HortScience 60(10):1793–1801. 2025. https://doi.org/10.21273/HORTSCI18797-25

LED Supplemental Light Intensity and Root Zone Heating, but Not Far-red Light, Influence Morphology, Leaf Color, and Anthocyanin Concentration of *Petunia ×hybrida* during Rooting

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Keywords. greenhouse, light-emitting diodes, supplemental lighting, vegetative propagation

Abstract. **In high-latitude regions (e.g., ≥40°), commercial greenhouse growers use supplemental lighting (SL) and root zone heating to decrease rooting time and increase the quality of young plants during periods of low solar irradiance. Light-emitting diode (LED) fixtures are increasing in popularity, but some growers have reported the development of purple foliage of select vegetatively propagated crops under LEDs, especially petunia (*Petunia ×hybrida*), during rooting. The objective of this study was to mitigate petunia leaf purpling by modifying the photon spectrum and flux density (light quality and quantity) of SL and delivering root zone heating. Shoot-tip cuttings of SureShot 'Dark Blue' and 'White' petunia were propagated in 72-cell trays inside glass-glazed greenhouses with a root zone temperature (RZT) of 21 or 25 °C. Cuttings were grown under sunlight supplemented by LEDs emitting a blue:green:red:far-red (B:G:R:FR) light ratio of either 10:7:82:1 (FR_{low}) or 10:18:59:13 (FR_{high}) at a total photon flux density (TPFD; 400–800 nm) of 70 or 120 μmol·m^{-2}·s^{-1}. Cuttings of both cultivars grown under the FR_{low} were generally more compact. Additionally, cuttings of both cultivars grown under a supplemental TPFD of 70 μmol·m^{-2}·s^{-1} were typically taller with thinner stems than those grown under 120 μmol·m^{-2}·s^{-1}. Cuttings of 'Dark Blue' grown under the higher TPFD featured more positive chromametric a* values and less positive b* values or displayed leaves that were overall more red and blue in color and also had higher total anthocyanin concentrations in their leaves compared with those under the lower TPFD. Cuttings of 'White' grown with a RZT of 25 °C were generally taller than those with a RZT of 21 °C, whereas cuttings of 'Dark Blue' grown with the higher RZT had relatively low anthocyanin concentrations in their leaves. Furthermore, cuttings of both cultivars generally had higher root dry masses when grown with the higher RZT. These results indicate that the severity of foliage purpling developed during propagation under LEDs, although cultivar-dependent, is not related to the percentage of FR in the SL. In addition, pigment accumulation can be mitigated by root zone heating or limiting the TPFD (<120 μmol·m^{-2}·s^{-1}) of SL during periods of low solar irradiance.**

Traditionally, greenhouse supplemental lighting (SL) has been delivered with high-pressure sodium (HPS) lamps (Ciolkosz et al. 2001). However, because of improvements in light-emitting diode (LED) technologies, the use of high-intensity LED fixtures to provide SL has been increasing (Mitchell et al. 2012). Although LED fixtures are generally more expensive, they emit less heat, have longer life spans, and more efficiently convert electricity into photosynthetically active radiation (*PAR*; 400–700 nm) compared to HPS lamps (Bourget 2008; Haitz et al. 2000; Mitchell et al. 2015; Nowakowska et al. 2023). Additionally, although LED fixtures can emit a variety of spectra, for horticultural applications, most commercial fixtures are populated by red (R; 600–699 nm), blue (B; 400–499 nm), and/or white (W) LEDs. Consequently, many fixtures emit little to no green (G; 500–599 nm) and far-red (FR; 700–750 nm) light. In contrast, HPS lamps radiate relatively high percentages of G and FR light and proportionally less B and R light (Bourget 2008; Nelson and Bugbee 2014).

Plants grown under LEDs can display differences in leaf color and pigmentation, plant morphology, and rooting when compared with those grown under HPS lamps. These differences tend to be more obvious and exacerbated as the percentage of total light delivered by SL increases beyond ≥40% (Craver et al. 2019; Randall and Lopez 2014, 2015). However, the influence of the SL spectrum on these quality metrics varies across studies. Randall and Lopez (2015) found that the chlorophyll contents in 'Titan Red Dark' vinca (*Catharanthus roseus*), 'Super Elfin XP Blue Pearl' impatiens (*Impatiens walleriana*), 'Bullseye Red' geranium (*Pelargonium ×hortorum*), and 'Dreams Midnight' petunia (*Petunia ×hybrida*) were similar when grown under sunlight supplemented by HPS lamps or LEDs emitting a R:B light ratio of 87:13 at a photosynthetic photon flux density (*PPFD*) of 70 μmol·m^{-2}·s^{-1}. The same study found that bedding plants grown under HPS lamps were generally taller than those grown under LEDs. In contrast, some studies suggested that differences in the SL spectrum between HPS lamps and LEDs may not influence crop morphology. For example, Poel and Runkle (2017) reported the heights of seedlings of 'Pinto Premium Salmon' geranium, 'Single Dreams White' petunia, 'Wave Misty Lilac' petunia, 'Montego Yellow' snapdragon (*Antirrhinum majus*), and 'Supersweet' tomato (*Solanum lycopersicum*) were similar under SL provided by LEDs emitting different percentages of B, R, and/or G light from LEDs or HPS lamps. Furthermore, all crops yielded a similar shoot dry mass (SDM), leaf number, and leaf area across SL treatments, and there were no subsequent differences in crop quality or flowering after transplant of any crop.

Although the influence of the SL spectrum on leaf coloration and crop morphology is still being researched, commercial plant propagators are increasingly adopting LEDs for SL (Lee et al. 2020). As more growers use LEDs, more reports of the development of purple foliage in bedding plant cuttings propagated under a high *PPFD* (e.g., ≥80 μmol·m^{-2}·s^{-1}) are being published (Veazie and Whipker 2024). The severity and frequency of this foliage purpling varies across crop species and cultivars, but it has the potential to make liner trays unmarketable (Fig. 1). One of the most noteworthy crops exhibiting this purple pigmentation is petunia, whose market value accounted for nearly 11% of the $3.6 billion of all floral and foliage crop sales across all container types in 2022 (Miller 2024; US Department of Agriculture, National Agricultural Statistics Service 2023).

This leaf purpling could possibly be caused by the relatively higher percentage of B light and less G and FR light being radiated by most LED fixture types when compared with HPS lamps (Runkle 2024; Veazie and Whipker 2024). The B light can stimulate the biosynthesis of anthocyanins, which are blue and red plant pigments, in a multitude of crops, including lettuce (*Lactuca sativa*) and petunia (He et al. 2021). The accumulation of these anthocyanins can lead to the development of red, blue, or purple tissue, which in some crops can be undesirable (Alvarez-Suarez et al. 2021). The synthesis and accumulation of anthocyanins

Fig. 1. Petunia propagated under light-emitting diodes displaying undesirable purpling in a greenhouse.

may be triggered by stressors that can cause oxidative damage because they possess free radical scavenging capabilities and are able to stabilize or neutralize reactive oxygen species (Ding et al. 2020; Gould et al. 2002). Because high-energy B light can cause oxidative stress and subsequent upregulation of the expression of genes controlling anthocyanin production, the increased percentage or intensity of B light emitted by LEDs could at least partially cause this development of purple foliage (Craver et al. 2020; Jia et al. 2024).

