1 Far-red Radiation Promotes Growth of Seedlings by Increasing Leaf Expansion and Whole-plant
2 Net Assimilation

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4 Yujin Park and Erik S. Runkle *

5 Department of Horticulture, Michigan State University, 1066 Bogue Street, East Lansing, MI
6 48824, USA

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8 *Corresponding author. Tel.: +1 517 353 0350; fax: +1 517 353 0890.

9 E-mail address: runkleer@msu.edu (E.S. Runkle).

Abstract

By definition, photosynthetically active radiation (PAR) includes wavelengths between 400 and 700 nm and thus, far-red radiation (FR, 700 to 800 nm) is excluded when the photosynthetic photon flux density (PPFD) is measured and reported. However, FR radiation [and the ratio of red (R; 600 to 700 nm) to FR] regulates phytochrome-mediated morphological and developmental plant responses to promote radiation capture and survival under shade. We postulated that the inclusion of FR in a radiation spectrum would have little effect on photosynthesis but would increase radiation capture and plant growth, while accelerating the subsequent flowering of shade-avoiding species. Geranium (*Pelargonium ×hortorum*), petunia (*Petunia ×hybrida*), snapdragon (*Antirrhinum majus*), and impatiens (*Impatiens walleriana*) were grown at 20 °C under an 18-h photoperiod provided by sole-source lighting from light-emitting diodes that included 32 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of blue and the following intensities of R and FR radiation: R₁₂₈ (128 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R), R₁₂₈+FR₁₆, R₁₂₈+FR₃₂, R₁₂₈+FR₆₄, R₉₆+FR₃₂, and R₆₄+FR₆₄. Plant height in all species studied and total leaf area of geranium and snapdragon linearly decreased as the R:FR (or the estimated phytochrome photoequilibrium) of each treatment increased. In geranium and snapdragon, the increase in total leaf area (by 7 %) with the addition of FR to the same PPFD subsequently increased shoot dry weight (DW) (by 28-50%) while the increase in total leaf area (by 30-40%) with the partial substitution of R with FR partly compensated for the reduction in PPFD (by 40%), producing a similar shoot DW. Whole-plant net assimilation of geranium, snapdragon, and impatiens increased with additional FR radiation, showing a linear relationship with the calculated yield photon flux density of each radiation treatment. In addition, inclusion of FR during seedling growth promoted flowering in the long-day plant snapdragon. We conclude that FR radiation increases plant growth indirectly through leaf expansion and directly through whole-plant net assimilation and in at least some species, promotes subsequent flowering.

Keywords: controlled environments; dry mass accumulation; flowering; phenotypic plasticity; photosynthesis; shade-avoidance response

Abbreviations: B, blue radiation; FR, far-red radiation; LEDs, light-emitting diodes; PAR, photosynthetically active radiation; PPE, phytochrome photoequilibrium; PPFD, photosynthetic

41 photon flux density; Pr, R-radiation-absorbing phytochrome; Pfr, FR-radiation-absorbing
42 phytochrome; PSI, photosystem I; PSII, photosystem II; R, red; TPDF, total photon flux density;
43 YPDF, yield photon flux density.

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1. Introduction

Plants use several classes of photoreceptors including red (R; 600-700 nm) and far-red (FR; 700-800 nm) radiation-absorbing phytochromes and blue (B; 400-500 nm) and UV radiation absorbing cryptochromes to perceive different aspects of the radiation environment (Possart et al., 2014; Keuskamp et al., 2010). Plant shading reduces the amount of photosynthetically active radiation (PAR) and changes the spectral composition. Photosynthetic pigments strongly absorb photosynthetically efficient B and R radiation but reflect or transmit most photosynthetically inefficient FR radiation (Casal, 2013a). Therefore, radiation under a plant canopy has a low R:FR (Gommers et al., 2013). The R:FR ratio is about 1.2 in daylight and is reduced to 0.2 under a typical canopy (Heraut-Bron et al., 1999). While FR photons are weakly or not effective at promoting the photosynthetic reaction, the low R:FR perceived by the phytochrome photoreceptors can induce rapid changes in gene expression and physiological processes, which regulate the phenotypic plasticity (e.g., extension growth) of plants and enable them to better compete with neighboring plants (Keuskamp et al., 2010).

The shade-avoidance response of plants is mediated by phytochromes and typically includes elongation of internodes, petioles, and hypocotyls (often associated with a reduction in leaf development), apical dominance, and early flowering (Keuskamp et al., 2010; Ruberti et al., 2012). These growth and developmental responses can enable plants to outgrow shade and capture more photosynthetic radiation. In contrast, for plants that tolerate shade (a shade-tolerance response), leaf expansion increases to intercept more radiation with a concomitant reorganization of chlorophyll to improve photosynthetic efficiency (Dale, 1988). All of these plant responses to shade can promote radiation capture and subsequent survival under shade conditions (Nozue et al., 2015).

