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An intermediate phytochrome photoequilibria from night-interruption lighting optimally promotes flowering of several long-day plants

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ABSTRACT

Flowering of long-day (LD) plants is promoted by low-intensity (photoperiodic) lighting during an otherwise long night. Conventional lamps that emit a relatively low red (R; 600–700 nm) to far-red (FR; 700–800 nm) light ratio (e.g., incandescent lamps) create an intermediate phytochrome photoequilibria (PPE) and are sometimes more effective at promoting flowering of LD plants (LDP) than lamps that emit a higher R:FR (e.g., fluorescent lamps) and establish a higher PPE. Thus, we postulated that flowering of several LDPs would be increasingly promoted as the fraction of FR radiation increased relative to R light and the established PPE decreased. *Rudbeckia* (*Rudbeckia hirta*), snapdragon (*Antirrhinum majus*), *Fuchsia* (*Fuchsia* × *hybrida*), and three cultivars of *Petunia* (*Petunia* × *hybrida*) were grown at 20 °C under a truncated 9-h ambient photoperiod with or without 4-h NI lighting by incandescent lamps or light-emitting diodes that emitted seven different R:FR and created estimated PPE from 0.16 to 0.89. For all three *Petunia* cultivars and snapdragon, flowering was earliest under an NI with an intermediate PPE and delayed under short days (SDs) or an NI that elicited the highest or lowest PPE. For *Rudbeckia* and *Fuchsia*, all NI treatments promoted flowering except for the highest PPE NI, which was perceived as an SD. There were relatively subtle effects of the NI treatments on extension growth except in *Petunia*, in which all three cultivars showed a quadratic response to the PPE under the NI treatments, where plants were tallest at flowering under intermediate PPE. We conclude that an NI that establishes an intermediate PPE optimally promotes flowering of a variety of LDPs. These results are not consistent with the established paradigm for how light quality regulates flowering of LDPs, particularly in *Arabidopsis*, suggesting that the paradigm is not necessarily applicable to plants outside of the Brassicaceae.

1. Introduction

The flowering of many horticultural crops – especially ornamentals – is influenced by photoperiod (Erwin and Warner, 2002; Mattson and Erwin, 2005; Runkle and Heins, 2003). Photoperiodic flowering responses are determined primarily by the length of the dark period, also known as the critical night length (Thomas and Vince-Prue, 1997). Plants have been categorized into photoperiodic classes, depending on how they respond to the critical night length, including long-day (LD) plants (LDPs), in which flowering is most rapid when uninterrupted dark periods are shorter than some genotype-specific critical night length (Vince, 1969). When the ambient photoperiod is short, commercial growers accelerate flowering of LDPs and inhibit flowering of short-day (SD) plants by using low-intensity (photoperiodic) lighting during the beginning or middle of the night.

Light quality, or the distribution of wavelengths, can cause a broad range of morphological and developmental changes in plants. It is detected by three identified families of photoreceptors in plants, including the phytochromes (Kami et al., 2010). The phytochromes exist in red- [R (600–700 nm); peak absorption at 660 nm] and far-red- [FR (700–800 nm); peak absorption at 730 nm] absorbing forms, P_R and P_{FR}, respectively (Hayward, 1984; Sager et al., 1988). Phytochromes have the potential to control a wide variety of plant responses, including seed germination, plant architecture, flowering, tuberization, bud dormancy, and shade-avoidance responses such as extension growth (Smith, 2000). The ratio of R to FR light (R:FR) incident on the plant influences the phytochrome photoequilibria (PPE) within the plant, although not in a linear manner (Fig. 1). Upon absorbing R light, P_R converts mainly to the P_{FR} form. The P_{FR} form largely converts back to the P_R form under FR light, or through a natural, gradual conversion during the dark period (Thomas and Vince-

Abbreviations: FR, far-red light; LD, long days; LDP, long-day plants; LEDs, light-emitting diodes; NI, night interruption; PAR, photosynthetic active radiation; P_{FR}, far red-absorbing phytochrome; PPE, phytochrome photoequilibrium; P_R, red-absorbing phytochrome; R, red light; SD, short days.

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Prue, 1997). The total pool of phytochrome in light-grown plants is constant, but the relative amounts in the P_{FR} and P_R forms fluctuate with changes to the light environment and during darkness.

During the night, P_{FR} promotes flowering in LDPs when present in sufficient concentrations and is associated with a high PPE. During long nights (SDs), P_{FR} slowly converts to P_R, leaving insufficient P_{FR} to promote flowering. However, if this dark period is interrupted with light for a sufficient period of time (e.g., >2 h), the conversion of P_{FR} to P_R is also interrupted, leaving enough P_{FR} to effectively promote flowering. Although the flower-promoting P_{FR} form of phytochrome depends on the R light conversion of P_R to P_{FR}, several studies have shown that a moderate R:FR during the photoperiod is more effective at promoting flowering in some LDPs than a high R:FR (Kim et al., 2002; Runkle and Heins, 2003; van Haeringen et al., 1998).

