SUPPLEMENTAL LIGHT SOURCE AND INTENSITY AND AIR AND ROOT-ZONE TEMPERATURE INFLUENCE MORPHOLOGY, LEAF COLOR, ANTHOCYANIN AND NUTRIENT CONTENT OF PETUNIA DURING ROOTING

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ABSTRACT

Greenhouse growers use supplemental lighting (SL) and heating to account for low seasonal solar radiation, air daily temperature (ADT), and root-zone temperature (RZT) during propagation. Historically, high-pressure sodium (HPS) lamps have been sued to deliver SL. However, light-emitting diodes (LEDs) are increasing in popularity due to improved energy efficacy. As propagators adopt LEDs, reports of foliage purpling of some species, such as petunia (*Petunia ×hybrida*) are increasing. This work aimed to determine how ADT and RZT along with SL spectrum and intensity influence the growth, nutrient content, and coloration of petunia. In one study, cuttings of petunia were propagated inside a greenhouse at an ADT of 21 or 23 °C with an RZT of 21 or 25 °C. Cuttings were grown under HPS lamps or one of two types of LED fixtures at a photosynthetic photon flux density of 60 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the first 6 d, then 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the remaining 16 d. In another study, cuttings of petunia 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 or 10:18:59:13 moderate G at a total photon flux density of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with the aforementioned RZT treatments. Cuttings grown at higher ADTs and RZTs often had greater stem lengths than cuttings grown at lower temperatures. Cuttings grown under LEDs or lower ADTs and RZTs had higher anthocyanin content in their leaves and were more red and blue than those grown under HPS lamps or higher temperatures. Cuttings grown under more intense SL were overall more red and blue and had higher total anthocyanin concentrations in their leaves than those under the lower light intensities. These results suggest that undesirable pigment accumulation may be mitigated by root-zone heating or limiting SL intensity (<120 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during periods of low solar irradiance.

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Introduction

The production of annual bedding plants in the United States (U.S.) is an economically important segment of specialty crops. The total wholesale value of the U.S. floriculture industry was reported at $4.8 billion in 2020, an increase in value of 9% from 2019 (USDA, 2020). The wholesale value of bedding and garden plants accounted for 49% of this total and sales were up 13% from 2019. Among the commonly produced bedding plant crops are those of the Begoniaceae family, such as *Begonia* ×*hiemalis* (begonia) and *Petunia* ×*hybrida* (petunia). In 2020, the reported wholesale value of begonias was $92 million, an increase in value of 3.7% from 2019 (USDA, 2020). For petunia, the wholesale value in 2020 was $160 million, an increase in value of 1.9% from 2019 (USDA, 2020).

Greenhouse production of bedding plants aims to produce compact crops that are optimized for distribution while also meeting retail and consumer standards. It typically consists of two phases, the young-plant (propagation) and the finished (post-transplant) phase. The young-plant phase typically requires growers to produce relatively compact, uniform, and vegetative plants that are capable of surviving shipping and mechanical and manual transplanting. Young plants are defined as being high-quality if they are fully rooted, relatively short or compact, in a vegetative state, have dark green foliage, and have a high stem diameter and root dry mass (Currey et al., 2012; Randall and Lopez, 2014). Plant propagation typically begins in the late winter to early spring to meet the spring demand for bedding and garden plants. In areas where the winter months coincide with the onset of cold outdoor temperatures and low ambient light levels, such as in Northern latitudes, outdoor production of bedding plants is not feasible. As such, bedding plant production in temperate regions generally takes place inside

greenhouses or other controlled environments. Within these environments, parameters such as light intensity, photoperiod, light quality, air and root-zone temperature, irrigation quantity and frequency, humidity, and plant nutrition can be optimized.

Light intensity, quality, and photoperiod can be adjusted in a greenhouse through the addition of supplemental lighting (SL). Air and root-zone temperatures may also be controlled in a greenhouse. Both factors influence plant temperature. At higher temperatures, rates of photosynthesis, growth, and development typically increase to an optimal temperature, after which rates decrease (Blanchard and Runkle, 2011). Watering rates and nutrient delivery concentrations can also be controlled within a greenhouse, allowing for rapid rates of growth (Alem et al., 2015; Cabrera, 2005).

Propagation

Plant propagation is the process by which new individuals of a population arise. Plants may propagate naturally either sexually through seed or spores or asexually through a variety of structures including stolons, rhizomes, bulbs, and tubers. In a horticultural sfetting, plants may also be propagated asexually through the use of stem or leaf cuttings. Asexual propagation is necessary when it is impractical to use seed, such as when seed costs are high or germination rates are low (Dole and Hamrick, 2006). More importantly, asexual propagation allows for the production of individuals that are genetically identical to the mother plant, which can make it easier to produce a uniform crop (Adhikary et al., 2021). Shoot-tip cuttings are the most common means of asexual propagation used to produce floriculture crops (Dole and Hamrick, 2006).

