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Floral Initiation in *Lolium temulentum* L.: the Role of Phytochrome in the Responses to Red and Far-red Light

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Summary. The possibility that phytochrome is involved in the promotion of flowering by far-red light was investigated. The addition of far-red (FR) to a day extension with red (R) light promotes inflorescence initiation in *Lolium*. A 2-hour interruption with darkness also promoted flowering compared with the uninterrupted red light control; apex length was further increased by a 10-minute FR irradiation given before the 2-hour dark interruption and was decreased by 10-minutes of R light given in the middle: both FR promotion and R inhibition were reversed by R and FR respectively. Apex length increased approximately linearly with increasing duration of dark interruption up to at least $2^1/_2$ hours. When varying ratios of R:FR light were substituted for a 2-hour dark period, apex length was increasingly depressed as the % R was increased above 25%; no difference between 25% R/75% FR and 100% FR could be detected. Apex length was inversely linearly related to the calculated [Pfr]/[P] ratios above about 40% Pfr.

FR promoted flowering when given during a 5-hour interruption of a day extension with R light but, between 0.25 and 0.90 J m^2 s^{-1}, there was no effect of intensity of FR; at 0.11 J m^{-2} s^{-1} apex length was shorter than at 0.25 J m^{-2} s^{-1} but longer than in darkness. When the duration of FR (from the beginning of a dark interruption of a day extension with R) was varied, apex length increased with increasing duration of FR up to $1^1/_4$ to 2 hours but further increasing the duration of FR did not promote flowering more.

The results implicate phytochrome in the promotion of flowering by FR light. It has been demonstrated that a low [Pfr]/[P] ratio (less than present in 25% R/75% FR) is needed over a relatively long period of time: this explains why a relatively high proportion of FR light must be added to R for several hours in order to give maximum promotion of flowering. It is concluded that, in *Lolium*, the increased flowering response to FR light is brought about by a reduction of [Pfr]/[P] ratio at the appropriate time, although the possibility that another effect of far-red is also involved has not been rigorously excluded.

I. Introduction

While it is generally recognised that flowering in many long-day plants is accelerated when the light used to extend the daylight period contains some far-red (Friend, 1964; Vince *et al.*, 1964; Evans *et al.*, 1965; Vince 1965; Lane *et al.*, 1965; Schneider *et al.*, 1967; Aspinall, 1969) the mechanism by which far-red light promotes flowering is far from being understood. From time to time several explanations have

been advanced and, recently, the existence of a 'high-energy' reaction (which may be some variant of phytochrome action) has been favoured (Friend, 1964; Moshkov *et al.*, 1968; Aspinall, 1969): it has been proposed that flowering in long-day plants may be under the control both of such a 'high-energy' far-red reaction and by the 'simple' red/far-red reversible phytochrome system, which promotes flowering when Pfr is present (Schneider *et al.*, 1967). An alternative explanation is that only the reversible phytochrome system is involved, but that the [Pfr]/[P] ratio established by red light may be supra optimal at certain times (Vince, 1965, 1966a).

In *Lolium temulentum* Ba 3081, flowering is markedly promoted by addition of far-red light to a low intensity day extension with red light; it was found that the effect of far-red was dependent on the time when it was given (Vince, 1965), and preliminary experiments indicated that the 'simple' action of phytochrome was implicated (Vince, 1966a). We have, therefore, investigated further the question of whether the red/far-red reversible phytochrome reaction and/or a 'high-energy' far-red system is involved in the promotion of flowering by far-red light in this plant.

II. Materials and Methods

Plants were raised in 8-hour photoperiods under fluorescent lamps (colour TL 32) in a glasshouse. During the period between 16.00 and 08.00 h G.M.T., plants were enclosed in a darkened structure; during the 8-hour light period the black covers were removed to ensure adequate ventilation and to prevent over-heating; an aluminised terylene film was mounted above the lamps and at the sides so that little natural daylight reached the plants. The plants were, therefore, raised in light of similar spectral distribution and intensity throughout the year. The intensity under the lamps was approximately 16 J m^{-2} s^{-1}, falling to 11.6 J m^{-2} s^{-1} towards the ends of the tubes. The night temperatures were maintained at a minimum of 14–15°C and the glasshouse ventilated when the day temperature rose to 25°C; forced ventilation was given in the summer to maintain the day temperature below 35°C.

