Comparison of Sole-source and Supplemental Lighting on Callus Formation and Initial Rhizogenesis of Gaura and Salvia Cuttings

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Abstract. Variability in outdoor daily temperatures and photosynthetic daily light integrals (DLIs) from early spring to late fall limits the ability of propagators to accurately control propagation environments to consistently callus, root, and yield compact herbaceous perennial rooted liners. We evaluated and compared the effects of sole-source lighting (SSL) delivered from red (R) and blue (B) light-emitting diodes (LEDs) to supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps on herbaceous perennial cutting morphology, physiology, and growth during callusing and initial rhizogenesis. Cuttings of perennial sage (Salvia nemorosa L. ‘Lyrical Blues’) and wand flower (Gaura lindheimeri Engelm. and A. Gray ‘Siskiyou Pink’) were propagated in a walk-in growth chamber under multilayer SSL provided by LEDs with [R (660 nm)]:[B (460 nm)] light ratios (%) of 100:0 (R100:B0), 75:25 (R75:B25), 50:50 (R50:B50), or 0:100 (R0:B100) delivering 60 μmol·m⁻²·s⁻¹ for 16 hours (total DLI of 3.4 mol·m⁻²·d⁻¹). In a glass-glazed greenhouse (GH control), cuttings were propagated under ambient solar light and day-extension SL provided by HPS lamps delivering 40 μmol·m⁻²·s⁻² to provide a 16-hour photoperiod (total DLI of 3.3 mol·m⁻²·d⁻¹). At 10 days after sticking cuttings, callus diameter and rooting percentage were similar among all light-quality treatments. For instance, callus diameter, a measure of growth, of wand flower cuttings increased from an average 1.7 mm at stick (0 day) to a range of 2.7 to 2.9 mm at 10 days after sticking, regardless of lighting treatment. Relative leaf chlorophyll content was generally greater under SSL R75:B25 or R50:B50 than all other light-quality treatments. However, stem length of perennial sage and wand flower cuttings propagated under SSL R50:B50 at 10 days were 21% and 30% shorter and resulted in 50% and 8% greater root biomass, respectively, compared with those under SL. The herbaceous perennial cuttings propagated in this study under SSL R50:B50 were of similar quality or more compact compared with those under SL, indicating that callus induction and initial rooting can occur under LEDs in a multilayer SSL propagation system.

Herbaceous perennials are propagated from seed (plugs), cuttings (liners), divisions, and tissue-cultured plantlets, and in 2015 had a reported wholesale value of $120 million for the 15 top-producing states (U.S. Department of Agriculture, 2016). Although herbaceous perennials can be successfully and economically propagated by seed, many are vegetatively propagated by shoot-tip, stem, basal, rhizome, or root cuttings, thus maintaining genetic uniformity, producing sterile cultivars, and hastening propagation production. (Pilon, 2006). Rhizome and root cuttings rarely yield uniform results and are labor intensive (Scoggins, 2006); therefore, shoot-tip, stem, and basal cuttings are recommended for vegetative perennial propagation.

According to Owen and Lopez (2016), 29%, 39%, and 18% of U.S. propagators receive and root herbaceous perennial cuttings during spring (March–May), summer (June–August), and fall months (September–November), respectively. During these months, seasonal outdoor daily temperatures and photosynthetic DLIs differ greatly, hindering the ability to maintain consistent environmental parameters during propagation. For instance, in 2015, the national average daily temperatures during spring, summer, and fall months were 12.0 ± 3.5, 22.0 ± 0.6, and 14.0 ± 2.9 °C, respectively (NOAA, 2016). Seasonal temperatures influence the need to heat or cool the propagation environment to achieve recommended air and root-zone temperatures of 20 to 23 °C and 18 to 25 °C, respectively (Pilon, 2006). The rate at which callus and adventitious root (AR) initials develop is temperature-dependent, thereby affecting AR formation (ARF) in cuttings. In addition, outdoor DLIs during late winter to early spring months are relatively low (5 to 20 mol·m⁻²·d⁻¹) compared with summer and fall months (30 to 50 mol·m⁻²·d⁻¹; Korczyński et al., 2002) and can be reduced by 50% or more from the greenhouse glazing material (Hanan, 1998) with further reductions from greenhouse infrastructure shading, white wash, and shade curtains (Lopez and Runkle, 2008). Although low DLIs (<5 mol·m⁻²·d⁻¹) during the early stages of propagation may be beneficial for minimizing stress and developing callus, excessively low DLIs (<5 mol·m⁻²·d⁻¹) can result in little to no ARF in cuttings. Overall, these seasonal variations pose a challenge to maintain consistent callusing and rooting of herbaceous perennials (Owen and Lopez, 2016). Therefore, additional temperature management and SL may be necessary during propagation.