The size and terminology used to describe shoot-tip cuttings depends on the species and cultivar, how the stock plants are managed, how the cuttings are harvested, and the crop timing of the propagator. A terminal shoot-tip cutting features the stem apex as well as young leaves

and at least one mature leaf (Dole and Hamrick, 2006). A basal cutting is composed of stem tissue and crown tissue that has not elongated yet. A subterminal cutting is formed of at least one axillary bud and leaf yet lacks a terminal apex. (Dole and Hamrick, 2006).

Asexual propagation from shoot-tip cuttings is possible due to the ability of many plant species to produce adventitious roots (AR). AR development is the process by which new roots arise from non-root tissue, such as stems or leaves and is triggered at the cutting site in shoot-tip cuttings by a wounding response (Li et al., 2009). After wounding, a layer of suberin forms, replacing injured cells, to form a protective boundary from water loss and biotic stresses (Hartmann et al., 2011). After this, new areas of meristematic growth are produced from living cells through cellular division. This region of new meristematic growth may create a mass of undifferentiated cells known as a callus. Callus cells may then engage in organogenesis that can give rise to new shoot- or root-tissues. In the case of shoot-tip cuttings, shoot cells dedifferentiate into a callus and then redifferentiate into root cells, leading to the development and establishment of AR. Dedifferentiation is the process by which cells revert to a region of meristematic growth. AR formation can be defined as occurring in 4 steps: 1) the differentiation of shoot cells into new regions of meristematic growth, 2) the formation of root initials, 3) the organization of root initials into root primordia and 4) the development of root primordia and connection of vasculature between the cutting and primordia (Hartmann et al., 2011).

While some species yield shoot-tip cuttings that readily produce AR, such as *Petunia ×hybrida*, other species, such as *Cannabis sativa*, require the application of exogenous hormones or their precursors for successful propagation (Porras-García et al., 2023; Pizzatto et al., 2011). Commonly applied hormones include indole-3-butyric acid and indole-3-acetic acid, which are auxins. Auxins promote AR formation and the development of root primordia in cuttings by

regulating cell growth, the development of roots, and cellular division as well as facilitating the movement of resources, such as nutrients and sugars, to the cutting site (Sourati et al., 2022; Finet and Jaillais, 2012). While these hormones are widely used in commercial settings, their functions and influences during AR formation, as well as their impact on the signaling networks they are involved in, are not entirely understood (Druege et al., 2016).

In commercial greenhouse crop production, there are five stages for the propagation of shoot-tip cuttings: preparation before cutting arrival (0); arrival of cuttings and sticking (1); callusing (2); root development (3); and toning or acclimation (4) (Klopmeyer et al., 2011). Prior to cutting arrival (Stage 0), a sanitary propagation environment should be established. Tray size and type, as well as substrate composition, should be determined before the arrival of cuttings (Klopmeyer et al., 2011). Tray size, type, and substrate composition will all be dependent on the species and cutting type being propagated. Once cuttings arrive (Stage 1), they should be examined for any damage, as well as for the presence of any pests or pathogens. Prior to this stage, the propagation environment should be prepared for cuttings to be placed into the substrate. This process of placing unrooted cuttings into the substate is known as sticking. To prepare the environment, a relatively low photosynthetic daily light integral (DLI) of 3 to 5 $mol \cdot m^{-2} \cdot d^{-1}$, or an average photosynthetic photon flux density (PPFD) of 90 to 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, should be established to mitigate water loss, rise in plant temperature, and light stress (Hartmann et al., 2011; Lopez et al., 2017). A low vapor-pressure deficit (VPD) of 0.3 to 0.4 kPa should also be established to help prevent water loss (Runkle, 2019). If cuttings cannot immediately be stuck, it may be appropriate to place them in a climate-controlled storage area at an air temperature around 10 °C and a low VPD to prevent water loss (Klopmeyer et al., 2011). Maximum storage times will vary depending on the size and type of cuttings as well as the plant

material they are derived from, but usually this period should be kept to a minimum (e.g., a few days).

The VPD is the difference between the amount of water vapor that air can hold at saturation and the actual vapor pressure of the air (Grossiord et al., 2020). In most situations, VPDs range anywhere from 0.0 to 3.0 kPa. When the air is completely saturated with water vapor (i.e., 100% humidity), the VPD is equal to 0. The VPD rises as the content of water vapor in the air decreases and/or temperature increases. As the VPD increases, the evaporative demand of the air, and thereby the risk of water loss, drought stress, and desiccation, increases. As such, low VPDs are recommended during the propagation of unrooted cuttings to limit water loss. In a greenhouse environment, steam injection, fogging, and misting are ways to establish and maintain a low VPD.