Along with high-energy B light, high-intensity light can also induce the accumulation of anthocyanins. Under high-light intensities, anthocyanins may absorb excess B and G light, reducing light penetration to chloroplasts to prevent or limit photoinhibition (Neill and Gould 2003). Petunia grown under a *PPFD* of 50 to 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ amassed significantly less anthocyanins in their leaves than that grown under 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which exhibited purple tissue (Albert et al. 2009). Additionally, the anthocyanin concentration of butterhead lettuce 'Teodore' increased by up to 17.8-times during propagation as the *PPFD* increased from 60 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Das et al. 2024).

In addition to light stress, low temperatures can cause oxidative stress. Suboptimal tissue temperature can lead to the synthesis and accumulation of anthocyanins, possibly leading to the development of purple foliage. For example, 'Red Wave' red-leaf lettuce grown at a root zone temperature (RZT) of 10 °C amassed substantially more anthocyanins in its leaves than that when plants were grown at RZTs of 20, 25, and 30 °C (Sakamoto and Suzuki 2015). Plant temperatures under lower light intensities may also be lower than those under higher intensities, leading to oxidative stresses. For example, relative to air temperature, shoot-tip temperature of vinca under SL from HPS lamps increased by 1.2, 1.5, and 1.7 °C under *PPFD*s 50, 75, and 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Faust and Heins 1997). Additionally, differences in emission spectra across SL fixtures can lead to differences in plant temperature. Islam et al. (2012) reported that the leaf temperatures of various poinsettia (*Euphorbia pulcherrima*) cultivars grown under SL at a *PPFD* of 100 ± 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were approximately 1.5 °C lower under LEDs that emitted a B:R of 1:4 when compared with those grown under HPS lamps. The HPS lamps emit more radiant heat than LEDs; therefore, crops below usually have warmer plant temperatures compared with those under LED lamps (Ouzounis et al. 2015).

Limited research has been conducted to determine the influence of the SL spectrum on coloration and pigmentation of crops sold for their ornamental value. Somma et al. (2025) grew 'Satine' red-leaf lettuce and 'Lugano' green-leaf lettuce under LED SL and found that the anthocyanin content was unaffected when FR comprised 0.3% to 2.1% of the SL spectrum. Because these were low portions of FR light, it is possible that a higher percentage of FR light could influence anthocyanin concentrations in leaves. For example, adding FR light to sole-source lighting decreased the anthocyanin concentration of 'Red Cross' baby-leaf lettuce grown under W light with or without ultraviolet LEDs (Li and Kubota 2009). In contrast, anthocyanin concentrations of 'Yanzhi' lettuce and 'Red Butter' lettuce grown under LED sole-source lighting emitting various percentages of ultraviolet A, W, and FR light were not influenced by FR light, indicating that responses may be cultivar-dependent (He et al. 2021). The influence of FR light from SL on the coloration and accumulation of anthocyanins in asexually propagated bedding plants has not been established.

The objective of this study was to mitigate leaf purpling of petunia cuttings during vegetative propagation by quantifying the influence of FR light, light intensity, and RZT, as well as their interactions, on plant morphology, coloration, and anthocyanin concentration. We postulated that during propagation, leaf purpling of petunia cuttings would be attenuated by delivering a lower SL intensity, a higher SL percentage of FR light, and a higher RZT.

Materials and Methods

Plant materials

Vegetative, unrooted, 3-cm long stem-tip cuttings of two petunia cultivars of the SureShot series, Dark Blue and White, were received on 29 Nov 2023 and 10 Jan 2024 (Ball FloraPlant, Las Limas, Nicaragua), marking the start of the first and second replications, respectively. These cultivars were chosen because commercial greenhouse propagators with LED SL reported a high incidence and severity of foliage purpling in 'Dark Blue', but not in 'White'. On the day of arrival, 1152 cuttings of each cultivar were inserted into 5.1-cm-deep, 72-cell plug trays (PTT72-STD-BLK; East Jordan Plastics, Inc., Beaverton, MI, USA) filled with (by volume) 50% soilless media (containing 86% peatmoss and 14% perlite; Suremix; Michigan Grower Products Inc., Galesburg, MI, USA) and 50% medium-grade perlite (Horticultural Medium Perlite; Perlite Vermiculite Packaging Industries Inc., North Bloomfield, OH, USA). Then, two trays of each cultivar were placed into one of eight unique treatments delivered by two SL sources, two supplemental lighting intensities, and two RZT setpoints.

Greenhouse environmental conditions

Trays were set on propagation benches in glass-glazed sections of the Plant Science Research Greenhouses at Michigan State University (East Lansing, MI, USA; lat. 43°N). A vapor pressure deficit (VPD) setpoint of 0.3 kPa was established for the first 7 d of propagation by injecting steam as necessary, which was controlled by a relay controller connected to a datalogger (CR1000; Campbell Scientific Inc., Logan, UT, USA). The VPD was calculated by the datalogger using temperature measurements from two thermocouples within an aspirated box (Type E Thermocouple; Omega Engineering, Stamford, CT, USA). One thermocouple was dry and directly measured air temperature, while the other was placed within a wick saturated with water. The VPD setpoint was raised to 0.5 kPa on day 8 of propagation and maintained until day 14; thereafter, it was raised once more to 0.7 kPa on day 15 and sustained until the end of the study. Reverse-osmosis water (maintained at 21 °C by a 500-W heater with a submersible thermometer; Hygger Aquarium Heater; Hygger, Shenzhen, China) in the form of mist was used to uphold cutting turgidity. The *PPFD* was measured at plant height by a quantum sensor (LI-190R; LI-COR, Lincoln, NE, USA) connected to the datalogger and recorded data every 30 s. When the combined ambient and supplemental *PPFD* reached 0.20 $mol \cdot m^{-2} \cdot h^{-1}$, or after 60 min (whichever occurred first), mist irrigation was turned on for 5 s. Thus, the frequency of mist irrigation events increased as ambient radiation levels increased. Misting control frequency was decreased throughout propagation as cuttings callused and formed roots, with misting ceasing on day 12. Thereafter, manual irrigation was used until cuttings were harvested. The following nutrients were supplied by both mist and manual overhead irrigation (in $mg \cdot L^{-1}$): 60 nitrogen, 23 phosphorus, 60 potassium, 28 calcium, 4.6 magnesium, 1.3 iron, 0.6 manganese, 0.6 zinc, 0.6 copper, 0.4 boron, and 0.1 molybdenum (MSU Plug Special; Greencare Fertilizers, Inc., Kankakee, IL, USA).

