Growth Responses of Ornamental Annual Seedlings Under Different Wavelengths of Red Light Provided by Light-emitting Diodes

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Abstract. Light-emitting diodes (LEDs) are of increasing interest in controlled environment plant production because of their increasing energy efficiency, long lifetime, and color can be combined to elicit desirable plant responses. Red light (600–700 nm) is considered the most efficient wavelength for photosynthesis, but little research has compared growth responses under different wavelengths of red. We grew seedlings of impatiens (Impatiens walleriana), petunia (Petunia × hybrida), tomato (Solanum lycopersicum), and marigold (Tagetes patula) or salvia (Salvia splendens) at 20 °C under six sole-source LED lighting treatments. In the first experiment, a photosynthetic photon flux (PPF) of 160 μmol·m⁻²·s⁻¹ was provided for 18 h·d⁻¹ by 10% blue (B; peak = 446 nm) and 10% green (G; peak = 516 nm) lights, with the remaining percentages consisting of orange (O; peak = 596 nm)—red (R; peak = 634 nm)—hyper red (HR; peak = 664 nm) of 20–30–30, 80–0–0, 0–20–60, 0–40–40, 0–80–0, and 0–0–80, respectively. There were no consistent effects of lighting treatment across species on any of the growth characteristics measured including leaf area, plant height, or shoot fresh weight. In a second experiment, seedlings were grown under two light intensities (low, 125 μmol·m⁻²·s⁻¹, and high, 250 μmol·m⁻²·s⁻¹) consisting of 10% B and 10% G light and the following percentages of R–HR: 0–80, 40–40, 80–0. Shoot fresh weight was similar in all light treatments, whereas shoot dry weight was often greater under the higher light intensity, especially under the 40–40 treatments. Leaf chlorophyll concentration under 40–40 was 80–0, or both was often greater than that in plants under the high light treatments, indicating that plants acclimated to the lower light intensity to better use photons available for photosynthesis. We conclude that O, R, and HR light have generally similar effects on plant growth at the intensities tested when background G and B lights are provided and thus, selection of red LEDs for horticultural applications could be based on other factors such as economics and durability.

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Materials and Methods

Expt. 1. The effect of red light wavelengths on plant growth. Four popular bedding plant species with varying shade tolerances were chosen for study: tomato (Solanum lycopersicum ‘Early Girl’), marigold (Tagetes patula ‘Deep Orange’), impatiens (Impatiens walleriana ‘SuperElfin XP Red’), and petunia (Petunia × hybrida ‘Wave Pink’). Seeds were sown in 128-cell plug trays (12.0-mL cell volume) at a commercial greenhouse (C. Raker & Sons, Inc., Litchfield, MI) and transferred to research greenhouses at Michigan State University (East Lansing, MI) within 2 d. Seeds were kept in a propagation greenhouse at 23 °C until >70% germinated, which was 2 d (replication 1) or 7 d (replication 2) after seed sow. Each plug tray was then cut into six sections each with ≥20 seedlings, thinned to one plant per cell, and placed in the LED modules.

Light environments. Six LED modules were custom-designed and constructed for experimentation (Osmar OptoSemiconductors, Northville, MI) (Fig. 1). The white rigid plastic modules had four sides and were 80 cm deep, 27 cm wide, and 52 cm tall. The top of each module contained blue (B, peak = 446 nm), green (G, peak = 516 nm), orange (O, peak = 596 nm), red (R, peak = 634 nm), and hyper red (HR, peak = 664 nm) LEDs that were uniformly distributed, facing downward inside the module. Eighty LEDs of each color were mounted on fan-cooled driver boards that were open to the environment to allow for adequate cooling. The light output of each color of LED could be adjusted manually by a dimmer switch. The LEDs were mounted 25 to 33 cm from the foliage canopy due to crop height variation. To improve air circulation within the module, 33 holes (diameter = 4 cm) were cut in the bottom. The light modules were placed on open, metal mesh benches inside the same refrigerated walk-in growth chamber.

Six light treatments were randomly allocated to the light modules for each replication and the light quality treatments were set to the desired ratios using a portable spectroradiometer (StellarNet Inc., model PS-200; Apogee Instruments, Inc., Logan, UT) with a PPF constant at 160 μmol·m⁻²·s⁻¹. All treatments delivered 10% B and 10% G light, with the remaining light quality percentages consisting of O–R–HR of 20–30–30, 0–80–0, 0–60–20, 0–40–40, 0–20–60, and 0–0–80. Predicted phytochrome photoequilibrium (PFR/FR, where P = PFR + FR) values were similar among all light treatments (0.88–0.89) (Sager et al., 1988). To increase uniformity of light intensity within each module, wire mesh was placed in the middle half of the chamber, just below the LEDs. The plant trays were randomly rearranged daily to reduce any spatial variability inside each module. The spectral quality of the light treatments was evaluated at six positions inside each LED module with the spectroradiometer (Fig. 2A).