Callus formation should begin soon after cuttings are stuck (Stage 2). Depending on the species and cultivar being propagated, the type of cutting, as well as environmental conditions, callus formation can take anywhere from a few days to weeks. While the development of a callus does not guarantee rooting, it is a strong indication that rooting will occur (Dole and Hamrick, 2006). During the callusing stage, warm air temperatures of 21 to 27 °C should be maintained during the day, although some cold-tolerant species may callus at 15 °C. Recommended air temperatures at night vary depending on the plant material being propagated (Hartmann et al., 2011). Root-zone temperatures should be anywhere between 18 to 25 °C. In some cold-tolerant species, reduced air temperatures and increased root-zone temperatures during propagation can be an energy-saving strategy without negatively affecting crop quality or propagation time (Kohler and Lopez, 2021). At the end of callusing, light intensity can be increased over time to a DLI of 8 $mol \cdot m^{-2} \cdot d^{-1}$, or an average PPFD of 120 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Lopez et al., 2017).

Once root initials begin to form (Stage 3), root-zone temperatures can be lowered to 19 to 21 °C and night air temperatures may be increased (Dole and Hamrick, 2006). Air temperatures during the day can be maintained. Irrigation or mist frequencies should be lowered to reduce substrate moisture levels to promote root formation. A low VPD of 0.3 to 0.6 kPa should be maintained (Dole and Hamrick, 2006) Once roots begin to develop, a DLI of 10 $mol \cdot m^{-2} \cdot d^{-1}$ is recommended (Hutchinson et al., 2012; Currey et al., 2012).

Once a root structure has been established (e.g., roots are distributed throughout the liner), cuttings can then be acclimated (Stage 4) to prepare for shipping and transplanting (Dole and Hamrick, 2006). Irrigation or misting frequency should be reduced and VPD should be increased. Night air temperatures may be decreased depending on the species, but day air temperatures, root-zone temperatures, and light levels should be maintained as previously mentioned (Dole and Hamrick 2006).

Light

In a greenhouse environment, light is typically thought of in three dimensions: duration, intensity, and quality. The influence and interplay of these dimensions lead to variations in plant morphology, biomass accumulation, flowering responses, and overall crop quality (Faust, 2011). Biomass accumulation increases with light intensity or quantity. This rise in photosynthetic efficiency continues until the photosynthetic machinery of a plant becomes saturated, after which increases in light intensity will not yield an increase in the rate of photosynthesis (Faust, 2011). The saturating light intensity depends on the crop, crop density, temperature, and carbon dioxide concentration.

The duration of light is simply the amount of time that photosynthetically active radiation is being emitted onto a crop each day. This is typically defined as the photoperiod or daylength

and is measured in $h \cdot d^{-1}$. Light intensity can be quantified through measurements such as DLI, PPFD, or total photon flux density (TPFD). The DLI is measured in $mol \cdot m^{-2} \cdot d^{-1}$ and determines the total amount of photosynthetically active photons, or photosynthetically active radiation (PAR; 400 to 700 nm), that accumulate within a square meter over a day. The PPFD, measured in $\mu mol \cdot m^{-2} \cdot s^{-1}$, quantifies the amount of photosynthetically active photons that accumulate within a square meter within a second. This differs from the photosynthetic photon flux (PPF), measured in $\mu mol \cdot s^{-1}$, which effectively measures all the photons of the PAR waveband emitted by a light source (Runkle, 2015). Light quality describes the spectrum of radiation being emitted onto a surface or plant, or the number of photons of different wavebands being emitted by a light source. PAR is composed of blue (B) light (400 to 499 nm), green (G) light (500 to 599 nm) and red (R) light (600 to 699 nm). Light from any of these wavebands may be used to drive photosynthesis (Runkle, 2015). Photons of other wavebands, such as UV-B (280 to 315 nm), UV-A (315 to 400 nm), and far-red (FR, 700 to 750 nm) are also biologically active and can influence growth and development, directly and/or indirectly.

Plants perceive photons within and outside of the PAR spectrum through photoreceptors. While light can act as a source of energy to drive photosynthesis, it may also serve as a signal that triggers various morphogenetic processes during plant development. One major group of photoreceptors is the phytochromes, which absorb R and FR light (Thomas and Vince-Prue, 1997; Faikin, 2018). Phytochrome exists in two forms: the P_R form, which primarily absorbs R radiation, and the P_{FR} form, which primarily absorbs FR radiation. The ratio of R to FR radiation (R:FR) influences various developmental processes such as flowering and stem elongation (Heins et al., 2000; Thomas and Vince-Prue, 1997). A shade-avoidance response occurs when plants are grown under a low R:FR, such as dense crop spacing or under hanging baskets. Stem

elongation, an increase in mean leaf area, and a reduction in leaf mass on a per-unit-area basis are all characteristic of a shade-avoidance response (Thomas and Vince-Prue, 1997; Percival and Carver, 2024). Such a response has been documented in numerous shade-avoiding species, including petunia, but also occurs (usually to a lesser extent) in shade-tolerant species (Percival and Carver, 2024).