During the experimental photoperiodic cycles plants received natural daylight during the 8-hour period. Between 16.00 and 08.00 hours plants received various treatments with red and/or far-red light, and darkness. The temperature increase recorded directly beneath the lamps was about 2.5°C during far-red and 1.5°C during red lighting treatments. The treatments began when the plants had 5 expanded leaves and 4 experimental cycles were given. The plants were then returned to 8-hour days under the artificial light, and dissected 3 weeks from beginning the experimental treatments. Previously (Vince, 1965) 10 cycles were given to plants with 3–4 expanded leaves; older plants are most sensitive to photoperiodic induction and consequently fewer cycles can be used.

Spectral emission curves for the experimental light sources were similar to those used previously (Vince *et al.*, 1964; Vince, 1965). The far-red source (FR) was a 75-watt tungsten-filament lamp with internal reflector enclosed within a lantern made of 1 layer of Strand 'Cinemoid' primary blue filter (no. 20) and 2 layers of red cellophane (British Cellophane M.S.F.C. 300). This source begins to emit above 720 nm and about 7% of the total radiation lies between 700 and 800 nm. The red

source (R) consisted of 2×40 watt white fluorescent lamps (colour TL 32) surrounded by 1 layer each of Strand 'Cinemoid' primary red (No. 6) and yellow (No. 1); most of the emission is between 600 and 700 nm and the source is virtually free from far-red.

The intensity (measured at plant height) was approximately 0.45 J m^{-2} s^{-1} in red and in the 700–800 nm band in far-red. Plants receiving red and far-red simultaneously also received double the normal intensity, except in experiment 3. The switching of the lamps was done with a punched card programmer so that changes from R to FR and vice-versa were made instantaneously.

The results are presented in terms of apex length; the values have not been transformed to logarithms (cf Vince, 1965; Evans, 1960) as, over this range, apex lengths approximate as closely to a linear function of intensity of induction as to an exponential one. A floral stage was also assigned (Vince, 1965); it was found, as before, to be highly correlated with apex length and the values are, therefore, not presented. The experiments have usually been repeated on at least two occasions.

Phytochrome measurements (Experiment 3) were made with a model R2 ratiospect; the ΔA was measured between 815 and 735 nm, and phytochrome was driven from one form to the other by irradiating samples for 60 sec alternately with 665 and 725 nm light. Samples consisted of cotyledons from 8 dark-grown seedlings of *Phaseolus aureus* which, when packed tightly into a 12 mm diameter cuvette, gave a path length of 0.8 mm. All operations were carried out at 25° C in darkness or in dim green light.

III. Experimental Results

1. Reversibility of the Effects of Red and Far-red Light

A criterion for a phytochrome mediated response is red/far-red reversibility. In a preliminary report (Vince, 1966a) it was shown that initiation in *Lolium* was slightly increased when red light (R) given from 16.00–01.00 h was interrupted with darkness from 19.00–21.00 h: the effect of darkness was enhanced by giving 15-minutes far-red (FR) at the beginning, and the effect of FR was prevented when it was followed by 15 minutes R. In view of the importance of this observation in terms of the mechanism by which FR promotes flowering, this experiment was repeated and extended here. A 2-hour period of darkness was interpolated into a 9-hour day-extension with red light; R and/or FR were given at the beginning or in the middle of this 2-hour dark period. Although the effects were small, the results (Fig. 1 A and B) clearly show that apex length was increased by 10 minutes FR given at the beginning of a 2-hour dark period, and decreased when 10-minutes R was given in the middle; the promoting effect of FR and the inhibitory effect of R were reversed by R and FR respectively. The interaction between R and FR was significant at $P=0.01$ in A and $P=0.05$ in B. The experiments have been carried out on other occasions with similar results and the R/FR reversibility is clearly established, supporting the hypothesis that phytochrome is implicated in the enhancement of flowering by far-red light in *Lolium*.

