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USE OF LIGHTING AND TEMPERATURE STRATEGIES TO
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ERIK SANFORD RUNKLE

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Royal D. Heins
Major professor

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USE OF LIGHTING AND TEMPERATURE STRATEGIES TO CONTROL FLOWERING AND ARCHITECTURE OF SELECT HERBACEOUS PLANTS

By

Erik Sanford Runkle

A DISSERTATION

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ABSTRACT

USE OF LIGHTING AND TEMPERATURE STRATEGIES TO CONTROL FLOWERING AND ARCHITECTURE OF SELECT HERBACEOUS PLANTS

By

Erik Sanford Runkle

Successful production of many floricultural crops requires precise timing of flowering and a final plant height that meets preset specifications. To meet height requirements, one method of suppressing internode extension is to provide a day temperature cooler than that during the night. In addition to temperature fluctuations, the incident distribution of spectral radiation influences stem extension and flowering of many plants. In particular, light with a high red (R, 600 to 700 nm) to far-red (FR, 700 to 800 nm) ratio suppresses extension growth, but it can also delay flowering in some long-day plants. Experiments were performed to determine: 1) if phytochrome A mediated the reduction in stem extension from cool day-temperature treatments, 2) how flowering and stem extension of *Echinacea purpurea* L. were mediated by lighting duration and quality, and 3) extension growth and flowering responses of a variety of herbaceous species, particularly long-day plants (LDP), in environments deficient in blue (B, 400 to 500 nm), R, or FR light.

Transgenic potato over- or under-expressing phytochrome A (phyA) and tomato phyA mutants were grown at one of three temperature regimens with a daily mean of 20 °C. Compared with that under a constant 20 °C, an 8 °C temperature depression at the onset of the photoperiod or a 16 / 24 °C day / night temperature suppressed extension

growth of tomato, potato, or both, regardless of phyA level. Therefore, it appears that phyA does not control extension growth in relation to cool temperature treatments.

Experiments were performed to determine how light regulates growth and development of *Echinacea purpurea*, a herbaceous Asteraceae perennial grown for its reported medicinal properties and its aesthetic value in the landscape. Plants were exposed to a variety of photoperiods (9 to 24 h), night-interruption (NI) lighting durations (7.5 to 240 min), and photoperiods deficient in B, R, or FR light. Flowering was most complete and rapid under photoperiods of 13 to 15 h, which indicates that *Echinacea purpurea* is an intermediate-day plant. Plants flowered when 15-h dark periods were interrupted with low-intensity lighting for 7.5 min, but flowering was most rapid when lighted for 30 to 60 min. A model composed of two distinct mechanisms is proposed to explain the flowering behavior of intermediate day plants such as *E. purpurea.*

Finally, experiments with photoselective plastic filters were performed to determine how photoperiods deficient in B, R, and especially FR light influenced stem extension and flowering in a variety of herbaceous annual and perennial plants. Photoperiods deficient in B or R generally promoted extension growth but had little or no effect on time to flower. However, an FR-deficient photoperiod inhibited extension growth and flowering in some LDP, such as pansy (*Viola ×wittrockiana*). Further experiments were performed to determine if lighting strategies could be used to produce short, compact plants without a concomitant delay in flowering. Results with pansy indicate that extension growth and flowering can not readily be separated with lighting strategies. Results from these studies and their applications to the floriculture industry are discussed.

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NOTE TO GUIDANCE COMMITTEE

The paper format was adopted for this dissertation in accordance with departmental and university regulations. As of November 10, 2000, Chapter I (Phytochrome A does not mediate stem extension in relation to cool-temperature treatments) was published in *Physiologia Plantarum* (104:596-602). Chapter II (Photocontrol of flowering and stem extension of the intermediate-day plant *Echinacea purpurea* Moench.) has been submitted to *Physiologia Plantarum*. Chapter III (Specific functions of red, far-red, and blue light in flowering and stem extension of long-day plants) has been accepted for publication in the *Journal of American Society for Horticultural Science*. Chapter IV (Stem extension and subsequent flowering of seedlings grown under a film creating a far-red deficient environment) is to be submitted to *HortScience*. Chapter V (Photocontrol of flowering and extension growth of the long-day plant pansy) is to be submitted to the *Journal of American Society for Horticultural Science*.

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CHAPTER I

PHYTOCHROME A DOES NOT MEDIATE STEM EXTENSION IN RELATION TO COOL-TEMPERATURE TREATMENTS

Runkle, E. S. and Pearson, S. 1998. Phytochrome A does not mediate reduced stem extension from cool day-temperature treatments. Physiol. Plant. 104(4):596-602.

Phytochrome A does not mediate reduced stem extension from cool day-temperature treatments

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Key words – DIF, DROP, *fri* mutant, internode elongation, *Lycopersicon esculentum*, phytochrome, potato, *Solanum lycopersicon*, *Solanum tuberosum*, stem extension, tomato, *tri* mutant.

Abstract

Stem elongation can be suppressed by a temperature drop at the onset of the photoperiod (DROP) or with a cooler day than night temperature (DT and NT, respectively), commonly described as DIF (DT – NT). To test our hypothesis that phytochrome A (phyA) mediated the reduction of stem elongation caused by -DIF and DROP, we conducted experiments with photomorphogenic mutants of tomato (*Solanum lycopersicon* L.) and transgenic potato (*Solanum tuberosum* L.). The plants studied were tomato mutants *fri*^{1} (deficient in phyA) and *tri*^{3} (deficient in phytochrome B1 [phyB1]) and their isogenic wild-type (WT) cv. Moneymaker, nontransformed potato, and two lines each of antisense phyA (15-9 and 15-11) and overexpressed phyA (PS2 and PS4). Plants

were placed in three temperature regimens with a daily mean of 20°C: a constant 20°C (0 DIF), an 8°C DROP for 3 h, and a -8°C DIF. For all tomato genotypes, -DIF and DROP reduced internode length by ≥21% and stem elongation by 30% compared to that of plants at 0 DIF. Interactions between temperature treatment and genotype were nonsignificant. For potato, -DIF, but not DROP, significantly reduced internode length of WT (by 39%) and both antisense lines (by 36 or 48%) but only one of the two lines of overexpressed phyA plants (by 18%). The -DIF significantly reduced stem length for only antisense phyA (by 36 or 48%) and WT (by 35%) plants. Thus, at least for tomato and potato, it appears that phyA does not control stem extension in relation to cool-temperature treatments.

Introduction

For many species, stem extension is suppressed when the day temperature (DT) is lower than the night temperature (NT) (Erwin et al. 1989a, b, Erwin and Heins 1995, Myster and Moe 1995). The inverse of this is also true: a warmer DT than NT promotes stem elongation. Erwin et al. (1989b) quantified this phenomenon in relation to the sign and magnitude of the difference between DT and NT (DIF); e.g., a +6 DIF signifies that plants are grown with a day temperature 6°C warmer than the night temperature. For many but not all species that respond to DIF, stem elongation also can be suppressed by a transient (i.e., 2- to 4-h) drop in temperature (DROP), which is often most effective when timed with the onset of the photoperiod (Erwin et al. 1989a, Langton et al. 1992, Cockshull et al. 1995). Stem extension is perhaps more accurately related by the absolute values of the day and night temperatures, not the differences between the two (Langton and Cockshull 1997).

The physiological mechanism of the suppression of stem extension caused by DIF or DROP is not understood, but several studies have implicated gibberellin (GA) involvement. Application of GA_1 to DIF-sensitive *Begonia* ×*hiemalis* Fotch grown under a -10°C DIF caused internode-length development similar to that of untreated plants under a constant temperature regimen (Myster et al. 1995). Studies with tomato (*Solanum lycopersicon* L. [syn: *Lycopersicon esculentum* Mill.]), pea (*Pisum sativum* L.), and campanula (*Campanula isophylla* Moretti cv. Hvit) suggest that -DIF suppresses internode elongation by altering rates of gibberellin metabolism (Jensen et al. 1996, Langton et al. 1997, Grindal et al. 1998). However, in some instances, the effects of DIF on stem elongation could not be attributed completely to changes in gibberellin biosynthesis, metabolism, or sensitivity. In addition, application of GA to two *Lilium* spp. only partially overcame the reduced stem extension cause by a -8°C DIF (Zieslin and Tsujita 1988).

Light plays a key regulatory role in mediating DIF or DROP responses; cool temperatures limit stem extension only when delivered with the photoperiod, and effective DROP temperature regimens are usually most effective when delivered in coordination with the light-on signal (Langton et al. 1992, Cockshull et al. 1995). In addition, DROP treatments during the dark period did not reduce plant height (Bertram 1992, Gertsson 1992). Moe and Heins (1990) postulated that one of the major photoreceptors found in green plants, phytochrome, could mediate the effect of contrasting day and night temperatures on stem elongation.

The phytochromes mediate many physiological events, including germination, de-etiolation, the shade-avoidance response, and flowering. Phytochrome exists in a photoequilibrium of two interconvertible forms, P_r and P_{fr}, which absorb maximally in the red (R) (660 nm) and far-red (FR) (730 nm) regions of the spectrum, respectively. Thus, at any one time, depending on the spectral quality of intercepted light, the proportion of phytochrome in the P_{fr} (presumed active) state varies (Smith 1995). This proportion is referred to as the phytochrome photoequilibrium (P_{fr}/P), and light with a high R : FR yields a P_{fr}/P high value. Furthermore, phytochrome mediates stem extension in response to R and FR light. DIF affects stem elongation only when plants are grown with a high R : FR ratio, and plants grown with a low R : FR ratio are phenotypically similar to those grown with a +DIF (Moe and Heins 1990).

There are several members of the phytochrome family: e.g., five genes and their encoded proteins have been identified in tomato (Hauser et al. 1995). The two most abundant and best characterized types of phytochrome are phytochrome A (phyA) and phytochrome B (phyB). PhyA is a light-labile (type I) phytochrome that accumulates in the dark and is rapidly depleted in light. In contrast, phyB is a light-stable (type II) phytochrome and is present in roughly equal amounts in the light and dark. The P_{fr} form of phyA ($P_{fr}A$) is considered the active form and in etiolated tissues has a 100-fold higher turnover rate (half-life $\approx$1 to 2 h) than the P_r form of phyA (P_rA) (half-life $\approx$1 week) (Vierstra 1994, Clough and Vierstra 1997). In green plants, the turnover of phyA as P_{fr} probably occurs at a slower rate (Jabben 1980). In addition, the rate of P_{fr} degradation depends on temperature (Schäfer and Schmidt 1974).

Given the accumulation of phyA in darkness, its metabolic instability in light and the sensitivity of P_{fr} to temperature, we conducted experiments with photomorphogenic mutants of tomato and transgenic potato (*Solanum tuberosum* L.) to determine whether phyA could be implicated in mediating reduced stem elongation caused by -DIF and DROP. We postulated that phyA could be involved for the following reasons: (1) in potatoes, the overexpression of phyA reduces internode length (Heyer et al. 1995); (2) phyA is degraded by light at the onset of the photoperiod, when DROP treatments are effective; and (3) phyA degradation appears to be temperature-dependent, and reducing the temperature in the morning via DROP may reduce the rate of phyA degradation, thereby leading to reduction in internode elongation.

Tomato was selected as an ideal model plant to test this hypothesis since it responds to DIF and DROP (Heuvelink 1989, Gertsson 1992, Jacobsen et al. 1992), and phyA and phyB1 mutants exist (van Tuinen et al. 1995a, b, Kerckhoffs et al. 1996). Potato was selected since it responds to +DIF (Bennett et al. 1991) and transgenic plants that expressed very low (antisense) or high (overexpressed) levels of phyA have been constructed (Heyer et al. 1995). Furthermore, *PHYA* has been implicated in controlling stem extension in potato in relation to the R : FR ratio (Heyer et al. 1995).

In tomato, *PHYA* and *PHYB1* are the first and second most abundant mRNAs, respectively (Pratt et al. 1997). Tomato phyA mutants are insensitive to far-red light and hence named *fri* mutants; phyB1 mutants are temporarily insensitive to red light and named *tri* mutants (van Tuinen et al. 1995a, b). If our hypothesis were true, we would expect *fri* mutants and antisense-phyA potato to show negligible or no -DIF or DROP response and plants with overexpressed phyA to show an enhanced -DIF or DROP response compared to that of the WT. Our data show that phyA does control stem extension in light-grown WT plants but has no specific effect in cool-temperature treatments.

Abbreviations – DIF, day temperature – night temperature; +DIF, day temperature > night temperature; -DIF, day temperature < night temperature; 0 DIF, day temperature = night temperature; DROP, morning temperature drop; FR, far-red light; phyA, phytochrome A; phyB1, phytochrome B1; R, red light; WT, wild type.

Materials and methods

Tomato plant material

Seeds of *fri*[1] and *tri*[3] (subsequently referred to as *fri* and *tri*) and their isogenic WT cv. Moneymaker were sown on 6 October 1997 in 3-cm (18-ml volume) plug trays containing peat and vermiculite compost (SHL Professional Seed Sowing/Modular Compost, Lincoln, UK). Seedlings were grown in a constant 20°C controlled-environment room irradiated for 16 h at ca 65 μmol m^{-2} s^{-1} PPFD (400 to 700 nm) at canopy level from cool-white fluorescent lamps (L40W/23; Osram Sylvania, Wembley, UK), as measured with a Li-Cor quantum (model 03017-7901; Li-Cor, Lincoln, NE, USA) sensor attached to a DC microvoltmeter (type 1221; Comark Electronics, Ltd, Littlehampton, UK). Extra seedlings were grown so that plants could be selected for uniformity at transplanting.

Three-week-old seedlings were transplanted into 9.5-cm (370-ml volume) pots containing 75% (by volume) peat (SHL Professional Potting Compost) and 25%

medium-grade Perlite. Plants were fertilized at every irrigation with well water and 180N-80P-150K (mg l^{-1}) fertilizer (Sangral 111, SHL) applied by top watering with minimal leaching. Plants were transferred to growth cabinets three days later and grown at a constant 20°C with 12-h photoperiods. Twenty-five 27-day-old plants (with approximately three or four leaves >1.5 cm) of each genotype were apportioned randomly to each temperature treatment.

Potato plant material

Unless otherwise noted, materials and methods are identical to those described above.

A nontransformed control, two lines of antisense phyA (15-9 and 15-11), and two lines of overexpressed phyA (PS2 and PS4) potato plants (transformed as described by Heyer et al. [1995]) were received in agar on 31 Oct. 1997 and held at a constant 20°C. *PHYA* in overexpressed lines was approximately twice that in WT; antisense constructs had a 7- or 10-fold reduction (15-9 or 15-11, respectively) in *PHYA* (Heyer et al. 1995). For antisense constructs, there was some evidence for slightly lower *PHYB* mRNA levels. Plants were removed from agar 7 d later and grown for one week in enclosed propagation domes containing peat and vermiculite; the lowest three to four nodes were buried below the soil surface to facilitate adventitious rooting. Plants were then potted into 9.5-cm pots with peat and Perlite and transferred to growth cabinets set at a constant 20°C with 12-h photoperiods. After 10 d of acclimation, 8 to 10 plants of each line were apportioned randomly to each temperature treatment. Plants had generally developed 8 to 11 true leaves above the soil surface at the onset of temperature treatments.

Light and temperature treatments

For each experiment, three temperature regimens were assigned randomly to growth cabinets, each with a daily mean of 20°C. Cabinet 1 was a constant 20°C; cabinet 2 was 13°C from 0600 to 0900 h and 21°C from 0900 to 0600 h (an 8°C DROP for 3 h); and cabinet 3 was 16 or 24°C from 0600 to 1800 h or 1800 to 0600 h, respectively (a −8°C DIF) (Fig. 1). Temperature fluctuations were completed within 10 min.

Air temperatures at canopy level were monitored with 36-gauge (0.127-mm-diameter) type-E thermocouples connected to a datalogger (Datataker DT500; Data Electronics, Letchworth Garden City, UK). The datalogger collected temperature data every 15 s and recorded the average every 10 min. Cabinet settings were adjusted regularly to maintain air temperatures at canopy level to those desired. Actual average temperatures were calculated and varied to within 0.2°C of the desired settings.

Each cabinet was illuminated from 0600 to 1800 h at ca 170 $\mu mol\ m^{-2}\ s^{-1}$ PPFD at canopy level from a mixture of cool-white fluorescent lamps (VHOF48T12; Osram Sylvania, Wembley, UK) and incandescent lamps (18% incandescent calculated by nominal wattage). Using the model of Hayward (1984), the calculated P_{fr}/P using a narrow-band absorption (1 nm) for each wavelength were 0.77, as measured with a spectroradiometer (Bentham 605 with dual Bentham TM300 monochrometers; Reading, UK).

Data collection and analysis

At the onset of the tomato experiment, the first proximal internode <1 mm in length was identified, and the node below it was tagged. After 10 days of temperature treatment, the following measurements were made: total plant height (from soil level to the apical meristem), hypocotyl length, length of the designated internode of interest, and stem length from and including that internode to the apical meristem. Total stem length from the hypocotyl to the apical meristem was calculated. In all temperature treatments and tomato genotypes, ca 5% of plants developed opposite or subopposite phyllotaxy at or near the internode of interest, and in such instances, internode lengths were not included in the results. The experiment was replicated with more mature plants and similar results were obtained (data not shown).

For the potato experiment, the most recent partially expanded leaf (>5 mm) was marked on each plant immediately before the beginning of the temperature treatments. After 14 days of temperature treatments, stem length from the marked leaf to the apex was measured and the number of developed nodes was counted. Average internode length was calculated by dividing the developed stem length by the number of nodes developed during temperature treatments.

A completely randomized design was used with 22 to 25 or 8 to 10 observations of tomato or potato, respectively, for each genotype and temperature treatment. Data were analyzed using SAS (SAS Institute, Cary, NC, USA) analysis of variance (ANOVA) and general linear models (GLM) procedures.

Results

Tomato

Compared to WT, light-grown photomorphogenic tomato mutants, especially phyB1 mutants, had increased hypocotyl lengths: hypocotyls were 49, 55, or 73 mm for WT, *fri*, or *tri* plants, respectively, significantly different at P <0.001 (data not shown).

Across all temperature treatments, total plant height (not including the hypocotyls) of *fri* and *tri* were ca 1 cm shorter than WT plants (statistically different at P = 0.009) (data not shown). The interaction between temperature treatment and genotype was nonsignificant.

For all genotypes, -DIF and DROP reduced the internode length of interest by 30 or 21%, respectively (Fig. 2A). Regardless of temperature treatment, internode length of *fri* plants was ca 3 mm shorter than that of the WT (significantly different at P = 0.043). The interaction between temperature treatment and genotype was statistically nonsignificant. Similarly, -DIF and DROP reduced stem elongation of WT, *fri*, and *tri* during the temperature treatments by 30%, or 2.5 cm (significantly different at P <0.001) (Fig. 2B). Developed stem length did not differ among genotypes.

Potato

Combined across all temperature treatments, antisense phyA plants (15-9 and 15-11) developed stems and internodes that were 22 or 19% longer, respectively, than that of WT or overexpressed phyA plants (PS-2 and PS-4) (Fig. 3). Measured parameters of overexpressed phyA plants were statistically similar to that of WT. However, for all measured parameters, there was a strong interaction between genotype and temperature treatments ($P \leq 0.014$), primarily from the response of the PS-4 overexpressed phyA line.

The -DIF significantly ($P < 0.001$) reduced average internode length by 39% for WT and 36 or 48% for 15-9 or 15-11 antisense phyA plants, respectively, but the overexpressed lines showed a proportionately reduced response, with only one of the two (PS-2) showing significantly reduced (27%) internode elongation (Fig. 3A). The DROP treatment did not reduce average internode length for any genotype. Regardless of *PHYA* level, average internode length under -DIF was 9.1 to 11.2 mm. At 0 DIF or DROP, internodes of antisense phyA plants were ≥26% longer than those of overexpressed phyA plants.

Compared to that of plants at 0 DIF, -DIF significantly reduced stem length of antisense phyA (by 36 or 48%) and WT (by 35%) plants, but for overexpressed phyA plants, the 27 or 16% reduction in stem length was not significant at the $P = 0.05$ level (Fig. 3B). All potato genotypes grown with a -DIF developed similar stem lengths, ranging from 5.7 to 6.7 cm. Unexpectedly, the DROP treatment did not reduce stem length for any genotype.

Discussion

We tested the hypothesis that phyA mediated the reduction in stem extension caused by cool day-temperature treatments. The -DIF treatment effectively reduced internode and stem extension (by at least 30%) for WT, *phyA* and *phyB1* tomato and WT and antisense phyA potato. The overexpressed phyA potato plants were apparently less responsive to the temperature regimens than WT. The DROP treatment reduced internode elongation of all genotypes of tomato but did not reduce stem extension of potato, which was surprising, given their genetic similarity. Thus, at least for tomato and potato, it appears that phyA does not control stem extension in relation to cool day-temperature treatments. In addition, phyB1, which is thought to control primarily stem extension in tomato (Kerckhoffs et al. 1997), can be eliminated as the only mediator of DIF and DROP responses in tomato. However, phyB1 is believed to be partially redundant because of the other phyB in tomato, phyB2 (Kerckhoffs et al. 1996, Kendrick et al. 1997), so phyB cannot be eliminated as mediating temperature-influenced stem extension.

Contrary to our hypothesis, our results suggest that DIF responsiveness in potato and tomato increases as *PHYA* expression decreases. Potato stem growth was reduced by -DIF the least in overexpressed phyA plants (16 or 27%), moderately in WT (35%), and the most in antisense phyA plants (36 or 48%). Potato internodes responded similarly. Comparably, -DIF suppressed stem extension more in phyA tomato mutants (38%) than in WT (22%) and DROP reduced stem extension by 35% in phyA mutants but only by 16% in WT.

Results with WT tomato are very similar to that of other DIF and DROP studies. The -8°C DIF temperature regimen suppressed tomato internode elongation by 30%; at a similar temperature regimen, internode extension was reduced by 26 or 22% for cvs Moneymaker or Metador, respectively (Jacobsen et al. 1992, Langton and Cockshull 1997). A 3-h 4.8°C DROP at the beginning of the light-on signal reduced stem elongation of tomato cvs Solentos or Elin by 22 or 14%, respectively (Gertsson 1992), which compares favorably to our 30% reduction in stem extension with a 3-h 8°C DROP.

Although the tomato internodes of interest were not completely elongated at measurement, our data provide an adequate measurement of the initial elongation rate.