Plants were grown under an 18-h photoperiod (0500–2300 HR) as controlled by a data logger (CR10; Campbell Scientific, Logan, UT). Air temperature of the growth chamber was set to 20 °C. Canopy temperature was measured by infrared sensors (Type K, OS36-01; Omega Engineering, Stamford, CT) positioned 17 cm from the module bottom and pointing downward toward the canopy of the closest plant tray, and air temperature was measured by shielded thermocouples (0.13-mm type E; Omega Engineering) inside each module at plant level.

Light intensity was measured continuously in each module by quantum sensors (LI-COR, Lincoln, NE) placed in the middle of each module at plug tray level. Environmental parameters were measured every 10 s and data were recorded by the data logger every 10 min throughout the duration of the experiments (Table 1). Plants were irrigated as needed, once or twice daily, through sub-surface irrigation with deionized water supplemented with a water-soluble fertilizer to provide the following (mg L⁻¹): 50N–19P–50K–23Ca–4Mg–1.0Fe–0.5Mn, Zn, and Cu–0.3B, and 0.1Mo (MSU Plug Special; GreenCare Fertilizers, Inc., Kankakee, IL).

Data collection and analysis. The experiment was performed twice and 10 plants of each species and treatment were selected at random and harvested the following number of days after seed sow (replication 1, 2): tomato (33, 31), marigold (34, 33), impatiens (43, 38), and petunia (45, 39). The following data were collected on plants in each treatment: leaf number (total leaf number including axillary branches on impatiens and petunia), leaf area [using a leaf area meter (LI-3000; LI-COR), stem height (from media level to apical meristem), shoot fresh weight, shoot dry weight (dried at ≥66 °C for ≥5 d), number of visible flower buds (if present), and flower bud fresh weight (if applicable). Effects of species and light treatments were compared by analysis of variance using SAS (SAS Institute, Cary, NC)
NC) PROC MIXED or PROC GLIMMIX (Poisson distribution for count data), with an additional program (Arnold M. Saxton, University of Tennessee, Knoxville, TN) that provided pairwise comparisons between treatments using Tukey honestly significant test at $P \leq 0.05$.

Expt. 2. The effects of red light ratios at two intensities. Experimental procedures and data collection were followed as reported in Expt. 1 unless otherwise noted. One 128-cell tray of the same tomato, impatiens, and petunia varieties in addition to salvia (*Salvia splendens* ‘Vista Red’) were obtained from the same commercial greenhouse. A low (125 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) or high (250 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) intensity was delivered with three light quality treatments. All treatments delivered 10% B and 10% G light, with the remaining light quality percentages consisting of R–HR: 0–80, 40–40, and 80–0 (Fig. 2B). Ten randomly selected plants were harvested the following number of days after germination (replication 1, 2): tomato (32, 33), impatiens (35, 34), petunia (37, 35), and salvia (39, 36). The total leaf number, including axillary branches, was counted on impatiens.

**Chlorophyll assay.** Chlorophyll concentration was measured using the procedure described by Richardson et al. (2002) 28 d after seed sow. Fresh leaf samples of 100 ± 2 mg, measured using a scale (Denver Instrument APX-320; Bohemia, NY), were placed in disposable culture glass tubes (16 × 100 mm; WMR International, West Chester, PA) and 7 mL of dimethylsulfoxide (DMSO; EMD Millipore, Billerica, MA) was added using an electronic pipette (Eppendorf Easypet; Hamburg, Germany) and heated in a deionized water bath (Isotemp 210; Fisher Scientific, Pittsburg, PA) at 65°C for 40 min. Three mL of DMSO was added to each sample tube and the electronic pipette was used to place 1.5 mL of the extraction solution into foil-wrapped 1.7 mL tubes (Posi-Click; Denville Scientific Inc., South Plainfield, NJ) to prevent photo- or thermo-degradation. Each sample was poured into a 1.5 mL polystyrene cuvette (Semimicro; Generation Biotech, Lawrenceville, NJ) and the absorbance of each sample was measured against a blank standard (DMSO) at 645 and 663 nm using a spectrophotometer (BioSpec 1601; Shimadzu, Kyoto, Japan). Chlorophyll $a$, $b$, and total chlorophyll concentrations were determined using the equations by Arnon (1949).