Phytochrome-regulated responses are often expressed in relation to the estimated phytochrome photoequilibrium (PPE), which is an indicator of the relative amount of active phytochrome in plants (Sager et al., 1988; Hogewoning et al., 2010). Phytochromes are photochromic biliproteins that exist in two photo-convertible isoforms: Pr, an R-radiation-absorbing form, and Pfr, a FR-radiation-absorbing form (Ciolfi et al., 2013). Upon absorption of R radiation, Pr is converted into the Pfr form (generally considered the biologically active form) that can absorb FR radiation and switch back to Pr (Ruberti et al., 2012). Following conversion to the Pfr form, phytochromes translocate to the nucleus to control several aspects of

photomorphogenesis (Li et al., 2011). The total pool of phytochrome in light-grown plants is constant, but the relative amounts in the Pfr and Pr forms (or PPE) depend on the R:FR ratio and darkness incident on the plant. A high R:FR ratio creates a high PPE, and vice versa. Models based on the distribution of incident spectral radiation have been developed to estimate the PPE and stem extension of a wide range of species and show an inverse linear relationship with estimated PPE. Therefore, the estimated PPE is useful in quantifying photomorphogenic responses to radiation quality (Smith, 1994; Runkle and Heins, 2001).

However, studies on the relationship between photomorphogenic response and estimated PPE have focused on shade-induced elongation. Overall plant growth and development in response to the estimated PPE is difficult to predict because the direction and extent of plastic responses to R:FR can differ among phytochrome-regulated plant responses. For example, unlike the inverse linear relationship between stem extension growth and the estimated PPE, the leaf expansion response to R:FR varies, ranging from inhibition to promotion, depending on growth conditions and species (Casal, 2013b; Demotes-Mainard et al., 2016). In addition, the acceleration of flowering was not correlated with the enhancement of elongation growth under a low R:FR in more than 100 accessions of arabidopsis (*Arabidopsis thaliana*), showing that ecotypes that responded strongly in elongation did not necessarily respond strongly in floral acceleration, and vice versa (Botto and Smith, 2002). Recent molecular studies have demonstrated that responses in different organs triggered by the same light signal are from distinct signaling cascades downstream of the activated photoreceptors in distinct tissues (Montgomery, 2016). Therefore, increased stem elongation does not necessarily correlate with leaf expansion or stimulation of flowering.

In many previous studies on plant responses to R:FR or shade, technically it was difficult to vary the R:FR ratio while maintaining other environmental constants, especially intensities of other radiation wavebands and the photosynthetic photon flux density (PPFD) (Dale, 1988). Thus, plant responses to R:FR often involved complex interactions with the radiation intensity and other wavebands, potentially confounding results. We utilized sole-source lighting from light-emitting diodes (LEDs) and highly controlled growth environments to determine how inclusion of FR in a radiation spectrum during seedling stage influences leaf area, radiation capture, and whole-plant net assimilation while also regulating characteristics relevant to the commercial production of seedlings such as plant habit (compactness) and subsequent growth

and flowering after transplant. The wavelength specificity of LEDs technically enables us to investigate how a specific combination of wavelengths regulates plant growth and development without unintentionally confounding effects from differences in radiation intensities and other wavebands (Massa et al., 2008; Morrow, 2008). LEDs are increasingly being used for sole-source lighting in commercial indoor plant production systems (Mitchell et al., 2015). One of the most cost-effective applications of LED sole-source lighting is young-plant production, in which high-value plants are grown at a high density and crop cycles are relatively short (e.g., 4 to 5 weeks). The vast majority of research with LED sole-source lighting has been published with only B and R radiation, and few studies have tested the benefits of including additional radiation wavebands (Mitchell et al., 2012; Randall and Lopez, 2014; Wollaeger and Runkle, 2015).

To determine the effects of FR addition on plant growth, quantified plant growth by measuring dry weight (DW) gain, which is determined by the product of net assimilation and total leaf area (Gregory, 1917; Vernon and Allison, 1963; Radford, 1967; Hunt et al., 2002; Casal, 2013b; Snowden et al., 2016). In plant growth analysis, DW gain can be separated into two components: net assimilation and leaf area. Net assimilation rate, which is defined by Gregory (1917) as the rate of increase of DW per unit leaf area, is a useful measure of the photosynthetic efficiency of plants (Vernon and Allison, 1963; Radford, 1967; Hunt et al., 2002; Casal, 2013b; Snowden et al., 2016). We postulated that the addition of FR radiation would have a negligible impact on whole-plant net assimilation, but as PPE decreased, changes in plant architecture (including leaf expansion and stem elongation) would increase radiation capture, potentially promoting plant growth. We also postulated that the inclusion of FR radiation during the seedling stage would promote the subsequent flowering of shade-avoiding species. We previously reported a subset of data for snapdragon (*Antirrhinum majus*) (Park and Runkle, 2016) which showed the impacts of FR radiation on plant architecture, whole-plant net assimilation, and shoot DW. Here we present a more comprehensive study with full results of snapdragon and other species. We anticipated results from this study would shed light on whether including FR radiation in a sole-source lighting spectrum elicited desirable plant responses including accelerated flowering and growth promotion.