Greenhouses maintained a 12-h day/12-h night air temperature setpoint of 22/19 °C for

Received for publication 17 Jun 2025. Accepted for publication 5 Aug 2025.
Published online 12 Sep 2025.
We gratefully acknowledge support by Michigan State University's Project GREEEN, the Michigan Specialty Crop Block Grant Program, the Metro Detroit Flower Growers Association, and the Western Michigan Greenhouse Association for funding; Ball Horticultural Co. for cuttings; and Nathan DuRussel and Sarah Armstrong for technical assistance.
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an average daily temperature of 21 °C. Air temperature was measured by a thermocouple connected to the data logger. Plant temperature was measured by an infrared sensor (Type T, OS36-01-T-80F; Omega Engineering) placed approximately 2.5 cm above the plant canopy at a downward angle of approximately 45°.

Lighting treatments

Both cultivars were propagated under one of two SL intensities by one of two LED fixture types emitting the same percentage of B light, but different fractions of FR light. The low FR treatment (FR_{low}) delivered a B:G:R:FR of 10:7:82:1 (Philips Green Power TopLighting Linear DRWMB; Philips, Eindhoven, the Netherlands), and the high FR treatment (FR_{high}) delivered a B:G:R:FR of 10:18:59:13 (Philips Green Power TopLighting Linear DR/W/FR_2 MB; Philips). The SL delivered a total photon flux density (TPFD; 380–780 nm) of 120.2 ± 0.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. To deliver the lower light intensity treatment, aluminum mesh covered the LED fixtures and delivered a TPFD of 70.3 ± 2.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. A 16-h photoperiod (0600–2200 HR) was maintained throughout the study using a combination of sunlight and SL from either LED fixture type. The SL was delivered throughout the photoperiod when the solar *PPFD* outside the greenhouse was <440 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as measured by an integrated outdoor weather station, and SL fixtures were controlled by an environmental control system (Integro 725 3030; Priva North America, Vineland Station, Ontario, Canada). The daily light integrals (DLIs) were calculated based on hourly *PPFD* averages measured by the aforementioned datalogger (Cambell Scientific). The average DLIs were approximately 8 to 10 $mol \cdot m^{-2} \cdot d^{-1}$ and approximately 5 to 7 $mol \cdot m^{-2} \cdot d^{-1}$ for the 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ TPFD treatments, respectively. The SL spectra were measured by a spectrometer (LI-180 Spectrometer; LI-COR Biosciences) at plant height without sunlight (at night) before each replication of the experiment to establish consistent lighting treatments between repetitions (Fig. 2).

Root zone temperature treatments

Benches within each greenhouse section were equipped with bench-top microtube root zone heating (RZH) systems that circulated heated water (Biotherm Benchwarmer Kit; TrueLeaf Technologies, Petaluma, CA, USA) to maintain an RZT setpoint of 25 °C. Tubes were insulated with boards of cellofoam-expanded polystyrene and overlain with a 2-mm-thick sheet of galvanized metal to evenly disperse heat throughout the bench. Half of the trays of each cultivar were placed on the bench with RZH, and half were placed on a portion of the bench without RZH to establish two RZT treatments. The RZT of trays with or without RZH were measured by thermistors (ST-100; Apogee Instruments, Logan, UT, USA) and thermocouples (Omega Engineering) inserted in individual tray cells containing substrate. The average root zone, plant, and air temperatures as well as DLIs and weekly VPDs during both replications are provided in Table 1.

Data collection

On day 22 of propagation, 10 cuttings of both cultivars in each treatment were removed from their trays and washed to remove growing media from their roots. Cuttings were then dried via blotting using paper towels, and root and shoot tissue were subsequently separated using a razor blade. The stem length of each shoot (from the bottom of the cutting to the apical meristem) was measured with a ruler, and the stem caliper (at the base) was measured using a digital caliper (GMI-SHG-006; Stead & Fast, Kowloon, Hong Kong). Then, shoot and root tissues were desiccated in a drying oven at 70 °C for 3 d; thereafter, the SDM and root dry mass (RDM) were recorded.

Anthocyanins were extracted and measured as described by Darby et al. (2024). Extraction and analysis were performed at the University of Tennessee. Foliage tissue of both cultivars and each treatment were freeze-dried and then ground into a powder with a ceramic mortar and pestle filled with liquid nitrogen. Then, 100 mg of tissue per cultivar and treatment was weighed and inserted into a 15-mL polypropylene centrifuge tube and subsequently mixed with 5 mL of 95% ethanol/1.5 N HCl (85:15 v:v). Then, mixtures were homogenized by an orbital shaker for 15 min at 200 rpm. Subsequently, samples were placed into an opaque container filled with ice in complete darkness for 24 h at 4 °C. Then, solution was put in a 25-mL Erlenmeyer flask, and a 200-µL aliquot of sample was pipetted into a 96-well assay plate. From this, samples were measured by a microplate reader (Biotek PowerWave XS; Agilent Technologies, Santa Clara, CA, USA). Optical density was recorded at 530 nm, and total anthocyanin concentrations were calculated based on the calibration curve of cyanidin-3-o-glucoside chloride (Millipore Sigma, Burlington, MA, USA). Additionally, leaf color (average of three leaves per cutting) of 15 cuttings per cultivar was measured using a colorimeter (CR-20 Color Reader; Konica Minolta, Inc., Tokyo, Japan) to quantify the L* (lightness), a* (redness to greenness), and b* (yellowness to blueness) values. Leaf color was measured both on the day of arrival and on day 12 after stick on three leaves of each of five cuttings per cultivar and treatment.

Experimental design and statistical analysis

The experiment was performed with a complete block design and replicated twice over time. Plants were blocked by the SL spectrum, SL intensity, and RZT, with 10 to 25 experimental units (individual plants) of each cultivar per treatment combination per replication. Data were analyzed using the SAS (version 9.2; SAS Institute, Cary, NC, USA) mixed model procedure (PROC MIXED) for the analysis of variance (ANOVA), and means were separated by Tukey's honestly significant difference test at $P \leq 0.05$. Data across replications were pooled because there were no significant differences between replications.