In nature, the light environment changes during the day, with the R:FR ranging from 1.15 under full sun to 0.70 at twilight (Lund et al., 2007). The R:FR can also vary significantly within layers of a plant canopy. These differences can occur between leaf layers of the same plant or between layers of a complex plant community (Smith and Holmes, 1977). Leaves at the top of a canopy receive unfiltered sunlight with a relatively high R:FR. As light passes through a plant canopy, the plant tissues absorb most of the photosynthetic light, whereas FR light is primarily transmitted through or reflected to the lower canopy (Smith, 1994). The R-depleted light under a plant canopy has a reduced R:FR, which can be as low as 0.05 under a dense canopy (Smith, 1982). Changes in the R:FR are a more dependable indicator of the proximity of potentially competing neighbors than associated reductions in light intensity (Smith, 2000). In commercial greenhouses, the R:FR of light is commonly altered by human-imposed factors such as plant spacing (plant density), canopy shading from plants in hanging baskets, use of electric lighting, and use of light-filtering films. Plants detect a low R:FR ratio and respond by increasing extension growth to compete for photosynthetic light (Morgan and Smith, 1978). This shade-avoidance response enables them to react to potential competition for light before it actually occurs. If elongation growth fails to bring a plant into an unshaded environment, other aspects of the shade-avoidance response can cause early flowering and seed production, thus increasing the chance of perpetuating the plant (Smith, 2000).

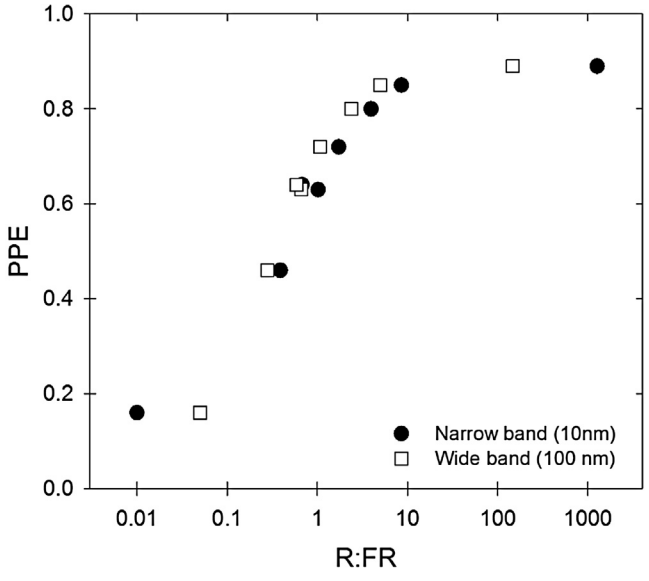

Fig. 1. The relationship between narrow- or wide-band ratios of red (R) to far-red (FR) light and phytochrome photoequilibrium (PPE). See Table 1 for additional information.

In greenhouses, horticultural crops are produced in controlled environments in which environmental factors such as temperature, light intensity, light quality, and photoperiod are manipulated beyond the realm of natural conditions. The characteristics of the light environment can have significant effects on plant growth, morphology, and flowering. The ability to elicit desirable plant responses to the R:FR and photoperiod in greenhouses allows commercial growers to produce ornamental plants that are in flower on predetermined market dates. In temperate regions, peak production of annual bedding plants and ornamental herbaceous perennials begins when the natural day lengths are short (<12 h). Many ornamental crops [e.g., *Petunia* (*Petunia* × *hybrida*) and snapdragon (*Antirrhinum majus*)] have an LD flowering response, and therefore lighting is commonly provided to accelerate flowering of LDPs. NI lighting is typically provided to LDP for 4 h because shorter durations can be less effective (Runkle et al., 1998).

Commercial growers traditionally used incandescent lamps to deliver photoperiodic lighting but only about 10% of their energy consumption is emitted as visible light and their longevity is relatively short (Kanter, 2009; Thimijan and Heins, 1983). With the phaseout of incandescent lamps, growers need more efficient sources of light to control flowering of photoperiodic crops. Compact fluorescent lamps are one alternative; they can promote flowering in some LDPs and are more energy efficient than incandescent lamps (Whitman et al., 1998). However, fluorescent lamps, which emit a relatively high R:FR and create a high PPE, were was less effective at promoting flowering in *Petunia* than incandescent lamps, which emit a moderate R:FR and create an intermediate PPE (Runkle et al., 2012).