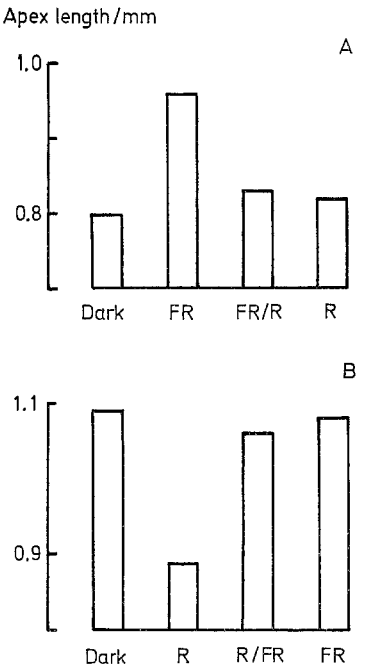

Fig. 1 A and B. Reversibility of the effect of a brief light treatment given during a dark interruption to a day extension with red light. Plants received red light from 16.00–01.00 h, interrupted in the middle by a 2-hour period of darkness. The 10-minute red (*R*) and/or far-red (*FR*) treatments were given at the beginning (A) or in the middle (B) of the dark period

2. The Rate of Conversion of Pfr to Pr in Darkness

The effect of a short dark period and its enhancement by a brief pre-irradiation with FR suggests that flowering is promoted when the [Pfr]/[P] ratio is lowered by thermal or photochemical conversion of Pfr to Pr. Varying the duration of darkness may, therefore, give information about the rate of dark Pfr reversion to Pr. Consequently different durations of darkness, either with or without a prior 10-minute FR irradiation, were interpolated into a 9-hour day extension with red light: all dark treatments ended at 21.00 hrs so that the major part of any dark period was during the time when an interpolation with two hours of FR was most effective in accelerating flowering (Vince, 1965). The flowering response was found to increase with increasing duration of darkness and, when a pre-treatment with FR was given, apex length was further increased (Fig. 2A). Each set of data shows a reasonably good fit to the

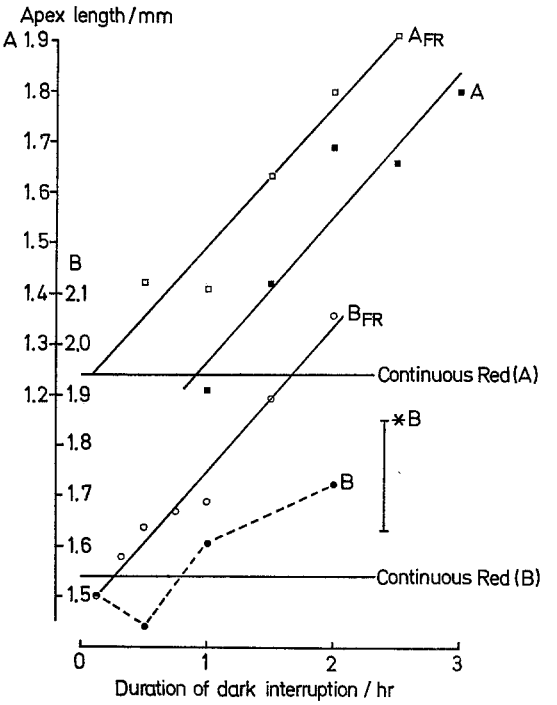

Fig. 2 A and B. Effect of the length of a dark interruption given during a day extension with red light. A_{FR} and B_{FR} received 10 minutes of far-red prior to darkness. A and B received only dark interruptions. Plants otherwise in red light from 16.00–01.00 h. * L.S.D. at $P = 0.05$

calculated linear regression line, and the slopes of the two lines with and without FR are the same; they lie approximately 45 minutes apart.

When the lines are extrapolated they cross the value for uninterrupted red light at 5 minutes with prior FR, and about 55 minutes without. In a further series of experiments with shorter periods of darkness the lines joining observed points crossed the value for uninterrupted red light at about 15 minutes (with prior FR) and 50 minutes (no FR) respectively (Fig. 2B). Thus the rate of reversion of Pfr to Pr in darkness at above 14–15° C appears to be such that within about 50–60 minutes the [Pfr]/[P] ratio is lowered sufficiently to increase flowering: increasing the duration of lowered [Pfr]/[P] ratio up to at least 2.5 hours further increased the flowering response. If [Pfr]/[P] is lowered photochemically by far-red light, the promotion of flowering begins within 5–15 minutes.