Historically, FR radiation has not been considered as being photosynthetically active and thus the waveband of 700 to 750 nm is not included in that of PAR. Research has shown that FR radiation alone, particularly >730 nm, does not drive photochemistry (Emerson and Lewis, 1943; McCree, 1971). Contemporary research shows that light sources emitting 30% to 40% FR radiation, when also emitting photons within the PAR waveband, drive photosynthesis at a similar rate to those only of the PAR waveband (Zhen and Bugbee, 2020). This research suggests that FR radiation, when accompanied by light between 400 to 700 nm, may be more effective at driving photosynthesis than previously understood and, perhaps, should be included in the definition of PAR.

Phototropins and cryptochromes are both groups of photoreceptors that absorb UV-A and B radiation (Wang et al., 2020). Both of these photoreceptors regulate the opening and closing of stomata, or stomatal conductance (Wang et al., 2020). Cryptochromes are involved in the regulation of circadian rhythms as well as developmental processes such as stem elongation and leaf expansion (Fraikin and Belenikina, 2022; Eskins, 1992). Increasing the percentage of B light emitted onto a crop may lead to changes in stem and leaf morphology. These changes typically manifest as reductions in stem length and leaf surface area. For example, seedlings of *Arabidopsis thaliana* displayed smaller leaves and shorter petioles when grown under increasing amounts of B light (Eskins, 1992).

Additionally, high energy radiation, such as UV-A, UV-B, and B light, encourages the biosynthesis and accumulation of anthocyanins in plant tissues (Lee et al., 2010; Son and Oh, 2013). Along with high energy light, a high amount of light may also lead to an increase in the production of and accumulation of anthocyanins as anthocyanins help to protect photosynthetic machinery from photoinhibition by absorbing excess light (Zhao et al., 2022). Anthocyanins are naturally occurring pigments found in plants that, when accumulated, lead to the development of red, blue, or purple tissue (Alvarez-Suarez et al., 2021). Flowers and fruits tend to accumulate these pigments under optimal growing conditions but leaves and other tissues may accumulate them under times of stress. Researchers have found that increasing the amount of B light emitted onto *Vitis vinifera* (grape) increases the anthocyanin content within the fruit (Cheng et al., 2015). Other research has found that red leaf coloration of red-leaf lettuce intensities as the fraction of B light being radiated onto the crop increases (Meng and Runkle, 2023).

Supplemental lighting

During peak bedding plant propagation months (e.g., December to February) in Northern latitudes, outdoor DLIs typically average from 5 to 10 $mol \cdot m^{-2} \cdot d^{-1}$, and may be even lower depending on weather conditions such as cloud coverage (Korczynski et al., 2002). Greenhouse glazing material, the age of the glazing material, and obstructions that cause shading, such as hanging baskets and heating pipes, can further reduce ambient light levels within a greenhouse by over 50% (Fisher and Runkle, 2004). A single-layer glass glazing typically allows for a maximum of 90% of ambient light to transmit through it whereas a double-layered polyethylene may only allow up to 80% maximum light transmittance (Both and Faust, 2004). While DLI responses are species and cultivar specific, a DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ is typically recommended to produce high quality crops of shade-intolerant plants such as petunia,

Catharanthus roseus (vinca), *Salvia coccinea* (salvia), and *Zinnia elegans* (zinnia) (Torres and Lopez, 2012; Runkle, 2006; Faust et al., 2005). In contrast, shade-intolerant plants, such as impatiens, can be grown at lower DLIs, such as 5 $mol \cdot m^{-2} \cdot d^{-1}$, without sacrificing crop quality (Runkle, 2006; Faust et al., 2005). Because ambient light levels are not conducive for the production of high-quality plants during the winter and early spring, the addition of SL is critical to produce marketable plants. An increased DLI may produce plants that are more compact, have higher root masses, have greater branching, and feature wider stems than those grown under lower DLIs (Runkle, 2006).