No previous DROP or -DIF studies with potato were available for comparison, but the remarkable suppression of stem extension we observed was unexpected. Compared to that of plants grown with 0 DIF, 90 days with +8°C DIF promoted stem extension of potato cvs. Norland and Denali by no more than 16% (Bennett et al. 1991). In contrast, the -8°C DIF reduced stem elongation of our WT potato by 35% compared to those at 0 DIF. The difference in the magnitude of these responses is surprising, since +DIF generally promotes stem extension more than -DIF with the same absolute value suppresses it (Erwin et al. 1989a). Our data with stem extension in phyA transgenic potatoes are consistent with that of Heyer et al. (1995): antisense phyA potatoes were taller than WT, and overexpressed phyA lines were similar to or slightly shorter than WT. For overexpressed lines, the difference in the -DIF response could reflect different amounts of phyA in the lines.

Phytochrome A in tomato is very similar to that in potato (Pratt et al. 1997). Thus, we expected stem extension responses of *phyA* tomato to be similar to that for antisense phyA potato, but this did not occur. These differences could reflect differences in GA status or in control of GA biosynthesis. The contrast between these species is of considerable interest, given their genetic similarity, and may indicate that the DROP and DIF responses are completely separate phenomena with different physiological bases. However, contrary to our original hypothesis, it is clear that DROP responses in potato are not associated with the action of phyA.

Our data show that phyA affects internode elongation; stem extension was enhanced in tomato or potato plants with little or no phyA and was reduced in potato with high levels of phyA. Studies with photomorphogenic mutants show that light and GAs interact to affect stem extension (Chory and Li 1997). Overexpression of oat phyA in transgenic tobacco (*Nicotiana tabacum* L.) (Jordan et al. 1995) and hybrid aspen (Olsen et al. 1997) reduced levels of active GAs and produced dwarfed phenotypes, suggesting that overexpression of phyA reduces GA biosynthesis. In addition, light (presumably mediated through phytochrome) affects the accumulation of GA 20-oxidase mRNA, which catalyzes the conversion of inactive GA_{19} to the active GA_1 (Wu et al. 1996). Thus, compared to that of the WT, the suppression of internode elongation in overexpressed phyA potato could be attributed to reduced GA biosynthesis, and vice versa for the phyA mutant tomatoes and antisense phyA potatoes.

In summary, our results indicate that, while phyA affects internode elongation, it alone does not mediate thermomorphogenic reduction of stem extension. However, other phytochromes, alone or in synergy, may be the mediators, which in turn could influence gibberellin biosynthesis or responsiveness and affect stem elongation. The use of phytochrome double mutants (e.g., the *fri,tri* mutant; Kendrick et al. 1997) or chromophore mutants (e.g., the *au* tomato mutant; Casal and Kendrick 1993) lend themselves to further thermomorphogenic studies. Alternatively, other photoreceptors (e.g., cryptochrome) may mediate temperature-regulated stem extension directly.

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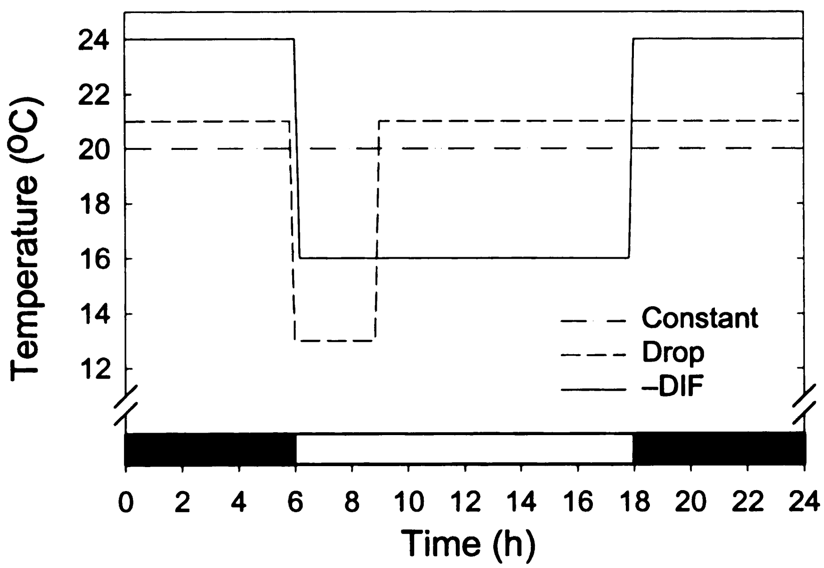

Fig. 1. Air temperature regimens at plant canopy level. Actual temperatures measured at plant height were within ±0.2°C of the indicated settings. The dark and light periods are indicated by closed and open bars, respectively.

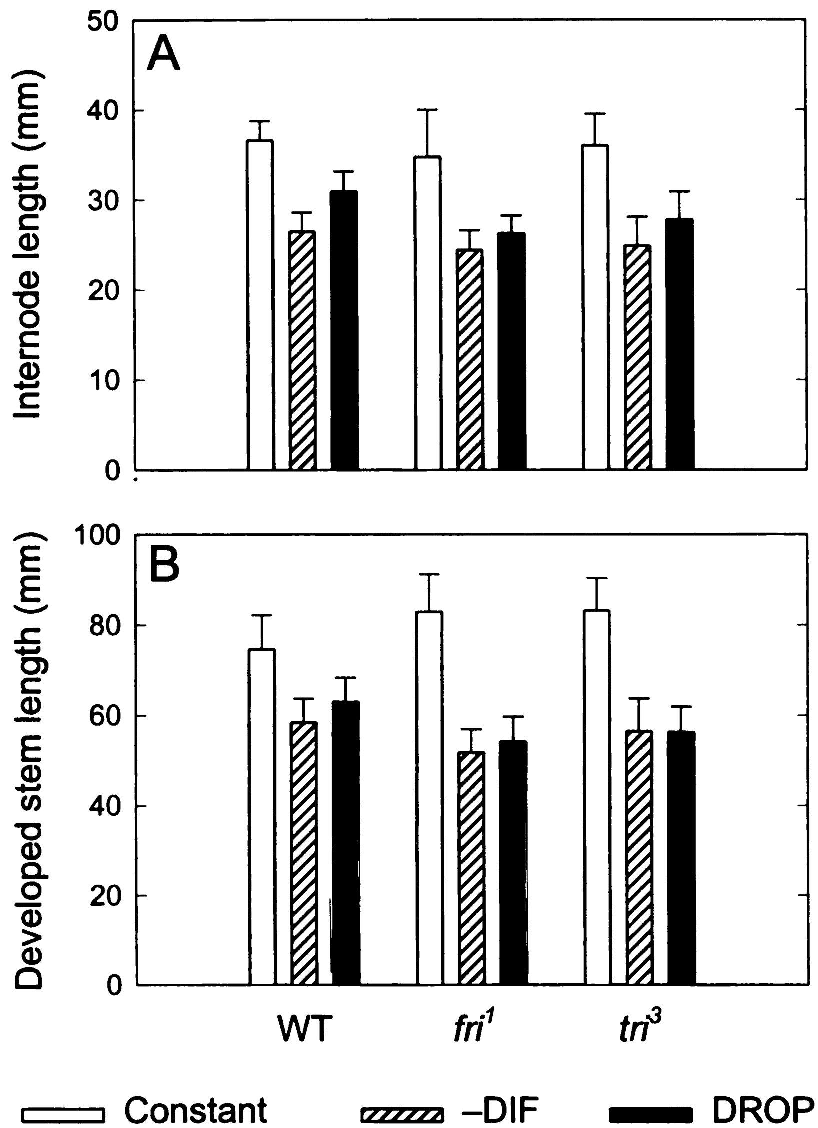

Fig. 2. Internode length (Fig. 2A) and developed stem length (Fig. 2B) of tomato WT and *fri*- and *tri*-mutant plants after 10 days of temperature treatments. Error bars are 95% confidence intervals (n = 22 to 25).

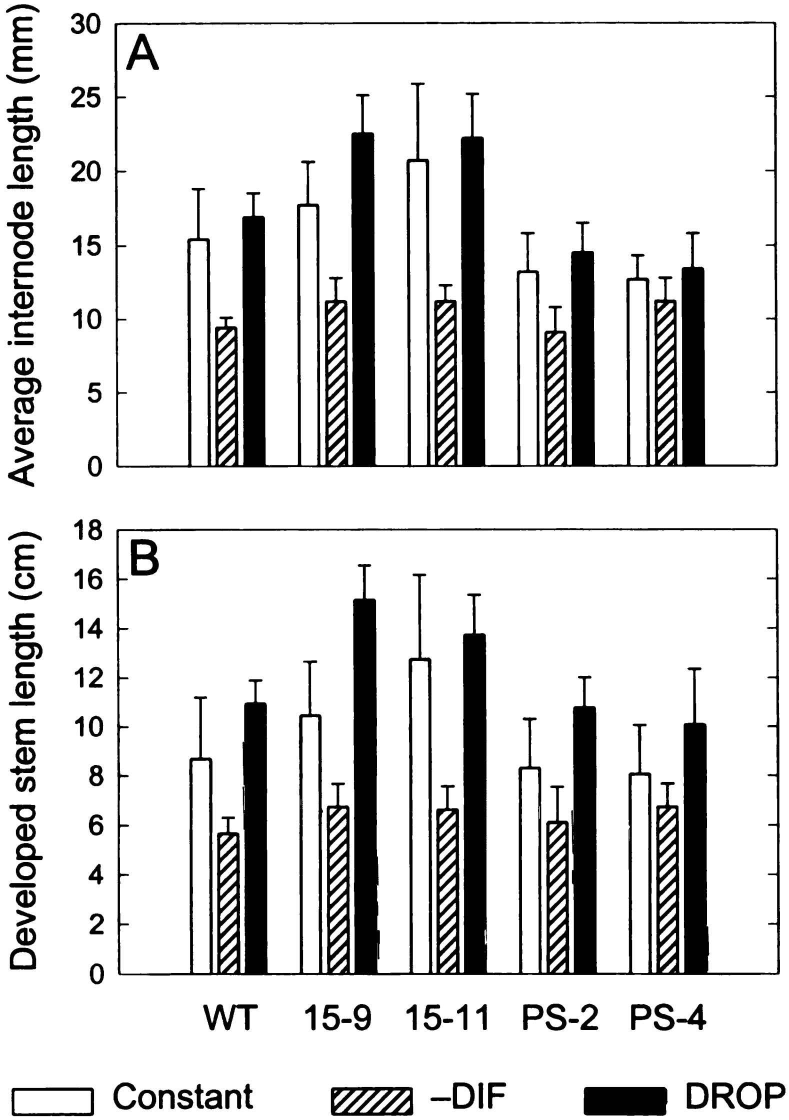

Fig. 3. Average internode length (Fig. 3A) and developed stem length (Fig. 3B) of WT (n = 10) and four lines of transgenic potato: 15-9 and 15-11 are antisense phyA (n = 9); PS2 and PS4 are with overexpressed phyA (n = 8). Measurements were taken after 14 days of temperature treatments. Error bars are 95% confidence intervals.

CHAPTER II

PHOTOCONTROL OF FLOWERING AND STEM EXTENSION OF THE INTERMEDIATE-DAY PLANT *ECHINACEA PURPUREA* MOENCH.

Runkle, E.S., R.D. Heins, A.C. Cameron, and W.H. Carlson. Photocontrol of flowering and stem extension of the intermediate-day plant *Echinacea purpurea* Moench. Submitted to Physiol. Plant.

Photocontrol of Flowering and Stem Extension of the Intermediate-Day Plant *Echinacea purpurea*

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Abstract

Intermediate-day plants (IDP) flower most rapidly and completely under intermediate photoperiods (e.g., 12 to 14 h of light), but few species have been identified and their flowering responses are not well understood. We identified *Echinacea purpurea* Moench as an IDP and, based on our results, propose a novel mechanism for flowering of IDP. Two genotypes of *Echinacea purpurea* ('Bravado' and 'Magnus') flowered most completely (≥ 79%) and rapidly and at the youngest physiological age under intermediate photoperiods of 13 to 15 h. Few (≤ 14%) plants flowered under 10- or 24-h photoperiods, indicating *E. purpurea* is a strongly quantitative IDP. Plants were also induced to flower when 15-h dark periods were interrupted with as few as 7.5 min of low-intensity lighting (night interruption, NI). Flowering was progressively earlier as the NI increased to 1 h, but was delayed when the NI was extended to 4 h. Stem length increased by ≥ 230% as the photoperiod or NI duration increased, until plants received a saturating duration (at 14 h or 1 h, respectively). Flowering was inhibited when 16-h photoperiods were deficient in red (R, 600 to 700 nm) light, [creating a low phytochrome photoequilibrium (P_{fr}/P)] and was promoted when photoperiods were deficient in far-red

(FR, 700 to 800 nm) light (creating a high P_{fr}/P). Because of our results, we propose the flowering behavior of IDP such as *E. purpurea* is composed of two mechanisms: a dark-dependent response operating through a high P_{fr}/P in which flowering is promoted by a short night, and a light-dependent response operating through a low P_{fr}/P in which flowering is inhibited by a long day.

Abbreviations - B, blue light (400 to 500 nm); B_d, blue-deficient light; DE, day-extension lighting; FR, far-red light (700 to 800 nm); FR_d, far-red deficient light; FR_n, far-red light (725 to 735 nm); IDP, intermediate-day plant; LD, long-day; LDP, long-day plant; N, neutral-density filter; NI, night-interruption lighting; R, red light (600 to 700 nm); R_d, red-deficient light; R_n, red light (655 to 665 nm); SD, short day; SDP, short-day plant; VB, visible bud.

Introduction

Plants have been classified into flowering response groups based on how photoperiod influences flowering (Vince-Prue 1975). Day-neutral plants flower irrespective of photoperiod, and short-day plants (SDP) and long-day plants (LDP) flower, or flower most rapidly, when photoperiods are less than or greater than some genotype-specific "critical" photoperiod, respectively. Some species have more specialized photoperiodic responses, such as intermediate-day plants (IDP) in which flowering is promoted most under intermediate photoperiods.

Under SD, an interruption of the dark period with light [known as night interruption (NI) lighting] promotes flowering in LDP and suppresses it in SDP. Night interruption is usually most effective at or near the middle of the night when the dark duration is long (e.g., 15 hours) (Lane et al. 1965). Short durations (e.g., 30 min) of NI lighting are often sufficient to keep SDP vegetative, but most LDP require a long (e.g., ≥ 2 h) duration of NI to promote rapid reproductive development (Lane et al. 1965, Vince-Prue 1975).

A distinction has been made between plants in which flowering is controlled primarily by dark processes (dark-dominant) or light processes (light-dominant; Thomas and Vince-Prue 1997). Light-dominant plants show a more or less quantitative relationship between irradiance of the night break and the magnitude of the flowering response, until a saturation light intensity, duration, or both, are reached. In contrast, dark-dominant plants are those in which a short lighting duration (e.g., ≤ 30 min) during a long night regulates plant development. In most instances, SDP and LDP are dark- and light-dominant plants, respectively, but a few exceptions exist. For example, *Fuchsia ×hybrida* Hort. ex Vilm. 'Lord Byron' has been classified as a dark-dominant LDP (Thomas and Vince-Prue 1997).

Phytochrome is a family of photoreceptors that mediate flowering and stem extension in many plants. There are several phytochromes found in plants (e.g., at least five have been identified in tomato and *Arabidopsis thaliana* Heynh.), each with distinct functions in some physiological processes and overlapping roles in others (Clack et al. 1994, Hauser et al. 1995). For any one phytochrome, there exists a photoequilibrium of two interconvertible forms: the red (R, 600 to 700 nm) and far-red (FR, 700 to 800 nm) absorbing forms, which are known as P_r and P_{fr}, respectively. A molecule is synthesized as P_r in darkness but is converted to the active P_{fr} form in light. P_r becomes the predominant form in darkness or upon FR exposure, but intermediate forms exist. Thus, depending on the R : FR of light, a photoequilibrium (known as $P_{fr}/(P_r + P_{fr})$, or P_{fr}/P) is established, where a high R : FR creates a high P_{fr}/P, and vice versa.

For flowering in light-dominant LDP, various experiments suggest that R light (or a high P_{fr}/P) is required at least during the early part of the photoperiod, and FR light (or a lower P_{fr}/P) is required toward the end of the photoperiod (Lane et al. 1965, Thomas and Vince-Prue 1997). The low P_{fr} requirement is supported by many studies in which NI and day-extension (DE) lighting containing R and FR more effectively induce flowering (i.e., flowering is more complete and rapid) than light deficient in FR. Some exceptions exist: Whitman et al. (1998) found that lamps with various R : FR (from 0.7 to 8.8) induced flowering similarly in three species that Runkle et al. (1998) found were light-dominant LDP. Light quality is not critical for day-extension or NI lighting to be effective in dark-dominant plants (Thomas and Vince-Prue 1995). In addition, environments with a low R : FR, thus a low P_{fr}/P, promote stem elongation, while a high R : FR suppresses it. Therefore, FR causes rapid flowering in LDP but also promotes stem extension.

Brassicaceae are especially sensitive to blue (B, 400 to 500 nm) light. Recently, two B photoreceptors, cryptochromes, were identified in the Brassicaceae member *Arabidopsis*, through which some specific roles of B light have been identified. Cryptochrome acts throughout the *Arabidopsis* life cycle, including in the promotion of flower induction and inhibition of stem extension (Mozley and Thomas 1995, Bagnall et al. 1996, Lin et al. 1996). Some of the cryptochrome actions are independent of phytochrome, while others are interactive (Poppe et al. 1998).

We performed experiments to determine how light regulates growth and development of *Echinacea purpurea*, a herbaceous Asteraceae perennial grown for its reported medicinal properties and its aesthetic value in the landscape. Our objectives were to determine (1) the differential sensitivity of plants to R, FR, and B light, (2) the photoperiodic flowering response, and (3) if flowering was dark- or light-dependent. Here, we report that *E. purpurea* is an IDP with an optimum reproductive photoperiod of 13 to 15 h. Short durations of NI (as little as 7.5 min) during 15-h dark periods induced flowering in most plants, indicating that *E. purpurea* is a dark-dominant plant. Light deficient in B or R reduced flowering percentages, suggesting specific, independent roles of phytochrome and cryptochrome in flower initiation of *E. purpurea*.

Materials and methods

Plant material

Seed were sown into 128-cell plug trays (10-ml volume) by a wholesale plug producer (Rakers Acres, Litchfield, Michigan) and grown at 22.5 ± 1.5 °C. Seedlings were initially grown under photoperiods ≥ 14 and ≤ 16 h, since under short photoperiods, leaf size is small and development is slow. Seeding, shipping, and forcing dates are provided in Table 1. Following shipping, plugs were thinned to one plant per cell and were held at 20 °C. At the onset of experiments, plants were transplanted into 13-cm square plastic containers (1.1-l volume) and node counts were recorded (Table 1).

Plant culture, 1994 to 1996

Plants were grown in a commercial soilless medium composed of composted pine bark, horticultural vermiculite, Canadian sphagnum peat moss, processed bark ash, and washed sand (MetroMix 510, Scotts-Sierra Horticultural Products Co., Marysville, Ohio). At every irrigation, plants were fertilized with well water (EC of 0.65 mS cm^{-1} and 105, 35, and 23 (mg L^{-1}) of Ca, Mg, and S, respectively) acidified (two parts H_3PO_4 plus one part H_2SO_4, which provided P at ≈80 mg L^{-1}) to a titratable alkalinity of ≈130 mg $CaCO_3$ L^{-1}. The nutrient solution (200-155 N-K mg L^{-1} from KNO_3 and NH_4NO_3) was applied by top-watering with minimal leaching. Micronutrients were added with a commercially available blended chelated material [Compound 111 (1.50 Fe-0.12 Mn-0.08 Zn-0.11 Cu-0.23 B-0.11 Mo), Scotts, Marysville, Ohio] at a constant 50 mg L^{-1}.

Plant culture, 1997 to 1999

Plants were grown in a commercial soilless medium composed of composted pine bark, vermiculite, Canadian sphagnum peat, coarse perlite with a wetting agent, and lime (High Porosity Mix, Strong-Lite Products, Pine Bluff, AR). Plants were fertilized at every irrigation with a nutrient solution of well water acidified with H_2SO_4 to a titratable alkalinity of ≈130 mg $CaCO_3$ L^{-1} and water soluble fertilizer [125-12-125 N-P-K mg L^{-1} plus 1.0-0.5-0.5-0.5-0.1-0.1 (Fe, Mn, Zn, Cu, B, Mo) mg L^{-1} (MSU Special, Greencare Fertilizers, Chicago, IL)].

Greenhouse temperature control

All plants were grown in a glass greenhouse at 20 °C. Air temperatures on each bench were monitored with 36-gauge (0.127-mm-diameter) type E thermocouples connected to CR10 dataloggers (Campbell Scientific, Logan, Utah). To provide uniform night temperatures, dataloggers controlled 1500-W electric heaters under each bench, which provided supplemental heat as needed throughout the night. The dataloggers collected temperature data every 10 s and recorded the hourly averages. For each experiment, actual average daily air temperatures from the beginning of forcing until the average date of flowering for every treatment were calculated (Table 1).

General lighting conditions, Expts. 1 and 2

Opaque black cloth was pulled at 1700 h and opened at 0800 h every day on all benches so plants received a similar daily light integral within cold treatment or replication. From 0800 to 1700 HR, high-pressure sodium (HPS) lamps provided a supplemental photosynthetic photon flux (PPF) of ≈75 $\mu mol\ m^{-2}\ s^{-1}$ at plant level when the ambient PPF outside the greenhouse was < 200 $\mu mol\ m^{-2}\ s^{-1}$ and were shut off when the ambient PPF was > 400 $\mu mol\ m^{-2}\ s^{-1}$. Incandescent (tungsten-filament) lamps, which delivered 1 to 3 $\mu mol\ m^{-2}\ s^{-1}$ at canopy level, were used in the photoperiodic and NI studies.

Photoperiod experiment (Expt. 1)

To determine the photoperiodic flowering response, noncooled and cooled plants were grown under one of eight photoperiods. The cold treatments consisted in holding half of the plugs in a controlled-environment chamber for 10 ('Magnus') or 15 ('Bravado') weeks at 5 °C. The chamber was illuminated by cool-white fluorescent lamps (VHOF96T12; Philips, Bloomfield, N.J.) from 0800 to 1700 h at 20 to 40 $\mu mol\ m^{-2}\ s^{-1}$ at canopy level, as measured with a LI-COR quantum sensor (model LI-189; LI-COR, Inc., Lincoln, Nebr.). While in the cooler, plants were watered with well water acidified (93% H_2SO_4) to a titratable alkalinity of $CaCO_3$ at ≈100 mg L^{-1}.