Compared to conventional lamps, light-emitting diodes (LEDs) have many desirable characteristics, including a very long operating life, full instantaneous irradiance when powered, and improving electrical efficiencies (Bourget, 2008; Morrow, 2008; Nelson and Bugbee, 2014). Here, LEDs were used to quantify the effect of the PPE of night-interruption (NI) lighting on flowering of several LD ornamental crops, with comparisons to plants under incandescent lamps. We postulated that flowering in LDPs would be increasingly promoted as the R:FR decreased and thus the PPE decreased. Preliminary results showed that NI lighting that created an intermediate PPE was the most effective at promoting flowering of one *Petunia* and one snapdragon cultivar (Craig and Runkle, 2012). Here, we present a more comprehensive study that quantifies how the PPE controls flowering responses of a range of LDPs without the possibly confounding effects of other light wavebands or environmental parameters.

2. Materials and methods

2.1. Plant material and culture

Seven- to 10-d-old seedlings of the LDPs *Petunia* 'Shock Wave Ivory', 'Easy Wave White', and 'Wave Purple Improved'; *Rudbeckia* (*Rudbeckia hirta*) 'Denver Daisy'; and snapdragon 'Liberty Classic Cherry' grown in 288-cell (6-mL) plug trays were received from a commercial greenhouse (C. Raker and Sons, Inc., Litchfield, MI, USA). In addition, rooted cuttings of *Fuchsia* (*Fuchsia* × *hybrida*) 'Trailing Swingtime' grown in 36-cell (32-mL) liner trays were received from the same source. These varieties were selected according to their commercial popularity, as well as previous photoperiod research experience. The young plants were subsequently grown under noninductive SDs (natural day length truncated to a 9-h photoperiod with blackout cloth) in a research greenhouse at 20 °C until transfer to the NI treatments. On the day of transfer, the young plants were transplanted into 10-cm (430-mL) round plastic pots containing a commercial peat-perlite medium (Suremix; Michigan Grower Products, Inc., Galesburg, MI,

USA). All species were thinned to one plant per pot at transplant. The experiment was repeated approximately 10 weeks later with the same propagation procedure and greenhouse environment as previously described.

2.2. LED lamps and NI treatments

Opaque black cloth enclosed all greenhouse benches from 1700 to 0800 HR, creating a 9-h SD (control). Above the remaining benches, NI lighting was delivered from 2230 to 0230 HR by either 40-W incandescent lamps or customized LED fixtures containing three R and/or FR LED diodes per lamp (CCS Inc., Kyoto, Japan). Lamps were paired to produce a total of six diodes, and thus seven R:FR ratios and PPE were created. Peak emission of the R and FR LEDs was 661 nm and 737 nm, respectively, which correspond closely to peaks of phytochrome absorption (Sager et al., 1988). Because photon flux from the R LEDs was approximately twice that from the FR LEDs, all R diodes were filtered with two layers of aluminum mesh.

Light spectra under each treatment were measured by two portable spectroradiometers (LI-1800, LI-COR, Inc., Lincoln, NE, USA; and PS-200, StellarNet-Inc., Tampa, FL, USA). Spectral measurements were taken at regular intervals across the bench area of each treatment. Mean photon flux from 600 to 800 nm was 1.3 to 1.6 $\mu mol\,m^{-2}\,s^{-1}$ for all NI treatments, and plants were positioned on benches only where it was $\geq$0.7 $\mu mol\,m^{-2}\,s^{-1}$, which was the saturating photoperiodic lighting intensity for a range of ornamental crops grown under four conventional lamp types (Whitman et al., 1998). The R:FR was measured and described with 10- or 100-nm wavebands ($R:FR_{narrow}$ and $R:FR_{wide}$, respectively) and the PPE was estimated for each NI lighting treatment following Sager et al. (1988) (Table 1).

2.3. Greenhouse environment

The experiment was conducted in a glass-glazed, environmentally controlled greenhouse at a constant temperature set point of 20 °C. In late April, whitewash was applied externally to the greenhouse glazing to reduce light transmission by 30–40% and thus decrease radiant heating. All treatments received supplemental lighting from 0800 to 1600 HR provided by high-pressure sodium lamps delivering photosynthetic active radiation (PAR) of 60–90 $\mu mol\,m^{-2}\,s^{-1}$ at plant height. The lamps were operated by an environmental control computer and were switched on when the irradiance outside the greenhouse was <185 $\mu mol\,m^{-2}\,s^{-1}$ and switched off when ambient irradiance was >370 $\mu mol\,m^{-2}\,s^{-1}$. Line quantum sensors that each contained 10 diodes (Apogee Instruments, Inc., Logan, UT, USA) were positioned on benches at plant height throughout the greenhouse. The sensors measured PAR every 10 s, and hourly averages were recorded by a data logger (CR10; Campbell Scientific, Logan, UT, USA). The mean photosynthetic daily light integrals were 15.2 and 14.5 $mol\,m^{-2}\,d^{-1}$ during the first and second experimental replications, respectively.