### Results

**Expt. 1.** The mean leaf number was similar among treatments for all species and was 25, 10, 8, and 29 for impatiens, marigold, tomato, and petunia, respectively (data not shown). Total leaf area was similar among treatments in impatiens, marigold, and petunia, whereas in tomato, it was lower under the

### Table 1. Air and canopy temperatures during Expts. 1 and 2 for all light quality treatments reported in percentage of the PPF emitted from orange (O), red (R), and hyper red (HR) light-emitting diodes (LEDs). All treatments also received 10% blue and 10% green light.

<table>
<thead>
<tr>
<th>LED (%)</th>
<th>Expt.</th>
<th>O</th>
<th>R</th>
<th>HR</th>
<th>PPF (( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} ))</th>
<th>Temperature (°C)</th>
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<tr>
<td></td>
<td></td>
<td>Air</td>
<td>Canopy</td>
<td>Air</td>
<td>Canopy</td>
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<td>1</td>
<td>0</td>
<td>80</td>
<td>0</td>
<td>152.5 ± 1.4$^4$</td>
<td>21.3$^3$</td>
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<td></td>
<td>0</td>
<td>60</td>
<td>20</td>
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<td>156.0 ± 2.8</td>
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<td>80</td>
<td>155.7 ± 0.9</td>
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<td>20</td>
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<td>150.2 ± 2.1</td>
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<td>134.0 ± 1.0</td>
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<td>40</td>
<td>249.4 ± 9.8</td>
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<td>80</td>
<td>253.2 ± 5.1</td>
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$^4$Standard deviation.

$^3$All temperatures had a standard error ±0.1 °C.
0–0–80 (O–R–HR) treatment than under three of the four treatments that delivered ≥30% R light (Fig. 3). Marigold developed dark purple spotting on leaves within 2 to 4 d in all treatments and in both replications (Fig. 4). In addition, tomato seedlings in all treatments developed edema, a purple leaf coloration particularly on the abaxial surface, and interveinal chlorosis.

Seedling height of impatiens was similar under all light quality treatments. Marigold and tomato plants were 13% or 18% shorter under the 0–40–40 than the 0–80–0 treatment, respectively, but plant height in both treatments was similar to that in the other light quality treatments. Shoot fresh weight was similar among treatments in impatiens and marigold. Tomato grown under the 0–60–20 treatment had ≥13% greater shoot fresh weight than plants under treatments that delivered less R and more HR light. Fresh weight of petunia was 27% or 22% greater under the 0–20–60 treatment than treatments 0–80–0 or 20–30–30, respectively. Lighting treatments had no effect on shoot dry weight of impatiens, but in marigold it was greater under 0–80–0 than under 0–40–40 or 0–0–80. Dry weight of tomato was 25% to 40% greater under 0–60–20 than three of the four treatments that delivered less R and more HR light.

Expt. 2. The mean leaf number was similar among treatments and was 20, 5, 11, and 11 for impatiens, tomato, salvia, and petunia, respectively (data not shown). Total leaf area was highly variable in tomato and to a lesser extent in petunia, and there were no statistically significant differences among treatments. Impatiens had 48% larger leaves under 80–0low (R–HR) than 80–0high and salvia had 43% larger leaves under 80–0low than 0–80high (Fig. 5). As in Expt. 1, tomato developed edema, chlorosis, necrotic leaf margins, and purple pigmentation in all treatments in both replicates. Shoot growth of the other crops appeared normal.

There was no effect of lighting treatment on plant height of impatiens or tomato, but seedlings of salvia under the 40–40low and 0–80low were 14% or 22% taller than those under the 40–40high and 0–80high, respectively. There were no consistent effects of lighting treatments on fresh shoot weight among the species studied. In contrast, there was consistently one light quality treatment for each species in which plants under the higher light intensity had a greater shoot dry weight than under the low intensity. For example, dry weight under 40–40high was greater in salvia (by 30%) and petunia (by 62%) than under 40–40low.

Chlorophyll concentration was greatest for impatiens, tomato, and petunia under the 0–80low treatment (83.8, 119.0, and 90.5 mg g⁻¹ fresh tissue, respectively) and was the greatest for salvia under the 40–40low treatment (138.0 mg g⁻¹ fresh tissue). Chlorophyll concentration under these treatments was set to 100% and relative concentrations were calculated for the other treatments. Chlorophyll concentration was relatively high in plants grown under 0–80low for all

Fig. 4. Dark purple spotting on the abaxial surface of marigold leaves (left) and edema and purple coloration of tomato leaves (right). Symptoms were present in all light quality treatments in Expt. 1.