Traditionally, SL has been most commonly delivered through high-intensity discharge (HID) lamps such as high-pressure sodium (HPS) or metal halide (MH) lamps (Ciolkosz et al., 2001). As of 2019, 98% of lighting sources in supplemented greenhouses in the U.S. were HPS or MH lamps (Lee et al., 2020). More recently, light-emitting diodes (LEDs) have been adopted by commercial growers to deliver SL. As of 2019, only 2% of lighting sources in supplemented greenhouses in the U.S. were LEDs (Lee et al., 2020), but commercial implementation is occurring at a rapid pace. While the purchase cost of LED lighting is relatively high, LED fixtures offer many advantages over HPS and MH lighting such as long life spans, compactness, and the ability to emit specific wavelengths of light, especially from 400 to 750 nm, which is the most effective at increasing photosynthesis (Haitz et al., 2000; Mitchell et al., 2015). LEDs also emit less heat than other light sources (Nowakowska et al., 2023). Most notably, LEDs are highly efficient at converting electricity into photosynthetically active photons, when compared to HID lamps (Bourget, 2008; Mitchell et al., 2015). Estimates suggest that, by using LEDs instead of HPS or MH lamps, operations may reduce their electricity consumption by 31% to 35% (Lee et al., 2020). As of 2019, if all SL sources used in the horticulture industry were LEDs,

annual energy consumption of SL would decrease by 34% or $350 million (Lee et al., 2020). Most commercially available and affordable models primarily emit a static spectrum of B or white and R light. These LEDs typically emit little to no G light or FR light. In contrast to LEDs, HPS lamps emit FR and G light while emitting proportionately less B and R light (Craver et al., 2019).

Limited research has been published directly comparing differences in floriculture crop responses to light quality when plants are grown under HPS lamps versus LEDs (Craver et al., 2019). This research has indicated that few differences in crop yield, morphology, and quality exist between bedding plants grown under SL from LEDs and HPS lamps (Currey and Lopez, 2013; Randall and Lopez, 2014). Researchers growing *Pelargonium* ×*hortorum* (geranium), petunia, *Antirrhinum majus* (snapdragon), and *Solanum lycopersicum* (tomato) reported similar dry shoot weights, plant heights, leaf area, leaf number, and no significant, consistent differences in crop morphology or flowering for plants grown under either LEDs or HPS lamps emitting 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Poel and Runkle, 2017). This being said, as more growers adopt LEDs over traditional light sources such as HPS lamps, they are reporting differences in crop responses such as the purpling of foliage and development of chlorosis (Smith et al., 2023; Veazie and Whipker, 2024).

Temperature

Temperature affects many aspects of plant growth and development. These facets include the rates of seed germination, callusing, photosynthesis, respiration, and transpiration as well as the growth and developmental rates of plant structures such as roots and shoots (Blanchard et al., 2006). The mean daily temperature, or average daily temperature (ADT), is a common way in which temperature is quantified for crops grown in controlled environments. The maturation rate

of root, shoot, leaf, and flower tissue is dependent on the rate that biochemical reactions may occur within a plant (Heins et al., 2000). The main factor dictating the rate at which these reactions occur is the amount of thermal energy present in the system. As such, an increase in ADT, which corresponds to an increase in thermal energy, will increase the rate of crop development up to a point (Heins et al., 2000). Note that while air temperature may be a strong indicator of the rate at which reactions are occurring within a plant, the actual plant temperature is the primary determinant. The plant temperature is often different than its surrounding air temperature due to the influence of energy conduction, convection, and radiation as well as transpiration (Lambers et al., 2008; Blanchard et al., 2011).

A plant's base temperature (T_b) is the lowest temperature at which cellular division may occur. Below the T_b, growth does not occur (Vincent and Gregory, 1989). While a plant may be able to survive at its base temperature, exposure to temperatures at or below a plant's T_b may cause chilling or freezing stresses to a crop. Notably, low temperature exposure may cause an accumulation of reactive oxygen species in a plant that, if unchecked, will lead to cellular damage and possibly to the death of the entire plant (Inzé and Van Montagu, 1995). Specifically, hydroxyl radicals, due to their high level of reactivity, may immediately cleave covalent bonds within various macromolecules leading to damage to enzymes, cell membranes, and nucleic acids (Inzé and Van Montagu, 1995; Prior, 2015).

Low temperatures may also lead to an increase in the expression of genes responsible for anthocyanin biosynthesis and a subsequent increase in the accumulation of anthocyanins in stem and leaf tissue (Lo Piero et al., 2005; Naing et al., 2018). Anthocyanins possess free radical scavenging capabilities such that an accumulation of anthocyanins increases a plant's ability to tolerate oxidative stress through the stabilizing and neutralization of reactive oxygen species

(Gould et al., 2002; Ding et al., 2020). From this, plants exposed to low temperatures may reduce oxidative stresses by producing and accumulating more anthocyanins in their tissues, allowing them to continue to effectively carry out photochemical reactions (Zhang et al., 2019). For example, researchers found that leaves of *Mikania micrantha* (bittervine) that had a higher concentration of anthocyanins were able to photosynthesize at significantly higher rates and accumulate significantly more biomass than those with a low concentration of anthocyanins after exposure to a 4 °C chilling treatment for 12 h (Zhang et al., 2019).