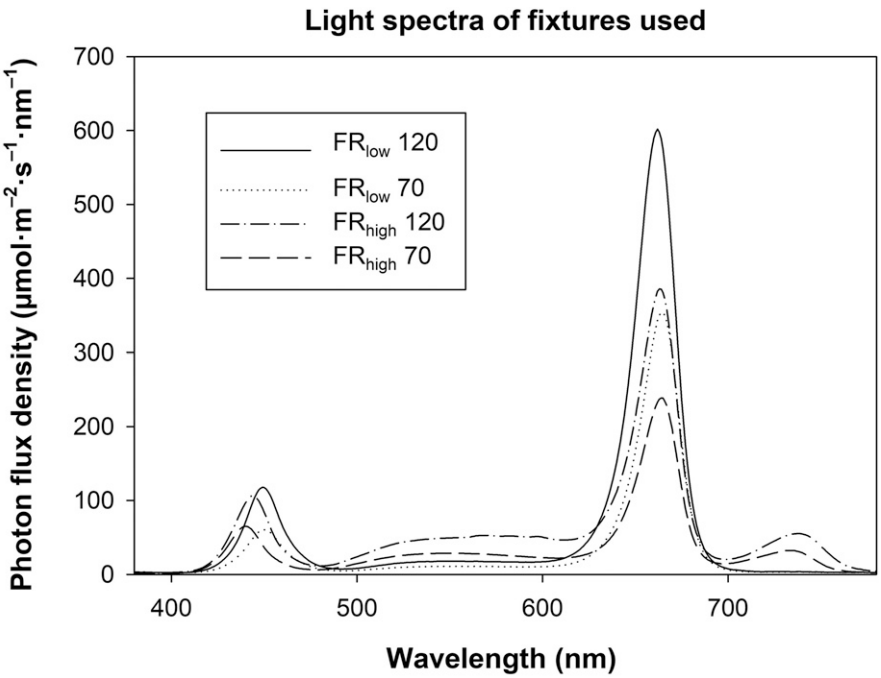

Fig. 2. Spectral distributions of four supplemental lighting treatments delivered by light-emitting diodes emitting either a low percentage of far-red (FR) light (FR_{low}) (10:7:82:1 of blue:green:red:FR light) or a relatively high percentage of FR light (FR_{high}) (10:18:59:13 of blue:green:red:FR light) at a total photon flux density of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Results

'Dark Blue'

Stem length and caliper. The spectrum and intensity of SL influenced both the stem

Table 1. Average daily light integral (DLI), root zone, plant, and air temperatures and vapor pressure deficit (VPD) for callusing and postcallusing of 'Dark Blue' and 'White' petunia during replications (reps.) 1 and 2. Cuttings were rooted under light-emitting diode fixtures providing supplemental lighting (SL) at a total photon flux density (TPFD) of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and on benches with or without root zone heating (RZH). Far-red$_{low}$ (FR_{low}) and FR_{high} fixtures emitted light ratios (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light, respectively.

SL	TPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	RZH	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Temp (°C)			VPD (kPa)		
				Root zone	Plant	Air	Week 1	Week 2	Week 3
FR_{low}	70	On	6.0 ± 0.3	24.9 ± 2.7	26.1 ± 2.2	20.9 ± 1.6	0.27 ± 0.02	0.45 ± 0.05	0.63 ± 0.04
		Off		19.9 ± 2.6					
	120	On	8.7 ± 0.4	25.5 ± 1.0	24.9 ± 1.9	21.0 ± 1.8	0.25 ± 0.26	0.41 ± 0.05	0.66 ± 0.17
		Off		20.9 ± 1.8					
FR_{high}	70	On	6.5 ± 0.3	23.8 ± 1.7	24.6 ± 2.5	20.9 ± 1.6	0.27 ± 0.02	0.45 ± 0.05	0.63 ± 0.04
		Off		20.1 ± 2.5					
	120	On	7.9 ± 0.4	23.4 ± 1.7	25.5 ± 2.2	21.0 ± 1.8	0.25 ± 0.26	0.41 ± 0.05	0.66 ± 0.17
		Off		20.4 ± 2.4					
FR_{low}	70	On	6.9 ± 0.4	24.1 ± 0.4	25.6 ± 2.3	21.2 ± 1.7	0.28 ± 0.08	0.50 ± 0.05	0.67 ± 0.11
		Off		19.2 ± 0.6					
	120	On	9.4 ± 0.4	23.0 ± 1.8	25.8 ± 2.3	20.7 ± 2.3	0.26 ± 0.05	0.50 ± 0.02	0.64 ± 0.02
		Off		19.9 ± 2.6					
FR_{high}	70	On	7.9 ± 0.4	22.9 ± 2.1	25.0 ± 2.5	21.2 ± 1.7	0.28 ± 0.08	0.50 ± 0.05	0.67 ± 0.11
		Off		19.2 ± 1.9					
	120	On	8.9 ± 0.3	23.8 ± 2.5	24.8 ± 2.5	20.7 ± 2.3	0.26 ± 0.05	0.50 ± 0.02	0.64 ± 0.02
		Off		20.2 ± 2.8					

length and stem caliper of 'Dark Blue' and, in addition, interactively influenced stem length (Table 2). In general, the stem length of 'Dark Blue' propagated under FR_{high} LEDs was greater than or equal to that grown under FR_{low} LEDs (Fig. 3). The stem caliper of cuttings of 'Dark Blue' grown under an SL intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was typically greater than or equal to that grown with SL at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4). Stem lengths of 'Dark Blue' grown under an SL intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were generally greater than or equal to those grown under an SL intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For example, stem lengths of 'Dark Blue' grown under FR_{low} LEDs at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 25% greater than those grown with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In addition, the stem lengths of cuttings of 'Dark Blue' grown under FR_{high} LEDs at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 26% greater than those grown under FR_{low} LEDs at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The RZH had no effect on the stem length or stem caliper of 'Dark Blue'.

Shoot dry mass and root dry mass. The SDM of 'Dark Blue' was unaffected by any treatments (Table 2, Fig. 5). Both light intensity and RZH affected the RDM of 'Dark Blue'. The RDM was also interactively influenced by multiple treatments. The RDM of 'Dark Blue' grown with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was generally greater than or equal to that grown with SL at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 6). The RDM of 'Dark Blue' grown with an RZT of 25 °C was typically greater than or equal to that grown with an RZT of 21 °C. Furthermore, the RDM of 'Dark Blue' grown with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at an RZT of 25 °C was 36% greater than that of cuttings grown with SL at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and an RZT of 21 °C.

Table 2. Analyses of variance of the effects of the supplemental lighting (SL) spectrum and total photon flux density (TPFD) output, root zone heating (RZH), and their interactions on stem length and caliper, root dry mass, shoot dry mass, total anthocyanin concentration, L*, a*, and b* values of 'Dark Blue' and 'White' petunia.

Treatment	Stem length (cm)	Stem caliper (mm)	Shoot dry mass (g)	Root dry mass (g)	Total anthocyanin concn ($mg \cdot g^{-1}$ DW)	L*	a*	b*
Dark Blue								
SL spectrum	***	*	NS	NS	NS	***	*	NS
TPFD	***	***	NS	***	***	***	***	***
RZH	NS	NS	NS	*	***	NS	***	NS
SL spectrum × TPFD	**	NS	NS	NS	NS	**	**	**
SL spectrum × RZH	NS	NS	NS	***	NS	NS	*	NS
TPFD × RZH	NS	NS	NS	*	NS	**	**	*
SL spectrum × TPFD × RZH	NS	NS	NS	NS	NS	*	*	NS
White								
SL spectrum	***	*	NS	NS	**	NS	NS	NS
TPFD	**	***	**	***	*	**	**	NS
RZH	***	NS	NS	***	NS	NS	NS	**
SL spectrum × TPFD	NS	NS	NS	NS	*	NS	NS	NS
SL spectrum × RZH	NS	NS	NS	NS	NS	NS	NS	NS
TPFD × RZH	NS	NS	NS	NS	**	NS	NS	NS
SL spectrum × TPFD × RZH	NS	NS	NS	NS	*	NS	NS	NS

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.
DW = dry weight.