2. Materials and Methods

2.1. Plant materials

Geranium (*Pelargonium ×hortorum* 'Pinto Premium Orange Bicolor'), petunia (*Petunia ×hybrida* 'Wave Blue'), snapdragon (*Antirrhinum majus* 'Trailing Candy Showers Yellow'), and impatiens (*Impatiens walleriana* 'Super Elfin XP Red') were chosen for study based on commercial significance, shade tolerance, and photoperiodic flowering characteristics. Geranium, petunia, and snapdragon are shade-avoiding species while impatiens is shade-tolerant; petunia and snapdragon are long-day plants while geranium and impatiens are day-neutral plants. Seeds of each species were sown in 128-cell (2.7 × 2.7-cm; 12.0-mL volume) plug trays by a commercial young plant producer (C. Raker and Sons, Inc., Litchfield, MI), and trays were moved within 7 d to a research greenhouse at Michigan State University (East Lansing, MI) with a 16-h photoperiod at 20 °C (see finish stage conditions below). Upon emergence of the first true leaves, each plug tray was then cut into four sections, each with ≥30 seedlings, thinned to one plant per cell. The experiment was performed twice in time and for each replication, the days from seed sow until first true leaf emergence was (rep 1, 2): geranium (7, 7), snapdragon (9, 10), petunia (9, 10), and impatiens (13, 10).

2.2. Radiation treatments and growth conditions

The six LED modules were placed on open, metal mesh benches inside a refrigerated walk-in growth chamber. The top of each of six LED modules [described by Wollaeger and Runkle (2013) but with new LED panels] contained a fan-cooled driver board with 80 LEDs each emitting B (peak = 447 nm), R (peak = 660 nm), and FR (peak = 731 nm) radiation and faced downward. Wire mesh was placed just below the middle half of the LED boards to provide a more uniform radiation intensity within each module. The intensities of the three LED types were adjusted manually by dimmer switches on the driver boards to create six radiation treatments based on an average of ten measurements from a spectroradiometer (PS-200; StellerNet, Inc., Tampa, FL) made at seedling-tray height at predetermined horizontal positions inside each module. The six radiation treatments were designed to determine the impacts of adding FR radiation to a typical B+R radiation spectrum (Table 1). The control treatment included 32 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of B and 128 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of R ($\text{B}_{32}+\text{R}_{128}$). The PPFD of 160 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was the target radiation intensity to achieve a photosynthetic daily light integral (DLI) of 10 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which is the recommended minimum DLI to produce high-quality young plants (Runkle, 2007). The B radiation intensity (32 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was kept constant for all radiation

treatments so that responses were not confounded by differences in cryptochrome-mediated responses (Pierik et al., 2004; Keuskamp et al., 2011; Pedmale et al., 2016). The second and third treatments were created by partial substitution of R radiation with FR radiation ($B_{32}+R_{96}+FR_{32}$, and $B_{32}+R_{64}+FR_{64}$) while keeping total photon flux density (TPF, 400-800 nm) identical to the control treatment. The other three treatments were created by the addition of FR radiation to a constant B+R radiation spectrum ($B_{32}+R_{128}+FR_{16}$, $B_{32}+R_{128}+FR_{32}$, and $B_{32}+R_{128}+FR_{64}$), and thus PPFD (400-700 nm) was identical to the control. At each replication, the six radiation treatments were randomly allocated to the LED modules. The random assignment of radiation treatments was carried out separately in each replication to decrease any positional effects of each LED module inside the growth chamber. In addition, plug trays were rotated daily to reduce any positional effects inside each LED module. For each radiation treatment, the yield photon flux density (YPPFD), which is the product of radiation intensity and relative quantum efficiency (in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), was calculated based on McCree (1972) and Sager et al. (1988); the R:FR was calculated with 100-nm wavebands; and the PPE was estimated with the spectra in Fig. 1, as described by Sager et al. (1988) (Table 1).

Fig. 1. The spectral distribution of six radiation treatments from blue (B), red (R), and far-red (FR) light-emitting diodes (LEDs). The values after each LED type indicate their intensity in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Table 1. Spectral characteristics of six radiation treatments. The values after each light-emitting diode type (B=blue; R=red; FR=far red) indicate their intensity in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Symbols are those used in Fig. 2 and 3.

Radiation treatment	Symbol	PPFD ¹	TPFD ²	YPFD ³	R:FR ⁴	PPE ⁵
B ₃₂ +R ₁₂₈ (control)	■	160	160	146	1:0	0.88
B ₃₂ +R ₉₆ +FR ₃₂	●	128	160	122	3:1	0.81
B ₃₂ +R ₆₄ +FR ₆₄	●	96	160	96	1:1	0.69
B ₃₂ +R ₁₂₈ +FR ₁₆	▽	160	176	149	8:1	0.85
B ₃₂ +R ₁₂₈ +FR ₃₂	▽	160	192	150	4:1	0.83
B ₃₂ +R ₁₂₈ +FR ₆₄	▽	160	224	157	2:1	0.78

¹PPFD: Photosynthetic photon flux density (photon flux integral between 400 and 700 nm, in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

²TPFD: Total photon flux density (photon flux integral between 400 and 800 nm, in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

³YPFD: Yield photon flux density, which is the product of TPFD and relative quantum efficiency (in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) based on McCree (1972) and Sager et al. (1988).

⁴R:FR: Ratio of photon flux integral of red (R; 600-700 nm) and far red (FR; 700-800 nm) radiation.

⁵PPE: Phytochrome photoequilibria, which is the estimated $P_{\text{FR}}/P_{\text{R+FR}}$ following Sager et al. (1988).