As the temperature rises above the T_b, the rate of plant development increases linearly until the optimum temperature (T_{opt}) is reached (Vincent and Gregory, 1989). At this temperature, the rate of plant development is as high as possible. Above this optimal value, as temperature increases, the rate of development decreases as the plant experiences heat stress. This reduction in developmental rate continues with increases in temperature until the maximum temperature (T_{max}) is reached. The T_{max} is the highest temperature at which cellular division occurs. Above T_{max}, plant development ceases and the plant may suffer irreversible damage depending on the magnitude of the heat and the duration of the exposure (Sage and Kubien, 2007).

The T_b, T_{opt}, and T_{max} vary greatly between species and thus, plants may be categorized based on their developmental response to low and/or high temperatures. Cold-tolerant species are considered to have a T_b <4 °C but may grow and develop at temperatures at or below 0 °C (Sage and Kubien, 2007; Blanchard and Runkle, 2011). Cold-sensitive plants have a T_b >7 °C and some could be as high as 15 °C (Sage and Kubien, 2007; Blanchard and Runkle, 2011). A cold-intermediate species has a T_b between 4 and 7 °C. These base temperatures vary between floriculture crops and across cultivars. For example, petunia, which is a cold-tolerant crop, may

have a T_b as low as 2 °C depending on the cultivar whereas the T_b of *Impatiens hawkeri* (New Guinea impatiens), a cold-sensitive crop, is around 10 °C (Blanchard et al., 2006).

Recommended temperatures for production can also differ between developmental or production stages. For example, the air temperature during the rooting stage of vegetative cuttings is generally recommended to be between 21 and 23 °C whereas the air temperature after root establishment for most species is generally recommended to be 18 to 21 °C (Blanchard et al., 2006). However, a grower may want to grow a crop below its T_{opt} depending on crop scheduling, light levels, desired crop quality, and the developmental status of the crop itself. For example, if a crop is growing faster than desired, a grower may decrease the temperature to slow the rate of growth.

A common technique to control crop morphology by influencing the rate of internode elongation, thereby influencing cutting length and overall crop height, is to manipulate the day and/or night air temperatures (Blanchard et al., 2006). This difference between the day and night temperature is commonly referred to as DIF and is calculated by subtracting the night temperature from the day temperature. When the day temperature is higher than the night temperature, a positive DIF (+DIF) is achieved. When the day temperature is lower than the night temperature, a negative DIF (-DIF) is achieved. A +DIF promotes stem elongation and produces a taller crop whereas a -DIF suppresses stem elongation and yields a shorter crop (Blanchard et al., 2006). The degree to which DIF affects a crop depends on the magnitude of DIF. For example, as a +DIF value increases, so will the internodal length (Blanchard et al., 2006). By manipulating this DIF, growers may be able to effectively control crop height with less or no use of plant growth regulators.

Root-zone heating

The establishment of a desirable root-zone temperature is critical for successfully germinating seed or propagating vegetative cuttings. Suboptimal root-zone temperatures will slow germination and rooting rates and may lead to an increase in the presence of pests and pathogens (Blanchard et al., 2006; Fonteno and Dole, 2006). The optimal root-zone temperature varies between species and perhaps different growing stages and, when propagating from vegetative cuttings, may also be dependent on the type and size of the cuttings used (Blanchard et al., 2006; Blanchard and Runkle, 2011). To promote root growth while minimizing excessive shoot growth, root-zone temperatures from 23 to 25 °C are often used in tandem with air temperatures from 18 to 21 °C. It is important to note that the root-zone temperature is usually different than the temperature of the surrounding air. As such, growers often utilize temperature probes implanted in the substrate, such as thermocouples or thermistors, to directly measure the root-zone temperature (Blanchard et al., 2006).

Within a greenhouse, it is often difficult to achieve the desired root-zone temperature by heating the air alone, and root-zone heating (RZH) may be necessary. RZH is commonly applied through substrate, bench-top, or floor systems that recirculate hot water throughout tubes embedded within or beneath the root-zone, which warm the substrate through conduction (Hartmann et al., 2011; Gerovac and Lopez, 2014). RZH may also be applied to a root zone fixated on top of a bench by installing an aluminum fin or steel pipe radiator under the bench that heats the above substrate through radiation (Bartok, 2013).

By utilizing RZH, rates of energy consumption for heating may be reduced by up to 50% when air temperature is decreased without deleteriously affecting crop quality (Sachs et al., 1992). For example, Kohler and Lopez (2021), when applying RZH and cooler air temperatures to cold-tolerant species, found that propagated *Calibrachoa* ×*hybrida* (calibrachoa), *Nemesia*

fruticans (nemesia), *Nepeta ×faassenii* (nepeta), and *Osteospermum ecklonis* (osteospermum) could be grown at an air ADT of 16 °C with a root-zone temperature setpoint of 24 °C without negatively impacting crop quality. The same study found that high-quality campanula, petunia, and phlox could be produced by propagating them at an air ADT of 16 or 21 °C and a root-zone temperature setpoint of 21 or 27°C, respectively.