Anthocyanin concentration and coloration. Light intensity and RZH independently influenced the total anthocyanin concentration of 'Dark Blue' (Table 2). The total anthocyanin concentration of 'Dark Blue' grown with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was generally greater than or equal to that grown with the lower SL intensity (Fig. 7). For example, when propagated under FR_{low} LEDs at an RZT of 21 °C, the anthocyanin concentration of 'Dark Blue' cuttings grown under SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was 49% greater than that grown at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, the total anthocyanin concentration of 'Dark Blue' grown with an RZT of 21 °C was generally greater than or equal to that grown with an RZT of 25 °C. For example, when propagated under FR_{low} LEDs with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the total anthocyanin concentration of 'Dark Blue' cuttings grown at an RZT of 21 °C was 132% greater than that grown under the same conditions but with SL at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The SL spectrum did not affect the total anthocyanin concentration of 'Dark Blue'.

The SL spectrum independently as well as interactively with SL intensity influenced the foliage lightness (L*) of 'Dark Blue' (Table 2). The lightness of 'Dark Blue' grown with SL at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was generally greater than or equal to that grown with SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (data not shown). The RZH did not affect 'Dark Blue' L*.

All treatments, especially SL intensity and RZH, influenced the foliage greenness and redness (a*) of 'Dark Blue' (Table 2). The a* value of 'Dark Blue' grown under FR_{low} LEDs was generally less negative (i.e., more red) than that grown under FR_{high} LEDs (Fig. 8). In addition, the a* of leaves of 'Dark Blue' propagated under SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was less negative than that under the lower SL intensity, especially those grown without RZH. Specifically, the a* of 'Dark Blue' grown under SL at 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an RZT of 21 °C was 46% more positive than that at the lower intensity and with an RZT of 25 °C.

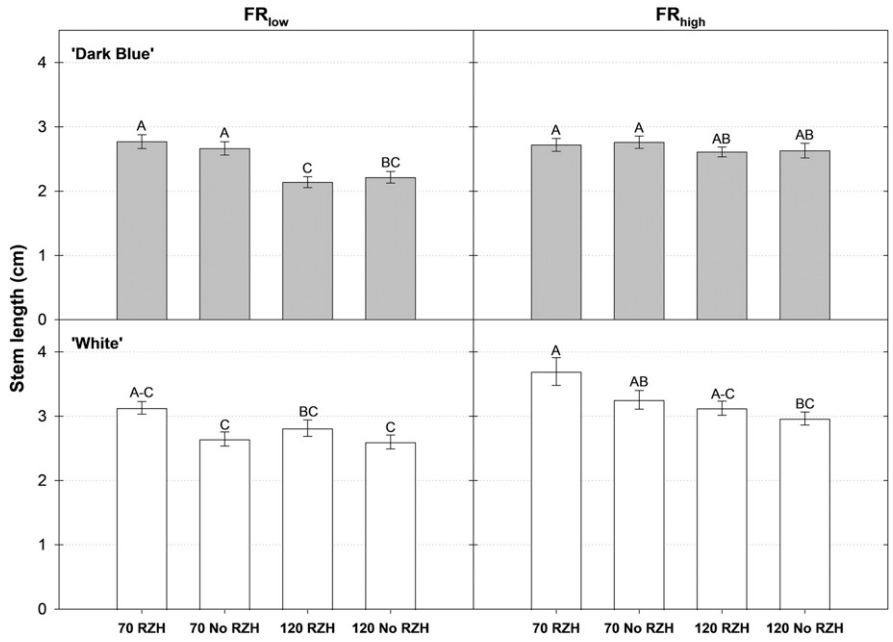

Fig. 3. Average stem length of 'Dark Blue' and 'White' petunia. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively. Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

The SL intensity influenced the foliage blueness and yellowness (b*) of 'Dark Blue' (Table 2). The SL spectrum and intensity as well as SL intensity and RZH interactively influenced the b* value of 'Dark Blue'. Cuttings of 'Dark Blue' propagated under both spectra of SL at 70 μmol·m^{-2}·s^{-1}, with or without RZH, had a higher B* value than those grown under the FR$_{low}$ SL at 120 μmol·m^{-2}·s^{-1} without RZH (Fig. 9). There was no main effect of the SL spectrum or RZH on the b* of 'Dark Blue'.

'White'

Stem length and caliper. The RZH influenced the stem length of 'White', while the SL spectrum and intensity affected both the stem length and stem caliper (Table 2). In general, cuttings grown with RZH at an RZT setpoint of 25 °C were as tall or taller than those grown without RZH. Furthermore, cuttings propagated under FR$_{high}$ LEDs or under an SL intensity of 70 μmol·m^{-2}·s^{-1} had stem lengths that were greater than or equal to those grown under FR$_{low}$ LEDs or under an SL intensity of 120 μmol·m^{-2}·s^{-1}, respectively (Fig. 3). For example, cuttings that were propagated without RZH at an SL intensity of 70 μmol·m^{-2}·s^{-1} were 40% taller when grown under FR$_{high}$ LEDs when compared with those grown under FR$_{low}$ LEDs. The RZH did not influence the stem caliper of 'White'. Additionally, across treatments, the stem caliper of cuttings grown at an SL intensity of 120 μmol·m^{-2}·s^{-1} were, on average, 8% greater than those grown under an SL intensity of 70 μmol·m^{-2}·s^{-1} (Fig. 4).

Shoot dry mass and root dry mass. The SL intensity significantly influenced the SDM of 'White' (Table 2). In general, the SDM of 'White' grown with SL at 120 μmol·m^{-2}·s^{-1} was greater than or equal to that grown with SL at 70 μmol·m^{-2}·s^{-1} (Fig. 5). The SL intensity and RZH affected the RDM of 'White'. The RDM of 'White' propagated at an SL intensity of 120 μmol·m^{-2}·s^{-1} was typically greater than or equal to that grown with SL at 70 μmol·m^{-2}·s^{-1} (Fig. 6). The RDM of 'White' grown with an RZT of 25 °C was usually greater than or equal to that grown with an RZT of 21 °C. The SL spectrum did not influence the SDM or RDM of 'White', and RZH did not influence the SDM.

Anthocyanin concentration and coloration. The anthocyanin concentration of 'White' was generally unaffected across treatments (Fig. 7). Additionally, the a* and L* of 'White' were not significantly influenced by any treatments (Fig. 8). The RZH influenced the b* of 'White', with cuttings grown with RZH at an RZT setpoint of 25 °C being greater than or equal to those grown without RZH, indicating that cuttings grown with RZH were more yellow and less blue than those grown without RZH (Fig. 9). However, these differences were minimal.