Plants were grown at a constant 20 °C under an 18-h photoperiod as controlled by a data logger (CR10; Campbell Scientific, Logan, UT). In each treatment, air and plant canopy temperature were measured by thermocouples (0.13-mm type E; Omega Engineering, Stamford, CT) above the plant canopy and infrared (IR) sensors (Type K, OS36-01; Omega Engineering) positioned 20 cm above the module bottom and pointing at a downward angle toward the canopy of the closest plant tray, respectively. Radiation intensity was measured continuously in each module by quantum sensors (LI-190R, LI-COR, Lincoln, NE) at plug tray level. The IR sensors, thermocouples, and quantum sensors were connected to the same data logger and environmental data were recorded every 10 s. The data logger recorded means every 10 min throughout the duration of the experimental replications. Average air/canopy temperatures during the experiment periods were 20.4/20.4°C, 20.4/20.5°C, 20.2/20.2°C, 20.4/20.5°C, 20.5/20.5°C, and

20.5/20.7°C for the B₃₂+R₁₂₈, B₃₂+R₁₂₈+FR₁₆, B₃₂+R₁₂₈+FR₃₂, B₃₂+R₁₂₈+FR₆₄, B₃₂+R₉₆+FR₃₂, and B₃₂+R₆₄+FR₆₄ treatments, respectively. All temperatures had standard deviations (SD) ≤ ±0.8°C. Plants were irrigated as needed, every two or three days, through subsurface irrigation with deionized water supplemented with a water-soluble fertilizer providing (in mg·L⁻¹) 50 N, 19 P, 50 K, 23 Ca, 4 Mg, 1 Fe, 0.5 Mn, 0.5 Zn, 0.5 Cu, 0.3 B, and 0.1 Mo (MSU Plug Special; GreenCare Fertilizers, Inc., Kankakee, IL).

2.3. Growth conditions during the finish stage

At the end of the seedling stage, ten seedlings from each treatment and replication were randomly selected and transplanted into 10-cm pots containing a 70% peatmoss, 21% perlite, and 9% vermiculite potting media (SUREMIX; Michigan Grower Products, Inc., Galesburg, MI) to determine whether the sole-source radiation treatments during the seedling stage had any subsequent effects on flowering when grown in a common greenhouse environment. Pots were randomly placed on benches in a glass-glazed, environmentally controlled greenhouse at a constant temperature set point of 20 °C. Supplemental lighting provided by 400-W high-pressure sodium lamps delivered a PPFD of 77 (SD ±13) μmol·m⁻²·s⁻¹ at plant height and a 16-h photoperiod. The high-pressure sodium lamps were controlled by an environmental control computer and were automatically switched on from 0600 to 2200 HR when the ambient solar PPFD was <185 μmol·m⁻²·s⁻¹ and off when it was >370 μmol·m⁻²·s⁻¹.

2.4. Data collection and analysis

The experiment was performed twice. In each replication, 20 random plants of each species in each treatment were used for data collection: ten plants at the end of the seedling stage and ten plants at the finishing stage. At the end of the seeding stage, ten random plants of each species in each treatment, usually excluding outer guard rows, were harvested the following number of days after seed sow (rep 1, 2): geranium (29, 30), snapdragon (35, 37), petunia (31, 31), and impatiens (37, 35). The following data were collected on plants in each treatment: leaf (at node) number, total leaf area [using a leaf-area meter (LI-3000; LI-COR)], SPAD index [using a portable chlorophyll meter (SPAD-502, Minolta corporation, Ltd., Osaka, Japan)], plant height (from media level to apical meristem), and shoot and root DW (after plants were dried in an oven at ≥66 °C for ≥5 d and using a Mettler Toledo PG5002 scale, Columbus, OH). Whole-

plant net assimilation was calculated by dividing shoot DW by total leaf area ($\text{g}\cdot\text{m}^{-2}$) for each plant (Gregory, 1917; Vernon and Allison, 1963; Radford, 1967; Hunt et al., 2002; Casal, 2013b; Snowden et al., 2016). A visible leaf that was 25% or greater unfolded was counted in leaf number. SPAD index measurements were taken at the central point of the leaflet between the midrib and the leaf margin of the second or third fully expanded leaves from the apical shoot. Three readings per leaf per plant were taken and averaged to a single SPAD value for each plant. During the finishing stage, data collected on ten plants of each species in each treatment included date of first flowering, flower bud or inflorescence number, and length of the primary stem at first flowering.

The experiment used a randomized complete block design with two blocks and ten subsamples per block. Each replication was regarded as a block. Each LED module was regarded as the experimental unit for the radiation treatment. Within each LED module, ten individual seedlings per species were the sub-samples or observational units. Data were analyzed with the SAS (version 9.4; SAS Institute, Inc., Cary, NC). Regression analysis was performed with the PROC REG procedure to relate the plant data parameters to the estimated PPE and YPFD of the radiation treatments. In regression analysis, the mean for each replication was treated as a single data point and included 12 data points for the six radiation treatment effects (2 replications $\times$ 6 treatments) in Fig. 2, 3, and 4; 8 data points for the FR addition effects (2 replications $\times$ 4 treatments) in Fig. 3; and 6 data points for the FR substitution effects (2 replications $\times$ 3 treatments) in Fig. 3. Flowering data were analyzed with the PROC MIXED procedure [with a fixed factor for radiation treatments, a random factor of blocks (or replications), and a random factor for interaction between blocks and radiation treatments] that provided pairwise comparisons between treatments by using Tukey's honestly significant test at $P \leq 0.05$.

