminary cool environments flowered sooner than predicted (solid symbols in Figs 4 and 6). Similarly, in Laird, at least, those plants which had experienced long photoperiods (16 h) flowered later than predicted (open symbols in Fig. 6). But it has already been shown (Figs 1 and 2) that photoperiod during the initial cool period had no effect on the time taken to flower after the plant had been transferred to warmer environments. These anomalies in Figs 4 and 6 which are apparently due to initial photoperiod are, paradoxically, almost certainly due to the fact that initial photoperiod had no effect. As argued earlier, most of the development which occurred in the preliminary environments would have been during the pre-inductive phase which is insensitive to photoperiod. Equation (1), and the model derived from it, does not recognize an initial photoperiod-insensitive phase and consequently it calls for greater delays in flowering than actually occur when short days are experienced during early development. By the same token, it calls for a greater advancement in flowering than actually ocurs when long days are experienced during early development.

The prediction error associated with the photoperiod experienced in the preliminary environment is likely to be greater in Laird in which the pre-inductive, photoperiod-insensitive phase is twice as long as in Precoz (Roberts *et al.*, 1986); and this was what was found (cf. Fig. 6 with 4). Furthermore, if this explanation is correct, prediction errors are likely to be exacerbated when cooler temperatures were applied for longer periods in the preliminary environment – the extreme case being 1 °C for 60 d – for, as explained earlier, the photoperiod-insensitive phase is likely to be longer under such circumstances. Conversely, the error is likely to be least when preliminary treatments were allowed to continue for only 10 d. A careful comparison of Fig. 5 with 4 shows that the greatest error of prediction tended to coincide with those temperatures which included preliminary environments which continued for 60 d or, to a lesser extent, 30 d at temperatures of 1 °C and, to a lesser extent, at 5 °C; i.e. those points which are identified as circles or triangles in Fig. 5, providing they are also identified as squares or triangles in Fig. 4. Where the errors of prediction are greatest, i.e. in Laird, it is clear that they are generally larger after 60 d in preliminary environments at 1 or 5 °C, especially in long days. This can be seen by identifying those points which are represented by circles in Fig. 7, especially if at the same time they are also represented as either squares or triangles in Fig. 6.

CONCLUSIONS

Equation (1) was originally used to describe the time taken for lentil to flower in various combinations of photoperiod and mean diurnal temperature when these determinants were maintained constant throughout development (Summerfield *et al.*, 1985*b*). We have shown here that eqn (1) can also be used to derive photothermal units (°C d), which can be used to measure progress towards the photothermal time θ, required for flowering. This approach enables the actual time taken to flower to be predicted in circumstances when the environment changes during the course of development. The main error arises from the fact that there is an initial pre-inductive phase of development which, although probably sensitive to temperature, is insensitive to photoperiod (Roberts *et al.*, 1986). But eqn (1), and therefore the model arising from it, does not recognize this initial period of photoperiod insensitivity. It is not obvious how to overcome this difficulty since the duration of the pre-inductive phase varies with genotype and probably also with temperature. Time-consuming, sequential, reciprocal-transfer treatments at several temperatures would be required to establish the duration of the pre-inductive phase for each genotype under various circumstances (Roberts *et al.*, 1986). These sorts of investigation would not be feasible for screening large numbers of germplasm accessions, which is the ultimate goal of this research programme.

However, in the experiment described here the prediction error, which is due to the false assumption that the initial stages of development are sensitive to photoperiod, was encountered in an exaggerated form since, in order to expose any possible short-day vernalization effect, extreme photoperiods (8 and 16 h) were used in the preliminary environments.

Most important lentil-growing areas are between latitudes 20° and 40° (Muehlbauer *et al.*, 1985) and for this species there is some evidence that the best estimate of daylength as perceived by the plant under natural conditions is the time from sunrise to sunset (Summerfield and Roberts, 1987). Almanacs show that, within these latitudes, minimum mid-winter daylength defined in these terms varies with latitude from 9 h 46 min to 10 h 34 min. But the daylength will usually be longer than these values immediately after sowing in the autumn, and so it may well be that most lentil crops will have completed their photoperiod-insensitive pre-inductive phase before the shortest daylengths arrive. Lentil crops are also grown at higher latitudes, up to 45° or 50°, but in these cases they are normally spring-sown, so that the daylengths which are experienced initially are likely to be in the region 12 h 30 min to 13 h. Thus, in most agricultural circumstances, the errors in applying the model based on eqn (1) are likely to be less than those encountered here, because they will be minimal when the initial daylengths during the pre-inductive phase approach 12 h. It is expected therefore that the errors deriving from this source when using the model to predict time of flowering in the field may not be too severe.

Variations in the coefficient values of eqn (1) in different experiments on what was meant to be the same genotype (Fig. 3) is of greater concern, especially as the individual seeds were graded by weight and the seedlings thinned to leave uniform plants. Nevertheless, in spite of these difficulties, a preliminary investigation has suggested the coefficients determined in artificial environments can, in fact, be used to predict flowering times in neutral environments with reasonable acuracy (Summerfield *et al.*, 1985*a*). But further verification in a wide range of natural environments is needed and is, indeed, in progress.

Now that large collections of germplasm are available in reliable storage a pressing need is to characterize accessions so that plant breeders are more fully aware of the genetic resources available (Williams, Anishetty and Konopka, 1988). We suggest that the demonstration here that the eqn (1) applies to variable as well as constant environments lends support to using it as a basis for screening for sensitivity to photoperiod and temperature as previously suggested (Summerfield *et al.*, 1985*b*).

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