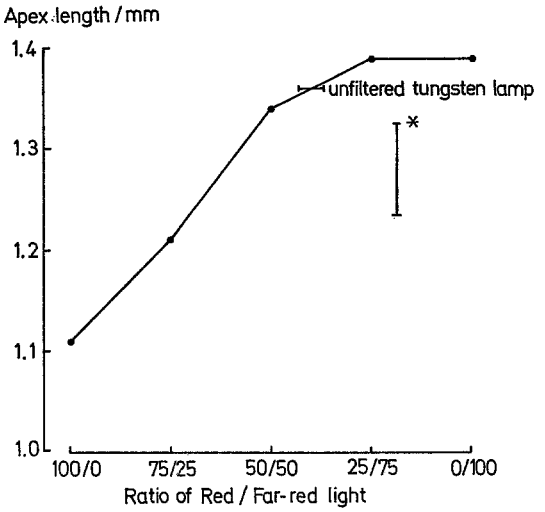

Fig. 3. Effect of the ratio of red to far-red light on flowering in *Lolium*. The various ratios of red/far-red were given for 2 hours from 19.00–21.00 h; plants otherwise in red light from 16.00–01.00 h. * L.S.D. at $P = 0.05$

3. The Ratio of R : FR Light

In the previous experiment flowering was accelerated by lowering [Pfr]/[P] between 19.00 and 21.00 h, but the results do not indicate whether there is an optimum ratio of [Pfr]/[P] or whether the amount of Pfr must be below a certain threshold. Varying ratios of [Pfr]/[P] were, therefore, established during the period from 19.00–21.00 h by giving mixtures of R and FR light obtained by screening parts of the two sources with black paper; an unfiltered tungsten lamp of the kind used in the FR source was also included. There was no indication of an optimum ratio of R:FR light (Fig. 3). Maximum flowering occurred when the ratio of R:FR was 25:75 or below; above this, flowering was increasingly depressed as the percentage of red light increased. The effect of the unfiltered tungsten-filament lamp was consistent with its ratio of R:FR light (approximately 40:60) despite the fact that the intensity was twice that from the other sources.

Attempts to measure phytochrome *in vivo* in *Lolium* leaves proved, as expected, to be unsuccessful. Estimates of the photostationary states established by the varying mixtures of R and FR were, therefore, made using etiolated seedlings of *Phaseolus aureus*. Seedlings were exposed to the R source for 10 or 20 minutes and the percentage of Pfr in the cotyledons was then immediately determined. Four seedling samples were

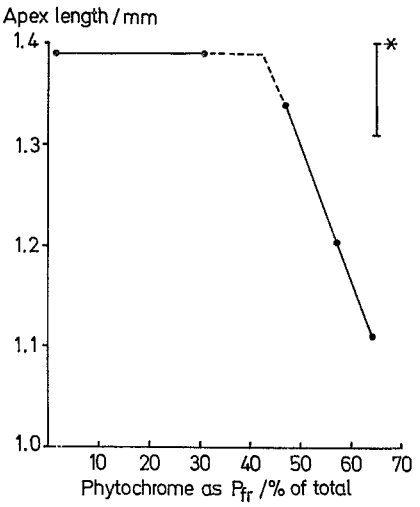

Fig. 4. Relationship between apex length and the fraction of phytochrome present as Pfr. * L.S.D. at $P = 0.05$

used on each of two days and the means for the amount of phytochrome present as Pfr on each day were 65% and 63.5%: the duration of exposure to red light did not affect the result. The amount of Pfr present in the FR source, which emitted only wave-lengths longer than 720 nm, was assumed to be zero. The approximate [Pfr]/[P] ratios in the mixed R and FR treatments were calculated from these values using $\varepsilon_R \phi_R$ at 660 nm $= 1.42 \times 10^7$ and $\varepsilon_{FR}\phi_{FR}$ at 730 nm $= 0.67 \times 10^7$ (Butler *et al.*, 1966) where ε_R and ε_{FR} are the extinction coefficients ($cm^2\ mol^{-1}$) for Pr and Pfr respectively and ϕ_R and ϕ_{FR} are the quantum efficiency coefficients (mol $Einstein^{-1}$) for the conversion of Pr to Pfr and Pfr to Pr respectively.

Although these calculations can give only very rough approximation to the conditions in the *Lolium* leaf, the results (Fig. 4) indicate that flowering begins to be depressed when the percentage of total phytochrome present as Pfr increases above a value which may be in the region of 40%; above this threshold flowering is inversely linearly related to the amount of phytochrome present as Pfr.