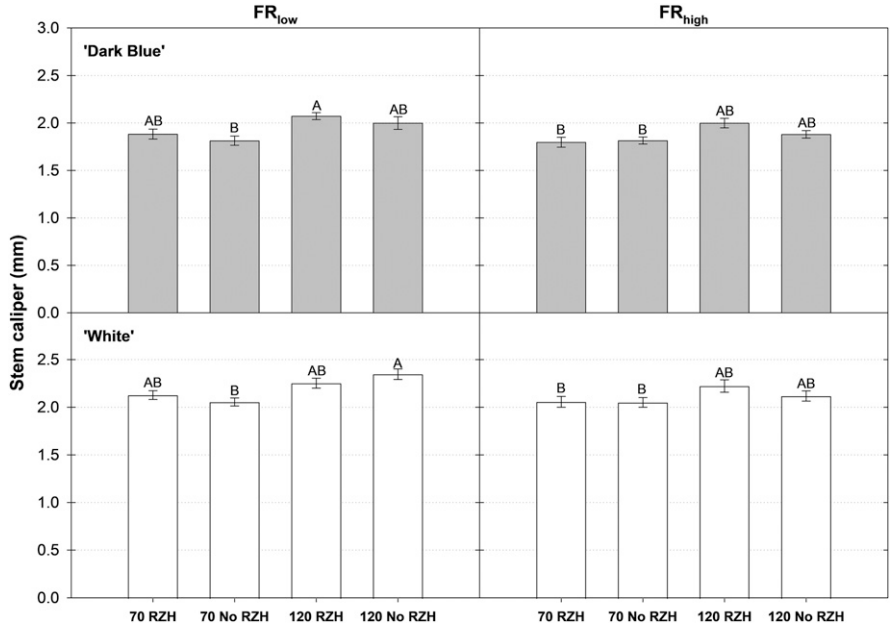

Fig. 4. Average stem caliper of 'Dark Blue' and 'White' petunia. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

Discussion

A shade-avoidance response, characterized partially by increased stem elongation, occurs in a variety of plants when light levels are low and/or when the percentage of FR light increases. For example, Brown et al. (1995) reported that 'Hungarian Wax' pepper (*Capsicum annum*) was significantly taller when grown under LEDs emitting 17% FR light (the rest as R light) than when grown under LEDs emitting only R light. This response has also been documented in various bedding plant species, including petunia (Drummond et al. 2015; Park and Runkle 2018; Percival and Craver 2024). Not surprisingly, cuttings of both 'Dark Blue' and 'White' grown under LEDs emitting a higher percentage of FR light were generally as tall or slightly taller than those grown under LEDs emitting much less

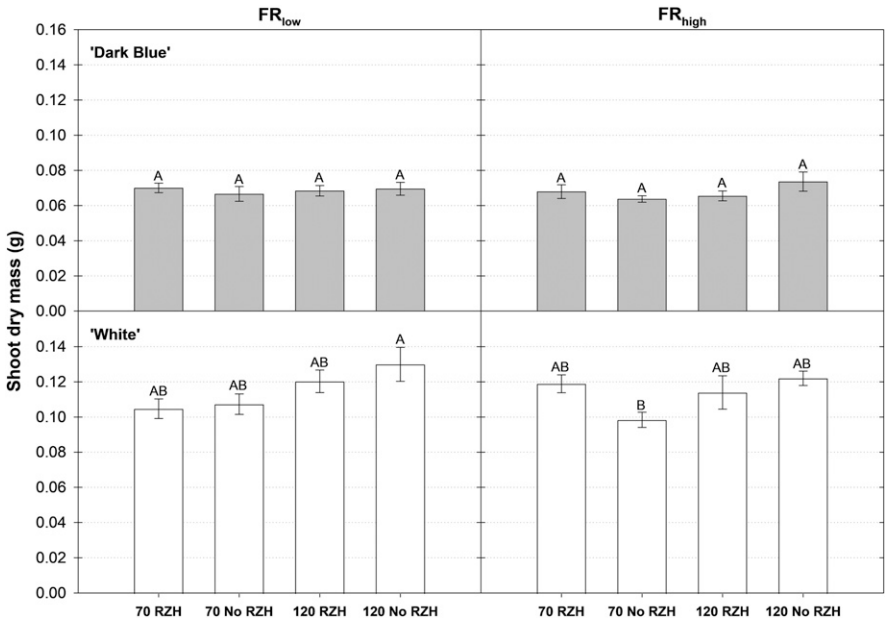

Fig. 5. Average shoot dry mass of 'Dark Blue' and 'White' petunia. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

FR light. Limited work has been conducted to determine the influence of supplemental FR light on the accumulation of anthocyanins and coloration of bedding plants during propagation. In the present study, the total anthocyanin concentration of 'White' grown under FR$_{high}$ LEDs was greater than or equal to that grown under FR$_{low}$ LEDs, while spectral differences in SL fixtures did not affect the total anthocyanin concentration of 'Dark Blue'. This suggests that the impact of FR light on anthocyanin accumulation may be cultivar-dependent.

It is well-understood that young plants tend to be more compact and have stronger stems as the DLI during propagation increases. For example, stem elongation of 'Tiny Tunia Violet Ice' petunia rooted cuttings was reduced by 35% as DLI increased from 1.2 to 3.9 mol·m^{-2}·d^{-1} (Lopez and Runkle 2006). Similarly, in the present study, cuttings grown under a supplemental TPFD of 120 μmol·m^{-2}·s^{-1} (DLI of 8.7 mol·m^{-2}·d^{-1}) tended to exhibit similar or shorter stems than those grown under a TPFD of 70 μmol·m^{-2}·s^{-1} (DLI of 7.1 mol·m^{-2}·d^{-1}) (Tables 1 and 2, Fig. 3). For example, cuttings of 'Dark Blue' grown under FR$_{low}$ LEDs at an RZT of 21 °C exhibited 13% shorter stems when grown at a DLI of 8.7 mol·m^{-2}·d^{-1} (TPFD of 120 μmol·m^{-2}·s^{-1}) compared with those grown under a DLI of 7.1 mol·m^{-2}·d^{-1} (TPFD of 70 μmol·m^{-2}·s^{-1}).