Ten noncooled or cooled plugs of each cultivar were planted and placed under one of eight photoperiods: 10, 12, 13, 14, 15, 16, or 24 h of continual light or 9 h with a 4-h NI. For the continual photoperiodic treatments, lamps provided DE; they were turned on at 1700 h and turned off after each photoperiod was completed. The NI was delivered from 2200 to 0200 HR.

NI duration experiment (Expt. 2)

To further characterize the NI response of *E. purpurea*, plants were provided with one of six NI durations: 0, 7.5, 15, 30, 60, or 240 min. All NI were provided during the middle of the 15-h dark periods. An additional treatment was a 14-h photoperiod with DE lighting. Twenty plants of 'Bravado' and 'Magnus' were placed under each lighting regimen.

Spectral filter experiment (Expt. 3)

A reciprocal transfer experiment with four different light quality environments was conducted to determine the specific effects of modified light wave bands from sunlight on flowering and stem extension of *E. purpurea*. Cladding materials used were neutral (N) density or plastics that selectively reduced the transmission of B (B deficient, B_d), R (R deficient, R_d), or FR (FR deficient, FR_d) light. Filter treatments were designed to transmit a similar PPF. The following filters (one layer each) enclosed greenhouse benches to provide the light quality treatments: N, OLS50 (Ludvig Svensson, Charlotte, NC) + PLS Clear (Ludvig Svensson); B_d, Lee filter 101 (Andover, UK) + OLS40 (Ludvig Svensson); R_d, Lee filter 115; FR_d, experimental FR filter (van Haeringen, 1998) + PLS Clear.

Spectral transmissions from 400 to 800 nm were measured by a spectroradiometer (LI-1800, LI-COR Inc.) and are shown in Fig. 1. Quantum ratios (R : FR, B : R, and B : FR) and the estimated P_{fr}/P (Sager et al. 1988) were calculated (Table 2).

A 16-h photoperiod was delivered with a combination of sunlight and HPS lamps positioned above filters. From 0600 to 2200 HR, HPS lamps provided a supplemental PPF of ≈35 μmol m^{-2} s^{-1} at plant level when the ambient greenhouse PPF was < 200 μmol m^{-2} s^{-1}, and was terminated when the ambient PPF was > 400 μmol m^{-2} s^{-1}. Since HPS lamps emit a high proportion of R light, an additional lamp was placed above the R_d filter so that the supplemental PPF was similar among treatments. The light quantum ratios under the filters were calculated when the lamps were the only light source (Table 2). Under each filter treatment, the average daily light integral was measured at canopy level with line quantum sensors connected to a CR10 datalogger (Campbell Scientific), each of which was independently calibrated under the filters by using the spectroradiometer (Table 2).

Twenty plants were placed under each of the B_d, R_d, and FR_d filter treatments, and 40 under the N filter. At VB, ten plants under the B_d, R_d, and FR_d environments were transferred to the N filter, and ten were transferred at VB from the N filter to each photoselective filter. Filter effects on flowering and stem extension before and after flower initiation could thus be separated.

Data collection and analysis

Experiments were replicated in time (Table 1) and were arranged in a completely randomized design. The date the first flower bud was visible (without dissection) and the date the terminal flower reached anthesis (flowering) were recorded for each plant. At flowering, visible inflorescences and nodes on the main stem were counted, and total plant height (not including the container) was measured. Plant height at VB was also measured in Expts. 2 and 3. Plants that did not have visible inflorescences after 15 weeks (Expts. 1 and 2) or 18 weeks (Expt. 3) of forcing were considered nonflowering and discarded. Days to VB, days from VB to flower, days to flower, and node-count increase from the start of forcing were calculated.

Data were analyzed using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA), general linear models (GLM) procedures, and a mean separation procedure for unequal observation numbers (pdiff) with $P = 0.05$. Regression analysis was performed by Sigma Plot (SPSS, Inc., Chicago). Data were pooled when cold treatment (Expt. 1) did not significantly influence the parameters measured.

Results

Photoperiod experiment

Flowering percentage for *E. purpurea* 'Bravado' and 'Magnus' was greatest under photoperiods of 13 to 15 h or a 4-h NI (Fig. 2A). Less than 20% of the plants under 10 or 24 h of light reached VB within 15 weeks of forcing. Cold treatment did not influence flowering percentage (data not shown). Further data for 'Bravado' under 10-h and 'Magnus' under 10- and 24-h photoperiods are not presented, since the low number of flowering observations prevented statistical analysis.

Under photoperiods of 13 to 16 h or a 4-h NI, 'Bravado' flowered in 87 to 95 days, and 'Magnus' in 95 to 100 days (Fig. 2B). Flowering was significantly delayed (by

≥ 14 days) for both cultivars under 12-h photoperiods and, for 'Bravado', under 24-h. Cold treatment did not significantly affect time to flower. Time from VB to flowering was not consistently influenced by photoperiod and averaged 29 days for 'Bravado' and 27 for 'Magnus' (data not shown).

From the start of forcing under NI or photoperiods of 14 to 16 h, flowering plants of both cultivars developed 13 to 15 nodes below the inflorescence (Fig. 2C). Plants under shorter or longer photoperiods developed significantly more nodes before flowering. The effect of cold treatment on node count before flowering was not significant at $P = 0.05$.

'Bravado' plant height at flowering increased from 23 to ≥ 58 cm as the photoperiod increased from 12 to ≥ 14 h (Fig. 2D). Similarly, plant height of 'Magnus' reached a plateau under photoperiods ≥14 h. Flowering height under NI was similar to that under ≥ 14-h photoperiods. Cold treatment did not modify plant height.

NI duration experiment

Flowering percentage of 'Bravado' and 'Magnus' was < 25 without NI and ≥ 74 when the 15-h night was interrupted with a 7.5 min or longer NI (Fig. 3A). There was a quadratic ($P < 0.001$) effect of NI duration on flowering percentage, and flowering percentage was highest under 30 or 60 min of NI or a 14-h photoperiod.

The duration of NI lighting had a quadratic ($P < 0.001$ for 'Bravado', $P = 0.017$ for 'Magnus') effect on time to flower and was most rapid under 30 or 60 min of NI (Fig. 3B). Flowering of both cultivars was less uniform (as indicated by the large 95% confidence intervals) and delayed (by ≥ 14 days) under the 4-h NI. Flowering of

'Bravado' was also delayed (by $\geq$ 20 days) and was less uniform when the NI was $\leq$ 15 min. Night interruption duration had no consistent effect on time from VB to flowering and averaged 25 and 27 days for 'Bravado' and 'Magnus', respectively (data not shown).

The effect of NI duration on node development before flowering was negatively correlated with the NI effect on flowering time (Fig. 3C). Night interruption duration had a quadratic ($P < 0.001$ for 'Bravado', $P = 0.010$ for 'Magnus') effect on the increase in node number, with the fewest nodes formed under the 60-min NI. Both cultivars under $\leq$ 15 min of NI developed $\geq$ 3.0 more nodes before flowering compared with plants under the 60-min NI.

Plant height at VB and flowering was markedly influenced by NI duration. Inflorescence height at VB was < 5 cm for both cultivars under $\leq$ 15 min of NI (data not shown). 'Bravado' height at VB increased from 4.0 to 21.6 cm as NI increased from 15 to 60 min and reached a maximum with $\approx$90 min of NI, when VB height leveled ($\approx$26 cm). Similarly, 'Magnus' height at VB increased from 4.3 to 18.3 cm as NI increased from 15 to 60 min, and longer NI durations did not increase stem elongation. At flowering, an increase in NI from 15 to 60 min increased 'Bravado' and 'Magnus' flowering height by 105% and 119%, respectively (Fig. 3D).

Spectral filter experiment

Flowering percentage was highest under the N and FR_d filters (Fig. 4). Significantly fewer plants flowered under the B_d filter (43%), and flowering percentage was lowest (10%) under the R_d filter. Plants reached VB 14 days (17%) earlier under the FR_d filter than under the N filter. Plant height at VB and flower, time from VB to flower, and the node count increase of flowering plants were not significantly influenced by filter type or transfer (data not shown).

Discussion

Both cultivars of *E. purpurea* had the highest flowering percentage and flowered most rapidly under intermediate photoperiods of 13 to 15 h, and flowering was strongly inhibited under short ($\leq$ 12 h) photoperiods or continual light. In addition, plants flowered at the youngest physiological age (e.g., node count) under these intermediate photoperiods. The photoperiodic response was similar in cooled and noncooled plants. Therefore, *E. purpurea* can be classified as an IDP. Few IDP have been identified: only 13 of the > 500 species classified into photoperiodic responses by Thomas and Vince-Prue (1997) were IDP.

Echinacea purpurea is native to the east central United States (lat. 32-42° N), and flowers naturally in June, when biological photoperiods are 15 to 16 h. Bolting, and hence flower initiation, occurs 6 to 8 weeks earlier, when photoperiods are approximately 60 to 90 min shorter. Thus, the optimum reproductive photoperiod ($\approx$13 to 15 h) in our studies is similar to the natural photoperiod when *Echinacea* is induced to flower.

Many of the identified IDP have a weakly quantitative flowering response, where plants flower under a broad range of photoperiods but flowering occurs slightly earlier under intermediate day lengths. In contrast, *E. purpurea* has a strong quantitative photoperiodic flowering response, where flowering is primarily restricted to intermediate photoperiods. Flowering of plants under short (10 h) or long (24 h) photoperiods could be at least partially attributed to exposure to inductive photoperiods at the seedling stage.

The most inductive photoperiod for many IDP is similar to that of *E. purpurea*: ≥ 13 and < 16 h (Allard 1938, E.A. Clough 1999. Thesis, Michigan State Univ., East Lansing, MI, USA). For some species, however, the most inductive photoperiod is slightly shorter (e.g., 12 h in *Capsicum frutescens* L.; Cochran 1942).

Compared to SDP and LDP, few details are known about flowering of IDP (Thomas and Vince-Prue 1997). Takeno et al. (1995) concluded that the quantitative intermediate-day behavior of *Salsola komarovii* Iljim was a type of SD response, since all plants under SD eventually flowered and at the same physiological age (e.g., node count) as those under the most inductive intermediate photoperiods. Sachs (1956) suggested that IDP are actually plants that require dual day length (e.g., SD followed by LD) in which intermediate photoperiods satisfy the requirements of both SD and LD. In our studies, nearly all plants remained vegetative under short (≤ 10 h) or long (24 h) photoperiods, and of those that did become reproductive, flowering occurred at an older physiological and chronological age. Thus, the flowering behavior of *E. purpurea* cannot be considered a type of SD or LD response. In addition, *E. purpurea* was induced to flower relatively rapidly when a light break interrupted a long night. Since SD were never provided, *E. purpurea* cannot be a plant that requires dual day length. Therefore, we conclude that a true intermediate photoperiodic flowering response exists.

Short-day plants and LDP are generally dark or light dominant, respectively, but to our knowledge, the roles of light and dark processes on IDP have not been described. We reported the sensitivity of *E. purpurea* 'Bravado' to short (30 min) durations of NI (Runkle et al. 1998). To our knowledge, no NI studies have been performed on any other IDP. In this study, we found that even shorter (7.5 min) durations of low-intensity lighting induced flowering of both cultivars during 15-h dark periods. Increasing the NI duration to 30 min increased flowering percentage and decreased time to flower and node count at flowering. Thus, *E. purpurea* can be labeled a dark-dominant IDP, but the response has some similarities to those of SDP and some LDP. A short NI regulates development of most SDP, but the response is an inhibition, not promotion, of flowering. In contrast, short NI durations are not sufficiently long to induce a flowering response in most LDP, since they are light dominant.

As with light-dependent plants, the spectral distribution of light delivered during the photoperiod had a marked effect on the reproductive status of *Echinacea*. Flowering of light-dominant plants is most rapid when long photoperiods contain at least some FR light, particularly toward the end of the photoperiod (Thomas and Vince-Prue 1995). When *Echinacea* was exposed to 16-h photoperiods deficient in FR light (e.g., under the FR_d filter), flowering was more rapid than plants under the N filter. When photoperiods were extended to $\geq$ 16-h with light rich in FR (e.g., day-extension lighting with incandescent lamps), flowering was inhibited. Thus, our data suggest that a light dominant response, operating through a low P_{fr}/P, inhibits flowering in *Echinacea*. Furthermore, the 4-h NI was repeatedly less inductive than shorter NI durations, which also suggests that flowering is inhibited by a light-dependent response. Flowering was strongly suppressed under the R_d filter (which created a low P_{fr}/P), as would be expected with an inhibitory light-dominant response. However, this could also be attributed to a lack of flowering promotion through the dark-dominant response, which is known to require P_{fr}.

We propose that the intermediate day behavior of *E. purpurea* is the result of two mechanisms: a dark-dependent response in which flowering is promoted by a short night, and a light-dependent response in which flowering is inhibited by a long day. Flowering occurs when conditions allow the dark-dominant mechanism to promote flowering but do not allow the light-dominant mechanism to inhibit flowering. Thus, flowering occurs when the duration of darkness is less than the critical night length of 11 h, but is decreased when the photoperiod is ≥ 16 h and the light-dominant mechanism inhibits flowering. A short NI prevents the inhibitory effect of a long night and induces flowering. However, when the NI is sufficiently long, the light-dominant mechanism becomes effective and flowering is depressed.

We have repeatedly observed a high flowering percentage (≥ 85%) when *Echinacea* was forced under natural day lengths extended to 16 h with HPS lamps at 75 $\mu mol^{-2}\, s^{-1}$ PPF (unpublished data). These lamps create a higher predicted P_{fr}/P (0.850, Table 2) than under incandescent lamps (0.645, data not shown) used in our photoperiod and night interruption studies. In accordance with our theory, the inhibitory light-dominant mechanism did not operate in plants under the HPS lamps, since a high P_{fr}/P was maintained. However, under the incandescent lamps, the P_{fr}/P was low and the light-dominant mechanism inhibited flowering.

Flower induction of 'Bravado' was also inhibited when photoperiods were deficient in B light. While the absolute quantity of FR light was greater under the B_d filter compared to the N filter, the R : FR and P_{fr}/P under the B_d treatment were nearly identical to that under the N treatment in which flowering occurred (Table 2). Thus, the reduced flowering in the absence of B light could be attributed to the lack of positive action of cryptochrome. This response is consistent with delayed flowering of the cryptochrome mutants of *Arabidopsis* (Mozley and Thomas 1995, Bagnall et al. 1996, Lin et al. 1996), and suggests that cryptochrome could be involved in the promotion of flowering outside Brassicaceae.

Here, we present a newly identified IDP for flower initiation, with a suggested mechanism, arguments against previous mechanistic proposals in the literature, and reasons to support our hypothesis. *Echinacea purpurea* showed a strongly quantitative flowering response with an optimum reproductive photoperiod of 13 to 15 h of light. Plants also initiated flowering when 9-h days/15-h nights were interrupted with short durations of light. Stem extension increased with an increase in photoperiod or NI, until a saturating lighting duration of about 60 min was reached. Thus, our data suggest that a true intermediate photoperiodic response type exists and has similarities to both SDP and LDP. We propose that in IDP such as *E. purpurea*, a dark-dependent response promotes flowering under a short night and flowering is inhibited by a long day in a light-dependent response operating through a low P_{fr}/P. However, further experiments are warranted to confirm that these mechanisms are indeed involved.

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Table 1. Seed, shipping, and forcing dates, initial node counts, and average air temperatures from date of forcing to average date of flowering (Expts. 1 and 2) or visible bud (Expt. 3) for each photoperiod and cold treatment of *Echinacea purpurea*. The average air temperature during 100 days of forcing is provided when no plants flowered within a treatment. NI = 4-h night interruption; — (long dash) = not included in experiment.

Expt.	Cultivar	Rep.	Weeks at 5 °C	Date: Seed	Date: Shipping	Date: Forcing	Initial nodes	Average air temperature during forcing (°C)							
								Photoperiod							
								10	12	13	14	15	16	24	NI
1	Bravado	1	0	10/17/94	12/16/94	12/19/94	4.2	20.6	20.4	—	20.8	—	20.9	20.6	20.5
			15	10/17/94	12/16/94	4/4/95		21.5	21.0	—	21.1	—	22.0	21.4	20.7
		2	0	10/9/95	12/12/95	12/15/95	4.0	20.8	20.7	20.7	21.0	—	20.3	20.8	20.7
			15	10/9/95	12/12/95	3/29/96		21.6	21.6	22.4	21.9	—	21.3	23.1	22.1
		3	0	10/12/98	10/31/98	12/2/98	5.4	21.0	20.8	20.7	20.9	20.3	20.6	21.0	21.2
			15	8/31/98	9/18/98	2/8/99		20.7	21.6	20.9	21.2	20.1	20.4	20.4	21.5
	Magnus	1	0	9/29/97	11/24/97	1/20/98	4.9	20.7	20.8	20.6	21.3	21.4	21.8	21.9	20.9
			10	9/29/97	11/24/97	3/29/98		22.1	22.0	21.8	23.3	22.4	23.1	23.9	21.6
		2	0	10/12/98	10/31/98	12/2/98	4.7	21.0	21.0	20.7	20.9	20.3	20.6	21.1	21.2
			10	10/12/98	10/31/98	2/10/99		20.7	21.6	21.2	21.2	20.1	20.4	21.1	21.7
								NI duration (min)							
								0	7.5	15	30	60	240	14-h	
2	Bravado	1	0	2/16/98	3/9/98	4/22/98	5.2	21.7	22.8	22.7	22.9	22.4	23.0	22.6	
		2	0	5/11/98	5/29/98	7/8/98	5.8	21.9	22.3	22.5	22.8	23.0	22.5	22.5	
	Magnus	1	0	2/16/98	3/9/98	4/22/98	4.9	22.1	22.8	22.7	23.0	22.7	23.1	22.8	
		2	0	5/11/98	5/29/98	7/8/98	5.2	22.1	22.3	22.5	22.4	22.6	22.2	22.2	
								Spectral filter							
								N	R_d	FR_d	B_d				
3	Bravado	1	0	5/11/98	5/29/98	7/9/98	5.8	21.8	22.1	22.2	21.8				
		2	0	8/31/98	9/19/98	10/26/98	6.3	20.4	20.7	20.1	20.5				

Table 2. Light quantity, quantum transmission percentage (from 400 to 800 nm) of red (R), far-red (FR), and blue (B) light, and calculated phytochrome photoequilibria (P_{fr}/P) (Sager et al. 1988) under filters with sun or high-pressure sodium (HPS) lamps as the sole light source. The average daily light integral was measured under each filter and calculated from date of forcing to average time to visible bud for each filter treatment. B = 400 to 500 nm; FR = 700 to 800 nm; FR_n = 725 to 735 nm; R = 600 to 700 nm; R_n = 655 to 665 nm.

	Average daily light integral (mol m^{-2} d^{-1})		Light source									
			Sun					HPS lamps				
Filter	Rep I	Rep II	% B	% R	% FR	$R_n : FR_n$	P_{fr}/P	% B	% R	% FR	$R_n : FR_n$	P_{fr}/P
Neutral	5.8	4.2	21	28	26	1.06	0.715	7	38	9	2.82	0.850
R_d	5.7	3.8	36	1	31	0.04	0.399	27	4	26	0.12	0.624
FR_d	5.4	4.1	23	25	14	8.22	0.798	6	38	7	9.96	0.873
B_d	5.8	3.8	2	35	34	1.04	0.723	0	42	11	2.74	0.851

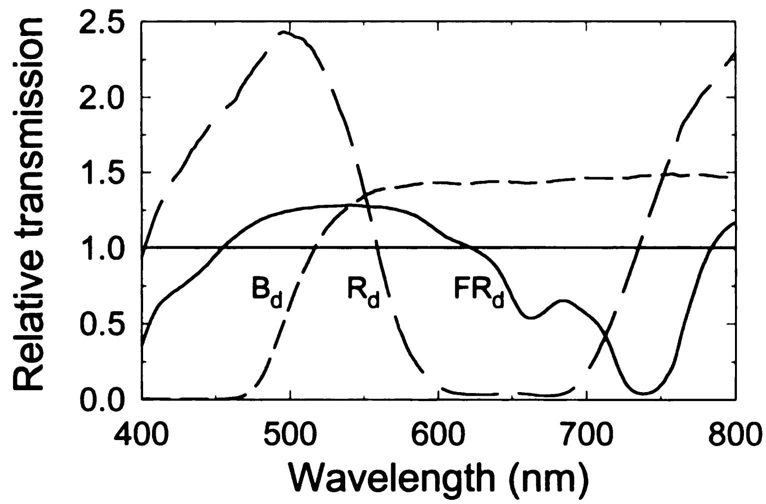


Fig. 1. Spectral transmissions relative to that under a neutral-density filter (N) under sunlight. R_d, red (600 to 700 nm) deficient filter; B_d, blue (400 to 500 nm) deficient filter; FR_d, far-red (700 to 800 nm) deficient filter. See Table 2 for light wave band ratios.

Fig. 2. Flowering of *Echinacea purpurea* 'Bravado' and 'Magnus' under continual photoperiods consisting in 9-h natural days extended with light from incandescent lamps (NI = 4-h night interruption). At first open flower, the number of nodes on the main stem below the inflorescence was counted and total plant height was measured. Data for noncooled and cooled plants were pooled, since cold treatment effects were insignificant. Legend in D applies to all figures. Values with the same letter ('Bravado' in uppercase, 'Magnus' in lowercase) are not statistically different at $P = 0.05$.