Phosphorus

Phosphorus is a key macronutrient found ubiquitously throughout plant tissues. It is involved in various essential physiological processes, such as nutrient transport, energy reactions, and photosynthesis (Armstrong, 1999). Phosphorus is required for the production of chemical energy as it is a structural component of both adenosine triphosphate and adenosine diphosphate. It is also an integral part of numerous nucleic acids, enzymes, phosphoproteins, and phospholipids (Armstrong, 1999). It is involved in all stages of plant growth and development, from the establishment and growth of roots to the maturation of fruit and development of seeds (Swiader and Ware, 2002).

Phosphorus is absorbed by plant root hairs and epidermal root cells (Armstrong, 1999). Acidic soils with a pH <6 affect the oxidation state and solubility of phosphorus, limiting its availability to the plant (Swiader and Ware, 2002; Lambers et al., 2008). In such soils, the activity of microbes is restricted. This may limit the facilitation of phosphorus uptake by mycorrhizal fungi, further reducing the ability of a plant to absorb phosphorus and possibly lead to the onset of nutrient deficiency (Armstrong, 1999; Swiader and Ware, 2002). Some substrate components, such as clay, strongly bind to phosphorus and may also reduce the availability of phosphorus to a plant (Swiader and Ware, 2002).

Crops that are deficient in phosphorus can have stunted growth, delayed flowering and/or

fruiting, abortion of flower buds, and foliage discoloration (Swiader and Ware, 2002). Other noteworthy signs of phosphorus deficiency include a decrease leaf size and number and a decrease in root mass (Armstrong, 1999). Phosphorus is readily mobilized throughout the plant and, as such, phosphorus deficiency first appears in older leaves (Armstrong, 1999). Plants deficient in phosphorus may develop dark green foliage, particularly in older leaves, as leaf unfolding and expansion slow and the production of chlorophyll increases. Some species, such as petunia, may develop red or purple foliage in response to phosphorus deficiency. This is caused by leaf chlorosis along with the accumulation of anthocyanins (de Mello Prado, 2021; Calkins, 2022). Symptomology and severity of leaf discoloration from phosphorus deficiency varies across species and cultivars.

Traditionally, purpling in vegetatively propagated plant material has been attributed to phosphorus deficiency (Gibson, 2006; Calkins, 2021). The application of low phosphorus to stock plants used for cutting production may be used to suppress the internode length of cuttings, making them more suitable for shipping (Calkins, 2022). Unrooted cuttings also lack the root structure necessary to readily uptake phosphorus from the substrate after they are stuck (Armstrong, 1999). Combined, these factors increase the likelihood of vegetatively propagated plant material to have a phosphorus deficiency early in their production cycle.

Contemporary research suggests that foliage purpling during propagation may be less related to phosphorus deficiency than previously reported. When comparing propagated echinacea irrigated with 4, 12, or 36 mg/L phosphorus concentrations during each irrigation event, the concentration of phosphorus had negligible effects on the development of foliage purpling (Dickson R and Harris C, unpublished information). All treatments were irrigated at the

same time and with the same volume of water. This study was conducted using plants propagated from both seed and tissue culture, suggesting that the findings may also be applicable to echinacea propagated from vegetative cuttings. Other potential causes for purpling in plants propagated from cuttings include a high intensity of blue light, suboptimal light intensities, low air temperatures, and excessively cool or wet root-zones (Runkle, 2021; Veazie and Whipker, 2024.

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SECTION II

TEMPERATURE MANAGEMENT AND SUPPLEMENTAL LIGHTING STRATEGY EFFECTS ON THE PROPAGATION OF *Petunia ×hybrida* PART I: GROWTH AND MORPHOLOGY

Temperature management and supplemental lighting strategy effects on the propagation of *Petunia ×hybrida* Part I: Growth and morphology