Fig. 5. Plant growth characteristics and relative chlorophyll content for impatiens, marigold, tomato, and petunia grown under three light quality treatments (R: red, HR: hyper red) where all treatments received 10% blue and 10% green light (Expt. 2). The PPF was 125 or 250 μmol m⁻² s⁻¹ (low or high, respectively). Means sharing a letter are not statistically different by Tukey’s honestly significant difference at P = 0.05. Error bars indicate ±SE and lack of mean separation indicates nonsignificance.
species. In addition, chlorophyll was similar to or reduced under high light within each light quality treatment, especially in petunia. Chlorophyll concentration was statistically similar within each crop under the high light treatments, whereas in the low light treatments, impatiens and petunia had a relatively low amount of chlorophyll under the 0–80 treatment.

Discussion

Light-emitting diodes emit a wide range of wavelengths, including those within the photosynthetically active waveband. Our objective was to determine whether young plants grew differently under one, two, or three different peaks of O or red light. In two different experiments, plants generally grew similarly and there were few consistent treatment effects between replicates and among species. When three ratios of R and HR were delivered at two intensities in Expt. 2, plants grown under twice the light intensity were similar to or shorter than plants at the lower intensity. Because an increase in extension growth is common in shade-intolerant species under low light intensities, it is not surprising that the magnitude of the difference tended to be greater for sun-adapted species such as salvia and tomato than in the shade-tolerant impatiens (Runkle and Heins, 2006; Smith, 1994).

The relative photosynthetic QE of the treatments was calculated according to McCree (1972) and Sager et al. (1988), and was marginally greatest for the 80% R light treatment (0.89) and least for the 80% HR light treatment (0.88). Therefore, at an intensity of 160 mol·m⁻²·s⁻¹, the effective irradiance of the R and HR light treatments only differed by 1% (141 vs. 142 mol·m⁻²·s⁻¹). Not surprisingly, biomass accumulation was often similar under the light quality treatments at the same PPF. Exposure to LEDs with peak emissions of 634 nm (R) and 664 nm (HR) likely resulted in similar stomatal conductances and photosynthetic rates because their peak wavelengths are below the critical threshold of 680 nm, above which decreased growth rates have been previously reported because of an inequality of photons between PSs I, II, and the electron transport chain (Tennen et al., 1994; Zeiger and Hepler, 1977). When salvia, ageratum (Ageratum houstonianum), and marigold were grown under 90 mol·m⁻²·s⁻¹ from R (peak = 650 nm) + B (peak = 470 nm), B + FR (peak = 720 nm), or R + FR LEDs, those under B or R with the addition of FR light had ≥30% to 60% less dry weight than those grown without wavebands ≥680 nm (Heo et al., 2006). Consistent with McCree (1972), Heo et al. (2006) reported that plants grown with 720-nm light had lower photosynthetic rates and, therefore, accumulated less biomass than those only grown with photons between 400 and 700 nm.

The well-established paradigm is that increasing light intensity increases photosynthesis, biomass accumulation, and harvestable yield. For example, Japanese mint (Mentha arvensis) had up to a 50% increase in photosynthesis when grown under white fluorescent lamps at a PPF of 200 μmol·m⁻²·s⁻¹ compared with 100 μmol·m⁻²·s⁻¹ (Malayeri et al., 2011). Spinach (Spinacea oleracea) grown under B, R, or white fluorescent lamps at 300 μmol·m⁻²·s⁻¹ had 10% to 80% greater leaf area and stem biomass than spinach grown under 100 μmol·m⁻²·s⁻¹, depending on the cultivar, when the light quality was kept consistent (Li et al., 2011). However, our study and related studies depart from this trend. We found that shoot fresh weights (and in many cases, dry weights) of seedlings under a PPF of 125 μmol·m⁻²·s⁻¹ were similar to those grown at 250 μmol·m⁻²·s⁻¹. Similarly, strawberry was grown under different combinations of R (peak = 660 nm) and B (peak = 450 nm) LED lighting at a PPF of 45, 60, or 75 μmol·m⁻²·s⁻¹ for 45 d. Plants grown under 60 μmol·m⁻²·s⁻¹ had 7% greater shoot fresh weight than those grown under 75 μmol·m⁻²·s⁻¹ (Nhut et al., 2003). These counter-intuitive results may result from plant acclimation responses to low light. One acclimation response to low light intensity is an increase in leaf area, such as that observed in strawberry (Jurik et al., 1979). In Expt. 2, leaf area at a PPF of 125 μmol·m⁻²·s⁻¹ was similar to or greater than that of plants grown at 250 μmol·m⁻²·s⁻¹.