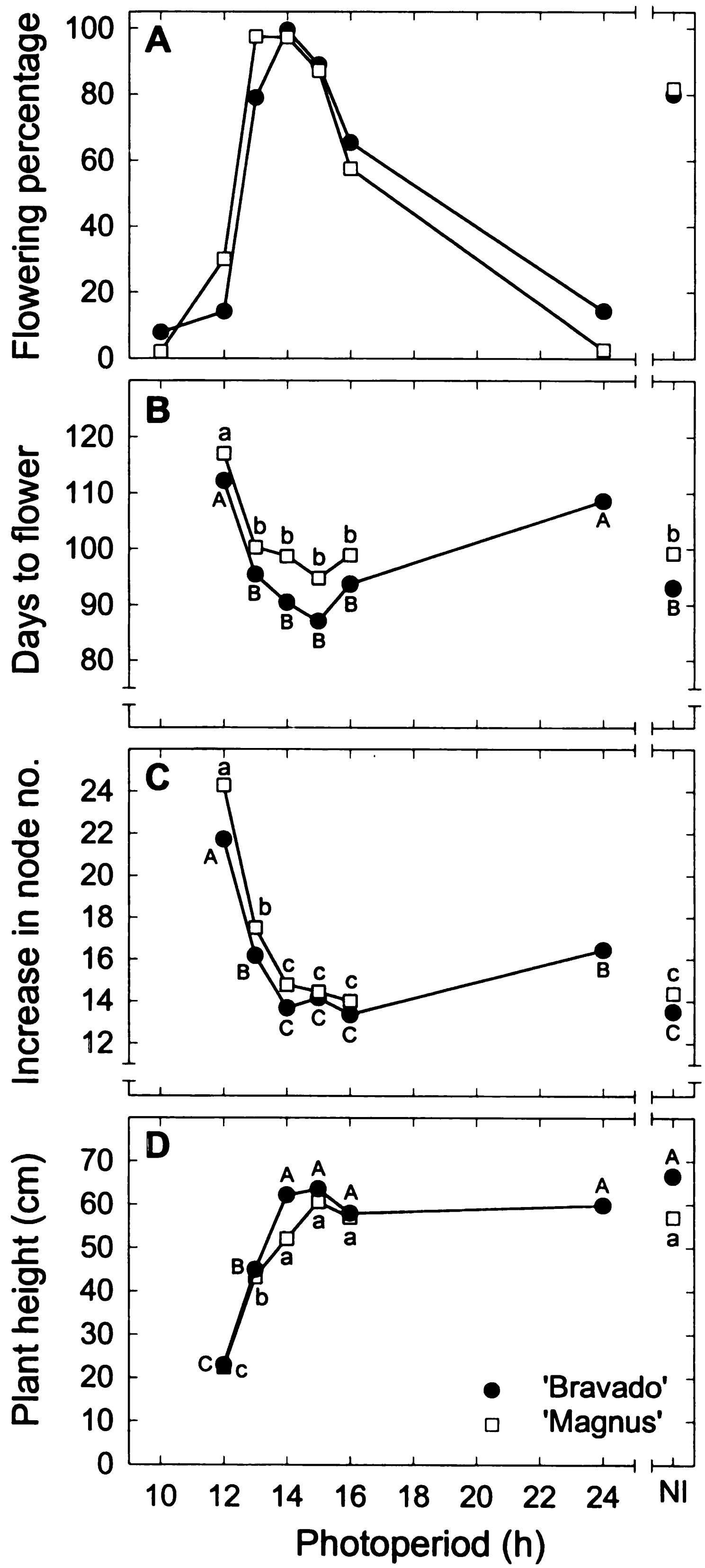
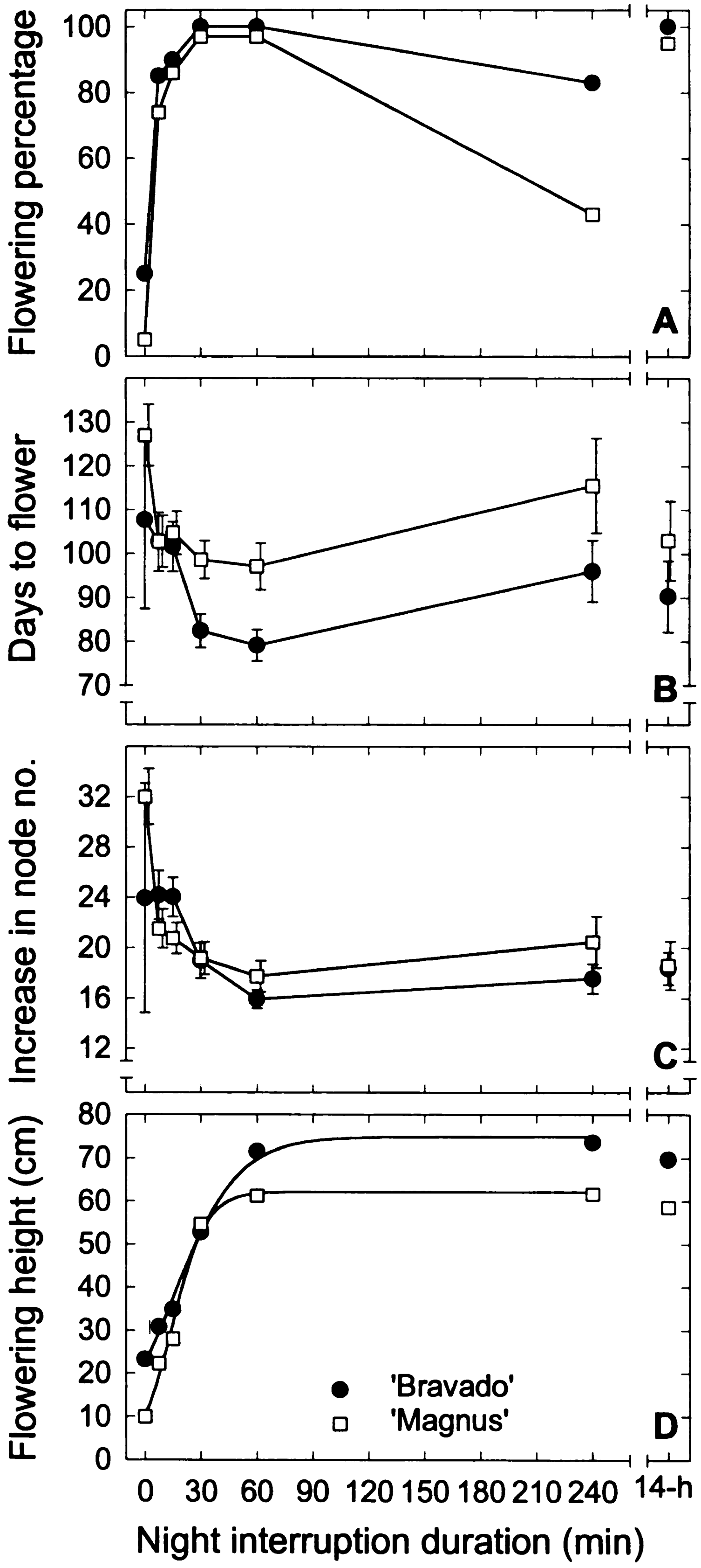

A
B
C
D
Flowering percentage
Days to flower
Increase in node no.
Plant height (cm)
Photoperiod (h)
NI
'Bravado'
'Magnus'

Fig. 3. Flowering of *Echinacea purpurea* 'Bravado' and 'Magnus' under various durations of night interruption lighting or with a 5-h day extension (14-h photoperiod). Photoperiods consisted in 9-h natural days with light from incandescent lamps during the middle of the dark period (night interruption) or following the natural photoperiod for 5 h (14-h photoperiod). Error bars are 95% confidence intervals. Legend in D applies to all figures. Nonlinear regression analysis was used to describe plant height (cm) as a function of night interruption duration (minutes) with the equation in the form $y = a/(1 + \exp(-(x - x_0)/b))$. For 'Bravado' and 'Magnus', respectively, a = 74.87 and 62.13; b = 17.37 and 9.06; x_0 = 15.01 and 14.73; and R^2 = 0.994 and 0.989.



Flowering percentage
100
80
60
40
20
0
A
Days to flower
130
120
110
100
90
80
70
B
Increase in node no.
32
28
24
20
16
12
C
Flowering height (cm)
80
70
60
50
40
30
20
10
0
'Bravado'
'Magnus'
D
0
30
60
90
120
150
180
210
240
14-h
Night interruption duration (min)

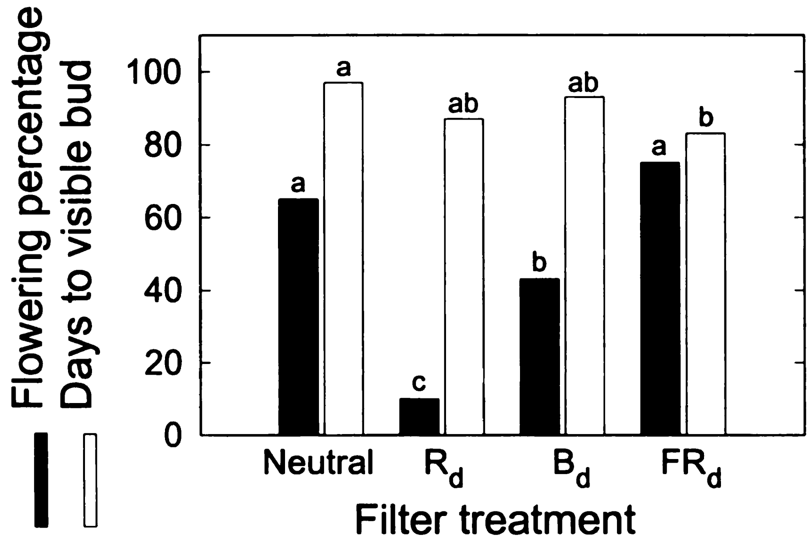


Fig. 4. Flowering of *Echinacea purpurea* 'Bravado' under a neutral-density filter or filters that selectively reduced transmission of red (R_d, 600 to 700 nm), blue (B_d, 400 to 500 nm), or far-red (FR_d, 700 to 800 nm) light. See Fig. 1 and Table 2 for filter transmission properties. Photoperiods consisted in day lengths extended with light from supplemental high-pressure sodium lamps positioned above filters from 0600 to 2200 HR. Values with the same letter are not statistically different at $P = 0.05$.

CHAPTER III

SPECIFIC FUNCTIONS OF RED, FAR-RED, AND BLUE LIGHT IN FLOWERING AND STEM EXTENSION OF LONG-DAY PLANTS

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Specific Functions of Red, Far-Red, and Blue Light in Flowering and Stem Extension of Long-Day Plants

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Abstract

For many long-day plants (LDP), adding far-red light (FR, 700 to 800 nm) to red light (R, 600 to 700 nm) to extend the day or interrupt the night promotes extension growth and flowering. Blue light (B, 400 to 500 nm) independently inhibits extension growth, but its effect on flowering is not well described. Here, we determined how R-, FR-, or B-deficient (R_d, FR_d, or B_d, respectively) photoperiods influenced stem extension and flowering in five LDP species: *Campanula carpatica* Jacq., *Coreopsis ×grandiflora* Hogg ex Sweet, *Lobelia ×speciosa* Sweet, *Pisum sativum* L., and *Viola ×wittrockiana* Gams. Plants were exposed to R_d, FR_d, B_d, or normal (control) 16-hour photoperiods, each of which had a similar photosynthetic (400 to 700 nm) photon flux. Compared with that of the control, the R_d environment promoted extension growth in *C. carpatica* (by 65 %), *C. ×grandiflora* (by 26 %), *P. sativum* (by 23 %), and *V. ×wittrockiana* (by 31 %). The FR_d environment suppressed extension growth in *C. ×grandiflora* (by 21 %), *P.*

sativum (by 17 %), and *V.* ×*wittrockiana* (by 14 %). Independent of the R : FR ratio, the B_d environment promoted stem extension (by 10 % to 100 %) in all species, but there was little or no effect on flowering percentage and time to flower. Extension growth was generally linearly related to the incident wide band (100 nm) R : FR or estimated phytochrome photoequilibrium except when B light was specifically reduced. A high R : FR (i.e., under the FR_d filter) delayed flower initiation (but not development) in *C. carpatica* and *C.* ×*grandiflora* and inhibited flower development (but not initiation) in *V.* ×*wittrockiana*. Therefore, B light and the R : FR independently regulate extension growth by varying magnitudes in LDP, and in some species, an FR_d environment can suppress flower initiation or development.

Introduction

Plants detect light quality by at least three families of photoreceptors: phytochromes, cryptochromes, and an unidentified UV light receptor. Phytochrome absorbance peaks are in the red (R, 600 to 700 nm) and far-red (FR, 700 to 800 nm) light, and to a lesser extent in blue (B, 400 to 500 nm) light. For any one phytochrome, there exists a photoequilibrium of two interconvertible forms: the R and FR absorbing forms, which are known as P_r and P_{fr}, respectively. P_{fr} is considered to elicit physiological responses but P_r could be the active form of phytochrome A (Shinomura et al., 2000). In addition, intermediate short-lived forms between P_r and P_{fr} exist.

Depending on light quality, a phytochrome photoequilibrium [$P_{fr}/(P_r + P_{fr})$, or P_{fr}/P] is established, where a high R : FR creates a high P_{fr}/P, and vice versa. Models have been developed (Hayward, 1984; Sager et al., 1988) to estimate the P_{fr}/P based on the distribution of incident spectral radiation from 300 to 800 nm. These models are based on cross-section phytochrome A data from etilated oats, but its validity in estimating P_{fr}/P in light-grown plants or for other phytochromes is unknown. Nevertheless, these estimates and R : FR ratios are useful in associating photomorphogenic responses with light quality (Smith, 1994).

In addition to phytochromes, B light is absorbed by cryptochromes. Cryptochromes participate in the inhibition of stem extension, particularly those in Brassicaceae (Kigel and Cosgrove, 1991). The suppression of extension growth by B light has been well documented, especially in hypocotyls and epicotyls (Casal and Smith, 1989; Laskowski and Briggs, 1989; Liscum et al., 1992; Warpeha and Kaufman, 1989). In addition to inhibiting stem extension, B light participates in flower induction in some LDP, such as *Hyoscyamus niger* L. (Schneider et al., 1967; Stolwijk and Zeevaart, 1955) and *Arabidopsis thaliana* Heynh. (Bagnall et al., 1996; Lin et al., 1996; Mozley and Thomas 1995). Some of the cryptochrome effects are independent of phytochrome, while others are conditionally interactive (Casal and Mazzella, 1998; Poppe et al., 1998). Compared with data on phytochrome, however, relatively little is known about how B light regulates flowering.

Plants are classified by their photoperiodic flowering response [e.g., short-day plants (SDP) or LDP]. Alternatively, plants that are controlled primarily by light or dark processes can be classified as light- or dark-dominant plants, respectively (Thomas and Vince-Prue, 1997). Most light- and dark-dominant plants are LDP and SDP, respectively, but some exceptions exist. Light-dominant LDP show two general characteristics: 1) a quantitative relationship exists between the irradiance of the night break and the magnitude of the flowering response, until a saturation light intensity, duration, or both, are reached; 2) flowering is often most rapid when photoperiods contain some minimal amount of FR. In contrast, flowering of dark-dominant plants, which include most SDP, can be regulated by a short night break (e.g., $\leq$ 30 min) containing little or no FR light.

Artificial long days (LD) can be created by lighting at the end of the natural photoperiod or by interrupting the dark period with a light break. Flowering of LDP is promoted most when artificial lighting contains R and FR (creating a moderately low P_{fr}/P) compared with light deficient in FR (creating a high P_{fr}/P; Downs and Thomas, 1982; Lane et al., 1965). However, extension growth of a wide range of species shows an inverse linear relationship with estimated P_{fr}/P (Smith, 1982, 1994). Therefore, incident light creating a moderately low P_{fr}/P simultaneously promotes flowering and stem extension, and that creating a high P_{fr}/P is inhibitory to both responses.

Plastic filters that selectively reduce transmission of red light have been used experimentally to promote extension growth in various herbaceous plants (Kubota et al., 2000; Murakami et al., 1996; Rajapakse et al., 1999). To limit stem extension, flexible plastic filters have been developed to reduce transmission of FR radiation (Oyaert et al., 1999; Rajapakse et al., 1999; van Haeringen et al., 1998). These FR_d filters have reduced extension growth in a variety of species, including vegetables and ornamental plants. However, an FR_d environment can delay flowering in some plants, but in many reports, this delay has not been addressed.

The primary objective of our experiments was to determine how flowering and stem extension in LDP were regulated by photoperiods deficient in R, FR, and B. First, specific wave band effects were identified by comparing plant responses under photoselective filters with those under a neutral (N) filter that transmitted a similar photosynthetic photon flux *(PPF)*. Two subsequent experiments were performed to further quantify how light deficient in FR regulated flower initiation and development. These studies illustrate the variability in species' responses to light quality and underscore the complexity of how light regulates flowering and stem extension in LDP. In addition, while an FR_d environment can suppress extension growth in plants, it can also delay flowering in some LDP.

Materials and Methods

Stem extension and flowering under photoselective filters (Expt. 1)

Plant material. Seed of *Campanula carpatica* 'Blue Clips', *Coreopsis ×grandiflora* 'Early Sunrise', and *Lobelia ×speciosa* 'Compliment Scarlet' were sown into 128-cell (10-mL) plug trays and that of *Viola ×wittrockiana* 'Crystal Bowl Yellow' into 288-cell (6-mL) plug trays by a wholesale plug producer (Rakers Acres, Litchfield, Mich.). Seedlings were grown under photoperiods ≤12 h at 22.0 ± 2 °C. *Pisum sativum* 'Utrillo' seed were sown into 50-cell (85-mL) plug trays. Seeding, shipping, and forcing (the onset of treatments) dates are provided in Table 1. Plants were thinned to one per plug and held at 20 °C until plugs were mature. At the onset of experiments, plants were transplanted into 13-cm (1.1-L) square plastic containers and node counts were recorded (Table 1).

Plant culture. Plants were grown in a commercial soilless medium composed of composted pine bark, vermiculite, Canadian sphagnum peat, coarse perlite with a wetting agent, and lime (High Porosity Mix, Strong-Lite Products, Pine Bluff, Ark.). Plants were fertilized at every irrigation with a nutrient solution of well water acidified with H_2SO_4 to a titratable alkalinity of ≈130 mg $CaCO_3 \cdot L^{-1}$ and water soluble fertilizer [125N–12P–125K ($mg \cdot L^{-1}$) plus 1.0Fe–0.5Mn–0.5Zn–0.5Cu–0.1B–0.1Mo ($mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago, Ill.)].

Lighting and filter treatments. A reciprocal transfer experiment with four different light quality environments was conducted using a neutral (N) density metalized woven fabric or plastics that selectively reduced the transmission of B (B deficient, B_d), R (R deficient, R_d), or FR (FR deficient, FR_d) light. Filter treatments were designed to transmit a similar *PPF*. The following filters (one layer each) enclosed greenhouse benches to provide the light quality treatments: N, OLS50 (Ludvig Svensson, Charlotte, N.C.) + PLS Clear (Ludvig Svensson); B_d, Lee filter 101 (Andover, UK) + OLS40 (Ludvig Svensson); R_d, Lee filter 115; FR_d, experimental FR filter (van Haeringen et al., 1998) + PLS Clear. Solar spectra transmissions from 400 to 800 nm were measured under filters by a spectroradiometer (LI-1800, LI-COR, Inc., Lincoln, Nebr.) and are shown in Fig. 1. Quantum ratios (R : FR, B : R, and B : FR), the estimated P_{fr}/P (Sager et al., 1988), and the relative quantum efficiency (McCree, 1972) were quantified for each light treatment (Table 2).

Twenty plants of each species were placed (≈8 cm apart) under each of the B_d, R_d, and FR_d treatments, and 40 under the N filter. When flower buds were visible [visible bud (VB)], 10 plants under the B_d, R_d, and FR_d environments were transferred to the N light treatment, and 10 were transferred at VB from the N environment to each other light environments. Filter effects on flowering and stem extension before and after flower initiation could thus be separated. The experiment was performed twice.

A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium (HPS) lamps positioned above filters. From 0600 to 2200 HR, HPS lamps provided at plant level a supplemental *PPF* of ≈35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when the ambient greenhouse *PPF* was < 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and were shut off when the ambient *PPF* was > 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The light quantum ratios under the filters were calculated at night when the lamps were the only light source (Table 2). Under each filter treatment, the average photosynthetic daily light integral (DLI) was measured at canopy level with line quantum sensors that included 18 G2711 photodiodes (Hamamatsu Co., Hamamatsu, Japan) connected to a CR10 datalogger (Campbell Scientific, Logan, Utah). Each line quantum sensor was independently calibrated under the filters by using the spectroradiometer (Table 1).

Greenhouse temperature control. All plants were grown in a glass greenhouse at 20 °C. Air temperatures under each filter treatment were monitored with 36-gauge (0.127-mm-diameter) type E thermocouples connected to CR10 dataloggers (Campbell Scientific). To provide uniform night temperatures, dataloggers were used to control 1500-W electric heaters under each bench, which provided supplemental heat as needed throughout the night. To improve temperature uniformity under filters during the day, dataloggers were used to control portable fans to vent each bench as needed. The dataloggers collected temperature data every 10 s and recorded the hourly averages. For each experiment, average daily air temperatures from the beginning of treatment until the average date of VB under each filter were calculated (Table 1).

Data collection and analysis. Experiments were replicated in time and were arranged in a completely randomized design. The date the first flower bud was visible (without dissection) and the date the first flower reached anthesis (flowering) were recorded for each plant. At flowering, visible flower buds or inflorescences and nodes on the main stem were counted. Except for *V. ×wittrockiana*, plant height (from soil level to the top of inflorescence) at VB was measured. Total plant height at flowering was measured for all species except *P. sativum*. Plants were considered nonflowering if flower buds were not visible after 32 or 63 d of treatments for *P. sativum* and *V. ×wittrockiana*, respectively, and after 15 weeks for *C. carpatica*, *C. ×grandiflora*, and *L. ×speciosa*. Leaves and stems of *P. sativum* were weighed after 32 d of treatments, and dry weight was measured following two days at 55 °C. Days to VB, days from VB to flower, days to flower, and node-count increase from the start of treatments were calculated. Data were analyzed by using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA), general linear models (GLM) procedures, and a mean separation procedure for unequal observation numbers (pdiff) with $P = 0.05$. Unless otherwise stated, all comparisons made are relative to responses under the N filter.

Viola reciprocal transfer experiment (Expt. 2)

A separate experiment was performed with 'Crystal Bowl Yellow' to determine how plant age influenced the sensitivity of *V. ×wittrockiana* to the flowering inhibition under the FR_d filter. Plants were placed under the N and FR_d filters, then were transferred to the other filter after 5, 10, 15, 20, 25, 30, 35, or 40 d. The corresponding average node numbers at transfer times were 1.9, 2.8, 4.0, 5.0, 5.9, 6.9, 7.3, and 8.8. Experimental conditions were as described above unless otherwise stated. Seeding, shipping, and forcing dates, average temperatures, and DLI are provided in Table 1. The following data were recorded: date of flowering (anthesis), node count increase to the first VB and first open flower, and whether first flowering was on the primary or an axillary shoot. Flowering percentage, days to flower, node-count increase to the first VB and flowering, undeveloped buds below the first open flower, and axillary flowering percentage were calculated. The experiment was terminated 21 weeks after initiation.