Air temperature was measured at canopy height on each greenhouse bench by a shielded and aspirated thermocouple [36-gauge (0.127-mm diameter) type E] every 10 s and hourly averages were recorded by the data logger. When the nighttime air temperature at bench level was <18.9 °C, a 1500-W electric heater, controlled by the data logger, provided supplemental heat during the night. The actual mean daily temperatures were 19.9 °C and 21.9 °C during the first and second experiments, respectively. Plants were irrigated as necessary with reverse-osmosis water supplemented with a water-soluble fertilizer providing (in $mg\,L^{-1}$) 125 N, 12 P, 100 K, 65 Ca, 12 Mg, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU RO Water Special; GreenCare Fertilizers, Inc., Chicago, IL, USA).

Table 1
Emission characteristics of incandescent bulbs or red (R) and far-red (FR) light-emitting diodes (LEDs) used to deliver night-interruption lighting to long-day plants. $R:FR_{wide}$ = 600–700:700–800 nm; $R:FR_{narrow}$ = 655–665:725–735 nm. The estimated phytochrome photoequilibria (PPE) is calculated from Sager et al., 1988.

Light source	Diode ratio (R:FR)	Measured		Estimated PPE
		$R:FR_{wide}$	$R:FR_{narrow}$	
Incandescent	–	0.59	0.68	0.64
LEDs	6:0	147.29	1270.42	0.89
	5:1	4.99	8.56	0.85
	4:2	2.38	3.95	0.80
	3:3	1.07	1.73	0.72
	2:4	0.66	1.02	0.63
	1:5	0.28	0.39	0.46
	0:6	0.05	0.01	0.16

2.4. Data collection and analysis

Ten plants were randomly assigned to each treatment in each experimental replicate. Plant height (from medium surface to shoot tip) was measured on the day of transplant and nodes were counted on each plant. The date of first flowering was recorded. *Rudbeckia* was considered flowering when at least 50% of the ray flowers of an inflorescence were reflexed. At flowering, the total number of visible flower buds or inflorescences (VBs), plant height, and number of nodes below the first flower (replicate 2 only) were recorded. Plants that did not have an open flower within 150% of the average flowering time were considered nonflowering. For experimental replicate 1, this cutoff date occurred 59, 65, 67, 78, 90, and 116 d after transplant for *Petunia* 'Shock Wave Ivory', 'Easy Wave White', and 'Wave Purple Improved', and snapdragon, *Fuchsia*, and *Rudbeckia*, respectively. In replicate 2, flowering occurred earlier and the cutoff dates were 50, 54, 52, 64, 72, and 87 d after transplant, respectively. The number of nodes formed below the first flower was calculated for each plant (replicate 2 only). Data were analyzed with SAS (version 9.1; SAS Institute, Cary, NC, USA) and data were pooled between replications if the statistical interactions between treatment and replication were not significant ($P \geq 0.05$). Regression analysis was performed with SAS to relate the data parameters to the estimated PPE of the NI.

3. Results

All *Petunia* 'Easy Wave White' plants flowered in all treatments (Fig. 2). Flowering of this plant occurred earlier in the second experimental replicate, when the average daily temperature was 2.0 °C higher, but response trends to the NI treatments were similar (Table A.1). Flowering time exhibited a quadratic trend and was most rapid under NI lighting that created a PPE = 0.46–0.72. There was an opposite quadratic response for stem elongation; the NI treatments with intermediate PPE elicited the longest stems at first flowering, whereas plants under the lowest PPE (FR-only) or highest PPE (R-only) NI treatments or SDs were the most compact. The average node, VB, and lateral branch numbers were 17.4, 47.0, and 7.0, respectively, and were similar among all treatments (data not shown). Flowering time and extension growth under incandescent lamps and LEDs with a similar estimated PPE (0.64 or 0.63) were similar.

All *Petunia* 'Shock Wave Ivory' flowered in all treatments, with the exception of a few plants under the FR-only NI (PPE = 0.16) and SD treatments in replicate 2. Flowering time and stem elongation exhibited quadratic trends similar to those for 'Easy Wave White'. Plants grown under the FR-only NI or SDs flowered 14 d later than those grown under the incandescent NI. Extension growth was greatest in plants grown under a PPE = 0.63 or 0.64 and decreased as the PPE of NI lighting increased or decreased. Under SDs or FR-only NI, plants produced 24 nodes below the first flower, which