Another way that plants respond to low light intensity is by changing their chlorophyll concentration. By using a SPAD measurement, strawberry plants had the greatest chlorophyll content index when irradiated with a PPF of 60 μmol·m⁻²·s⁻¹, the second greatest under 75 μmol·m⁻²·s⁻¹, and the lowest under 45 μmol·m⁻²·s⁻¹ (Nhut et al., 2003). Similarly, in Expt. 2, leaves of plants grown under the low-intensity light treatments had up to 40% more chlorophyll than leaves of the high-intensity light treatment, especially under the 80% HR treatment, which could have increased the utilization of photons available for photosynthesis and at least partially explain the similarities in fresh and dry weight of plants under the two intensities. Relatively little has been published about the effects of different wavelengths of R light on chlorophyll concentration, although other wavebands are known to have an effect. For example, Saebo et al. (1995) examined how a PPF = 30 μmol·m⁻²·s⁻¹ emitted by colored fluorescent lamps influenced growth of silver birch (Betula pendula) under different ratios of B (410–510 nm), R (640–680 nm), and FR (700–750 nm) light. Plants grown under B light had ≥25% greater chlorophyll concentration per leaf area than plants grown under cool-white fluorescent light and ≥50% more than R light. In addition, the chlorophyll concentration of leaves in cucumber (Cucumis sativus) was ≥36% greater under 50% B LED (peak = 450 nm) light than without B light (measuring percentage of light provided by R LED (peak = 638 nm), while the leaf photosynthetic capacity was three times greater, respectively, at a PPF = 100 μmol·m⁻²·s⁻¹ (Hogewoning et al., 2010).

Tomato (in Expts. 1 and 2) and marigold (in Expt.1) developed leaf disorders in all of the lighting treatments studied. The purple pigmentation present on the abaxial leaf surface was likely not nutritionally related because media pH was tested and was within the normal range (5.5–6.2) (Nau, 2011) and plants received complete fertigation throughout the duration of experiments. Environments without ultraviolet radiation, specifically ultraviolet-B (280–315 nm) (Jenkins, 2009) (e.g., Lang and Tibbits, 1983; Jones and Burgess, 1977; Nilsen, 1971), without B light (Massa et al., 2008), without FR light (Morrow and Tibbits, 1988), or with high humidity (e.g., Balge et al., 1969; Warrington, 1990) have been associated with edema in some crops, especially those in the Solanaceae. Massa et al. (2006) reported that edema or intumescent development on cowpea plants (Vigna unguculata) when grown under <10% to 15% B light (peak = 440 nm) when in a R dominant (peak = 660 nm) environment. Similarly, pepper plants (Capsicum annuum) developed severe edema on the leaves and fruit, which negatively affected fruit productivity. However, tomato ‘Persimmon’ did not exhibit edema under the same environmental conditions. Morrow and Tibbits (1988) reported that wild tomato (L. hirsutum) developed edema on 63% of the sampled leaf area surface when under R fluorescent lamps whereas it was absent under B fluorescent lamps at a PPF of 25 μmol·m⁻²·s⁻¹. The development of edema on tomato in all R-dominant treatments is consistent with those of Morrow and Tibbits (1988), but is not consistent with Massa et al. (2006) who suggested that 10% B light (present in all our treatments) should have been sufficient to prevent edema.

Because there were no consistent differences in plant growth between different wavelengths of orange-red light, red LEDs could be chosen based on other factors such as electrical efficiency. We measured the energy consumed by our modules with a wattage meter (Kill a Watt meter; Arbor Scientific, Ann Arbor, MI) with the LEDs off but power supplies on, and again with each color emitting 50 μmol·m⁻²·s⁻¹. The B, G, O, R, and HR LEDs in our modules had the following efficiencies (μmol-W⁻¹): 2.39, 0.84, 0.72, 2.29, and 2.46. These measurements indicate that the HR LEDs were 7% more efficient than the R LEDs, while the O LEDs were less than one-third as efficient as the R or HR LEDs. The B LEDs were also relatively efficient whereas the G LEDs had a low efficiency. Therefore, horticultural lighting could use B, R, and/or HR LEDs for maximum energy efficiency. In addition, factors such as cost, longevity, and reliability should be considered when choosing LEDs for horticultural lighting applications.

Literature Cited