Coreopsis transfer experiment (Expt. 3)

Only approximately half of the *C. ×grandiflora* flowered in Expt. 1. We attributed the low flowering percentage to the relatively low DLI provided to plants. Therefore, a third experiment was performed to determine if plants could be induced under naturally high light then transferred to the N or FR_d filters until flowering. Plants were grown under unfiltered, natural photoperiods supplemented from 0600 to 2200 HR with HPS lamps (as described above) but with a *PPF* ≈100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 0, 2, 3, and 4 weeks under high light, 10 plants were transferred to the N and FR_d filters until flowering. Experimental conditions were as described above unless otherwise stated. Seeding, shipping, and forcing dates and average temperature and DLI from forcing to flowering are provided in Table 1. The dates of VB and flowering were recorded. At flowering, visible flower buds and nodes on the main stem were counted and total plant height was measured. Flowering percentage, days to flower, and node-count increase to flowering were calculated.

Results

Experiment 1

Stem extension. The R_d environment increased plant height from forcing to VB by 65 % in *C. carpatica* and 23 % in *P. sativum* compared with that in the N environment (Fig. 2). The B_d environment promoted internode elongation in all species studied. Compared with that under the N filter, the B_d filter increased stem length from forcing until VB by 100 %, 72 %, 17 %, and 16 % in *C. carpatica*, *C.* ×*grandiflora*, *L.* ×*speciosa*, and *P. sativum*, respectively.

The R_d treatment increased stem and inflorescence elongation from VB to flowering by 26 % or 30 % in *C.* ×*grandiflora* and *V.* ×*wittrockiana*, respectively (Fig. 3). The FR_d filter suppressed extension growth in only two species, *P. sativum* (stem length by 20 %) and *V.* ×*wittrockiana* (stem and peduncle length by 14 % and 33 %, respectively). Changes in R or FR light did not influence stem extension of *L.* ×*speciosa.* From VB to flowering, the B_d environment increased stem and inflorescence elongation in *C.* ×*grandiflora*, *L.* ×*speciosa*, and *V.* ×*wittrockiana* by 31 %, 11 %, and 10 %, respectively. In addition, the B_d filter increased peduncle length of *V.* ×*wittrockiana* by 27 %.

Flowering. The FR_d environment inhibited flowering in *V.* ×*wittrockiana*; 88 % and 65 % of plants under the FR_d filter reached VB and flowering, respectively, whereas all plants flowered in the other three light environments (data not shown). The B_d, R_d, and FR_d environments had no significant effect on flowering percentage of the other species studied. Essentially all *C. carpatica*, *L.* ×*speciosa*, and *P. sativum* reached VB and flowered under all light quality treatments, but irrespective of filter treatment (including that under the N filter), only 50 % and 42 % of *C. ×grandiflora* reached VB and anthesis, respectively (data not shown).

Light deficient in R delayed time to VB by 4 or 1 d in *C. carpatica* and *P. sativum* and hastened it by 4 d in *V. ×wittrockiana* (Figs. 4A and 5A). However, the delay in time to VB under the R_d filter was not accompanied by the formation of more nodes before VB appearance in any species (Fig. 4B). The R_d environment had no effect on time from VB to flowering for any species (data not shown). However, the R_d treatment reduced flower number by 54 % or 33 % in *C. carpatica* and *L. ×speciosa*, respectively, and reduced dry matter accumulation by 40 % in *P. sativum* (Figs. 4C and 4D).

The FR_d environment delayed appearance of VB in *C. carpatica* by 2 d and *C. ×grandiflora* by 14 d (Fig. 4A) and delayed flowering of *V. ×wittrockiana* by 21 d (Fig. 5B). *Viola ×wittrockiana* developed an average of 9.1 or 12.4 nodes before flowering under the N or FR_d treatments, respectively (significantly different at $P < 0.05$; data not shown). In addition, *C. carpatica* and *C. ×grandiflora* developed more nodes before flowering under the FR_d environment than those under the N filter (Fig. 4B). The FR_d environment increased flower number of *L. ×speciosa* by 44 % but not for any other species (Fig. 4C).

A deficiency in B light hastened time to VB in *P. sativum* (by 1 d) and *V. ×wittrockiana* (by 4 d) but did not influence node number (Figs. 4A, 4B, and 5C). The B_d environment had no effect on VB timing of the other three species. In addition, the B_d environment had no effect on time from VB to flowering for any species (data not shown). However, flower number of *C. carpatica* and *L. ×speciosa* and dry weight of *P.*

sativum were significantly greater under the B_d filter (by 15 %, 19 %, and 37 %, respectively, Figs. 4C and 4D).

Extension growth of a variety of species shows an inverse linear relationship with estimated P_{fr}/P (Smith, 1982, 1994). Using data in our studies, we compared how extension growth was related to R : FR ratios and the estimated P_{fr}/P under the filter treatments (Table 2; Fig. 6). Stem or internode length under the N, R_d, and B_d environments was compared with that under the FR_d filter, in which stems were shortest. Using results under the R_d, N, or FR_d environments, stem extension was not linearly related to narrow band (10 nm) R : FR ratios (Fig. 6A). However, stem extension was linearly related to a wide band (100 nm) R : FR ratio (Fig. 6B) or estimated P_{fr}/P (Fig. 6C). The promotion of stem extension in the B_d environment did not fit any of the relationships.

Experiment 2

In the reciprocal transfer experiment, all *V. ×wittrockiana* flowered under the N filter, in an average of 83 d. Under the FR_d environment only 81 % flowered, in an average of 108 d (Table 3). Plants initiated flowers at approximately the seventh node under both filter treatments, but under the N filter, the first flower developed to anthesis at least two nodes sooner than under the FR_d filter. Thus, the number of initiated but undeveloped buds below the first open flower was significantly higher under the FR_d filter.

Experiment 3

During the first two weeks of the experiment, the average DLI was 11.8 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ under the unfiltered, high light environment and 4.3 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ under the N filter. *Coreopsis ×grandiflora* that received ≥2 weeks of LD with a high DLI developed about two fewer nodes and flowered 11 or 12 d earlier than plants held under the same photoperiod but under the N or FR_d filters (Table 4). The FR_d filter reduced plant height by 21 % regardless of the duration of high light exposure but did not influence any other measured characteristic.

Discussion

Here, we show the variability in LD species' responses to environments deficient in R, FR, and B light. Relative to the five LDP studied, the sensitivity of extension growth to R : FR was small in *L. ×speciosa*, moderate in *P. sativum* and *V. ×wittrockiana*, and large in *C. carpatica* and *C. ×grandiflora*. Independently, stem extension was promoted in all species under the B_d environment, but the lack of B light had little or no effect on flowering percentage and time to flower. Although the R : FR had no effect on flowering time of *L. ×speciosa* and *P. sativum*, a high R : FR delayed flower initiation in *C. carpatica* and *C. ×grandiflora* and inhibited flower development in *V. ×wittrockiana*.

Although the average DLI was relatively low (Table 1), time to flower of the same cultivars of *C. carpatica*, *C. ×grandiflora*, *L. ×speciosa*, and *V. ×wittrockiana* was similar to that in published research using similar temperatures and identical photoperiods (Runkle and Heins, 1998; Runkle et al., 1998; Runkle et al., 1999; Whitman et al., 1997). In addition, a high daily light integral (> 6 $mol{\cdot}m^{-2}{\cdot}d^{-1}$) was required for complete flowering in *C. ×grandiflora*, since flowering was promoted when plants were provided with ≥2 weeks of high light.

Stem extension in *C. carpatica*, *C. ×grandiflora*, *P. sativum*, and *V. ×wittrockiana* showed an inverse linear relationship with a wide band R : FR or estimated P_{fr}/P, similar with that reported in other species (Ritchie, 1997; Smith, 1982, 1994). Narrow band R : FR ratios were not linearly related to stem extension of these species. However, the promotion of stem extension in the B deficient environment did not fit any of the relationships, including the estimated P_{fr}/P that accounts for phytochrome absorption in the B region. Thus, our data indicate that relating stem extension to R : FR ratios or estimated P_{fr}/P is invalid when B light levels differ significantly from that in the natural environment.

Blue light inhibits extension growth in a variety of plants [e.g., pea, pepper (*Capsicum annuum* L.), and mustard (*Sinapis alba*)] and tissues (hypocotyls, epicotyls, and stems; Brown et al., 1995; Casal and Smith, 1989; Laskowski and Briggs, 1989; Liscum et al., 1992). Our studies suggest that B light plays a role equal to or greater than that of R : FR in mediating stem extension in LDP; internode extension in all five LDP species studied was promoted under the B_d filter as well as or better than that under the R_d filter. The stem extension responses appear to be independent of phytochrome, since the R : FR and P_{fr}/P under the B_d treatment were nearly identical to that under the N treatment (Table 2). In *Arabidopsis* and *Hyoscyamus*, the absorption of B light accelerates flowering (Bagnall et al., 1996; Guo et al., 1998; Lin et al., 1996; Mozley and Thomas, 1995; Stolwijk and Zeevaart, 1955), but in the five LDP presented here, B light had little or no effect on flowering percentage or timing. Under the B_d filter, transmission of B light was < 10 % of that under the N filter. However, it is possible that enough B light penetrated the B_d filter to saturate any B-mediated effect on flowering without saturating a B-mediated effect on extension growth.

In LDP, various experiments suggest that flowering is promoted most when R light (or light creating a high P_{fr}/P) is delivered at least during the early part of the photoperiod and FR light (or light creating a lower P_{fr}/P) toward the end (Evans, 1976; Lane et al., 1965; Thomas and Vince-Prue, 1997). In our studies, R_d photoperiods had little or no effect on flowering percentage and timing and node count increase, which suggests that a high P_{fr}/P during the first half of the photoperiod may not necessarily be promotive to flowering in some light-dominant LDP. However, flowering was inhibited in three of the five species studied when the P_{fr}/P was high, and was specific to initiation in *C. carpatica* and *C. ×grandiflora* and development in *V. ×wittrockiana*. Flower initiation was delayed in an FR_d environment in the LDP snapdragon (*Antirrhinum majus* L.; van Haeringen et al., 1998). The requirement for FR light for rapid flower development, but not initiation, was reported in the *Hyoscyamus* (Downs and Thomas, 1982). These findings support the hypothesis proposed by Thomas and Vince-Prue (1997) that an FR response, especially toward the end of the photoperiod, is specific to postinductive flower development in some LDP rather than an effect on induction. In other LDP, an FR response is specific to flower initiation. Interestingly, flower development of the SDP chrysanthemum [*Dendranthema ×grandiflorum* (Ramat) Kitamura] was delayed under weakly inductive N photoperiods compared to the same photoperiod deficient in FR, which suggests that, depending on the timing, FR light can have opposite effects on flower development in SDP and LDP (McMahon, 1999; Rajapakse and Kelly, 1995).

Within the photosynthetically active wave band (400 to 700 nm), photons are not equally effective at producing photosynthesis. Red light, especially around 600 nm, is the most efficient wave band, whereas B light is ≈80 % as efficient (McCree, 1972; Sager et al., 1988). A model has been developed to quantify the relative quantum efficiency (RQE), which is useful in predicting the photosynthetic capacity of a given light quality (Sager et al., 1988). The RQE was lower (by 13 %) under the R_d filter and higher (by 5 %) under the B_d filter compared with that of the N filter (Table 2). Flower number of *C. carpatica* and *L. ×speciosa* and dry weight of *P. sativum* were reduced by 33 % to 54 % under the R_d filter and increased by 15 % to 37 % under the B_d filter. Similarly, dry weight of chrysanthemum was reduced under R_d films compared to N filters transmitting a similar PPF (Oyaert et al., 1999). Therefore, differences in flower number and dry weight among lighting treatments could be at least partially attributed to the different RQE and suggest that relatively small changes in RQE can have large effects on growth.

Plants absorb most visible light (400 to 700 nm) but reflect or transmit most FR light, and thus a low R : FR is created under a canopy. In response to such an environment, extension growth and flowering are promoted in shade-avoiding species (Smith, 1994). The ecological strategy of the shade-avoidance syndrome is to promote and direct extension growth in an attempt to better harvest available sunlight. In contrast, shade-tolerant species respond to a low R : FR without a significant change in extension growth. Not surprisingly, in our studies the species most adaptive to shade in the natural environment *(L. ×speciosa)* was the least responsive to R : FR, while the shade-avoiding species *C. carpatica* and *C. ×grandiflora* were sensitive to R : FR (Armitage, 1989). Light under a canopy is also deficient in B, and since a B_d environment promoted extension growth in all species we studied, an alternative shade-avoiding ecological strategy could be mediated by B light. These potentially redundant shade-avoiding strategies are consistent with studies demonstrating independent, interactive, and redundant actions of the B-absorbing and R- and FR- absorbing photoreceptors (cryptochrome and phytochrome, respectively) in *Arabidopsis* (Casal and Mazzella, 1998; Poppe et al., 1998).

The species in which flowering and extension growth was influenced the most by R : FR was *V. ×wittrockiana*. Plants under the R_d filter (e.g., low R : FR) were strongly apically dominant; stem extension and flowering were promoted, and in nearly all instances, first flowering occurred on the primary stem. In contrast, branching was promoted and flowering was inhibited under the FR_d filter (e.g., high R : FR). Of those that flowered under the FR_d filter, a significant proportion (25 % in Expt. 2) of plants first flowered on an axillary stem (Table 3).

In summary, our data illustrate the variability in how light quality influences flowering in LDP and demonstrate that although an FR_d environment can suppress stem extension, it can also delay flowering in some species. A deficiency in FR light can specifically delay flower initiation in some species (e.g., *C. carpatica* and *C. ×grandiflora*) and flower development in others (e.g., *V. ×wittrockiana*). In the former (but not latter) species, plants can be induced to flower under N photoperiods then transferred to an FR_d environment to facilitate rapid flowering with reduced stem extension. The filters used reduced, but did not completely eliminate, the transmission of certain wavebands of light. However, our results suggest that extension growth can be attributed to a wide (but not narrow) band R : FR or P_{fr}/P, except when the B component is significantly altered. Therefore, blanket statements are inappropriate when discussing flowering and stem extension responses of LDP to light quality. Currently, we are performing additional studies to determine if various lighting strategies can suppress stem extension in sensitive LDP without an inhibition in flowering. Further research is also merited to determine how B light interacts with the R : FR to regulate extension growth in plants outside the Brassicaceae.

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Table 1. Seed, shipping, and forcing dates, initial node counts, and average air temperature and photosynthetic daily light integral during experiments. Environmental data were calculated from date of forcing to average date of visible bud (Experiment I), average date of flowering (Experiment III), or 144 d from forcing (Experiment II) under each treatment. N = neutral-density; R_d = red (600 to 700 nm) deficient; B_d = blue (400 to 500 nm) deficient; FR_d = far-red (700 to 800 nm) deficient; na = not applicable; – (long dash) = incomplete data.

Experiment and species	Rep.	Date: Seed	Date: Shipping	Date: Forcing	Initial nodes	Average air temperature during forcing (°C)				Average daily light integral during forcing ($mol \cdot m^{-2} \cdot d^{-1}$)			
						Light environment							
Experiment I						N	R_d	FR_d	B_d	N	R_d	FR_d	B_d
Campanula carpatica	1	20 July 1998	26 Sept. 1998	29 Oct. 1998	8.3	20.2	20.3	20.0	19.9	4.5	3.8	4.0	3.7
	2	30 Nov. 1998	31 Dec. 1998	22 Feb. 1999	11.0	20.7	21.1	20.0	20.4	6.4	5.8	6.6	6.0
Coreopsis ×grandiflora	1	11 May 1998	29 May 1998	9 July 1998	6.1	22.3	22.7	22.5	22.1	5.3	5.8	5.0	5.0
	2	31 Aug. 1998	19 Sept. 1998	21 Oct. 1998	5.8	20.2	20.4	20.2	20.0	4.8	4.0	4.3	4.2
Lobelia ×speciosa	1	18 May 1998	12 June 1998	9 July 1998	5.9	22.5	22.5	22.6	22.4	5.7	5.9	4.7	5.2
	2	31 Aug. 1998	26 Sept. 1998	3 Nov. 1998	6.3	20.3	20.5	20.0	20.1	4.3	3.8	4.0	3.8
Pisum sativum	1	19 June 1998	-	9 July 1998	5.5	22.2	22.3	22.2	22.2	5.9	4.5	4.8	6.4
	2	10 Aug. 1998	-	30 Aug. 1998	6.1	21.3	21.4	21.5	21.2	5.7	6.0	6.1	6.3
Viola ×wittrockiana	1	15 June 1998	14 July 1998	18 July 1998	4.0	22.3	22.7	22.6	22.6	–	–	–	–
	2	20 July 1998	18 Aug. 1998	24 Aug. 1998	4.0	21.8	22.2	21.9	22.2	5.9	6.8	6.4	6.6
Experiment II													
Viola ×wittrockiana		28 Oct. 1998	2 Nov. 1998	9 Nov. 1998	1.0	20.1	na	20.1	na	4.8	na	4.9	na
						Weeks at high DLI							
Experiment III						0	2	3	4	0	2	3	4
Coreopsis ×grandiflora		30 Nov. 1998	1 Jan. 1999	29 Jan. 1999	4.8	20.3	20.6	20.7	20.7	5.7	7.5	8.5	9.6

Table 2. Quantum ratios of red (R), far-red (FR), and blue (B) light, calculated phytochrome photoequilibria (P_{fr}/P), and relative quantum efficiency (RQE) under filters with sun or high-pressure sodium (HPS) lamps as the sole light source (McCree, 1972; Sager et al., 1988). B = 400 to 500 nm; FR = 700 to 800 nm; FR_n = FR narrow band width (725 to 735 nm); R = 600 to 700 nm; R_n = R narrow band width (655 to 665 nm).

	Light source										
	Sun						HPS lamps				
Filter	$R_n : FR_n$	R : FR	B : R	B : FR	P_{fr}/P	RQE	$R_n : FR_n$	R : FR	B : R	B : FR	P_{fr}/P
Neutral	1.06	1.07	0.75	0.81	0.715	0.889	2.82	3.98	0.17	0.69	0.850
R_d	0.04	0.04	30.72	1.16	0.399	0.785	0.12	0.15	7.16	1.05	0.624
FR_d	8.22	1.74	0.94	1.63	0.798	0.849	9.96	5.73	0.15	0.86	0.873
B_d	1.04	1.05	0.06	0.06	0.723	0.934	2.74	3.82	0.02	0.09	0.851

Table 3. Flowering characteristics of *Viola ×wittrockiana* under the neutral (N) or far-red deficient (FR_d) filter. Plants were transferred from the N to the FR_d filter, or vice versa, following 5, 10, 15, 20, 25, 30, 35, or 40 d. Data were pooled by filter type because transfer time and filter × transfer time interaction had no significant effect on any measured characteristics.

	Final environment		
Characteristic	N	FR_d	Significance
Flowering (%)	100	81	***
Axillary flowering (%)	4	25	***
Days to flower	83	108	***
Node of first visible bud	7.2	6.8	NS
Node of open flower	11.7	14.1	***
Undeveloped flower buds[z]	4.6	7.4	***

[z]Below the first open flower.

NS, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

Table 4. Flowering responses of *Coreopsis* ×*grandiflora* transferred from an unfiltered 16-h photoperiod with supplemental high pressure sodium lamps [delivering a high daily light integral (DLI)] to a neutral (N) or far-red deficient (FR_d) filter.

	Flowering (%)	Days to flower	Increase in node no.	Flower no.	Height (cm)
Weeks at high DLI					
0	90	65.5	8.4	9.9	25.4
2	95	53.5	6.3	8.2	26.5
3	95	54.4	5.9	11.9	26.4
4	100	53.2	6.8	14.7	26.4
Final filter environment					
N	98	56.9	6.8	10.6	29.2
FR_d	93	56.4	6.8	11.7	23.1
Significance					
Weeks high DLI (WHDLI)		***	***	***	NS
Final filter (FF)		NS	NS	NS	***
WHDLI × FF		NS	NS	NS	NS

NS, *** Nonsignificant or significant at $P \leq 0.001$, respectively.

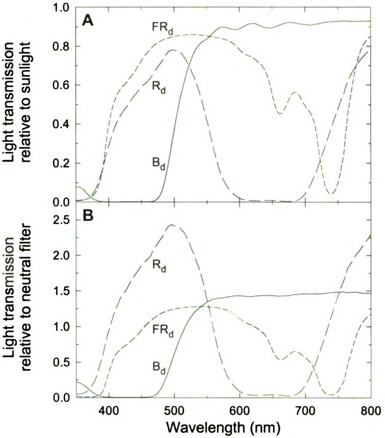


Fig. 1. Spectral transmissions of photoselective filters relative to sunlight (A) or relative to that under the neutral-density filter treatment with an equal photosynthetic photon flux (B). R_d, red (600 to 700 nm) deficient filter; B_d, blue (400 to 500 nm) deficient filter; FR_d, far-red (700 to 800 nm) deficient filter. See Table 2 for light wave band ratios.

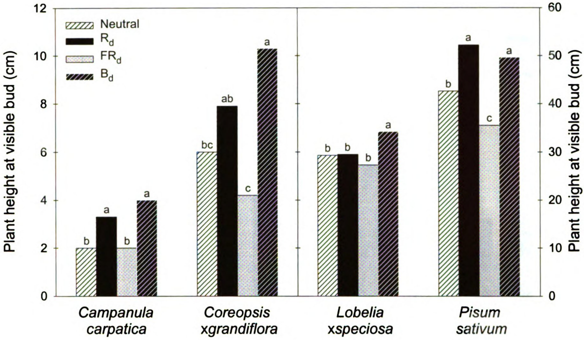


Fig. 2. Plant height at visible bud of *Campanula carpatica*, *Coreopsis ×grandiflora*, *Lobelia ×speciosa*, and *Pisum sativum* under a neutral filter or a light environment deficient in red (R_d, 600 to 700 nm), far red (FR_d, 700 to 800 nm), or blue (B_d, 400 to 500 nm). A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium lamps positioned above filters. Values with the same letter within species are not statistically different at $P = 0.05$.