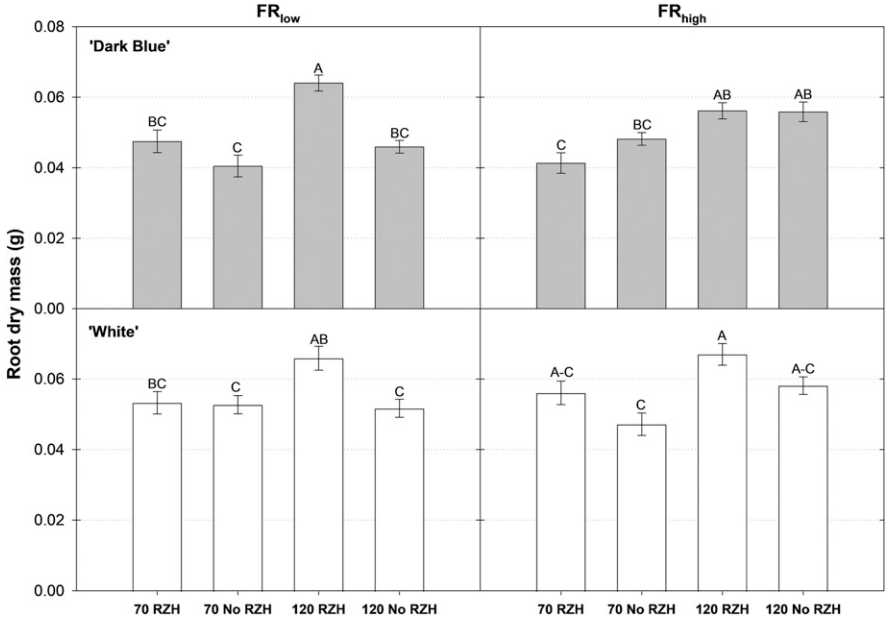

Fig. 6. Average root dry mass of 'Dark Blue' and 'White' petunia. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

Currey et al. (2012) reported that the stem calipers of 'AngelMist White Cloud' Angelonia (*Angelonia angustifolia*), 'Madeira Cherry Red' argyranthemum (*Argyranthemum frutescens*), 'Wink Coral' diascia (*Diascia barberae*), 'Aromatica Royal' nemesia (*Nemesia frticans*), 'Voltage Yellow' osteospermum (*Osteospermum ecklonis*), and 'Aztec Violet' verbena (*Verbena ×hybrida*) increased as the DLI increased from 1.2 to 12.3 mol·m^{-2}·d^{-1}. In the present study, light intensity was the most impactful variable that influenced stem caliper, whereby cuttings propagated under a higher DLI developed larger stem calipers (Fig. 4). In addition to stem caliper, SL intensity influenced the RDM of both petunia cultivars, with cuttings grown under higher DLIs yielding higher RDMs (Fig. 6). These results are consistent with those of Currey et al. (2012), who also reported increases in the RDM of all previously listed species in addition to 'Lucky Gold' lantana (*Lantana camara*), 'Blue Print' scaevola (*Scaevola hybrid*), and 'Abunda Giant White' bacopa (*Sutera cordata*) under increasing DLIs. These results are also consistent with those of Lopez and Runkle (2008), who reported that the RDM of 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' petunia increased by 680%, 2395%, and 108%, respectively, as the DLI increased from 1.2 to 8.4 mol·m^{-2}·d^{-1}. From this, one can conclude that the application of relatively high *PPFD*s may be necessary during cutting propagation to produce compact, fully rooted cuttings with thick stems suitable to handle the stresses of shipping and transplanting (Currey et al. 2012; Pramuk and Runkle 2005; Randall and Lopez 2014).

The SL source, intensity, and RZH did not significantly influence the SDM of 'Dark Blue' and 'White' petunia (Table 1, Fig. 5). This finding differed from those of Lopez and Runkle (2008), who reported that the SDM of various petunia cultivars increased as the DLI gradually increased from 1.2 to 8.4 mol·m^{-2}·d^{-1}. This suggests that the influence of higher DLIs on the SDM of petunia may be cultivar-dependent. However, because the increase in DLI was relatively smaller in the present study, it is possible that the difference in the DLI simply was not large enough to have an effect on SDM.

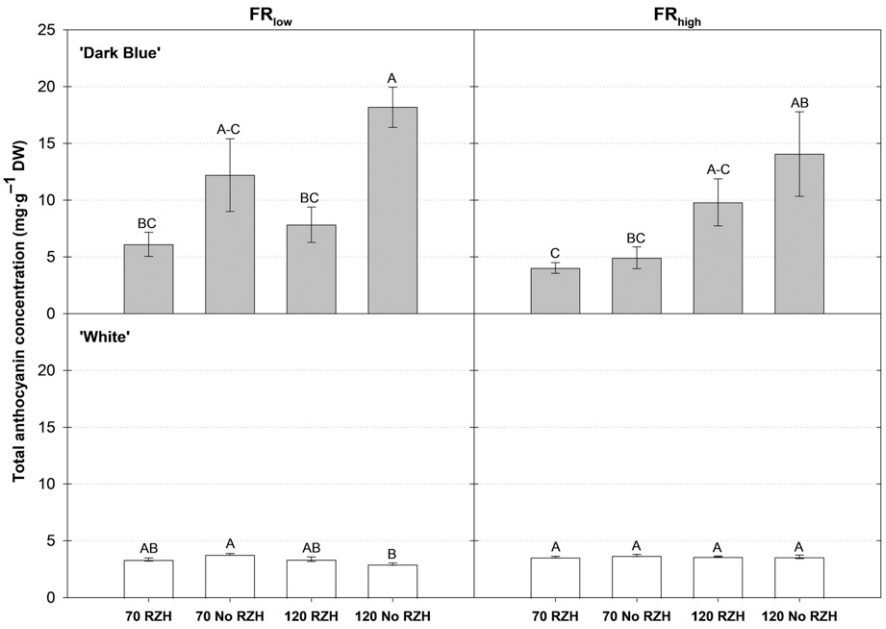

Fig. 7. Average total anthocyanin concentration of 'Dark Blue' and 'White' petunia leaves. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$). DW = dry weight.

'Rose of Heaven' petunia (*Petunia axillaris* ×*Petunia hybrida*) as well as *Lc* petunia from the 118C seed line accumulated higher amounts of anthocyanins when grown under metal halide SL providing a *PPFD* of 750 μmol·m^{-2}·s^{-1} as opposed to *PPFD*s of 50 to 350 μmol·m^{-2}·s^{-1} (Albert et al. 2009). Similarly, in the present study, the anthocyanin concentration of 'Dark Blue' petunia was greater in cuttings grown under a TPFD of 120 μmol·m^{-2}·s^{-1} compared to those grown under a TPFD of 70 μmol·m^{-2}·s^{-1} (Table 2, Fig. 7). Although direct reporting of the influence of supplemental light intensity on the redness, greenness, blueness, and yellowness of petunia is limited, it is likely that an increased concentration of anthocyanin in leaves could have an effect on foliage coloration, as expressed by the chromametric values of a* and b*. In the present study, 'Dark Blue' cuttings rooted under a higher light intensity had a higher a* (more red) and lower b* (more blue). This suggested that the undesirable purpling being reported by bedding plant propagators may, in part, be caused by the accumulation of anthocyanins in foliage as a stress response to high light intensities. However, light intensity did not influence the anthocyanin concentration, a*, or b* values of 'White'. This implies that the accumulation of anthocyanins in response to light intensity may be cultivar-dependent, as previously suggested by Smith (2025).