3. Results

Seedling stem length decreased linearly as the estimated PPE of each treatment increased from 0.69 to 0.88, although the magnitude of the increase varied by species (Fig. 2). Stem length of geranium, petunia, snapdragon, and impatiens decreased by 41, 95, 41 and 24%, respectively, as the estimated PPE of each treatment increased from 0.69 to 0.88. The largest increase in stem length of petunia under the lowest PPE (0.69) in this study was accompanied by an upright growth habit while the plants under the other treatments showed a rosette growth habit.

At least for geranium and snapdragon, total leaf area also decreased linearly as the estimated PPE of each treatment increased, but to a lesser extent than stem extension (Fig. 2). Treatment did not affect the number of leaves except for petunia, in which plants under light with a PPE=0.69 had 0.7-1.3 fewer leaves than those under other light treatments (data not shown). Thus, the decrease in total leaf area with increasing PPE can be attributed to a decrease in individual leaf size.

Fig. 2. Influence of the estimated phytochrome photoequilibria (PPE) of six radiation treatments on seedling stem length, leaf area, and SPAD reading. Six radiation treatments were categorized into far-red (FR; 700-800 nm) substitution treatments ($B_{32}+R_{96}+FR_{32}$ and $B_{32}+R_{64}+FR_{64}$), FR addition treatments ($B_{32}+R_{128}+FR_{16}$, $B_{32}+R_{128}+FR_{32}$, and $B_{32}+R_{128}+FR_{64}$), and control treatment ($B_{32}+R_{128}$). Each data point represents the mean and standard error of two replications with 10 subsamples (plants) per replication and species. Associated correlation coefficients (R^2) and regression equations are presented

when statistically significant (solid line) but not when not significant (dotted line). *, **, *** indicate significant at $P < 0.05$, 0.01 or 0.001, respectively.

SPAD value, which is an index of chlorophyll concentration per unit leaf area, was correlated positively with the estimated PPE of each treatment for geranium, petunia, and snapdragon, but not impatiens (Fig. 2). When chlorophyll content per leaf was estimated by multiplying SPAD value by total leaf area, the values were similar among treatments in all species (data not shown).

When the PPFD of each treatment was the same, shoot DW of geranium, petunia, and snapdragon showed similar inverse linear relationships with estimated PPE as those observed for stem elongation and leaf expansion (Fig. 3). In other words, DW increased with the addition of FR radiation. When the PPFD was reduced by up to 40% as R radiation was partially substituted with FR, shoot DW of these three species was similar among radiation treatments. In contrast, shoot DW of impatiens was similar when the total PPFD of each treatment was the same while shoot DW decreased under the lowest PPE (0.69) compared to under other treatments.

Shoot DW per unit leaf area or whole-plant net assimilation of geranium and snapdragon was negatively correlated with the estimated PPE of each treatment when PPFD of each treatment was the same (Fig. 3), indicating that the increase in shoot DW with additional FR radiation was related to an increase in whole-plant net assimilation. Under the FR substitution treatments, as both PPFD and PPE decreased, dry shoot weight per unit leaf area of geranium decreased linearly. In addition, shoot DW per unit leaf area of geranium, snapdragon, and impatiens showed a positive correlation with the calculated YPF_D of each treatment (Fig. 4).

There was little to no effect of FR addition or partial substitution for R radiation (and thus, PPE) on root DW (data not shown). With few significant effects of light treatments on root DW, total (root+shoot) DW showed a similar pattern as shoot DW in relation to the PPE (data not shown). The shoot to root ratio (S:R) decreased linearly as the estimated PPE of each treatment increased from 0.69 to 0.88 in snapdragon but the trend was not significant in other species (Fig. 3).

Fig. 3. Influence of the estimated phytochrome photoequilibria (PPE) of six radiation treatments on plant dry weight (DW). Six radiation treatments were categorized into far-red (FR; 700-800 nm) substitution treatments ($B_{32}+R_{96}+FR_{32}$ and $B_{32}+R_{64}+FR_{64}$), FR addition treatments ($B_{32}+R_{128}+FR_{16}$, $B_{32}+R_{128}+FR_{32}$, and $B_{32}+R_{128}+FR_{64}$), and control treatment ($B_{32}+R_{128}$). Each data point represents the mean and standard error of two replications with 10 subsamples (plants) per replication and species. Associated correlation coefficients (R^2) and regression equations are presented when statistically significant (solid line) but not when not significant (dotted line). NS, *, or ** indicate nonsignificant or significant at $P < 0.05$ or 0.01 , respectively.

Fig. 4. Influence of the calculated yield photon flux density of six radiation treatments on shoot dry weight (DW) per unit leaf area. Each data point represents the mean and standard error of two replications with 10 subsamples (plants) per replication and species. Associated correlation of coefficients (R^2) and regression equations are presented when statistically significant (solid line) but not when not significant (dotted line). NS, *, **, *** indicate nonsignificant or significant at $P < 0.05$, 0.01 or 0.001, respectively.

In the long-day plant snapdragon, subsequent flowering was promoted by 10 to 12 d when treatments included $\geq 16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR radiation (and the resulting PPE was ≤ 0.85) (Table 3). The earlier flowering of snapdragon was accompanied by a shorter stem length at flowering. In the long-day plant petunia, the number of visible buds at flowering under the lowest PPE (0.69) in this study was significantly less than those grown under a higher PPE, but flowering time and stem length at flowering were not influenced by the seedling treatments. In contrast, days to flower, flower bud or inflorescence number, and stem length at flowering in the day-neutral species geranium and impatiens did not show a treatment response.