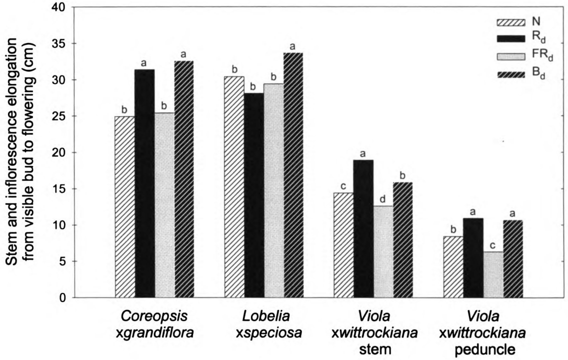
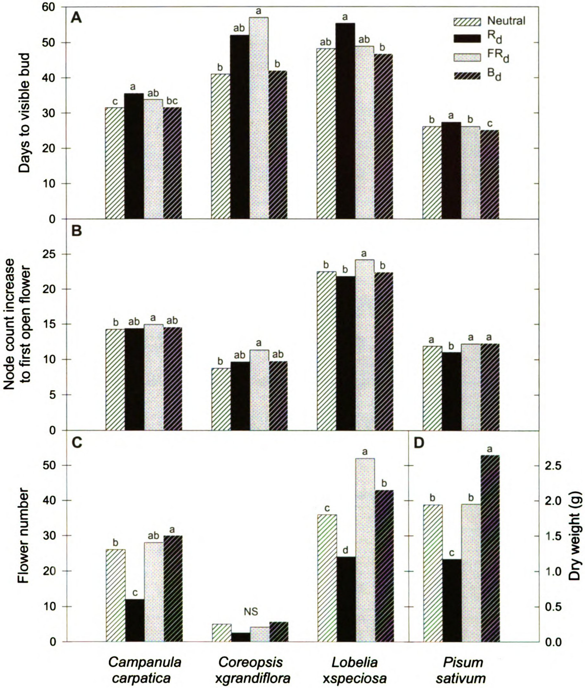


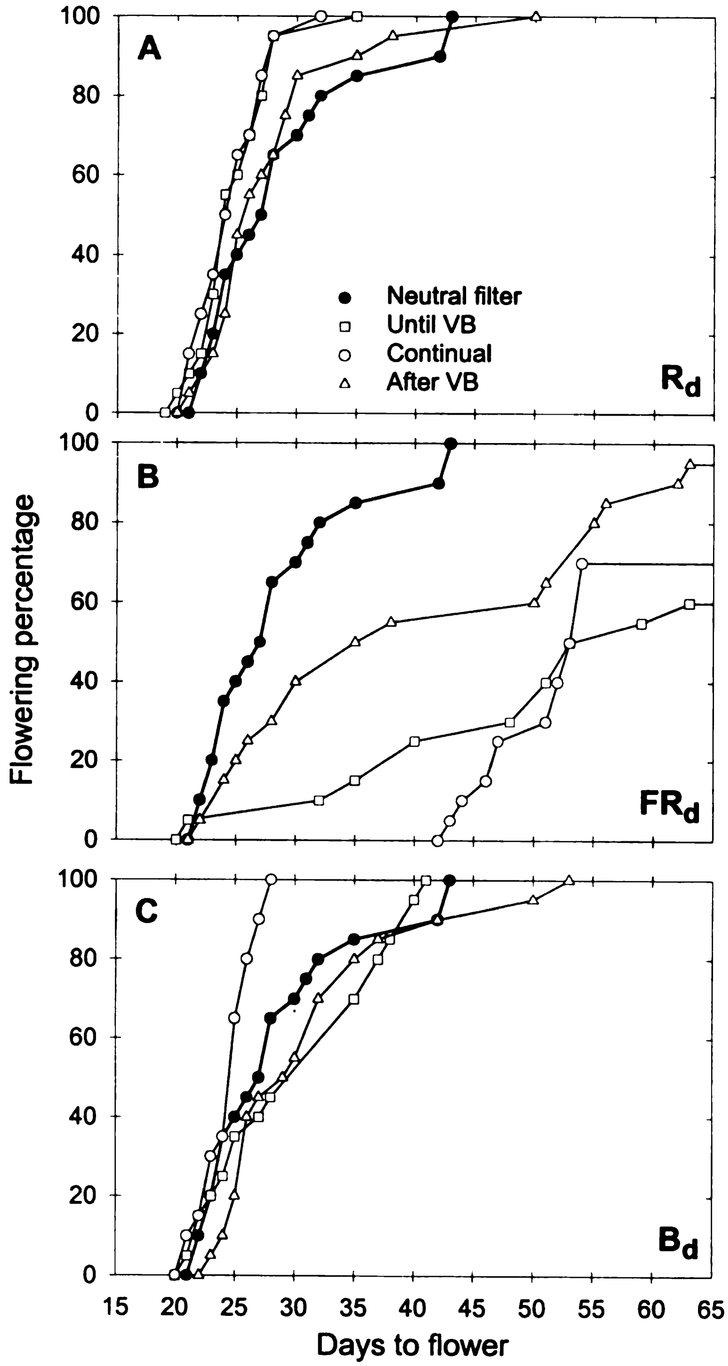
Fig. 3. Stem and inflorescence elongation from visible bud to flowering of *Coreopsis ×grandiflora*, *Lobelia ×speciosa*, and *Viola ×wittrockiana* under a neutral filter or a light environment deficient in red (R_d, 600 to 700 nm), far red (FR_d, 700 to 800 nm), or blue (B_d, 400 to 500 nm). A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium lamps positioned above filters. Values with the same letter within species and measurement are not statistically different at $P = 0.05$.

Fig. 4. Days to visible bud (A), node count increase to first open flower (B), and flower number (C) or dry weight (D) of *Campanula carpatica*, *Coreopsis ×grandiflora*, *Lobelia ×speciosa*, and *Pisum sativum* under a neutral filter or a light environment deficient in red (R_d, 600 to 700 nm), far red (FR_d, 700 to 800 nm), or blue (B_d, 400 to 500 nm). A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium lamps positioned above filters. Values with the same letter within species are not statistically different at $P = 0.05$. Legend in A applies to all figures. NS = not significant.



A
B
C
D
Neutral
R_d
FR_d
B_d
Days to visible bud
Node count increase to first open flower
Flower number
Dry weight (g)
NS
Campanula carpatica
Coreopsis xgrandiflora
Lobelia xspeciosa
Pisum sativum

Fig. 5. Flowering percentage of *Viola ×wittrockiana* under a neutral filter or a light environment deficient in red [R_d, 600 to 700 nm, (A)], far red [FR_d, 700 to 800 nm, (B)], or blue [B_d, 400 to 500 nm, (C)] light. Plants were held under the light treatments until visible bud (until VB), continually, or after visible bud (after VB). Plants were under the neutral filter at all other times. A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium lamps positioned above filters. Legend in A applies to all figures.



A
B
C
Neutral filter
Until VB
Continual
After VB
R_d
FR_d
B_d
Flowering percentage
Days to flower
0
20
40
60
80
100
15
20
25
30
35
40
45
50
55
60
65

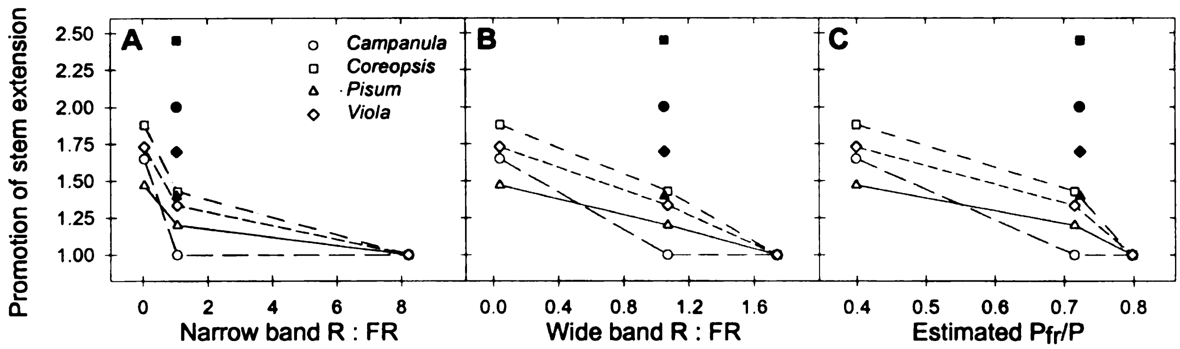


Fig. 6. Stem extension of *Campanula carpatica*, *Coreopsis ×grandiflora*, *Pisum sativum*, and *Viola ×wittrockiana* relative to that under the far-red (FR) deficient filter. Stem length was related to red (R) : FR ratios and the estimated phytochrome photoequilibria (P_{fr}/P) under the filter treatments. The R : FR ratios were determined using narrow [10 nm (A)] and wide [100 nm (B)] band widths. See Fig. 1 and Table 2 for spectral data. Open symbols represent plants exposed to a neutral filter or light deficient in R or FR. Closed symbols represent plants exposed to light deficient in blue (400 to 500 nm). Legend in A applies to all figures.

CHAPTER IV

STEM EXTENSION AND SUBSEQUENT FLOWERING OF SEEDLINGS GROWN UNDER A FILM CREATING A FAR-RED DEFICIENT ENVIRONMENT

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Stem Extension and Subsequent Flowering of Seedlings Grown under a Film Creating a Far-Red Deficient Environment

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Abstract

Photoselective plastic filters that reduce the transmission of far-red light (FR, 700 to 800 nm) have been developed to potentially reduce stem extension and thus plant height. However, an FR-deficient (FR_d) environment can also delay flowering, particularly in long-day plants. Our objective was to determine if seedlings could be grown under an FR_d filter to reduce extension growth without subsequently delaying flowering when grown under natural long photoperiods. Pansy (*Viola ×wittrockiana* Gams), petunia (*Petunia ×hybrida* Vilm.-Andr.), impatiens (*Impatiens walleriana* Hook.), snapdragon (*Antirrhinum majus* L.), and tomato [*Solanum lycopersicon* L. (syn.: *Lycopersicon esculentum*)] were placed under the FR_d filter or a neutral-density filter (N) that transmitted a similar photosynthetic daily light integral. Since a dense plant canopy promotes extension growth, a third treatment consisted in transferring plants from the N to the FR_d filter when leaves of each species began to touch (after 18 or 24 d under the N

filter). After 25 to 35 treatment days with a 16-hour photoperiod at 20 °C, seedlings were measured then grown under natural photoperiods (≥ 14.5 hours) at 20 °C until flowering. Compared with that of seedlings continually under the N filter, stem or petiole length under the FR_d filter was significantly reduced in impatiens (by 10 %), pansy (by 18 %), petunia (by 34 %), snapdragon (by 5 %), and tomato (by 24 %). Extension growth in plants held continually under the FR_d filter was similar to that of plants transferred from the N to FR_d filter when leaves first touched. Flowering of plants from plugs under the FR_d filter was delayed by two to three days in snapdragon, petunia, and pansy. At flowering, flower number and plant height of all species were similar among treatments. Therefore, the FR_d filter presents an attractive method of controlling seedling height with minimal impact on flowering when plants are subsequently grown under unfiltered light.

Introduction

Plant height is a commonly manipulated component of plant architecture, especially in the commercial production of floriculture crops. Promotion of stem extension is useful for production of cut flowers, but more often plant height is suppressed in a variety of bedding and potted plants, container ornamentals, and vegetables. Effectively controlling plant height is important, since purchasers are demanding strict plant morphological specifications, namely, narrow windows of acceptable plant heights.

A common method of reducing extension growth is to apply plant growth-retarding chemicals (e.g., ancymidol, chlormequat, daminozide, paclobutrazol, and uniconazole), which inhibit various steps in the gibberellin biosynthetic pathway (Rademacher, 2000). Although these chemicals can be a convenient method of retarding stem extension, efficacy varies by chemical and species, some chemicals add a considerable production expense, and use has become increasingly restricted.

A variety of cultural and environmental manipulations have been used to reduce plant height, including temperature (Erwin et al., 1989; Erwin and Heins, 1995), fertilization (Melton and Dufault, 1991), watering (Liptay et al., 1997), and mechanical stimulation (Johjima et al., 1992) regimens. Although these strategies are sometimes effective, each technique has limitations. For example, warm outdoor temperatures can prevent implementation of cool-temperature treatments. Therefore, alternative strategies to control extension growth are needed.

Plants absorb most visible light (400 to 700 nm) but reflect or transmit most far-red (FR, 700 to 800 nm) light. Therefore, a low red (R, 600 to 700 nm) : FR is created under a canopy or among closely spaced plants. In response to such an environment, extension growth in shade-avoiding plants is promoted as plants adapt to better harvest available sunlight (Smith, 1994). In contrast, shade-tolerant species respond to a low R : FR without a significant change in extension growth.

To limit stem extension, various materials have been used experimentally to reduce transmission of FR radiation. The recent development of flexible plastic FR filters [creating an FR-deficient (FR_d) environment] offers an alternative method of controlling plant height (Oyaert et al., 1999; Rajapakse et al., 1999; van Haeringen et al., 1998). These FR_d filters have reduced extension growth in a variety of species, including vegetables and ornamental plants (Rajapakse et al., 1999). An FR_d environment can delay flowering in some plants (Runkle and Heins, 2001), but in many reports, this delay has not been addressed.

In this study, we determined how an FR_d filter influenced seedling growth of five common bedding plant species and then quantified what residual effect, if any, the seedling environment had on subsequent flowering.

Materials and Methods

Seedling stage

Plant material. Seed of *Antirrhinum majus* 'Liberty Scarlet', *Impatiens walleriana* 'Accent Rose', *Petunia ×hybrida* 'Carpet Pink', *Solanum lycopersicon* 'Beefmaster' (syn: *Lycopersicon esculentum* Mill.), and *Viola ×wittrockiana* 'Crystal Bowl Yellow' were sown into 288-cell plug trays (6-mL volume) by a wholesale plug producer (Rakers Acres, Litchfield, Mich.) on 26 March 1999 and 5 May 1999 for Rep I and II, respectively. Plants were shipped and received six or eight days later, then were thinned to one per plug.

Lighting and filter treatments. Seedlings were grown on greenhouse benches enclosed by a neutral [N; PLS Clear (Ludvig Svensson, Charlotte, N.C.)] or FR_d (van Haeringen et al., 1998) filter, which transmitted similar photosynthetic photon fluxes *(PPF)*. Since extension growth is promoted most when a canopy becomes closed, a third treatment consisted in transferring plants from the N filter to the FR_d filter when leaves began to touch (after 18 d for tomato and 24 d for the other species). For each replication, three plug trays of each species were cut in half to yield two blocks for each of the three filter treatments. Solar spectra transmissions were measured under filters by a spectroradiometer (LI-1800, LI-COR, Inc., Lincoln, Nebr.; Fig. 1). The R : FR and estimated phytochrome photoequilibrium (P_{fr}/P; Sager et al., 1988) were 1.07 and 0.715

under the N filter and 1.74 and 0.798 under the FR_d filter, respectively. The narrow waveband R : FR [(655 to 665 nm) : (725 to 735 nm)] was 1.06 and 8.22 under the N and FR_d filters, respectively.

A 16-h photoperiod was delivered with a combination of sunlight and high-pressure sodium (HPS) lamps positioned above filters. During Rep I and II, the average natural photoperiod (lat. 43 °N) during the plug stage was ≈14 and 15.5 h, respectively. From 0600 to 2200 HR, HPS lamps provided at plant level a supplemental *PPF* of ≈40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when the ambient greenhouse *PPF* was < 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and were shut off when the ambient *PPF* was > 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average photosynthetic daily light integral, measured at canopy level with quantum sensors (LI-COR) connected to a CR10 datalogger (Campbell Scientific, Logan, Utah), was 9.4 and 7.3 $mol \cdot m^{-2} \cdot d^{-1}$ under the N filter and 9.1 and 7.2 $mol \cdot m^{-2} \cdot d^{-1}$ under the FR_d filter during Rep I and II, respectively.

Plant culture. Plants were fertilized at every irrigation with a nutrient solution of well water acidified with H_2SO_4 to a titratable alkalinity of ≈130 mg $CaCO_3 \cdot L^{-1}$ and water soluble fertilizer [125N–12P–125K ($mg \cdot L^{-1}$) plus 1.0Fe–0.5Mn–0.5Zn–0.5Cu–0.1B–0.1Mo ($mg \cdot L^{-1}$; MSU Special, Greencare Fertilizers, Chicago, Ill.)].

Greenhouse temperature control. All plants were grown in a glass greenhouse at 20 °C. Air temperatures on each bench were monitored with 36-gauge (0.127-mm-diameter) type E thermocouples connected to CR10 dataloggers (Campbell Scientific). To provide more uniform temperatures, dataloggers were used to control 1500-W electric heaters under each bench during the night and portable fans during the day to heat or vent each bench as needed. The dataloggers collected temperature data every 10 s and recorded the hourly averages. The average temperatures under the N and FR_d filters were 20.0 and 19.6 °C during Rep I and 20.8 and 20.9 °C during Rep II, respectively.

Data collection and analysis. Seedlings of each species were deemed ready for transplant after 26, 31, 32, 35, and 35 d for tomato, impatiens, snapdragon, petunia, and pansy, respectively. Typically, seedlings in the outer rows of a plug tray are shorter than those toward the inner rows. Therefore, node number and shoot (hypocotyl plus stem) or longest petiole (for pansy only) length were recorded from each block and treatment from 10 plants in the outer two rows (outside), the next inner two rows (middle), and the innermost rows (inside). Data were analyzed by using SAS's (SAS Institute, Cary, N.C.) analysis of variance (ANOVA), general linear models (GLM) procedures, and a mean separation procedure (pdiff) with $P = 0.05$.

Forcing stage

For each species, block, and repetition, 10 seedlings were removed from the middle two rows of each half-tray and transplanted into 10-cm round (470-mL vol.) pots containing a commercial soilless medium composed of composted pine bark, vermiculite, Canadian sphagnum peat, coarse perlite with a wetting agent, and lime (High Porosity Mix, Strong-Lite Products, Pine Bluff, Ark.). Plants were grown under unfiltered natural photoperiods ($\geq$ 14.5 h) in a glass greenhouse at 20 °C.

Data collection. Experiments were replicated in time and were arranged in a completely randomized design. The date the first flower bud was visible (without dissection) and the date the first flower reached anthesis (flowering) were recorded for each plant. At flowering, total plant height (not including the container) was measured, and visible flower buds (VB) or inflorescences and nodes on the main stem were counted. Days to VB, days from VB to flower, days to flower, and node-count increase from the start of forcing were calculated. All other experimental conditions were as described during the seedling stage.

Results

Seedling stage. In snapdragon, stem length of plants held continually under or transferred to the FR_d filter was similar to or shorter than that of plants under the N filter (Fig. 2). In impatiens, plants on the outer rows of the plug tray were similar under all treatments, but plants in the middle and inner rows were ≈15 % shorter when grown under the FR_d filter continually or upon transfer. Irrespective of position within the plug tray, pansy, petunia, and tomato were 18 %, 34 %, or 24 % shorter, respectively, than plants held continually under the N filter. Similarly, stem length of plants transferred from the N to FR_d filter was 16 %, 29 %, or 23 % shorter in pansy, petunia, and tomato, respectively, than that of plants under the N filter. Filter treatment had no significant effect on node number, except for snapdragon, in which plants transferred from the N to FR_d filter had an average of 0.11 fewer nodes than plants held continually under the N filter (data not shown).

Forcing stage. The average time to flower of impatiens and tomato was 49 and 60 d, respectively, and was not significantly influenced by seedling environment (data not shown). Under the N filter, pansy, petunia, and snapdragon flowered in 54, 51, and 62 d, respectively (data not shown). However, when pansy, petunia, and snapdragon seedlings were grown continually under the FR_d filter, subsequent flowering was delayed by 2, 2, and 3 d, respectively, compared to seedlings grown continually under the N filter.

Flowering of seedlings transferred from the N to FR_d filter was delayed (by one d) only in snapdragon and pansy. In petunia and snapdragon, the flowering delay in seedlings raised in the FR_d filter was accompanied by the formation of an average of 0.6 more nodes before flowering (data not shown). No other significant differences in node number at flowering existed. In addition, seedling treatment had no effect on time from VB to flowering or plant height and flower number at first flowering for any species tested (data not shown).

Discussion

The FR_d filter used in our study effectively controlled extension growth in all of the species studied, with little or no delay in flowering or reduction in flower number when plants were subsequently grown under unfiltered light. In addition, extension growth was suppressed similarly when seedlings were exposed to the FR_d environment continually or only from the time leaves first began to touch within the plug tray. The species least sensitive to FR light was snapdragon, in which plants were $\leq$ 12 % shorter than plants under the N filter, depending on the plug tray position. Petunia and tomato were the most sensitive species studied: the FR_d filter suppressed extension growth by $\geq$ 23 % in all plug tray positions. This result suggests that, of the species studied, snapdragon is the most shade-tolerant plant, and petunia and tomato are the most shade-avoiding species.

Extension growth reduction in our study is similar to that in other FR_d filter studies. In tomato, other FR_d filters reduced extension growth in other tomato cultivars, including 'Saturn' (9 % to 17 % reduction) and 'Mountain Pride' (25 % reduction; Li et al., 1999; Murakami et al., 1996, 1997; Rajapakse et al., 1999). In snapdragon, Kumai et al. (1998) did not observe a reduction in stem extension under an FR_d filter; in contrast, van Haeringen et al. (1998) reported a 30 % reduction in internode length. Kumai et al. (1998) and Kubota et al. (2000) reported a ≈60 % reduction in petunia stem extension, and Runkle and Heins (2001) reported a 14 % suppression in pansy peduncle length. To our knowledge, the effects of an FR_d filter on extension growth of impatiens, which is the best selling bedding plant species in the United States (Behe et al., 2000), have not been published.

The reduction in extension growth by the FR_d filter is comparable to that in studies with other nonchemical height control strategies. For example, extension growth of tomato was reduced by cool day-temperature regimens (by 22 % to 33 %), decreased N fertility (by ≈40 %), and mechanical stimulation (by 20 % to 33 %; Garner et al., 1997; Gertsson, 1992; Heuvelink, 1989; Johjima et al., 1992; Melton and Dufault, 1991). Each of these alternatives has limitations: cool temperature treatments cannot be used when outdoor temperatures are high, decreased N fertility significantly reduced dry matter accumulation and leaf area, and mechanical stimulation caused some damage to plants. The primary disadvantage of the FR_d filter is its reduction in PPF transmittance (by ≈25 %), which could limit use under low light conditions (e.g., during the winter).

A distinction has been made between plants in which flowering is controlled primarily by dark processes (dark-dominant) or light processes (light-dominant; Thomas and Vince-Prue 1997). Light-dominant plants show a more or less quantitative relationship between irradiance of the night break and the magnitude of the flowering response until a saturation light intensity, duration, or both are reached. In addition, flowering of light-dominant plants is most rapid when light contains some minimal FR

(Downs and Thomas, 1982; Lane et al., 1965; Runkle and Heins, 2001). Most light-dominant plants are long-day plants (LDP), but a few exceptions exist.