Previous research has investigated the effects of DLI and SL from high-pressure sodium (HPS) lamps and LEDs during AR development and subsequent rhizogenesis of numerous genera of vegetatively propagated annual bedding plants (Currey et al., 2012; Hutchinson et al., 2012; Lopez and Runkle, 2008). In controlled environments, the effects of SSL provided by LEDs during seedling (plug) propagation (Randall and Lopez, 2015; Wollaeger and Runkle, 2015) and in vitro propagation (Budiarjo, 2010; Gu et al., 2012; Jao et al., 2005) have been documented.

Ex vitro vegetative cutting propagation under SSL LEDs has been investigated for calibrachoa (Calibrachoa Llave and Lex. ‘MiniFamous Neo Royal Blue’; Olschowski et al., 2016), English lavender (Lavandula angustifolia Mill. ‘Hidcote’; Christiaens et al., 2015), garden mum (Chrysanthemum ×morifolium ‘Orlando’; Christiaens et al., 2015), sweet basil (Ocimum basilicum L.; Lim and Eom, 2013), and four genera of woody ornamentals (Christiaens et al., 2015; Van Dalsen and Slingerland, 2012). These studies examined the photomorphogenic responses into monochromatic and dichromatic light spectra. For instance, Christiaens et al. (2015) propagated garden mum cuttings under SSL LEDs delivering 60 μmol·m⁻²·s⁻¹ (DLI of 4.1 mol·m⁻²·d⁻¹) of R (660 nm):B (460 nm) light ratios (%) of R100:B0, R90:B10, R50:B50, R0:B100, or B0:R100. They determined root dry mass (RDM) of garden mum cuttings propagated under R0:B100 or B0:R100 to be 171% to 200% greater, respectively, than for cuttings propagated under all dichromatic ratios tested. In another study, Olschowski et al. (2016) propagated calibrachoa cuttings under SSL LEDs delivering 80 μmol·m⁻²·s⁻¹ of R100:B0 or B0:R100 or HPS lamps delivering 80 μmol·m⁻²·s⁻¹ to provide a DLI of 4.6 mol·m⁻²·d⁻¹ and maintained 95% relative humidity (RH) and 24 °C air temperature. After 21 d, they observed calibrachoa cuttings propagated under SSL LEDs exhibited significantly shorter roots and had smaller shoot dry mass (SDM) and RDM compared with cuttings propagated under HPS lamps.

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Although the foci of these experiments were only to determine ARF and subsequent root and shoot growth and development, literature determining the effects of SSL LEDs on callus formation and growth in vegetative cuttings has not been documented. Methods to assess in vitro callus formation and growth include fresh and dry callus mass, surface area and volume measurements, visual comparisons, cellular quantification, mitotic indices, and callus respiration (Mottley and Keen, 1987; Sathyarayana and Varghese, 2007). To date, scientific methods have not been established to measure and quantify ex vitro callus formation and growth.

There is little known about the effects of light quality from SSL on callus formation and growth and on initial ARF during vegetative propagation of herbaceous perennials. The recent interest in SSL LEDs for ornamental seedling production, combined with the potential use for vegetative cut propagation, provides a unique opportunity to investigate the impact of spectra-specific SSL applications. In addition, a multilayer vertical system for ex vitro cutting propagation provides propagators a controlled environment (light and temperature) to uniformly callus and root vegetative cuttings, maintain consistent inner quality, and maximize space efficiency. Therefore, our objectives were to quantify and compare the effects of SSL from LEDs providing four different light qualities to SL from HPS lamps on callus formation and growth and on early subsequent rhizogenesis of herbaceous perennial cuttings. In addition, we aim to establish new methodologies to quantify callus formation and growth in vegetatively propagated herbaceous perennial cuttings.