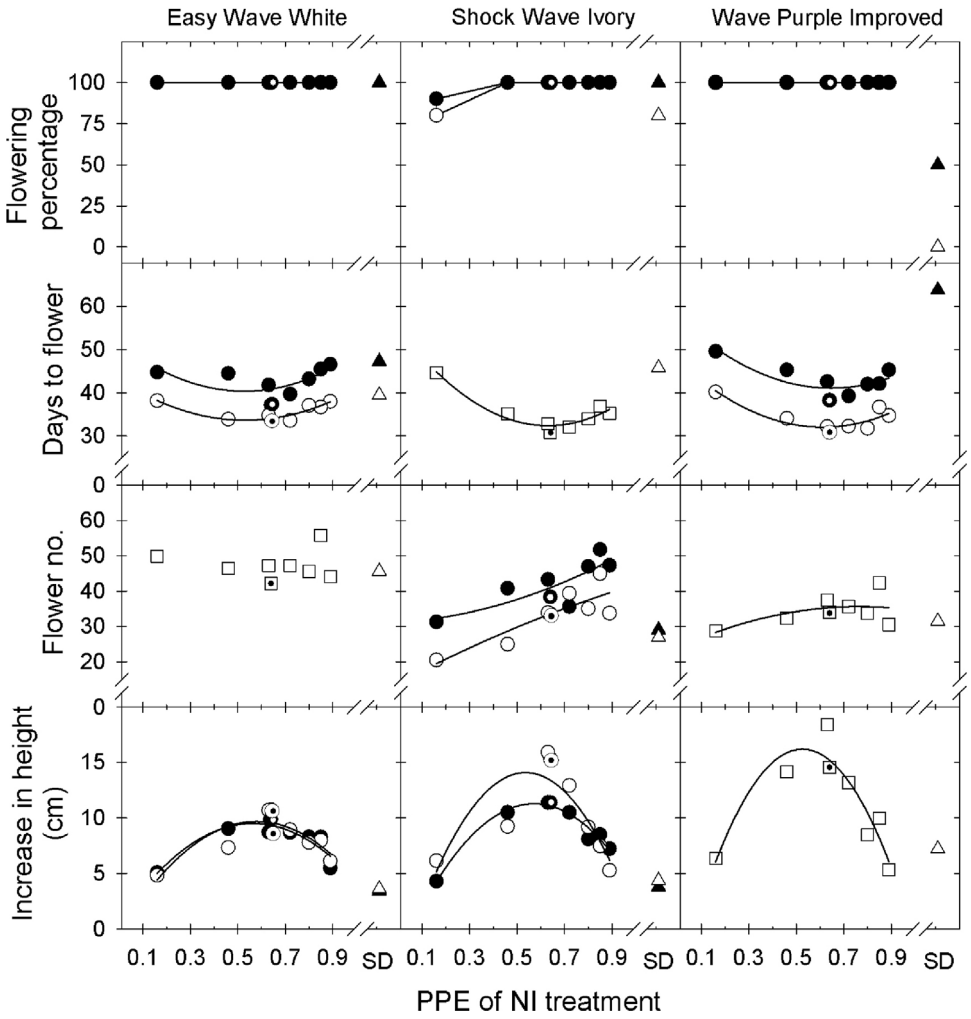

Fig. 2. The effects of the estimated phytochrome photoequilibria (PPE) of night-interruption (NI) lighting on flowering and extension growth of three *Petunia* cultivars. Open square symbols indicate pooled data; multiple circle plots indicate replicate 1 (solid symbols) and replicate 2 data (open symbols). Dotted symbols indicate the incandescent control treatment. SD = short-day control treatment. See Table A.1 for regression equations.

was seven more than in all other treatments (data not shown). In replicate 2, plants grown under SDs, R-only NI, or FR-only NI produced two more lateral branches at first flowering than those grown under an NI with a PPE = 0.64 (data not shown). VB number at first flowering increased as the NI PPE increased.

All *Petunia* 'Wave Purple Improved' plants that received NI lighting flowered, regardless of light quality, whereas flowering percentage under the SD treatment was reduced to 50 (replicate 1) or 0 (replicate 2). Flowering time and stem elongation exhibited quadratic trends similar to those for 'Easy Wave White'. In the plants that flowered under SDs in replicate 1, flowering was delayed by 26 d compared with that of plants grown under the incandescent NI. Flowering was also delayed by 9 to 11 d in plants grown under the FR-only NI compared to those grown under incandescent lamps. Stem extension was greatest under NIs with a PPE = 0.46–0.72. Under SDs, plants had produced two more lateral branches at flowering than those under incandescent lamps at first flowering (data not shown). The average node and VB numbers were 19 and 34, respectively, and were similar among all NI treatments.