Flowering is delayed in some LDP, such as *Coreopsis grandiflora* Hogg ex Sweet 'Early Sunrise' and pansy, when grown continually in an FR_d environment (Runkle and Heins, 2001). In petunia, an FR_d environment delayed flowering in some cultivars but not in others H.-H. Kim, personal communication; Kubota et al., 2000). In our studies, the species in which an FR_d environment delayed subsequent flowering were the LDP (pansy, petunia, and snapdragon), which are likely light-dominant; the day-neutral plants (impatiens and tomato) showed no flowering delay (Adams et al., 1997; Vince-Prue, 1975). Our study indicates that during the early phases of seedling development, light-dominant plants appear to be relatively insensitive to light quality with respect to flowering but are responsive with respect to extension growth. When plants develop the capacity to flower, light quality influences extension growth and flowering concomitantly. Therefore, if complete and rapid flowering is desired, light-dominant LDP can be exposed to an FR_d environment during the seedling stage for height control, then can be transferred to an unfiltered environment for normal flowering.

In summary, the FR_d filter presents an effective, easy-to-use, alternative method of controlling plant height. Unlike cool-temperature regimens, the filter could be used commercially in temperate and tropical locations throughout the year. However, the PPF is reduced (by ≈25%), and as with all height control methods, the magnitude of extension growth suppression varies by species. Use of an FR_d filter can delay flowering in sensitive species, particularly light dominant LDP, but the delay can be minimized if seedlings grown in an FR_d environment are subsequently grown under natural light.

Although a delay in flowering is generally considered undesirable, the promotion of vegetative growth under inductive conditions could be useful in some situations, such as during propagation or seedling establishment.

Acknowledgments

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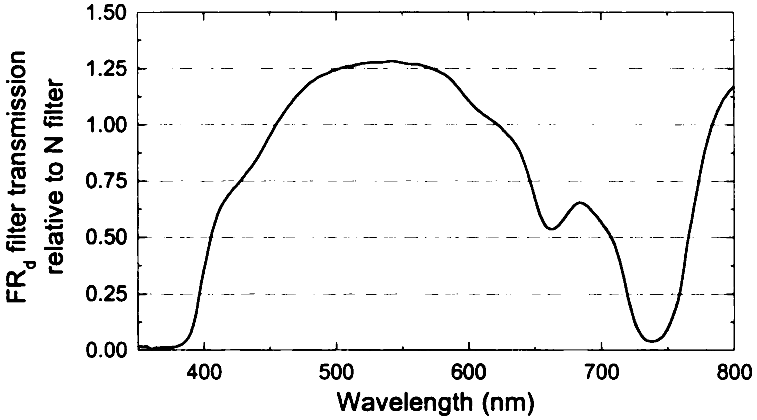
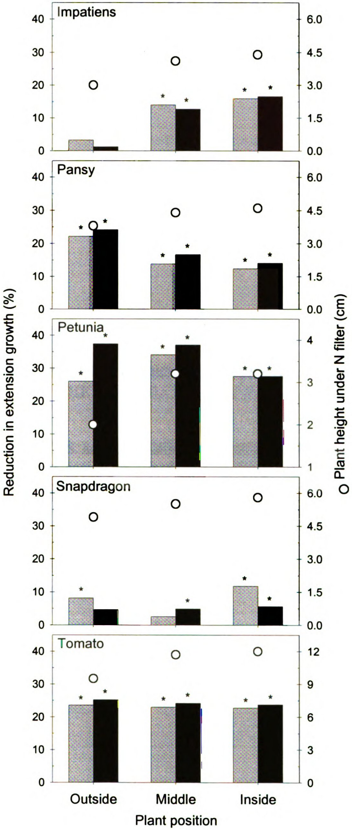


Fig. 1. Spectral transmission of sunlight under the far-red (700 to 800 nm) deficient filter relative to a filter that reduced the transmission of all wavelengths equally. The photosynthetic daily light integrals under the two filters were similar (see text).

Fig. 2. Percentage reduction in stem length (or longest petiole length for pansy) of seedlings transferred from the neutral (N) to far-red deficient (FR_d) filter when leaves of each species began to touch within the plug tray (□), or held continually under the FR_d filter (■), relative to that continually under the N filter (○). Seedlings were under filter treatments for 26, 31, 32, 35, and 35 d for tomato, impatiens, snapdragon, petunia, and pansy, respectively. Measurements were taken of seedlings from the outer two rows (outside), from the next inner two rows (middle), and from the innermost rows (inside) from each plug tray. Bars represent means with n = 40. Asterisks indicate that height was significantly ($P = 0.05$) less than of plants that were continually under the N filter.



Impatiens
Pansy
Petunia
Snapdragon
Tomato
Reduction in extension growth (%)
Plant height under N filter (cm)
Outside
Middle
Inside
Plant position

CHAPTER V

PHOTOCONTROL OF FLOWERING AND EXTENSION GROWTH OF THE THE LONG-DAY PLANT PANSY

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Photocontrol of Flowering and Extension Growth of the Long-Day Plant Pansy

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Additional index words. Far-red filter, night interruption, photoperiod, phytochrome, spectral filters, *Viola ×wittrockiana*

Abstract

Plastics that selectively reduce the transmission of far-red light (FR, 700 to 800 nm) have been recently developed to reduce extension growth of floricultural crops. However, an FR deficient (FR_d) environment delays flowering in some long-day plants (LDP), including 'Crystal Bowl Yellow' pansy (*Viola ×wittrockiana* Gams). Our primary objective was to determine if some additional amount of FR light could be added to an otherwise FR_d environment to facilitate flowering with minimal extension growth. In one experiment, plants were grown under a 16-h FR_d base photoperiod and FR-rich light was added during portions of the day or night. For comparison, plants were also grown with a 9-h photoperiod [short day (SD) control] or under a neutral (N) filter with a 16-h photoperiod (LD control). Apical flowering percentage was 52 or 98 under the SD or LD controls, respectively, and was reduced to 28 in 16-h FR_d photoperiods without supplemental FR-rich light. Flowering was promoted most (i.e., flowering percentage increased and time to flower decreased) when FR-rich light was added during the entire 16-h photoperiod, during the last 4 h of the photoperiod, or during the first or second 4 h

of the otherwise dark period. In a separate experiment, pansy was grown under an N or FR_d 9-h base photoperiod with 0, 0.5, 1, 2, or 4 h of night interruption (NI) lighting that delivered a red (R, 600 to 700 nm) to FR ratio of 0.56 (low), 1.28 (moderate), or 7.29 (high). Compared with that under the N filter, the FR_d filter reduced flowering percentage (by 20), delayed time to flowering (by 4 d) and reduced stem length (by 28 %). Under the N filter, the minimum NI duration that increased flowering percentage was 2 h with a low or moderate R : FR and 4 h with a high R : FR. Under the FR_d filter, 2, 4, or > 4 h of NI lighting with a moderate, low, or high R : FR, respectively, was required to increase flowering percentage. However, conditions that promoted flowering also promoted extension growth. Therefore, it appears that in LDP such as pansy, light duration and quality concomitantly promote extension growth and flowering, and cannot readily be separated with lighting strategies.

Introduction

Buyers of floricultural crops impose strict morphological specifications, especially plant height, on crops they purchase. Chemicals that inhibit various steps in the gibberellin biosynthetic pathway are often used to limit extension growth of herbaceous plants. However, their use can be expensive, is increasingly restricted, and is perceived by some as environmentally "unfriendly". Recently, flexible plastic filters that absorb far-red light (FR, 700 to 800 nm) have been developed as an alternative method of controlling plant height (Rajapakse et al., 1999; van Haeringen et al., 1998). Although an FR-deficient (FR_d) environment effectively retards stem extension in many herbaceous species, it can delay flower initiation or development in some long-day plants (LDP; Runkle and Heins, 2001). Here, we determined if a minimal amount of FR light could be provided to plants in an FR_d environment to promote rapid flowering without promoting stem extension.

Plants have been classified into flowering response groups based on how photoperiod influences flowering (Vince-Prue 1975). Flowering is promoted when LDP are exposed to photoperiods longer than some genotype-specific "critical" photoperiod. Under short days (SD), an interruption of the dark period with light [known as night interruption (NI) lighting] promotes flowering in LDP and suppresses it in SD plants (SDP). To promote rapid reproductive development, most LDP require a long (e.g., ≥ 2 h) duration of night break lighting, which is usually most effective at or near the middle of a long dark duration (e.g., 15 hours; Lane et al., 1965; Runkle et al., 1998; Vince-Prue, 1975).

While plants are often classified by their photoperiodic flowering response (e.g., SDP or LDP), the photo-regulation of flowering can perhaps be more accurately described by whether flowering is controlled primarily by light or dark processes. Plants in which flowering is controlled primarily by light processes, which include most LDP, are known as light-dominant plants (Thomas and Vince-Prue, 1997). Light-dominant plants show a more or less quantitative relationship between the irradiance of the night break and the magnitude of the flowering response, until a saturation light intensity, duration, or both, are reached. In addition, flowering in light-dominant LDP is often most rapid when photoperiods contain some minimal amount of far-red light (FR, 700 to 800 nm; Downs and Thomas, 1982; Lane et al., 1965; Runkle and Heins, 2001). While FR light promotes flowering in light-dominant plants, it also promotes internode elongation.

Therefore, in light-dominant LDP, a relatively low R : FR simultaneously promotes flowering and stem extension while a high ratio is inhibitory to both responses.

Red (R, 600 to 700 nm) and FR light are absorbed by the phytochrome family of photoreceptors, which in many plants regulate growth and development. For any one phytochrome, in the presence of light there exists a photoequilibrium of two interconvertible forms: the R and FR absorbing forms, which are known as P_r and P_{fr}, respectively. Depending on light quality, a phytochrome photoequilibrium [known as $P_{fr}/(P_r + P_{fr})$, or P_{fr}/P] is established, where a high R : FR creates a high P_{fr}/P, and vice versa. Models have been developed to estimate the P_{fr}/P based on the distribution of incident spectral radiation (Sager et al., 1988). Although these models are based on cross-section phytochrome A data from oats grown in darkness, these estimates and R : FR ratios are useful in associating phytochrome-mediated responses with light quality (Smith, 1994).

We performed lighting and filter experiments with pansy, which is one of the five best selling bedding plants in the United States (Behe et al., 2000). Pansy 'Crystal Bowl Yellow' was chosen for study because of its sensitivity to light quality with respect to extension growth and flowering (Runkle and Heins, 2001). Using pansy as a model plant, the objectives of our experiments were: 1) to quantify the photoperiodic flowering response, 2) determine if light rich in FR could be added during the day or night in a FR_d light environment to facilitate rapid flowering with minimal extension growth, and 3) to determine the minimum amount and duration of FR light delivered as an NI for rapid flowering under a neutral or FR_d filter.

Materials and Methods

Plant material and culture. Seed of pansy (*V. ×wittrockiana* 'Crystal Bowl Yellow') were sown into 288-cell plug trays (6-mL volume) by a wholesale plug producer (Rakers Acres, Litchfield, Mich.), germinated for ≈4 d in darkness, then grown under photoperiods ≥ 14 h at 20 to 24 °C. Plugs were thinned to one plant per cell. Propagation and forcing dates and node counts at the beginning of experimental treatments are provided in Table 1. Plants were transplanted into 10-cm (470-mL) round pots in Expts. 1 and 2 and into 11-cm (600-mL) round pots in Expt. 3. Media, irrigation, and nutrition were previously described [Runkle et al., 1998 (Expt. 1), Runkle and Heins, 2001 (Expts. 2 and 3)].

General greenhouse conditions. All plants were grown in glass greenhouses at 20 °C. Air temperatures were controlled and monitored as previously reported (Runkle and Heins, 2001) and are provided in Table 1. Nine-hour base photoperiods were created by opening and closing opaque black cloth at 0800 and 1700 HR, respectively. Sixteen-hour photoperiods were provided by a combination of sunlight and high-pressure sodium (HPS) lamps positioned above filters. From 0600 to 2200 HR, HPS lamps provided at plant level a supplemental photosynthetic photon flux *(PPF)* of 40 to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ when the ambient greenhouse *PPF* was < 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and were shut off when the ambient *PPF* was > 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The average photosynthetic daily light integral (DLI) was measured at canopy level with LI-COR quantum sensors (model LI-189; LI-COR, Lincoln, Nebr.) connected to a CR10 datalogger (Campbell Scientific Logan, Utah; Table 1).

Spectral filters. Spectral filters were used in Expts. 2 and 3 to provide two light quality environments [neutral (N) or FR_d] with similar daily light integrals. Filters were an N density metalized woven shading fabric [PLS Clear (Ludvig Svensson, Charlotte, N.C.)] or a plastic that selectively reduced the transmission of FR light (van Haeringen et al., 1998). Both filters reduced photosynthetic active radiation (PAR) by ≈25 %. Solar spectra transmissions through the filters were as previously reported (Runkle and Heins, 2001).

Photoperiod experiment (Expt. 1). To determine the photoperiodic flowering response of 'Crystal Bowl Yellow', eight or ten plants were apportioned to each of seven photoperiod treatments: 10, 12, 13, 14, 16, or 24 h of continual light or 9 h with a 4-h (2200 to 0200 HR) NI. Continual photoperiods consisted in 9-h base photoperiods completed by day-extension lighting; lamps were turned on at 1700 HR and turned off after each photoperiod was completed. Day-extension and NI lighting were provided by incandescent (INC) lamps at 1 to 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level. The experiment was performed twice.

Timing of FR light delivery (Expt. 2). An experiment was performed to determine if light rich in FR could be added to an otherwise FR_d LD to facilitate rapid flowering with minimal internode extension. Plants were exposed to one of nine filter and lighting treatments: a 9-h photoperiod, a 16-h photoperiod under the N filter, a 16-h FR_d photoperiod, or a 16-h FR_d photoperiod with lighting from INC lamps (delivering ≈2.3 and 4.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of R and FR light, respectively) positioned below filters from 0600 to 2200 HR, 0600 to 1000 HR, 1200 to 1600 HR, 1800 to 2200 HR, 2200 to 0200 HR, or 0200 to 0600 HR. The experiment was performed thrice with 20 plants per treatment and replication.

Night interruption lighting experiment (Expt. 3). An alternative lighting strategy was performed with 'Crystal Bowl Yellow' to determine if NI lighting could be used in an FR_d environment to promote rapid flowering with minimal stem extension. Twenty-six wooden chambers (55 cm × 75 cm × 64 cm) were constructed with an open top and southward-facing side. Half of the chambers were covered with the N filter and half with the FR_d filter. To minimize any temperature increase, chambers were continually ventilated with exhaust fans (model 4C548; Dayton Electric, Chicago) that provided ≈5.8 air exchanges·min. The outlet air temperature of each chamber was recorded (Table 1).

Inside each chamber, one of three light sources provided NI lighting that delivered a R : FR ratio of 0.56 (low), 1.28 (moderate), or 7.29 (high). The low, high, or moderate R : FR were provided by an INC lamp, a soft-white fluorescent (SWF) lamp, or an INC and a SWF lamp, respectively. Electrical timers were used to turn lamps on midway through 15-h dark periods (at 0030 HR) for 0, 0.5, 1, 2, or 4 h. All lamps were surrounded with a filter (Lee filter 101, Andover, UK) to reduce the transmission of blue (400 to 500 nm) light. In addition, an N filter (OLS50; Ludvig Svensson) surrounded the combined lamps to provide a more similar *PPF* among light quality treatments. The spectral qualities of lighting treatments are provided in Table 2.

Data collection and analysis. Experiments were replicated in time and treatments were arranged in a completely randomized design. Plants were considered nonflowering if pansy did not reach anthesis within 56 d, 95 d, or 65 d of forcing in Expts. 1, 2 and 3, respectively. The date the first flower bud was visible (without dissection) and the date the first flower reached anthesis on the apical stem [(apical) flowering] or a lateral stem (lateral flowering) were recorded for each plant. At flowering, visible flower buds above the first open flower and nodes on the main stem below the first open flower were counted. In Expts. 2 and 3, total plant height (from soil level) was measured. Node count increase to the first open flower and days to VB, days from VB to flower, and days to flower from the start of forcing were calculated. Internode length of flowering plants was calculated by dividing stem length by the increase in node count, and for nonflowering plants, by determining the average internode length of the first 10 nodes from the start of forcing. Data were analyzed by using analysis of variance (ANOVA), general linear models (GLM) procedures, and a mean separation procedure for unequal observation numbers (pdiff) with $P = 0.05$ (SAS Institute, Cary, N.C.). Regression analysis was performed by Sigma Plot (SPSS, Inc., Chicago)

Results

Expt. 1. Flowering percentage of 'Crystal Bowl Yellow' increased from 50 to 100 as the photoperiod increased from 10 to 16 h (Fig. 1). Time to flower decreased as the photoperiod increased, and was most rapid under continual (24 h) light or a 4-h NI. Flowering plants developed ≥ 10 nodes before flowering under photoperiods ≤ 13 h, and ≤ 8.4 nodes under longer photoperiods or NI (data not shown).

Expt. 2. Apical flowering percentage was 52 or 98 under natural 9- or 16-h photoperiods, but was reduced to 28 when 16-h photoperiods were deficient in FR (Figs. 2A and B). Half of the plants flowered on an apical stem when light was added to the 16-h FR_d photoperiod in the morning (0600 to 1000 HR) or mid-day (1200 to 1600 HR).

Apical flowering percentage was further increased (≥ 72) when light was added before or after the end of the 16-h base photoperiod, but was not as high as that under natural 16-h photoperiods. Lateral flowering was generally greatest under conditions that inhibited apical flowering.

Flowering was most rapid under natural 16-h photoperiods or when INC lighting was added to FR_d photoperiods after 1800 HR and before 1000 HR (Figs. 2C and D). Regardless of photoperiod or lighting treatment, plants initiated flowers at the same node (Figs. 2E and F). Conditions that were least favorable for flowering (e.g., 9-h natural or 16-h FR_d photoperiod) developed more nodes before anthesis compared with the most inductive treatments. Peduncles of flowering plants were shortest under 9-h days or FR_d photoperiods without or with lighting during the 16-h base photoperiod (Figs. 2G and H). Except for pansy grown under 9-h days or a 16-h FR_d environment with INC lighting from 0600 to 1000 HR, total plant height of flowering plants was similar (data not shown).

Expt. 3. Regardless of NI lighting, primary flowering percentage and stem length of pansy were reduced (by 20 and 28 %, respectively) when the 9-h base photoperiod was deficient in FR (Table 3). Of the plants that flowered under the FR_d base photoperiod, average time to flower was delayed (by 4 d) compared to flowering plants under the N filter. In general, flowering percentage increased as the NI duration increased, but the response varied by the quality of light provided (Figs. 3A and B). Relative to plants under the N filter without NI lighting, the minimum NI duration that increased flowering percentage was 2 h when the NI R : FR was low or moderate and 4 h when the ratio was high (Fig. 3A). Under the FR_d filter, 2 or 4 h of NI lighting with a moderate or low R : FR, respectively, was required to increase flowering percentage compared with plants without an NI (Fig. 3B). A 4-h NI with a high R : FR failed to promote flowering under an FR_d environment. Axillary flowering percentage was greatest under the high R : FR (13 %) compared with a low (7 %) or moderate (6 %) R : FR and the least when the NI was for 4 h (2.5 % compared with ≥ 7.5 % under shorter NI durations; data not shown).

Time to anthesis decreased as the NI duration increased, but the magnitude varied by the R : FR (Figs. 3C and D). One hour of NI lighting significantly accelerated flowering under the N filter when delivered with a low or moderate R : FR, but 4 h of lighting with a high R : FR was required to hasten flowering. A 4-h NI with a low or moderate R : FR hastened flowering by 18 d under the N filter and 14 d under the FR_d filter. Plants provided with a 4-h NI with a high R : FR flowered 7 or 8 d earlier than plants without an NI, regardless of filter type.

Light quality during the base photoperiod did not have a significant effect on the node at flower initiation or anthesis (Table 3), so data within filter treatments were pooled. Pansy initiated flowers at the same node, regardless of NI duration or quality (Fig. 4A). However, compared with plants without an NI, pansy developed fewer nodes before anthesis when the NI was ≥2 h with a low R : FR or 4 h with a moderate R : FR ratio (Fig. 4B). An NI with a high R : FR did not reduce the number of nodes developed before anthesis compared with plants under SD.

An NI of ≤ 1 h had little or no effect on stem extension, regardless of light quality (Figs. 3E and F). However, compared with plants without an NI, stem length increased by ≥ 138 % or ≥ 103 % when a 4-h NI was delivered with low or moderate R : FR, respectively, regardless of light quality during the base photoperiod. In contrast, a 4-h NI with a high R : FR promoted stem extension by ≤ 35 %.

Discussion

Half of the *Viola ×wittrockiana* 'Crystal Bowl Yellow' plants under unfiltered 9- or 10-h photoperiods reached anthesis within the experimental periods. Flowering percentage increased and time to flower decreased as the photoperiod increased, until ≈16 h, when essentially all plants flowered with natural photoperiods extended with INC or HPS lamps. A 4-h NI with INC lamps promoted flowering similar to that under continual photoperiods ≥ 16 h. Therefore, 'Crystal Bowl Yellow' is a quantitative LDP, which is similar to that reported in another pansy cultivar (Adams et al., 1997).

In all experiments, 'Crystal Bowl Yellow' initiated flowers at approximately the eighth node, regardless of the duration or spectral quality of the photoperiod. However, flower bud development was arrested when photoperiods were short or when long photoperiods were deficient in FR light. When 16-h photoperiods were deficient in FR, flowering was suppressed greater than that under unfiltered 9-h photoperiods. Therefore, pansy appears to be day-neutral with respect to flower initiation and a quantitative LDP with respect to flower development. In addition, pansy can be considered a light-dominant plant since 1) it required a long period (e.g., ≥ 2 h) of night break lighting to maximally promote flowering and 2) the promotion of flowering was dependent upon the spectral quality of incident radiation, with a low or moderate R : FR more effective than a high R : FR.

Experiments 2 and 3 were performed to determine if pansy could be exposed to a minimal amount of FR light to promote flower development without promoting stem extension. Light from INC lamps, which is rich in FR, added during the first 10 h of the base photoperiod had little or no promotive effect on flowering or stem extension.

Flowering was promoted when light from INC lamps was added at the end of or continually during the base photoperiod, or during the otherwise dark period. Plants lighted from 2200 to 0200 HR or from 0200 to 0600 HR received a 20-h photoperiod, and thus at least some of the promotion of flowering could be attributed to the increase in day length. However, regardless of the addition of FR light, the percentage of plants with a normal flowering phenotype (with apical flowering) was always lower under the FR_d filter compared with that under the N filter. In addition, when the addition of FR promoted flowering, it also promoted extension growth. Therefore, exposing plants to a FR-rich environment for portions of the day or night is not an effective strategy to promote flowering without promoting extension growth.