349 Table 3. Influence of the estimated phytochrome photoequilibria (PPE) of six radiation
350 treatments on flowering characteristics. The values after each LED type (B=blue; R=red; FR=far
351 red) indicate their intensity in $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Data represent the means of two replications (n =
352 20), and values with the same letter are not statistically different at $P = 0.05$.

Radiation treatment	Days to flower	Stem length (cm)	Flower bud or inflorescence no.
Geranium 'Pinto Premium Orange Bicolor'			
B ₃₂ +R ₁₂₈ (control)	56.1	19.7	3.2
B ₃₂ +R ₉₆ +FR ₃₂	54.6	19.6	3.0
B ₃₂ +R ₆₄ +FR ₆₄	54.8	18.5	3.7
B ₃₂ +R ₁₂₈ +FR ₁₆	54.9	19.2	3.0
B ₃₂ +R ₁₂₈ +FR ₃₂	54.0	19.8	2.9
B ₃₂ +R ₁₂₈ +FR ₆₄	53.6	19.2	2.9
Significance	NS	NS	NS
Petunia 'Wave Blue'			
B ₃₂ +R ₁₂₈ (control)	34.2	8.4	18.7 a
B ₃₂ +R ₉₆ +FR ₃₂	26.6	7.0	9.3 a
B ₃₂ +R ₆₄ +FR ₆₄	25.8	7.8	8.0 b
B ₃₂ +R ₁₂₈ +FR ₁₆	29.6	7.7	17.1 a
B ₃₂ +R ₁₂₈ +FR ₃₂	30.0	6.7	14.9 a
B ₃₂ +R ₁₂₈ +FR ₆₄	28.2	5.7	13.4 a
Significance	NS	NS	*
Snapdragon 'Trailing Candy Showers Yellow'			
B ₃₂ +R ₁₂₈ (control)	31.1 a	17.5 a	14.8
B ₃₂ +R ₉₆ +FR ₃₂	18.8 b	12.6 b	9.3
B ₃₂ +R ₆₄ +FR ₆₄	20.8 b	14.4 b	8.2
B ₃₂ +R ₁₂₈ +FR ₁₆	18.6 b	13.3 b	8.7
B ₃₂ +R ₁₂₈ +FR ₃₂	18.7 b	13.0 b	10.4
B ₃₂ +R ₁₂₈ +FR ₆₄	18.3 b	12.8 b	10.6
Significance	***	**	NS

Impatiens 'Super Elfin XP Red'

B ₃₂ +R ₁₂₈ (control)	17.2	4.9	11.8
B ₃₂ +R ₉₆ +FR ₃₂	16.7	5.1	13.6
B ₃₂ +R ₆₄ +FR ₆₄	17.8	5.5	15.6
B ₃₂ +R ₁₂₈ +FR ₁₆	18.1	5.9	13.6
B ₃₂ +R ₁₂₈ +FR ₃₂	19.5	5.6	18.9
B ₃₂ +R ₁₂₈ +FR ₆₄	16.2	5.1	14.9
Significance	NS	NS	NS

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

4. Discussion

Stem elongation in all species studied and leaf expansion of geranium and snapdragon showed an inverse linear relationship with the estimated PPE regardless of differences in the PPFD among treatments (Fig. 2). The linearity between PPE and plant responses suggests that R:FR and phytochrome directly regulated responses. Green leaves absorb radiation strongly in the range of PAR (400 to 700 nm) but reflect or transmit most FR radiation, and thus the extent of the reduction in R:FR or PPE is quantitatively related to the density and proximity of neighboring vegetation (Smith and Whitelam, 1997; Casal, 2013a). In response to the reduced R:FR or PPE, shade-avoiding species promote and direct extension growth in an attempt to better harvest available sunlight (Smith, 1994; Franklin, 2008), while shade-tolerant species typically display few or no shade-avoidance traits, such as elongation responses in stems and petioles, compared to shade-avoiding or open-habitat species (Gommers et al., 2013). In our study, in stem length and total leaf area, the shade-avoiding geranium, petunia, and snapdragon were sensitive to the changes in PPE, as indicated by the steep slopes of the regression lines, while shade-tolerant impatiens was the least responsive to the PPE, as indicated by relatively flat slope and no correlation with PPE (Fig. 2). In the shade-avoiding geranium and snapdragon, the slope of the line in both stem elongation and total leaf area was similar, showing similar sensitivity to R:FR or PPE. The inverse linear relationship between stem elongation and the estimated PPE has been reported in many species (Smith, 1994), and the magnitude of stem elongation mostly involves increased cellular expansion (Sasidharan et al., 2008). In contrast, FR radiation addition

(or a lower R:FR ratio) increased or decreased total leaf area, depending on species and growth conditions (Demotes-Mainard et al., 2016). In our study, decreasing R:FR or PPE increased the total leaf area of geranium and snapdragon and it was attributed to the increase in individual leaf size, not leaf number.