**Materials and Methods**

**Plant material and culture.** On 9 (Rep. 1) and 24 Mar. (Rep. 2) unrooted herbaceous cuttings of perennial sage (Salvia nemorosa L. ‘Lyrical Blues’) and wand flower (Gaura lindheimeri Engelm. and A. Gray ‘Siskiyou Pink’)) were received from a commercial cutting supplier (Darwin Perennials, Ball Horticultural Co., West Chicago, IL). Species were selected according to the survey conducted by Owen and Lopez (2016). For each species, cuttings with similar stem length, stem caliper, node, and leaf numbers were selected. At initiation (0 d), average stem length, stem caliper, node number, and total leaf area (TLA) for perennial sage were 23.6 mm, 2.5 mm, 4.4 nodes, and 27.6 cm², respectively. Average stem length, stem caliper, node number, and TLA of wand flower at initiation were 35.4 mm, 1.4 mm, 4.4 nodes, and 6.6 cm², respectively.

Industry standard, 72-cell propagation trays (54 cm×28 cm×3 cm; T.O. Plastics, Inc.) filled with triple-rinsed aggregates of a calcined, nonswellirig illite and silica clay (MVP®, Turface®; PROFILE Products LLC, Buffalo Grove, IL) with a pH of 5.9 and electrical conductivity (EC) of 0.2 mS·m⁻¹ (HI 9813-6; Hanna Instruments, Woosocket, RI). Each tray received 300 mL of deionized water with an average pH, EC, and alkalinity (HI775 Freshwater alkalinity handheld colorimeter; Hanna Instruments) of 5.6, 0.0 S·m⁻¹, and 52.3 mg·L⁻¹ CaCO₃, respectively. The trays were covered with a clear, plastic humidity dome (54 cm×28 cm×15 cm; Acro Dome, Acro Plastics, LTD., Edmonton, Alberta, Canada).

**Growth chamber propagation environment.** Trays were placed on a propagation bench without drainage holes (54 cm×28 cm×3 cm; T.O. Plastics, Inc.) filled with triple-rinsed aggregates of a calcined, nonswellirig illite and silica clay (MVP®, Turface®; PROFILE Products LLC, Buffalo Grove, IL) and 50% coarse perlite (Strong-Lite (Fafard 2; Sun Gro Horticulture, Agawam, MA) with 2-mm galvanized sheet metal. To prevent heat loss and moisture accumulation, high-temperature aluminum foil tape (3 m×9.1 m; 3M, St. Paul, MN) was used to attach the sheet metal to the bench. In addition, benches were covered with a 4-mil black construction film (3 m×30.5 m; roll; Blue Hawk, Poly-America, Grand Prairie, TX). Root-zone heating was controlled by a substrate thermistor probe (BioTherm® Benchwarmer Kit; TrueLeaf Technologies) and programmed for a propagation substrate temperature set point of 24 °C.

Cuttings were placed under fixed shade cloth providing 54% shade (Solaro 5620 O-R-FR; Ludwig Svensson, Inc., Charlotte, NC) under ambient daylight supplemented with a total PPFD of 0.8 ± 0.1 μmol·m⁻²·s⁻¹ at plant height [as measured with a quantum sensor (LI-190SL; LI-COR Biosciences, Lincoln, NE)] delivered from HPS lamps (PARSource Lighting Solutions, Petaluma, CA) from 0600 to 0800 and 1800 to 2200 h (16-h photoperiod). Woven shade curtains (OLS 50; Ludwig Svensson, Inc.) were retracted when the outdoor light intensity reached 500 μmol·m⁻²·s⁻¹. The target greenhouse DLI, air temperature, and RH set points were 3.3 mol·m⁻²·d⁻¹, 21 °C, and 80%, respectively.

**Environmental data collection.** Light quality and PPFD were measured at the beginning and end of each experimental replication by taking five individual spectral scans per treatment using a spectroradiometer (BLUE-Wave Miniature Spectrometer; StellarNet, Inc., Tampa, FL). Spectral quality of each SSL source and the GH control are provided in Fig. 1. For each species under each SSL LED and SL treatment, precision thermistors (ST-100; Apogee Instruments, Inc., Logan, UT) were used to measure the air temperature and substrate temperature under each humidity dome. Amplified quadratic sensors (LI-COR Biosciences) measured PPFD under each SSL treatment in the growth chamber and ambient and SL in the greenhouse. Measurements were recorded in the every 30 s and the average of each sensor was logged every 15 min by a data logger (Model CR1000; Campbell Scientific, Inc., Logan, UT). In the growth chamber, average air temperature, RH, and CO₂ concentration between treatments. Treatments were randomized within the two shelves between replications.