All snapdragon plants flowered in all treatments (Fig. 3). The time to first flowering exhibited a quadratic trend similar to that of the three *Petunia* cultivars. Plants grown under SDs or the R-only NI flowered 9 to 16 d later than those grown under the incandescent NI. Plants under R-only or FR-only NI were 6.5 cm taller at flowering than those grown under the incandescent NIs. Plants grown under the R-only NI or SDs developed approximately eight more nodes (data not shown) and eight more VBs than those grown under the incandescent NI. Under SDs, plants had two more lateral branches than those under other treatments (data not shown). The average VB number exhibited a quadratic trend and was least under NI treatments with a PPE = 0.46–0.80, which were the treatments that elicited the most rapid flowering.

No *Rudbeckia* plants flowered under the FR-only NI treatment or under SDs, whereas all plants flowered in the remaining treatments. When grown under an FR-only NI or SDs, the plants remained as rosettes and never showed signs of bolting that typically precede flowering. Under the remaining NI treatments, there was little effect of the NI on time to flowering, VB number, or plant height at flowering. Among the plants that flowered, the number of nodes formed below the first flower was similar (≈16 nodes) in all treatments (data not shown). Although data were not collected, leaf size was noticeably small under SDs, intermediate under the FR-only NI, and relatively large under the remaining NI treatments.

Under the FR-only NI, flowering percentage of *Fuchsia* was 0 and 60 in replicates 1 and 2, respectively. Few plants also flowered under SDs (0% and 20% for replicates 1 and 2, respectively). Flowering time and extension growth decreased slightly with

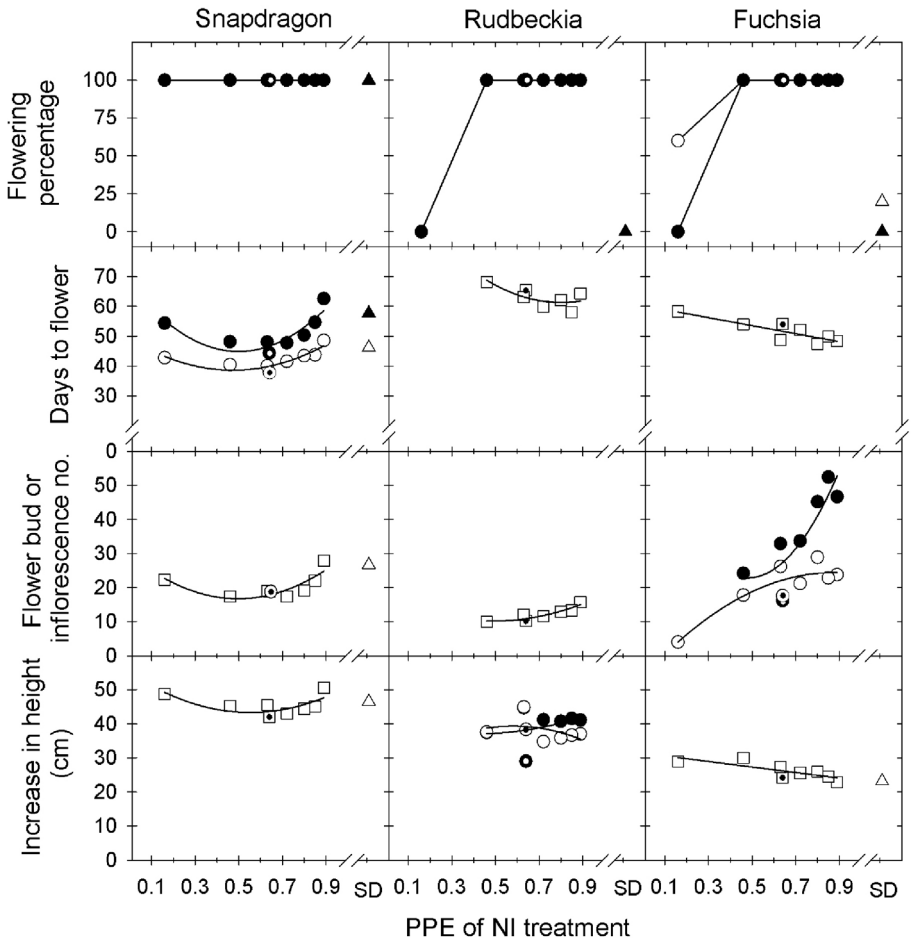

Fig. 3. The effects of the estimated phytochrome photoequilibria (PPE) of night-interruption (NI) lighting on flowering and extension growth of snapdragon 'Liberty Classic Cherry', *Rudbeckia* 'Denver Daisy', and *Fuchsia* 'Trailing Swingtime'. Open square symbols indicate pooled data; multiple circle plots indicate replicate 1 (solid symbols) and replicate 2 data (open symbols). Dotted symbols indicate the incandescent control treatment. SD = short-day control treatment. See Table A.1 for regression equations.

increasing PPE of the NI treatment. Node number below the first flower was similar in all treatments (data not shown). VB number increased with increasing PPE of the NI, especially in the first experimental replicate.