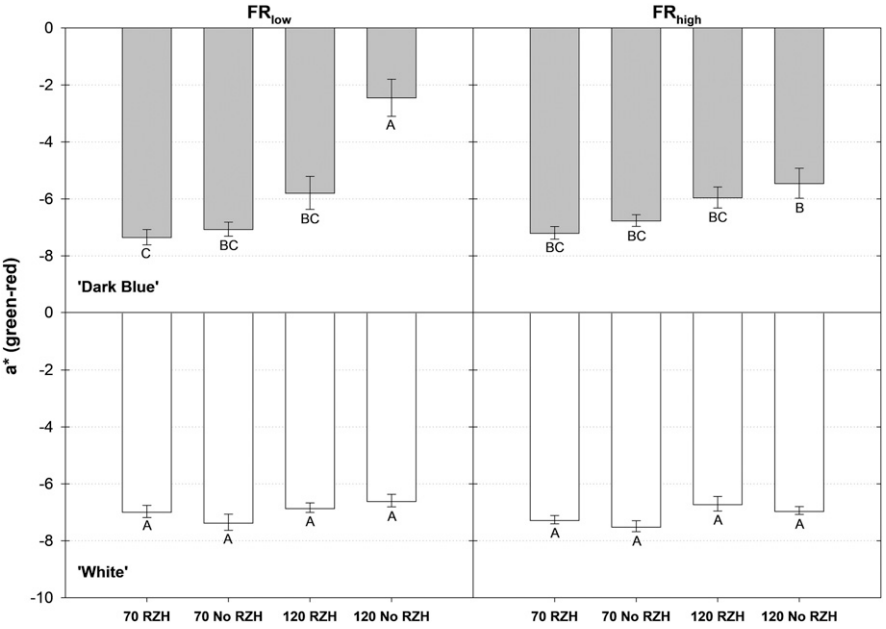

Fig. 8. Average a* value of 'Dark Blue' and 'White' petunia leaves. More negative values denote a more green coloration, while more positive values indicate a more red coloration. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 μmol·m^{-2}·s^{-1} and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR$_{low}$) and 10:18:59:13 (FR$_{high}$), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

The influence of the RZT on the stem length and RDM is described as being genus- and species-dependent (Cooper 1973). For instance, Owen (2017) reported that the RDM of 'Black Beauty' coral bells (*Heuchera hybrida*) was reduced as the RZT was raised from 20 to 28 °C. Kohler and Lopez (2021) observed that the RDM of 'Callie Coral' calibrachoa (*Calibrachoa* ×*hybrida*), 'Aromatica Royal Blue' nemesia, and 'Sanguna Patio Blue' petunia grown at an ADT of 21 °C decreased as the RZT was raised from 21 to 27 °C. In contrast, 'Dark Blue' and 'White' petunia developed a greater RDM when rooted at an RZT of 25 °C than when rooted with an RZT of 21 °C (Fig. 6). This suggested that the influence of RZT on RDM may be cultivar-dependent, or that cultivars have different optimal RZTs for root growth and development. It is known that the application of RZH raises the plant temperature, leading to increased stem elongation (Vogelezang and van Weel 1989). In the present study, 'White' petunia exposed to RZH with an RZT of 25 °C typically grew stems that were as long or longer than those grown over an RZT of 21 °C (Fig. 3). While the differences in plant temperature between cuttings that received and did not receive RZH were not measured, it is likely that the extended stem growth experienced by 'White' cuttings grown over warmer RZTs was attributable to an increase in plant temperature caused by higher RZTs. However, because the RZT did not influence the stem length of 'Dark Blue', this response is likely cultivar-dependent.

One might associate foliage purpling in young plants to phosphorus deficiency, especially during asexual propagation (Henry et al. 2019). However, preliminary and unpublished research suggested that the discoloration reported by growers who adopted LEDs is unrelated to phosphorus deficiency. Specifically, cuttings of petunia fertigated with the same nutrition regimen that was applied in the current study developed foliage purpling at varied severity and incidence rates (Smith 2025). This purpling developed in tissues regardless of age, occurred in tissues with measured phosphorus

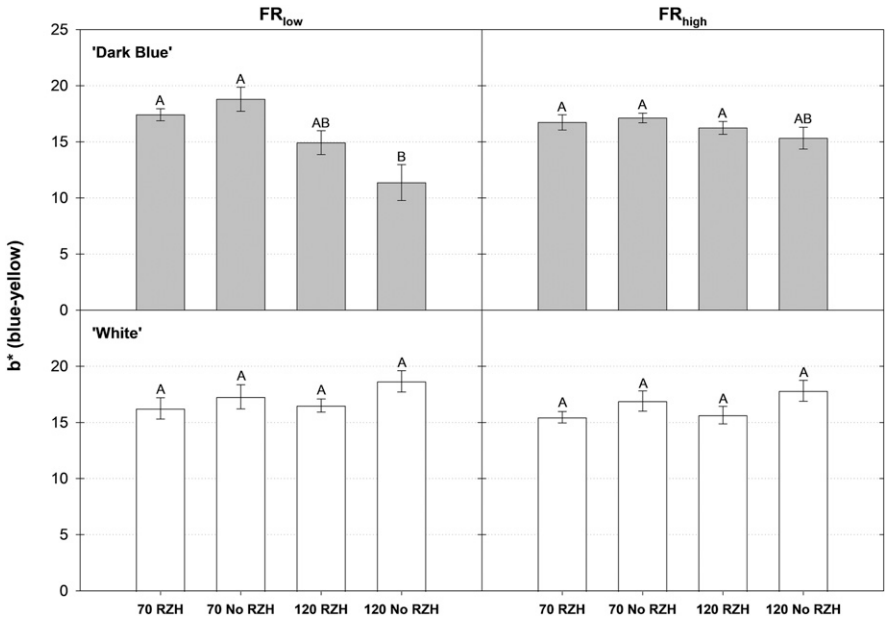

Fig. 9. Average b* value of 'Dark Blue' and 'White' petunia leaves. More positive values indicate a more yellow coloration, while less positive values denote a more blue coloration. Cuttings were rooted under supplemental light-emitting diode fixtures providing a total photon flux density of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and on benches with or without root zone heating (RZH). Fixtures emitted blue:green:red:far-red (FR) light ratios (%) of 10:7:82:1 (FR_{low}) and 10:18:59:13 (FR_{high}), respectively (combined reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters indicate significant differences within a cultivar according to Tukey's honestly significant difference test ($P < 0.05$).

concentrations within the recommended range to avoid deficiency, and did not occur in tissues with phosphorus concentrations below the recommended range, as suggested by Santos et al. (2011). Therefore, we suspect that the purpling is unrelated to phosphorus.

These results suggest that the purpling reported by propagators using LED supplemental lighting on their crops may be, in part, the result of high light intensities ($\geq$90 $\mu mol \cdot m^{-2} \cdot s^{-1}$) that commercial LED fixtures emit. These high SL intensities likely lead to an increase in the concentration of anthocyanins in the foliage of crops grown under them, thus leading to an increase in the redness and blueness of leaves. This increase in the amount of anthocyanins may be mitigated through the application of RZH to increase RZTs by approximately 4 °C or by lowering the SL intensity used during propagation. Therefore, further work using SL fixtures delivering a wider range of FR light percentages may be necessary to elucidate any relationships between FR light and the parameters measured in the present study.

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