**Greenhouse propagation environment.** Cuttings were placed on a propagation bench in a glass-glazed greenhouse (GH control). Exhaust fan and evaporative-pad cooling, radiant hot water heating, and retractable shade curtains were controlled by an environmental control system (Maximizer Precision 10; Priva Computers Inc., Vineland Station, ON, Canada). The propagation bench was insulated with cellofoam EPS boards faced with a reflective foil (1.2 m×2.4 m×2.3 cm; Polyshield®). A closed-loop bench-top root-zone heating system was installed with microtubing that circulated hot water (49 °C) across the bench (BioTherm® Benchwarmer Kit; TrueLeaf Technologies, Petaluma, CA). To evenly distribute heat across the bench, the microtubing was covered with 2-mm galvanized sheet metal. To prevent heat loss and moisture accumulation, high-temperature aluminum foil tape (6.3 cm×9.1 m; 3M, St. Paul, MN) was used to attach the sheet metal to the bench. In addition, benches were covered with a 4-mil black construction film (3 m×30.5 m; roll; Blue Hawk, Poly-America, Grand Prairie, TX). Root-zone heating was controlled by a substrate thermistor probe (BioTherm® Benchwarmer Kit; TrueLeaf Technologies) and programmed for a propagation substrate temperature set point of 24 °C.

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Table 1. Lighting manufacturer, spectral ratio (%) from sole-source light (SSL) from light-emitting diodes (LEDs) providing red (R) or blue (B) light, average photon flux from 400 to 700 nm ± SD derived from spectral scans for Rep. 1 (9 Mar.) and Rep 2 (24 Mar), R:B ratio, and the estimated phytochrome photoequilibrium (\( \phi \)); lighting treatment structural dimensions, array number, spacing, and height from heating mat or bench at which lighting treatments were above perennial sage (Salvia nemerosa L. 'Lyrical Blues') and wand flower (Gaura lindheimeri Engl. and A. Gray 'Siskiyou Pink') cuttings.

| Manufacturer | Ratio (%) | Photon flux (\( \mu \text{mol}\cdot \text{m}^{-2}\cdot \text{s}^{-1} \)) | R:B | \( \phi \) | Structural dimensions | LEDs per array* | No. arrays or lamps* | Array or lamp spacing (cm) | Ht (cm) |
|--------------|-----------|----------------------------------------------------------|-----|-----|---------------------|-----------------|----------------------|--------------------------|-------|
| PARsource1 | R100:B0 | 60.5 ± 3.4 | 60.0 ± 2.6 | — | 0.89 | 1.20 cm square, 1.22 m long x 1.30 mm wide | 28 red | 5 red | 10.5 | 48.5 |
| Orbitec | R75:B25 | 60.9 ± 3.7 | 60.2 ± 3.6 | 3.0 | 0.79 | 1.20 cm square, 1.22 m long x 1.30 mm wide | 28 red | 6 red | 6.5 | 48.5 |
| Orbitec | R50:B50 | 59.5 ± 2.8 | 59.1 ± 2.2 | 1.0 | 0.68 | 1.20 cm square, 1.22 m long x 1.30 mm wide | 28 red | 2 red | 13.5 | 49.5 |
| Orbitec | R0:B100 | 59.4 ± 10.0 | 59.0 ± 10.0 | — | 0.49 | 1.20 cm square, 1.22 m long x 1.30 mm wide | 24 blue | 3 blue | 13.5 | 37.5 |

1Ratio of R to B (R:B) determined from the average photon flux for monochromatic or dichromatic R or B LED SSL treatment.
2Phytochrome photoequilibrium (\( \phi = P_{\text{fr}}/P_{\text{r}} + P_{\text{fr}} \)) was estimated according to Sager et al. (1988).
3Number of individual LEDs per LED array.
4Number of LED arrays per lighting treatment.
5Center spacing between high-pressure sodium (HPS) lamps or LED arrays.
6Distance from canopy level to HPS lamps or LED arrays.
7PARsource = PARsource Lighting Solutions, Petaluma, CA.
8Information or no measurable data.
9Orbitec = Orbital Technologies Corporation, Madison, WI.
Variance, and means were separated between treatments using Tukey’s honestly significant (HSD) test at $P \leq 0.05$. A $t$ test was used to compare SSL treatment means with the SL treatment means.