In large white petunia (*Petunia axilaris*), leaf expansion in FR-treated plants (FR=52 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, PPFD=180 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was related to the late phase of leaf growth and larger cells (Casal et al., 1987). Low R:FR-grown (R:FR=0.1, PPFD=130 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) leaves of arabidopsis displayed enhanced cell expansion of all cell types, suggesting that the regulation of leaf growth was mediated primarily by changes in cell expansion (Patel et al., 2013). In a separate study, the reduced leaf size of arabidopsis grown under low R:FR (R:FR=0.1, PPFD=27 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was caused by a decreased cell number with a negligible effect on cell size (Carabelli et al., 2007). In addition, the inhibitory effect of FR radiation on leaf expansion in several species was, in part, attributed to the consequence of competition for resources with the elongated stem, which reduced the carbon supply to leaf growth (Casal et al., 1987). In general, the leaves deprived of photosynthate or nutrients developed fewer cells than plants with a greater carbon supply because cell division in plants has been correlated with carbohydrate supply (Van Volkenburgh, 1999). These results indicate that leaf expansion is promoted under low R:FR when the PPFD is sufficient for growth while it is inhibited under low R:FR when the PPFD is excessively low and thus, when the carbon supply for leaf growth is limited. This suggests that the contrasting effect of low R:FR in leaf expansion in previous reports can be attributed to the PPFD of the shade treatments and their effect on the subsequent carbon supply for leaf growth. The consistent increase in leaf size with decreasing R:FR and PPE in our study is therefore likely from an increase in the cell expansion because the PPFD was sufficient for growth and thus, the carbon supply for leaf cell division was adequate.

Dry mass accumulation of plants is a function of the incident radiation, the efficiency of incident radiation interception, and conversion of captured radiation into biomass through photosynthesis, as well as other environmental and cultural conditions (Heraut-Bron et al., 1999; Maliakal et al., 1999; Richards, 2000; Casal, 2013b). The efficiency of radiation capture is primarily affected by plant architecture and especially by leaf area because radiation capture increases with increasing leaf area (Cope et al., 2014). In plant growth analysis, growth has been interpreted as the product of net assimilation and total leaf area (Gregory, 1917; Vernon and

Allison, 1963; Radford, 1967; Hunt et al., 2002; Casal, 2013b; Snowden et al., 2016). Several studies showed that plant growth is determined more by parameters related to leaf area, such as total leaf area of plants, specific leaf area (the ratio of leaf area to leaf dry mass), and leaf area index (the leaf area per unit ground surface area), than by area-based photosynthesis, indicating that leaf area is an important parameter in determining plant growth (Klassen et al., 2003; Hogewoning et al., 2010; Weraduwege et al., 2015; Snowden et al., 2016). In our study, in geranium and snapdragon, the addition of FR to the same PPFD, which reduced the PPE, increased total leaf area (by 7%) and subsequently increased shoot DW (by 28-50%) (Fig. 2 and 3). Also, an increase in leaf area (by 30-40%) under FR substitution treatments partly compensated for the 40% decrease in PPFD, enabling plants to produce a similar shoot biomass (as measured by DW) under more light-limiting conditions (Fig. 2 and 3). The promotive effect of FR radiation on leaf expansion and subsequent dry mass accumulation has been previously reported including in lettuce (*Lactuca sativa*) (Li and Kubota, 2009; Stutte et al., 2009) and arabidopsis (Patel et al., 2013). Together, these results suggest that FR radiation can indirectly promote biomass accumulation by establishing a canopy with a larger effective leaf area and thus, better radiation interception.

Different wavelengths have different quantum yields for CO₂ fixation. R radiation generally has the highest quantum yield, but it decreases dramatically at longer red wavelengths (above 680 nm), and FR radiation alone is considered inefficient at driving photosynthesis (McCree, 1972). However, our results showed that the additional FR radiation increased whole-plant net assimilation (Fig. 3). YPFD has been suggested as a more accurate predictor of photosynthesis than PPFD; it includes a broader waveband (300 to 800 nm) and photons of each wavelength are weighed by the relative quantum efficiency (RQE) (McCree, 1972; Sager et al., 1988; Cope and Bugbee, 2013). In our study, the RQE value at the emission peak of the FR LED (731 nm) was 0.15 while it was 0.93 for the R LED (660 nm). Thus, compared to the calculated YPFD under B+R, YPFD decreased by 34% when 50% of R radiation was substituted with FR and increased by up to 8% when FR radiation was added to B+R radiation (Table 1). The whole-plant net assimilation in three species showed a positive correlation with the calculated YPFD, within the range from 96 to 157 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 4). When YPFD decreased by 34% in FR substitution treatments, whole-plant net assimilation decreased by 22-33%. When YPFD increased by 8% in FR addition treatments, whole-plant net assimilation increased by 20-37%.

437 Considering the increase in whole-plant net assimilation for FR addition treatments was caused
438 only by the increase in YPF_D by the addition of the photons in FR region, this result shows that a
439 small increase in quantum yield with FR photons can have large direct effects on whole-plant net
440 assimilation.