4. Discussion

Far-red light is known to promote flowering in LDPs, so we postulated that as the proportion of FR light relative to R light increased (as the PPE decreased), flowering in LDPs would be increasingly promoted. However, *Rudbeckia* and *Fuchsia* did not perceive the FR-only NI as an LD. In addition, flowering of several of the other LDPs studied was delayed when the NI treatment did not also contain R light. These findings are not consistent with a somewhat established paradigm that FR light is the most promotive to flowering of LDPs (Goto et al., 1991). This paradigm is partly based on previous experiments that used relatively primitive lighting treatments, including filters and lamps that included extraneous photons that could have confounded results (Lane et al., 1965; Schneider et al., 1967). Here, with the exception of *Fuchsia*, the most effective PPE for promoting flowering were intermediate values (0.63–0.80) This conclusion is supported by a study on baby's breath (*Gypsophila paniculata*) in which an R:FR between 0.23 and 0.71 (PPE not presented) was more effective at promoting flowering than FR alone (Nishidate et al., 2012). One of the objectives of this research was to establish an R:FR and resulting PPE of NI lighting that would promote flowering of a range of photoperiodic crops. We conclude that an intermediate PPE, similar to that emitted by incandescent lamps, optimally promotes flowering of LDPs.

Several studies have shown that the presence of FR light promotes flowering of LDPs during the photoperiod, during LD lighting, or both, although the studies usually tested only a few different R:FR (Downs et al., 1958; Lane et al., 1965; Runkle and Heins, 2003). For example, flower initiation in the LDPs snapdragon (van Haeringen et al., 1998), tussock bellflower (*Campanula carpatica*), tickseed (*Coreopsis grandiflora*) (Runkle and Heins, 2001), and *Petunia* (Kim et al., 2002) was delayed when grown under photoselective filters creating an FR-deficient environment during the entire day. Light quality during NI treatments can also affect flowering in LDPs: flowering of pansy (*Viola* × *wittrockiana*), *Petunia*, and lisianthus (*Eustoma grandiflorum*) was promoted most when NI lighting contained a mixture of R and FR light (e.g., from incandescent and fluorescent lamps) compared with FR-deficient (fluorescent) light (Runkle and Heins, 2003; Runkle et al., 2012; Yamada et al., 2011). These studies are consistent with results reported here and indicate that light containing R and FR light is more effective at promoting flowering in LDPs than either R or FR light.

Thomas and Vince-Prue (1997) proposed that flowering in LDPs could be controlled by P_{FR} in two ways, one in which a certain threshold amount promotes flowering, and another in which some greater amount inhibits flowering. A mixture of R and FR light (e.g., sunlight, incandescent light) generates an intermediate PPE within the plant that induces a flowering response. At the same time, this intermediate PPE has insufficient P_{FR} to induce the flower-inhibiting response. If the PPE decreases below this intermediate

level (i.e., if the light source is too rich in FR), there is insufficient P_{FR} to drive the promoting response. If the PPE increases above this intermediate level (i.e., if the light source has a high R:FR), the excess P_{FR} drives the inhibitory response. Our findings are consistent with this theory; there was little or no LD flower promotion under the FR-only NI (R deficient), and the R-only NI (FR deficient) delayed flowering in most crops studied.

Some of our understanding of the shade-avoidance response has come from experiments performed in growth chambers or greenhouses that used spectral filters or electric light sources. For example, Yamada et al. (2008) grew lisianthus under three NI treatments delivered by fluorescent or incandescent lamps with an R:FR that ranged from 0.01 to 5.00 (PPE not presented). The lamps with an R:FR of 0.01 and 0.43 increased internode length by 26% and 23%, respectively, compared to that of plants grown without an NI. Plants grown with an R:FR of 5.0 had 14% shorter internodes than those grown without an NI. In another study with lisianthus, different combinations of FR- and R-fluorescent lamps were used to deliver a range of R:FR. Mean internode length of lisianthus was shorter in plants that received an NI with an R:FR > 5.0, whereas an R:FR < 2.0 increased internode length compared to that of plants grown without an NI (Yamada et al., 2011). Similarly, we observed less elongation growth in the three *Petunia* varieties and *Fuchsia* under NI treatments with a high R:FR (high PPE). However, elongation growth was inhibited under the FR-only NI in *Petunia* and *Rudbeckia*, which could be attributed to lack of the extension growth that occurs simultaneously with flowering.