**Results**

**Morphology.** At initiation (0 d), average callus diameter of perennial sage and wand flower cuttings was 2.7 and 1.7 mm, respectively (Fig. 2A and B). Under each light-quality treatment, callus diameter of perennial sage exhibited a quadratic increase, whereas callus diameter of wand flower increased linearly from 2 to 10 d after sticking (data not shown). Regardless of light-quality treatment, callus growth of perennial sage reached a maximum diameter of 3.3 to 3.6 mm at 6 d (Fig. 2A). The largest measurable callus diameter of wand flower was recorded at 10 d and ranged from 2.7 to 2.9 mm (Fig. 2B).

Regardless of SSL treatment, rooting of perennial sage was similar and occurred 2 d earlier (at 8 d) than under SL (Table 3). At 10 d, 43% more perennial sage cuttings rooted under SSL R_{75}:B_{25} LEDs than under SL. Rooting of wand flower occurred at 4 d under all treatments, with the greatest percentage observed under SSL R_{100}:B_{0} LEDs; however, after 4 d, rooting was similar regardless of light quality.

For both species, stem length (Table 3) and node number (data not shown) increased as days after sticking increased, although the magnitude of response varied under each light-quality treatment. Stem length of perennial sage cuttings was generally shorter as the proportion (%) of R light decreased from 100% (R_{100}:B_{0}) to 50% (R_{50}:B_{50}). For instance, at 6, 8, and 10 d, perennial sage stem length was 24%, 28%, and 45% shorter under R_{50}:B_{50} than R_{100}:B_{0} LEDs, respectively; and 18%, 18%, and 27% shorter than cuttings propagated under SL, respectively (Table 3). Perennial sage TLA was unaffected by light-quality treatment (Table 3).

Under SL, wand flower stem length at 4 and 6 d was 23% and 26% shorter, respectively, than cuttings under SSL R_{100}:B_{0} LEDs; however, by 10 d, no differences were observed. At 10 d, average stem length of wand flower under SSL R_{50}:B_{50} LEDs was 24% to 27% shorter than cuttings propagated under SSL R_{100}:B_{0} and R_{75}:B_{25} LEDs; and was 30% shorter than cuttings propagated under SL. Stem caliper and node number were unaffected when cuttings were propagated under SSL LEDs (data not shown). At 10 d, LDM, RDM, and TDM of cuttings propagated under SSL R_{50}:B_{50} LEDs was 40% and 50% larger, respectively, compared with cuttings under SL (Table 3). SDM was significantly influenced by SSL and increased linearly throughout propagation for all light-quality treatments (Table 3).

At 2 and 6 d, LDM and SDM of wand flower cuttings were significantly different among SSL LED treatments, but at 10 d, were similar regardless of light quality (Table 3). Compared with cuttings under SL, LDM at 10 d was 7.4, 7.3, and 4.3 mg smaller when cuttings were propagated under SSL R_{100}:B_{0}, R_{50}:B_{50}, and R_{0}:B_{100} LEDs, respectively. RDM was similar among all SSL LED treatments and increased linearly from 2 to 10 d. At 10 d, cuttings propagated under SSL R_{50}:B_{50}, R_{0}:B_{100}, and R_{25}:B_{75} LEDs exhibited 17%, 8%, and 17% more root biomass, respectively, than under SL. TDM at 2 d was significantly smaller under R_{0}:B_{100} than all other SSL LEDs, but at 10 d, there were no significant differences (data not shown). However, TDM of cuttings propagated under SSL R_{100}:B_{0} and R_{50}:B_{50} LEDs were 9.7 and 10.2 mg smaller, respectively, than cuttings propagated under SL (data not shown).

**Discussion**

In the current study, the degree to which LED SSL elicited photomorphogenic responses varied among species, but was generally found to have no effect on callus diameter, rooting percentage, stem caliper, node number, or SDM of perennial sage or wand flower cuttings at 10 d after stick (Fig. 2; Table 3). We did, however, observe some variability in callus formation, per experimental replication were used. For destructive measurements, six samples (individual cuttings) per species per light-quality treatment per experimental replication were used. Species were analyzed independently and data were pooled across experimental replications. Effects of light quality within SSL were analyzed using SAS (version 9.2; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance, and means were separated between treatments using Tukey’s honestly significant (HSD) test at $P \leq 0.05$. A $t$ test was used to compare SSL treatment means with the SL treatment means.
rhizogenesis, and growth and development of cuttings from 2 to 8 d after stick.