The maximum predicted P_{fr}/P in an environment completely devoid of FR light is $\approx$0.89, since there is considerable overlap in the R- and FR-absorbing forms of phytochrome in the 650 to 700 nm waveband (Sager et al., 1988). The estimated P_{fr}/P under the FR filter was $\approx$0.78 to 0.80, which is significantly greater than that of unfiltered sunlight ($P_{fr}/P \approx 0.71$ to 0.72). Although the FR filter reduced the transmission of FR radiation, particularly around 730 nm, a substantial amount of FR radiation penetrated the filter when the ambient light intensity was high. However, stem extension and flowering were suppressed when the P_{fr}/P was increased even though plants were exposed to some FR light.

Our studies indicate that a moderately low P_{fr}/P (e.g., < 0.78) is required toward the end of the photoperiod for normal flower development in pansy (Expt. 2). A similar requirement for a high P_{fr}/P, especially toward the end of the photoperiod, has been reported for rapid initiation in other LDP (Carr-Smith et al., 1989). For example, in the

LDP *Lolium temulentum* L., FR promoted flowering during the first ≈6 h of a 16-h night and R was most promotive toward the end of the night (Evans, 1976; Vince, 1965). While most studies have described a FR requirement for flower initiation, our results with pansy are similar to that in *Hyoscyamus niger* L., where the primary effect of FR light is on flower development, not initiation (Downs and Thomas, 1982).

A high P_{fr}/P during the base photoperiod inhibited flowering, regardless of NI lighting duration or spectral quality (Expt. 3). This suggests that, in addition to the end-of-day FR requirement, a moderate or low P_{fr}/P (< 0.80) is required during the base photoperiod for rapid and complete flowering of pansy. This is supported by Expt. 2, in which flowering percentage under the FR_d filter was never as high as that under the N filter, regardless of lighting treatment.

Flowering is promoted most in light-dominant LDP when an NI is long (e.g., ≥ 2 h) and contains a minimal amount of FR light. In pansy, flowering was promoted most when the night break was for 4 h and the R : FR was relatively low (e.g., ≤ 1.28). An NI ≤ 1 h had no promotive effect on flowering percentage, regardless of the light quality provided during the day or night. Except for 1 h of NI with INC lamps, an NI ≤ 1 h did not significantly promote stem extension in any treatment. The conditions that were most promotive to flowering (e.g., a 4-h NI with a low or moderate R : FR) were also most promotive to extension growth. Together, this suggests that flowering and stem extension are promoted similarly by the quality and duration of NI.

To quantify the relationship between stem extension (SE) and flowering promotion (FP), indices were developed for each of the 26 treatments used in Expt. 3.

The FP index (adapted from Lange, 1993) and SE index of each treatment were determined by:

$$SE = I_t \cdot I_{max}^{-1} \quad [1]$$

$$FP = F \cdot FT_{min} \cdot FT_t^{-1} \quad [2]$$

where I_t = treatment average internode length (mm), I_{max} = maximum average internode length (mm), F = flowering (%), FT_{min} = minimum average flowering time (d), and FT_t = treatment average flowering time (d). In our studies, plants under the N filter with a 4-h NI from INC lamps flowered most rapidly and had the greatest internode length, and thus were the values used for I_{max} and FT_{min} (Figs. 3C and E).

Regardless of light quality during the photoperiod or the NI light quality or duration, the promotion of extension growth was linearly related to the promotion of reproductive development (Fig. 5). This suggests that in light-dominant LDP such as pansy, light duration and quality concomitantly promote extension growth and flowering, and it does not appear that they can readily be separated with lighting strategies.

A variety of phytochrome-mediated processes, including extension growth and flowering, are mediated by the gibberellin (GA) family of plant hormones. A close relationship between phytochrome, gibberellins, and extension growth has been demonstrated by several molecular studies. In transgenic aspen (*Populus tremula* × *tremuloides*), the over-expression of phytochrome A reduced internode length, which was correlated with low levels of GAs in apical stem and leaf tissue (Olsen et al., 1997). Similarly, transgenic tobacco and potato with over-expressed phyA induced dwarfism

(Heyer et al., 1995; Jordan et al., 1995). Phytochrome A mutants of *Arabidopsis thaliana* Heynh. are relatively insensitive to FR and LD, which suggests that this phytochrome participates in the regulation of extension growth and flowering (Reed et al., 1994). In the LDP *Nicotiana sylvestris* Speg. & Comes, phytochrome B overexpression concomitantly reduced internode extension and delayed flowering (J. Metzger, personal communication). Therefore, it appears that regulating the phytochrome status, using lighting or molecular approaches, is not an effective strategy to suppress extension growth without inhibiting flowering.

In summary, these experiments indicate that while an FR_d environment can limit extension growth, it can also delay flower initiation in light-dominant LDP such as pansy. Exposure of plants to periods of FR light can partially but not completely overcome a delay in flowering. However, when light conditions are promotive to flowering, they are also promotive to extension growth. Therefore, commercial use of an FR filter could be limited when rapid and complete flowering of LDP is desired.

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Table 1. Propagation and forcing dates, initial node counts, and average air temperatures and photosynthetic daily light integrals under treatments during experiments. N = neutral-density; FR_d = far-red (700 to 800 nm) deficient.

Expt. and species	Rep.	Date		Initial nodes	Average air temperature during forcing (°C)		Average daily light integral during forcing ($mol \cdot m^{-2} \cdot d^{-1}$)	
		Propagation	Forcing					
Expt. I								
Viola ×wittrockiana	1	19 Sept. 1995	31 Oct. 1995	nr[z]	20.8 ± 0.3		nr	
	2	16 Jan. 1996	29 Feb. 1996	nr	21.0 ± 0.5		nr	
Expt. II								
Viola ×wittrockiana	1	14 Sept. 1999	9 Oct. 1999	2.0	20.8 ± 0.6		5.5	
	2	1 Nov. 1999	24 Nov. 1999	2.0	20.8 ± 0.4		7.6	
	3	13 Dec. 1999	12 Jan. 2000	2.0	20.6 ± 0.9		9.4	
					Filter treatment			
Expt. III					N	FR_d	N	FR_d
Viola ×wittrockiana	1	13 Dec. 2000	18 Jan. 2000	3.0	20.3 ± 0.5	20.5 ± 0.5	5.8	5.5
	2	6 Mar. 2000	31 Mar. 2000	2.0	21.6 ± 0.8	21.7 ± 0.4	6.2	5.8

[z]nr = not recorded.

Table 2. Spectral radiation and estimated phytochrome photoequilibria (P_{fr}/P; Sager et al. 1988) under neutral (N) or far-red deficient (FR_d) filters and of incandescent (INC) and soft-white fluorescent (SWF) lamps alone or combined. All lamps were surrounded with a filter to reduce the transmission of blue light (400 to 500 nm) and an N filter surrounded the combined INC + SWF lamps to provide a more similar *PPF* among light quality treatments. R = red light (600 to 700 nm); FR = far-red light (700 to 800 nm).

	Filter		Lamp(s)		
Parameter	N	FR_d	INC	SWF	INC+SWF
% B^z	18	20	1	4	2
% R	29	28	31	51	41
% FR	27	19	55	7	32
P_{fr}/P	0.72	0.77	0.64	0.86	0.74
$\sum(400\text{ to }800\text{ nm})^y$			13.1	9.6	10.2

^{z}Percentage of light from 400 to 800 nm.

$^y\mu mol\cdot m^{-2}\cdot s^{-1}$.

Table 3. Analysis of variance (ANOVA) for various flowering and extension growth parameters of pansy as affected by filter treatment during the photoperiod and night interruption (NI) quality and duration.

Source of variation	df	Terminal flowering percentage	Node at flower initiation	Node at anthesis	Days to anthesis	Peduncle	Stem or internode length
Filter (F)	1	***	NS	NS	***	***	***
NI quality (Q)	2	**	NS	***	***	***	***
NI duration (D)	3	***	NS	***	***	***	***
F × Q	2	NS	NS	NS	NS	NS	NS
F × D	3	NS	NS	NS	NS	NS	NS
Q × D	6	*	NS	NS	**	NS	***
F × Q × D	6	NS	NS	NS	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

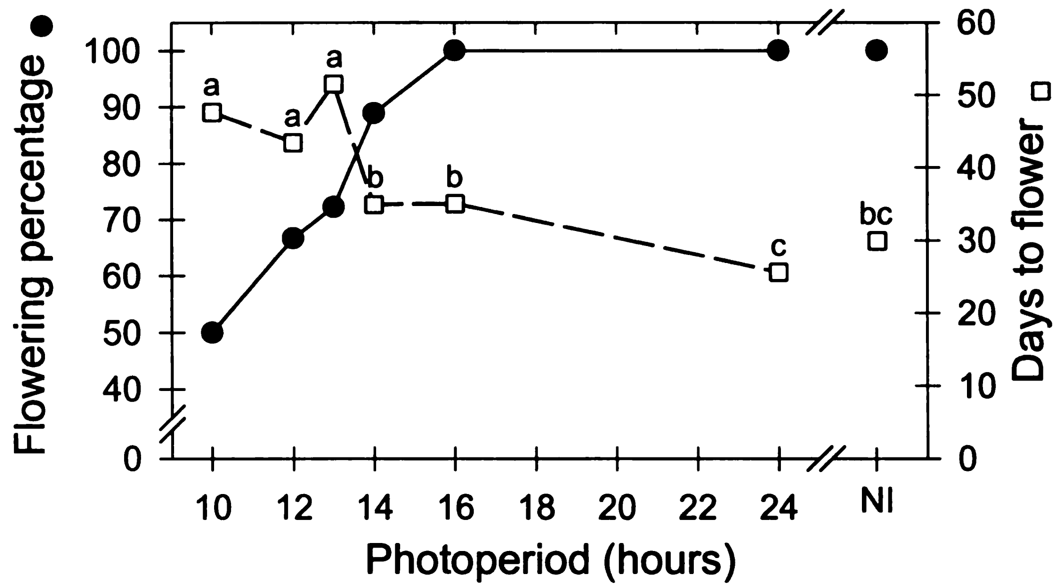
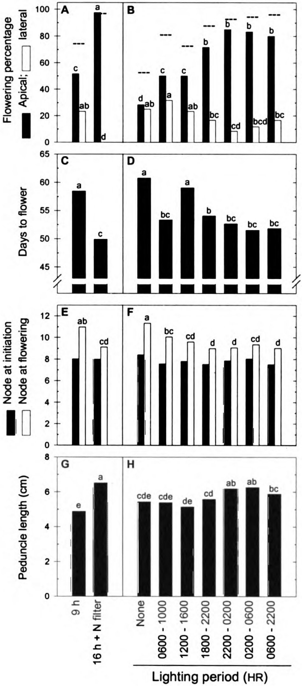


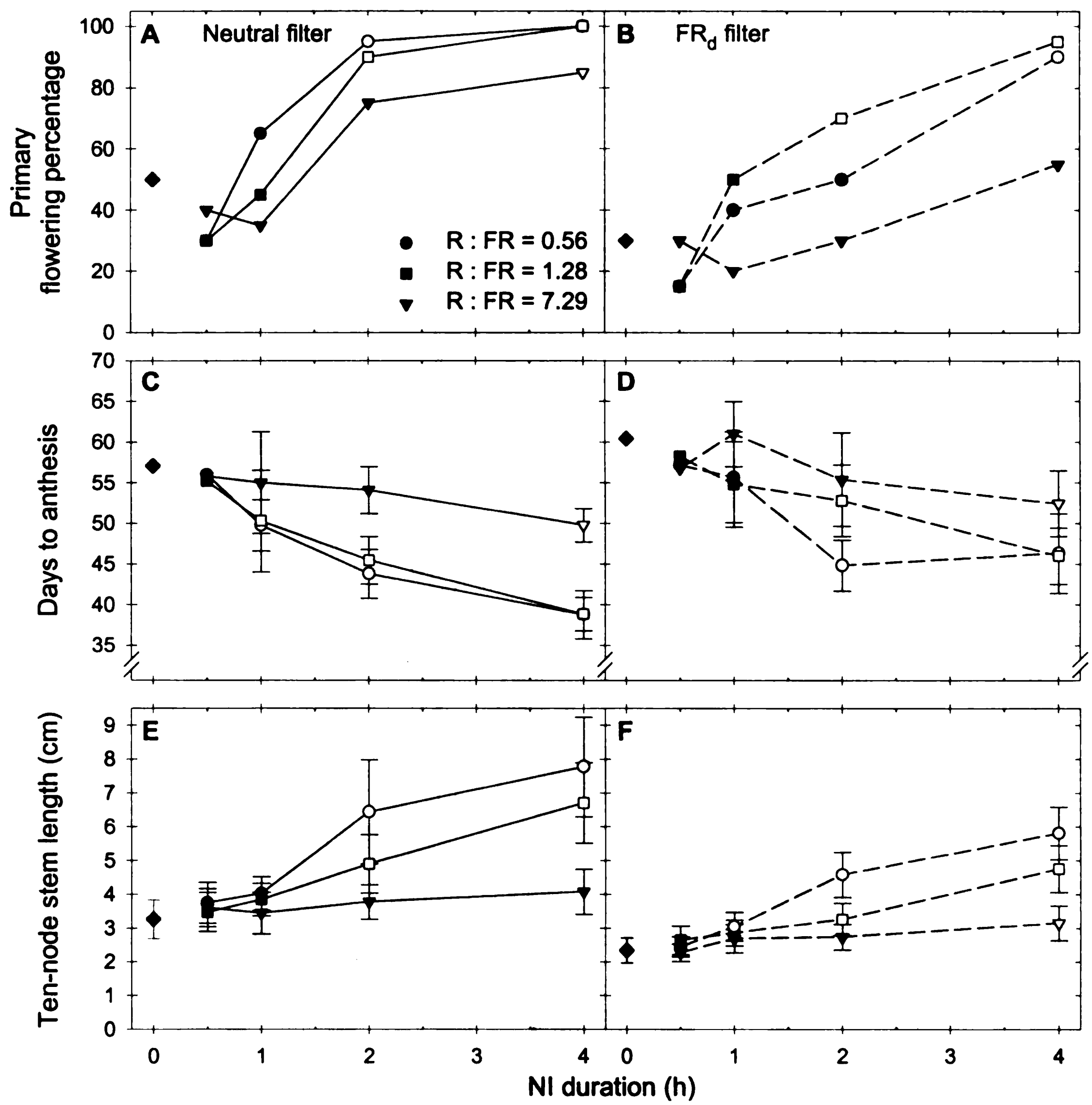
Fig. 1. Flowering of *Viola* ×*wittrockiana* 'Crystal Bowl Yellow' under photoperiods consisting of 9-h natural days extended with light from incandescent lamps. NI = 9-h photoperiods plus 4-h night interruption. Plants were considered nonflowering if they did not reach anthesis within 98 to 100 d from seed. Days to flower with the same letter are not statistically different at $P = 0.05$.

Fig. 2. Flowering and peduncle length of *Viola ×wittrockiana* 'Crystal Bowl Yellow' grown under a neutral (N) filter (Figs. A, C, E, and G) or one that selectively reduced the transmission of far-red (FR, 700 to 800 nm) light (Figs. B, D, F, and H). In Figs. A and B, --- represents the total flowering percentage. Except for the 9-h photoperiod, a 16-h base photoperiod was provided by natural photoperiods extended with light from high-pressure sodium lamps from 0600 to 2200 HR. Light rich in FR was provided under the FR filter for periods during the day or night, as indicated. Values with the same letter within measurement are not statistically different at $P = 0.05$. Letters are not provided when all treatments are statistically similar.



A
B
C
D
E
F
G
H
Flowering percentage
Apical; lateral
Days to flower
Node at initiation
Node at flowering
Peduncle length (cm)
9 h
16 h + N filter
None
0600 - 1000
1200 - 1600
1800 - 2200
2200 - 0200
0200 - 0600
0600 - 2200
Lighting period (HR)

Fig. 3. Flowering and stem length of *Viola ×wittrockiana* 'Crystal Bowl Yellow' grown under a 9-h neutral (N) filter (Figs. A, C, and E) or a filter that selectively reduced the transmission of far-red (FR, 700 to 800 nm) light (Figs. B, D, and F). Night interruption (NI) lighting was provided for varying durations by lamps delivering a low (0.56), moderate (1.28), or high (7.29) red (R, 600 to 700 nm) to FR ratio (Table 2). In each graph, open or dark symbols represent means are significantly different (at $P = 0.05$) from or similar to that without an NI, respectively. Error bars represent 95 % confidence intervals, and except for Figs. A and B, are not presented when NI lighting treatments were statistically similar.



A
Neutral filter
B
FR_d filter
Primary flowering percentage
R : FR = 0.56
R : FR = 1.28
R : FR = 7.29
C
D
Days to anthesis
E
F
Ten-node stem length (cm)
NI duration (h)

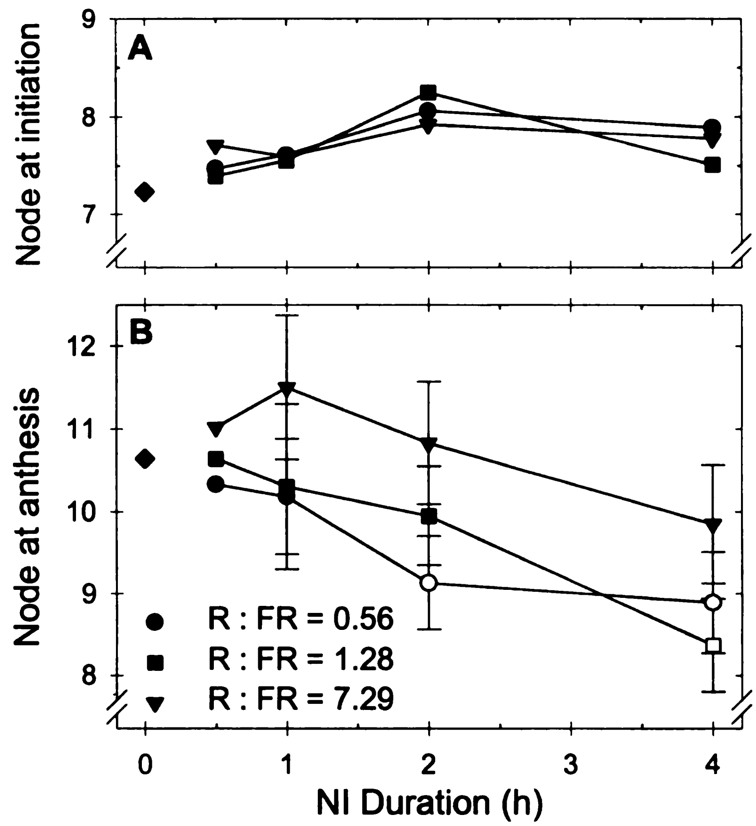


Fig. 4. Flowering and stem length of *Viola ×wittrockiana* 'Crystal Bowl Yellow' grown under a neutral (N) or far-red deficient (FR_d) filter with night interruption (NI) treatments as described in Fig. 3. Data for plants under the N and FR_d filters were pooled, since the effects of base photoperiod were insignificant. Error bars represent 95 % confidence intervals, and are not presented when NI lighting treatments were statistically similar.

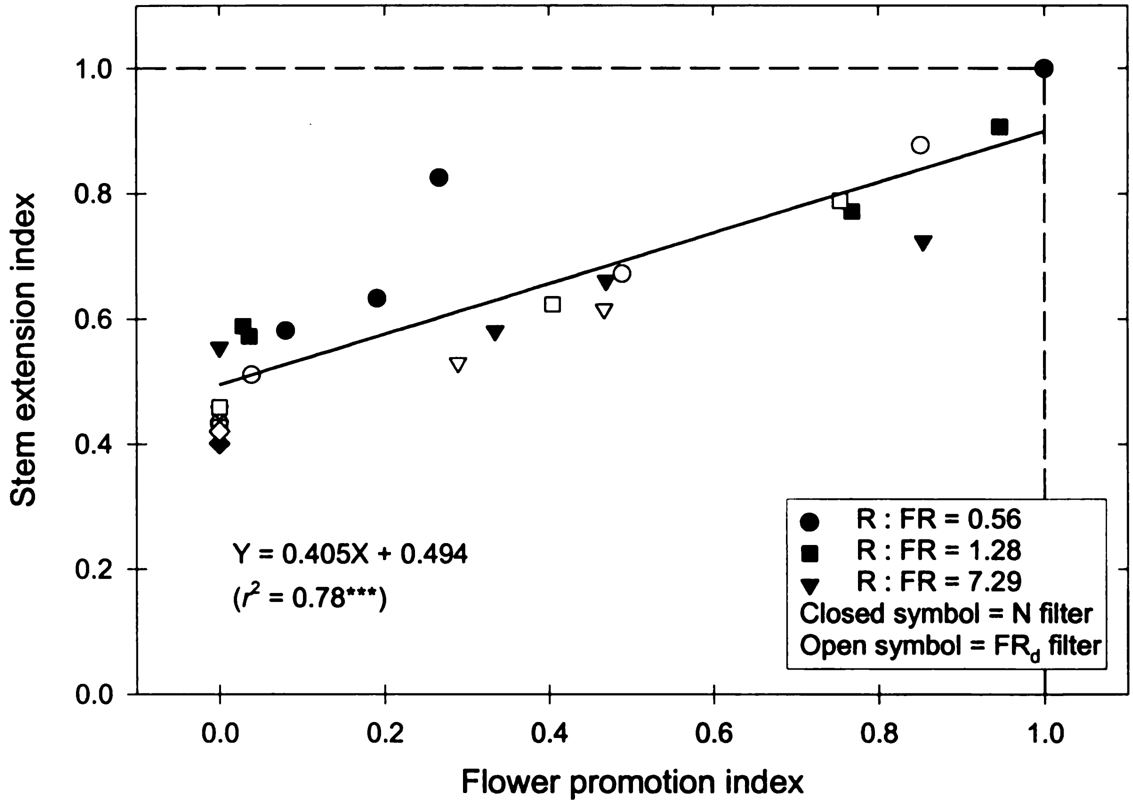


Fig. 5. Flowering and stem extension of *Viola ×wittrockiana* 'Crystal Bowl Yellow' grown under 26 combinations of filter and night interruption (NI) treatments, as described in Fig. 3. Linear regression analysis was used to relate the relative promotion of stem extension with flowering; see text for equations. *** = significant at P ≤ 0.0001.