4. Effects of Intensity and Duration of Far-red

Responses dependent on the photoconversion between Pfr and Pr are usually saturated at relatively low intensities (Vince, 1966b); if the *only* effect of far-red light in *Lolium* is to lower the [Pfr]/[P] ratio, a marked

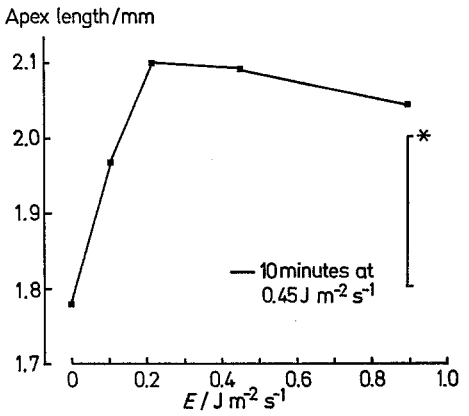

Fig. 5. Effect of intensity of far-red light on flowering in *Lolium*. Far-red light was given for two hours in the middle of a day extension: except during this period, plants received red light from 16.00–01.00 h. The 10-minute far-red treatment was followed by darkness for the remaining 110 minutes. * L.S.D. at $P = 0.05$

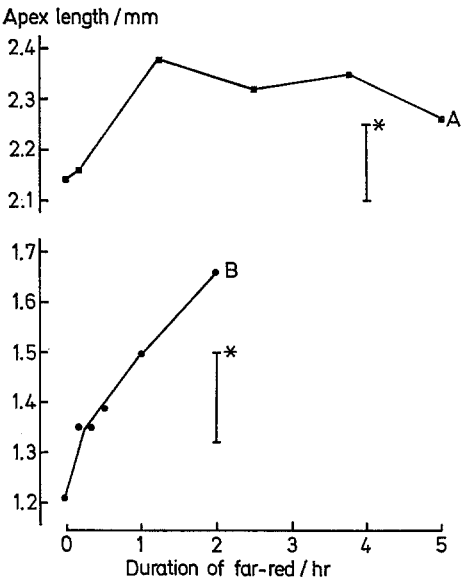

Fig. 6. A and B. Effect of duration of far-red light given from the beginning of a dark period interruption of a day extension with red light. A: far-red given from the beginning of a 5-hour dark period; B: far-red given from the beginning of a 2-hour dark period. Plants otherwise in red light from 16.00–01.00 h. * L.S.D. at $P = 0.05$

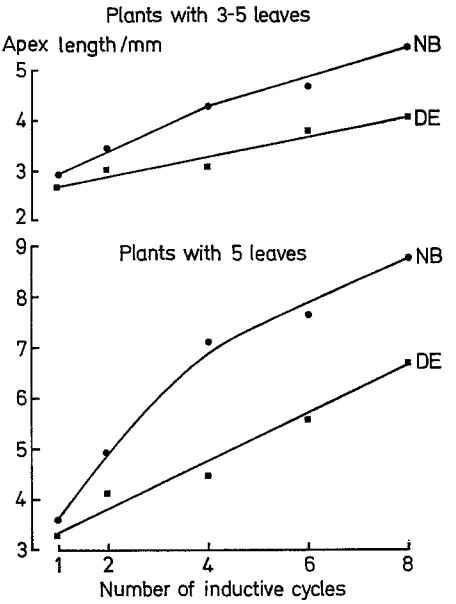

Fig. 7. The response to red light of plants of different ages. Red light was given as a night-break (*NB*) from 23.00–01.00 h. or as a day extenison (*DE*) from 16.00–01.00 h

intensity dependence would not be expected, particularly if far-red is used in conjunction with a long dark period which would allow [Pfr]/[P] to remain low subsequently. Consequently, a 2-hour period of FR at varying intensities was given at the beginning of a 5-hour dark period inserted into a red day-extension. The results (Fig. 5) are not entirely unambiguous: between 0.25 and 0.9 J m^{-2} s^{-1} apex length was increased in FR compared with darkness but, within this range, there was no effect of intensity; at 0.11 J m^{-2} s^{-1} the value was less than at higher FR intensities but more than in darkness. A 10-minute FR treatment at 0.45 J m^{-2} s^{-1} had almost no effect. Varying the duration of FR at this intensity given from the beginning of a 5-hour dark period (Fig. 6a) also gave somewhat puzzling results. Increasing the duration from 10-minutes (which had little effect) to 1.25 hours significantly increased apex length; a further increase in the duration of FR did not further promote flowering. When used in conjunction with shorter dark periods (Fig. 6b), apex length increased with increasing duration of FR up to 2 hours; the 10-minute FR treatment appeared to have a greater effect when combined

with 2 hours than with 5 hours of darkness, but the variability was rather high and only 30 minutes or more of FR resulted in apex lengths significantly greater than the dark value.