Callus induction of perennial sage and wand flower was not influenced by light quality, as all cuttings exhibited callus at 2 d after stick (Fig. 2). In contrast, Budiarto (2010) reported increased callus induction of anthurium ‘Violeta’ and ‘Pink Lady’ plants grown in vitro for 30 d under R100:B0 than R50:B50 and R25:B100 LEDs delivering 45 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) (DLI of 2.6 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)). In our study, perennial sage callus diameter, a measure of growth, was smallest at 2 d under R50:B50 compared with SSL R100:B0 LEDs (Fig. 2); however, from 4 to 10 d, callus formation was similar regardless of light-quality treatment (Table 3). Previous studies have investigated responsiveness of R, far-red (FR), and B light on AR. Pfaff and Schopfer (1974) reported hypocotyl cuttings of mustard (Sinapis alba L.) seedlings grown ex vitro to regenerate ARs at the cutting surface when treated with short pulses of R (568 nm; 3.1 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) or continuous FR (740 nm; 17.5 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) light. Further investigations by Pfaff and Schopfer (1980) revealed that phytochrome mediated the de novo formation of root primordia in mustard seedling hypocotyls near the cutting surface within 12 to 24 h after excision and that these seedlings exhibited increased primordium formation and ARs per cutting from 2 to 6 d and 3 to 7 d after excision, respectively. In another study, Fuernkranz et al. (1990) reported ARF of in vitro axillary shoots of black cherry (Prunus serotina Ehrh.) to be significantly reduced after 14 d under B light, delivering 15 to 22 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), and was completely inhibited at 36 \( \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). Similarly, Stoutemyer and O’Rourke (1946) found B light at \( \approx 75 \text{ mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) to reduce ARF in early forsythia (Forsythia ovata Nakai) cuttings. These studies suggest that at 4 d, ARF of wand flower cuttings propagated under SSL R100:B0 LEDs and SL were initially influenced by phytochrome. Although, with the absence of FR light under SSL LEDs, ARF under SSL R100:B0 LEDs may have been influenced by the pre-severance light quality of the stock plant environment previously described in the literature (Heins et al., 1980; Hoad and Leaky, 1996; Leaky and Storeton-West, 1992). For instance, Leaky and Storeton-West (1992) reported abachi (Triplochiton scleroxylon K. Schum.) cuttings harvested from stock plants grown under an R:FR ratio of 1.6 to have a greater number of roots per cutting compared with cuttings harvested from stock plants grown under an R:FR ratio of 6.3. However, reduced rooting of wand flower cuttings under LED SSL treatments
that provided ≥50% B light could be a result of reduced auxin signaling mediated by the presence of B light. Previous studies suggest B light receptors act through oxidative decarboxylation of auxin, resulting in a reduction of endogenous auxin, thereby delaying or inhibiting rooting (Fuernkranz et al., 1990).