441 FR radiation has a low quantum yield because of insufficient excitation of photosystem II
442 (PSII) with photosystem I (PSI) overexcitation (Emerson and Lewis, 1943; Duysens et al., 1961;
443 Pettai et al., 2005; Thapper et al., 2009). PSII, which preferentially absorbs wavelengths between
444 400-680 nm and maximally at around 680 nm, and PSI, which absorbs FR maximally at around
445 700 nm, operate in series to carry out photosynthesis in algae and higher plants (Duysens et al.,
446 1962; Evans and Anderson, 1987; Hogewoning et al., 2012; Laisk et al., 2014). Since PSII
447 determines the rate of electron supply to PSI, insufficient excitation of PSII only with FR
448 radiation strictly limits the overall quantum yield of photosynthesis (Duysens et al., 1961;
449 Blankenship, 2002; Pettai et al., 2005). It is postulated that for stable operation with high
450 efficiency, electron transport rates through these two photosystems should be balanced and any
451 imbalance in excitation of the two photosystems decreases quantum yield (Allen, 2003;
452 Pfannschmidt, 2005; Hogewoning et al., 2012). Although the peaks of the R LED (peak = 660
453 nm) and FR LED (peak = 731 nm) used in this study are slightly different from the paradigm
454 peaks of PSII and PSI, they are within the range of their absorption spectrum. Thus, the added
455 FR radiation possibly contributed to increased net assimilation by balancing the excitation
456 between PSI and PSII while without FR, B+R radiation overexcited PSII relative to PSI.

457 However, the increase of net assimilation by FR radiation could be a consequence of the
458 acclimation process of leaf architecture to a low R:FR. In our study, low R:FR or PPE increased
459 leaf area and decreased SPAD value (an index of chlorophyll concentration per unit leaf area)
460 (Fig. 2). In previous studies, although leaf area responses to R:FR have varied (ranging from an
461 increase to a decrease), generally a decrease in R:FR decreases chlorophyll content per unit area
462 (Heraut-Bron et al., 1991; Demotes-Mainard et al., 2016), increases specific leaf area, and
463 reduces leaf thickness (Smith and Whitelam, 1997; Gommers et al., 2013). In general, thinner
464 shade leaves showed lower light compensation points and lower maintenance respiration rates
465 compared to thicker sun-acclimated leaves, maintaining a higher net photosynthesis (gross
466 photosynthesis minus respiration) under lower light than sun leaves (Chapin et al., 2011;
467 Weraduwege et al., 2015). Also, under high R:FR, leaf mass per area and stomatal density

increased, increasing photosynthesis in response to a higher PPFD (Boccalandro et al., 2009). These results suggest that low R:FR signals plants to adjust their leaf architecture and subsequent photosynthetic properties to shade conditions (lower PPFD and R:FR). Considering that the photosynthetic radiation intensity ($PPFD = 96\text{-}160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in this study was relatively low for both shade-avoiding and shade-tolerant species, the leaves developed under low R:FR with FR radiation addition might contribute to the increase in whole-plant net assimilation under low PPFD conditions.

The acceleration of flowering by low R:FR is one characteristic of the shade-avoidance response (Smith and Whitelam, 1997; Morelli and Ruberti, 2000; Nozue et al., 2015). Our results are not consistent with this paradigm; while other shade-avoidance responses were regulated by low R:FR or PPE, flowering of three crops was not influenced by the R:FR or PPE within the range studied while in snapdragon, FR promoted flowering but the response was apparently saturated by $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (or $PPE \leq 0.85$; Table 3). This result is consistent with the existence of separate signaling mechanisms regulating phytochrome-mediated changes in plant architecture and floral induction (Botto and Smith, 2002; Cerdan and Chory, 2003; Nozue et al., 2015). In arabidopsis, while CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) and the four SUPPRESSOR OF PHYA-105 (SPA) genes are essential for hypocotyl and leaf petiole elongation in response to low R:FR, the acceleration of flowering in response to a low R:FR was normal in *cop1* and *spa* mutants, demonstrating that the pathways of flowering time in response to low R:FR were different from those of elongation responses (Rolauffs et al., 2012). Also in arabidopsis (a long-day plant), a low R:FR shade signal promoted flowering partially through activation of the photoperiodic pathway (Kim et al., 2008) and low R:FR had little effect on acceleration of flowering under a short day compared to a long day (Wollenberg et al., 2008). In our study, the photoperiod of the radiation treatments was 18 hours, and the short night length combined with a lower R:FR during the day maximally accelerates flowering of at least some long-day plants, such as snapdragon. Also, the values of the effective PPE for promoting flowering of snapdragon in our study (0.69 to 0.85; Table 3) were similar with those most effective for night interruption lighting (0.63-0.80) in long-day plants including snapdragon and petunia (*Petunia $\times$ hybrida*) (Craig and Runkle, 2016). These results suggest that including FR radiation in a light spectrum can regulate subsequent flowering independently from photomorphogenic responses such as extension growth of leaves and stems.

Here we showed that inclusion of FR promoted plant growth indirectly through leaf expansion and directly through an increase in whole-plant net assimilation. In addition, inclusion of FR during seedling growth promoted subsequent flowering in one long-day species. Although the effects of FR (and subsequent estimated PPE) on phytochrome-mediated responses was quantitatively different for some characteristics measured (such as between stem elongation and leaf expansion) or independent for others (such as between stem elongation and acceleration of flowering), the estimated PPE of the radiation spectrum showed an inverse linear relationship with several plant morphological traits including leaf expansion and stem elongation. In addition, the calculated YPFD positively correlated with whole-plant net assimilation. These results indicate that inclusion of FR radiation in a radiation spectrum, whether from conventional incandescent lamps or more modern LEDs, can promote plant size and growth while regulating morphological and flowering characteristics as desired.

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