5. The Inhibition of Flowering by Red Light

An experiment was carried out primarily to examine the relationships between age, number of inductive cycles, and the kind of illumination treatment given. The results (Fig. 7) clearly indicate that between 16.00 and 23.00 h red light is inhibitory to flowering; when red light was given continuously between 16.00 and 01.00 h, apices were considerably shorter than those of plants irradiated only between 23.00 and 01.00 h. There was a significant interaction between the number of inductive cycles and the time of illumination: for the night-break treatments, the biggest increase relative to the day extension treatments occurred between 1 and 4 cycles; between 4 and 8 cycles the slopes for the two light treatments were similar. Younger plants responded in a similar manner, but the effect of increasing the number of inductive cycles was smaller.

IV. Discussion

When considering the mechanism by which far-red light promotes flowering in *Lolium*, perhaps the most telling point is that the insertion of a period of darkness into a day extension with red light promotes flowering *without giving any far-red light*. When this period of darkness is short, a brief far-red treatment at the beginning and a brief red treatment in the middle increased and decreased apex length respectively: in both cases the effects were reversible establishing that by merely lowering the [Pfr]/[P] ratio at this time the flowering response is enhanced. It is, therefore, suggested that the photoperiodic mechanism in this long-day plant involves a 'low Pfr' reaction in the first part of the night; this must be followed by a 'high Pfr' reaction because, unless far-red light is followed by red, flowering does not occur (Holland, 1969). This concept is similar to the model suggested by Evans and King (1969) for the short-day plant, *Pharbitis nil*, except that the sequence of the 'high' and 'low' Pfr reactions is reversed. They concluded that the low Pfr reaction in *Pharbitis* begins when the proportion of Pfr phytochrome has fallen to that set by 5–20% red light: considering all the differences involved, the latter figure is remarkably close to the value of 25% red determined for *Lolium*. The 'low Pfr' reaction in *Lolium* may involve inhibition by supra-optimal amounts of Pfr because apex length was increasingly reduced over the range of 25–100% red light. If two reactions with differing Pfr requirements are implicated in the control of flowering in *Lolium*, both appear to occur at nearly the optimal rate when a mixture of red and

far-red light is given continuously as this treatment gives rise to the longest apices; however, they did not greatly exceed those obtained when a 4-hour far-red interruption was given in the early part of a red irradiation otherwise continued throughout the night (Holland, 1969). Under continuous 'night' irradiation, Lane *et al.* (1965) obtained optimum flowering with an approximately equal ratio of R:FR light; this contrasts with the threshold effect found here when only the 'low Pfr' reaction was examined at a time when this reaction is the most important one.

The question remains whether, in addition to the effect resulting from a lowered [Pfr]/[P] ratio, there is any evidence for the participation of a specific far-red promoted reaction. Unfortunately, the results of experiments designed to test this hypothesis were not unambiguous. Under conditions when [Pfr]/[P] was maintained constant and low the response both to intensity and duration of far-red irradiation was limited but, nevertheless, flowering was significantly increased with increasing intensities up to 0.25 J m^{-2} s^{-1}, and with increasing duration up to 1.25 or 2 hours. A comparison with the intensities and durations of FR light effective in the 'high-energy' reaction of etiolated seedlings would not lead one to expect these limits of response if the same reaction is implicated in flowering. It is possible that increasing the duration and intensity of FR may affect flowering by accelerating the conversion of Pfr to Pr. Most of the radiation emitted by the far-red lamps used in the experiments was at wavelengths longer than the absorption maximum of phytochrome at 735 nm; experiments with *Phaseolus aureus* showed that, when given after red light, 10 minutes at 0.45 J m^{-2} s^{-1} from these FR lamps was not sufficient to saturate the photoconversion and at least 30% of the phytochrome remained as Pfr; however after 1 hour the photoconversion was complete and no Pfr could be detected. As apex length is quantitatively related to the percentage of Pfr present above a certain threshold, the limited effect of intensity and duration of FR may be explained by the more rapid lowering of the Pfr level to the threshold value. The increased leaf temperature in far-red may also have influenced the results. The reversibility by red of the short far-red treatments establishes with certainty that flowering is increased by lowering [Pfr]/[P] at a particular time but one cannot completely exclude the possibility that the unexplained additional effect of far-red may be related to temperature rather than to light.

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