The biological phases that cuttings undergo during propagation were best characterized by Dole and Hamrick (2006). During propagation, unrooted cuttings are not actively growing during stages 0 to 2 (cutting harvest to callus ing), but are undergoing several cytological, histological, and physiological changes until stage 3 (rooting). Growth commences at stage 3 with the rise of AR primordia and subsequent ARF, thereby increasing shoot growth (Dole and Hamrick, 2006), and thus biomass accumulation. In the current study, statistical differences in morphology, physiology, and growth that occurred before ARF at 0 to 6 d and 0 to 2 d after sticking perennial sage and wand flower cuttings, respectively, could be attributed to the variability of initial cutting size; although cuttings were sorted by stem length, stem caliper, and node and leaf number as suggested by Dole and Hamrick (2006). Consistent differences were observed in both species at each day for stem length. Stem length of perennial sage measured at 2 to 10 d after sticking was shortest for cuttings propagated under SSL R0:B100 LEDs and SL, respectively. Wand flower stem length was comparable among SSL LEDs at 2 and 8 d, but by 10 d, were shortest when cuttings were propagated under SSL R100:B50 LEDs. Therefore, perennial sage and wand flower cuttings were most compact under R50:B50 than those propagated under all other light-quality treatments. Similarly, Gu et al. (2012) reported plantlet height of in vitro propagated anthurium ‘Alabama’ and ‘Sierra’ to be shortest under SSL R50:B50 than R100:B0 (658 nm) and R50:B100 (460 nm) LEDs delivering 40 μmol·m–2·s–1 (DLI of 1.7 mol·m–2·d–1) for 60 d. Randall and Lopez (2015) reported French marigold (Tagetes patula L. ‘Durango Yellow’) seedling height with a half-life of 1 to 2 h (Clough and Vierstra, 1997). It is speculated that the decrease in node number and thus, number of leaves, is associated with shorter stem lengths found in cuttings under SSL R50:B50 and R50:B100 LEDs at 10 d and may be a result of the absence of FR light that was provided pre-severance and a decreasing proportion of R light provided during propagation (Table 3). In general, phytochromes detect the ratio of R:FR light received by plants (Fukuda et al., 2008) and their levels are actively modulated during the life cycle of a plant to optimize light absorption and perception (Clough and Vierstra, 1997). A decrease in the ratio of R:FR, from that of sunlight (≈1:2) to values less than 1, may increase stem elongation (shade avoidance response) and reduce shoot production (Hoad and Leaky, 1996). This is consistent with our results where we found stem length of cuttings to be longer under SSL R100:B0 LEDs compared with SL. In addition, phytochrome isofroms present in the leaf are controlled by turnover of the photo repressor on photoconversion from the R-absorbing phytochrome (Pfr) to the FR-absorbing phytochrome (Pr) (Clough and Vierstra, 1997). The Pr form has a half-life of ≈1 week and the Pr form is rapidly degraded with a half-life of 1 to 2 h (Clough and Vierstra, 1997). It is postulated that stock plants were grown under high R:FR ratios, and when cuttings were excised and shipped from offshore production facilities to be received 2 d later for the experiment, the Pr form may have rapidly degraded from a half-life for ≈1 week to 5 d (Clough and Vierstra, 1997). Once cuttings were placed under their perspective light-quality treatments, Pfr had converted back to Pr, and in the presence of ≥75% R LED SSL, began to degrade back to the Pr form. Whereas under ≥50% B LED SSL, the conversion of Pfr to Pr did not occur and degradation continued, and thus, resulted in shorter cuttings with fewer nodes, and leaves.

In addition, we observed epinasty in leaves of all perennial sage cuttings propagated under SSL R100:B0 and R50:B25 LEDs, which has also been previously reported in banana (Musa ×paradisiaca L. ‘Nam Dinh’; Nhuat et al., 2002) and geranium (Pelargonium zonale (L.) L’Hér. Ex Aiton ‘Oobic White’; Fukuda et al., 2008). Nhuat et al. (2002) found in the absence of B light in vitro banana plantlets exhibited abnormal growth, whereas normal growth was clearly related to the presence of B light. Moreover, perennial sage cuttings propagated under SSL R0:B100 LEDs were found to have less RCC compared with all other light-quality treatments 6 to 10 d after sticking. This is inconsistent with previous literature, which reported B light from LEDs significantly promoted the accumulation of leaf chlorophyll content in calla lily (Zantedeschia jucunda ‘Black Magic’; Jao et al., 2005), florist mum (C. morifolium Ramat. ‘Ellen’; Kurilčík et al., 2008), and yarrow (Achillea millefolium L.; Alverenga et al., 2015). Previous literature indicates that plants generally use B light less efficiently for photosynthesis (Wollaeger and Runkle, 2015), thus limiting the production of photosynthates required for biomass accumulation (Currey and Lopez, 2013).
Table 3. Rooting percentage, stem length, total leaf area (TLA), leaf dry mass (LDM), shoot (stems and petioles) dry mass (SDM), and root dry mass (RDM) of perennial sage (*Salvia nemorosa* L. ‘Lyrical Blues’) and wand flower (*Gaura lindheimeri* Engelm. and A. Gray ‘Siskiyou Pink’) cuttings. Vegetative cuttings were propagated for 10 d in a greenhouse at 21 °C under ambient solar light and supplemental light (SL) delivered from high-pressure sodium (HPS) lamps or in a growth chamber at 21 °C under sole-source light (SSL) from light-emitting diodes (LEDs) with red/blue light ratios (%) of 100:0 (R100:B0), 75:25 (R75:B25), or 50:50 (R50:B50) with a 16-h photoperiod (0600 to 2200 h). Propagation substrate was heated to 24 °C.