Long-day and SD plants vary in their response to the spectral quality of NI lighting. In a similar study with SD plants (Craig and Runkle, 2013), an NI with a PPE ≥0.63 delayed flowering in chrysanthemum (*Chrysanthemum* × *morifolium*), African marigold (*Tagetes erecta*), and dahlia (*Dahlia hortensis*). For the LDPs *Petunia*, *Rudbeckia*, snapdragon, and *Fuchsia*, generally an NI with an intermediate PPE promoted flowering, whereas a higher PPE was less effective. For both SD plants and LDPs, the FR-only NI (PPE = 0.16) was usually perceived as an SD, indicating that some threshold amount of R light is necessary for both SD plants and LDPs to perceive an NI treatment. In other words, these results are not in agreement with the paradigm that a high R:FR light converts phytochrome to the P_{FR} form to elicit a biological response such as flowering.

The roles of phytochromes in flowering and extension growth have been identified in LDPs primarily according to research with *Arabidopsis thaliana*, a facultative LDP (Franklin and Quail, 2010). There are five phytochromes in *Arabidopsis* (phyA to phyE), and null mutants of one or more have established their roles in regulating flowering. phyA Mutants are insensitive to LD lighting with respect to flowering, indicating that phyA plays a role in photoperiodic perception (Johnson et al., 1994). In particular, the promotion of flowering by FR LD lighting is regulated by phyA (Mockler et al., 2003). Phytochromes B, D, and E act redundantly to mediate the shade-avoidance response and also inhibit flowering under a high R:FR at moderate temperatures (Franklin et al., 2003). Therefore, at least in *Arabidopsis*, FR light promotes flowering through phyA, whereas R light inhibits flowering through phyB (Mockler et al., 2003). Accordingly, plants grown under FR-only LDs should flower the earliest and R-only LDs should flower the latest. Here, an intermediate R:FR was the most promotive to flowering (as well as stem extension), and it was generally suppressed under an NI that emitted only R or FR light. In *Rudbeckia*, none of the plants under the FR NI flowered. Therefore, these results are not consistent with those reported with *Arabidopsis* and therefore may not necessarily be applicable to plants outside of the Brassicaceae.

Acknowledgments

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Table A.1
Parameters of regression analysis relating days to flower, flower bud or inflorescence number, or increase in height to the estimated phytochrome photoequilibrium of the night interruption.

Species	Parameter	Replicate	Regression equation	r^2
Petunia Easy Wave White	Days to flower	1	$y = 51.6 - 42.2x + 39.9x^2$	0.23***
	Days to flower	2	$y = 43.1 - 35.4x + 33.4x^2$	0.34***
	Flower no.	1 + 2	n.s.	n.s.
	Increase in height	1	$y = 1.8 + 28.2x - 28.9x^2$	0.17**
	Increase in height	2	$y = -0.3 + 34.4x - 29.7x^2$	0.23***
Petunia Shock Wave Ivory	Days to flower	1 + 2	$y = 54.8 - 71.0x + 56.3x^2$	0.37***
	Flower no.	1	$y = 31.2 + 4.6x + 16.8x^2$	0.22***
	Flower no.	2	$y = 14.0 + 35.4x - 7.6x^2$	0.32***
	Increase in height	1	$y = -2.2 + 47.2x - 41.3x^2$	0.34***
	Increase in height	2	$y = -4.0 + 67.7x - 63.1x^2$	0.44***
Petunia Wave Purple Improved	Days to flower	1	$y = 57.4 - 50.4x + 39.0x^2$	0.44***
	Days to flower	2	$y = 47.6 - 50.8x + 41.6x^2$	0.39***
	Flower no.	1 + 2	$y = 24.1 + 29.6x - 18.8x^2$	0.04*
	Increase in height	1 + 2	$y = -4.7 + 79.7x - 75.8x^2$	0.27***
Snapdragon	Days to flower	1	$y = 67.4 - 90.3x + 90.4x^2$	0.51***
	Days to flower	2	$y = 49.2 - 44.4x + 46.9x^2$	0.59***
	Inflorescence no.	1 + 2	$y = 29.8 - 52.5x + 52.9x^2$	0.15***
	Increase in height	1 + 2	$y = 55.1 - 42.5x + 38.4x^2$	0.13***
Rudbeckia	Days to flower	1 + 2	$y = 96.0 - 82.4x + 50.0x^2$	0.06*
	Inflorescence no.	1 + 2	$y = 16.7 - 27.5x + 28.8x^2$	0.15***
	Increase in height	1	$y = -18.6 + 157.5x - 103.4x^2$	0.74***
	Increase in height	2	$y = -22.8 + 190.7 - 142.7x^2$	0.73***
Fuchsia	Days to flower	1 + 2	$y = 60.3 - 13.2x$	0.06*
	Flower no.	1	$y = 67.4 - 182.6x + 187.4x^2$	0.24***
	Flower no.	2	$y = -6.1 + 69.8x - 39.7x^2$	0.31***
	Increase in height	1 + 2	$y = 31.8 - 8.7x$	0.03*

n.s., *, **, *** indicate nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Appendix A.

See Table A.1.

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