| Days | HPS | R100:B0 | R75:B25 | R50:B50 | R0:B100 | HPS | R100:B0 | R75:B25 | R50:B50 | R0:B100 |
|------|-----|---------|---------|---------|---------|-----|---------|---------|---------|---------|
| 2    | 0   | 0       | 0       | 0       | 0       | 0   | 0       | 0       | 0       | 0       |
| 4    | 0   | 0       | 0       | 0       | 0       | 0   | 0       | 0       | 0       | 0       |
| 6    | 0   | 0       | 0       | 0       | 0       | 0   | 0       | 0       | 0       | 0       |
| 8    | 0   | 10      | 30      | 50      | 30      | 0   | 100     | 100     | 100     | 100     |
| 10   | 35  | 30      | 50      | 30      | 30      | 0   | 100     | 100     | 100     | 100     |

Significance: L** Q** L*** Q** L*** Q** L*** Q** L*** Q** L*** Q** L*** Q** L*** Q** L*** Q** L*** Q**

| Significance | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** | L*** Q** |
|--------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 2            | 34.8     | 39.5 a   | 31.8 b   | 27.9 b   | 32.5 ab  | 24.2     | 40.8 a   | 39.1 a   | 38.9 a   | 38.7 a   |
| 4            | 39.8     | 44.3 a   | 34.9 b   | 31.4 b   | 38.5 ab  | 40.3     | 49.5 a   | 45.7 ab  | 39.7 b   | 39.6 b   |
| 6            | 43.6     | 45.7 a   | 38.7 ab  | 36.8 ab  | 50.1 a   | 40.6     | 51.3 a   | 44.7 ab  | 40.4 b   | 39.3 b   |
| 8            | 44.3     | 48.3 a   | 44.0 ab  | 37.7 b   | 47.0 ab  | 47.9     | 55.8 a   | 48.1 a   | 46.3 a   | 45.6 a   |
| 10           | 47.2     | 54.2 a   | 50.3 a   | 37.3 b   | 45.9 ab  | 65.7     | 63.8 a   | 61.1 a   | 46.3 b   | 59.8 a   |

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current study, perennial sage cuttings under SSL R0:B100 LEDs were likely not photosynthesizing efficiently. Thus, rather than partitioning carbohydrates into the leaves and stems, they were instead allocating their limited photosynthate supply into AR growth and development. Meanwhile, cuttings under LED SSL providing ≥50% R light were photosynthesizing efficiently and partitioning carbohydrates into both leaf and root biomass accumulation. This trend of reduced LDM and RDM resulted in lower TDM of perennial sage cuttings propagated under SSL R0:B100 LEDs.

Wand flower leaf, shoot, and total biomass accumulation increased linearly from 2 to 10 d under SL, whereas cuttings under LED SSL did not exhibit a statistical response of biomass accumulation to light quality. However, wand flower RDM increased linearly from 2 to 10 d after stick and was higher under LED SSL providing ≥25% B light compared with SSL R100:B0 LEDs and under SL. Increased RDM at 10 d may be contributed to larger or comparable TLA and RCC found in wand flower cuttings under LED SSL providing ≥25% B. This is likely because spectral energy distribution of R:B light coincides with that of chlorophyll absorption (Goins et al., 1997), thus increasing net photosynthetic rate (Gu et al., 2012), and as a result, wand flower cuttings were likely allocating photosynthates into root growth rather than stem (leaf and shoot) growth and development.

**Conclusion**

When our morphological, physiological, and growth data of cuttings at 10 d of
propagation under SSL LEDs are compared with SL, we can conclude that there are no negative effects of propagating herbaceous perennials under SSL. Callus growth and AR occurred and increased under all light-quality treatments for both perennial sage and waxflower; however, cuttings propagated under SSL LEDs providing R50:B50 exhibited shorter stem lengths and higher RDM, which is commercially desirable, as cuttings are less likely to be damaged during shipping and transplanting. Based on our results, we conclude that cutting propagators should establish a DLI of ~4 mol m⁻² d⁻¹ delivered from SSL R50:B50 LEDs during callusing and initial rooting of herbaceous perennial sage and waxflower shoot-tip cutting propagation in a growth chamber or SSL controlled-environment. Furthermore, we have established a method to quantitatively measure ex vitro callus growth in vegetative shoot-tip cuttings of herbaceous perennials, and with further research, we expect similar outcomes when applied to vegetatively propagated annual bedding plants. Collectively, our results expand the general understanding of light quality on ex vitro callus growth, ARF, and morphology of cuttings. Further investigation of these effects on vegetatively propagated annual bedding plants and other herbaceous perennials is warranted.

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