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Abstract

In Northern latitudes, commercial greenhouse growers utilize supplemental lighting (SL) and heating to offset low solar radiation, air average daily temperature (ADT), and root-zone temperature (RZT) during peak young-plant production. Growers have historically used high-pressure sodium (HPS) lamps to deliver SL, but are transitioning to light-emitting diode (LED) fixtures mostly because of their improved energy efficacy. However, many growers report changes in crop morphology and undesirable purple leaf pigmentation when cuttings of some species, especially petunia (*Petunia ×hybrida*), were grown under LEDs. The objective of this study was to quantify how light intensity during callusing, ADT, RZT, and SL sources influence the morphology, rooting, leaf pigmentation, and quality of petunia and to mitigate the purpling of leaves. Shoot-tip cuttings of petunia SureShot 'Dark Blue' and 'White' were inserted into 72-cell trays and propagated inside a greenhouse at an air ADT of 21 or 23 °C and with an RZT of 21 or 25 °C. Cuttings were grown under SL delivered by HPS lamps or LED fixtures proving different light qualities (low blue or moderate blue) at a photosynthetic photon flux density (PPFD) of 60 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the first 6 d, then 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the remaining 16 d. Cuttings of both cultivars grown at an air ADT of 23 °C often had greater stem lengths and shoot dry masses than cuttings grown at 21 °C. Cuttings of both cultivars grown with an RZT of 25 °C typically had longer stems than those grown with an RZT of 21 °C. Overall, cuttings of both cultivars propagated under LEDs were of greater quality (shorter stems, greater root dry mass) than those grown under HPS lamps. The light quality provided by the LED fixtures had no effect on quality metrics. These results indicate that growers may have to adjust other environmental parameters, such as light intensity, ADT, and RZT, to influence morphology and quality of cuttings.

Introduction

The commercial production of annual bedding plants is divided into two distinct phases: the young plant or propagation phase and the finished or post-transplant phase. For young-plant production, a common objective is to reduce the time to produce a marketable seedling or rooted cuttings. Finished young plants are considered high-quality if they are fully rooted, relatively compact, in a vegetative state, have dark green and relatively small leaves, and thick stems (Currey et al., 2012; Randall and Lopez, 2014). In commercial bedding plant production, crops are commonly propagated asexually through stem tip cuttings. Propagation from stem tip cuttings offers a shorter production time than from seeds and allows for the production of a uniform crop composed of individuals that are genetically identical to the mother plant (Erwin, 1995; Adhikary et al., 2021).

Young-plant production begins in the winter to meet the large spring demand for bedding and garden plants. During this period, in high-latitude regions (>40 °N), the outdoor photosynthetic daily light integral (DLI) typically ranges from 5 to 10 $mol \cdot m^{-2} \cdot d^{-1}$, but is often lower during extended cloudy weather (Korczynski et al., 2002). Inside a greenhouse, crops may receive DLIs as low as 2 $mol \cdot m^{-2} \cdot d^{-1}$, depending on the age and type of glazing material as well as shade cast by structural components or other overhead obstructions (Fisher and Runkle, 2004). Plant responses to DLI are species- and cultivar-specific. However, a target average DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ is generally recommended for shade-avoiding species such as petunia (*Petunia* ×*hybrida*) (Faust et al., 2005; Runkle, 2006; Torres and Lopez, 2011). The propagation period typically increases as the DLI decreases below 8-10 $mol \cdot m^{-2} \cdot d^{-1}$ (Lopez and Runkle, 2008).

To overcome the seasonally low solar radiation during propagation of bedding plants, high-intensity supplemental lighting (SL) is utilized to increase the average DLI. Historically, SL

has been provided by high-pressure sodium (HPS) lamps, but advancements in light-emitting diode (LED) technology have encouraged growers to utilize LEDs to emit SL (Ciolkosz et al., 2001; Mitchell et al., 2012). While the capital expenditure of LEDs is relatively high, they have longer life spans, emit less heat, and most importantly, are more efficient at converting electricity into photosynthetically active radiation than HPS lamps (Haitz et al., 2000; Bourget, 2008; Mitchell et al., 2015; Nowakowska et al., 2023).

While SL LED fixtures are capable of emitting light with a wide range of narrow- and broad-band wavelengths, the vast majority of commercially available greenhouse fixtures emit red (R; 600–700 nm) light with blue (B; 400–500 nm) or white light. They typically do not emit far-red (FR; 700–750 nm) light, and fixtures with only R and B LEDs emit little to no green (G; 500–600 nm).. In contrast to LED fixtures, HPS lamps emit FR and G light while emitting proportionately less B and R light (Bourget, 2008; Thimijan and Heins, 1982). When LED supplemental lighting fixtures provide ≥40% of the total DLI, plants may display differences in morphology, foliage color, biomass accumulation, and stem elongation when compared to those grown under HPS lamps due to differences in emission spectra (Randall and Lopez, 2014, 2015; Poel and Runkle, 2017; Craver et al., 2019). For example, seedlings of petunia ‘Plush Blue’, salvia ‘Vista Red’ (*Salvia splendens)*, and pansy ‘Mammoth Big Red’ (*Viola ×wittrockiana*) propagated under supplemental light from HPS lamps generally had longer and thinner stems when compared to LED fixtures with R light or without B light when SL provided accounted for ≈33 to 90% of the total DLI (Randall and Lopez, 2014).

In contrast, some studies report few or no differences in crop morphology, rooting, and quality between young plants grown under SL from LEDs and HPS lamps (Currey and Lopez, 2013; Poel and Runkle, 2017). For example, Poel and Runkle (2017) propagated seedlings of

geranium ‘Pinto Premium Salmon’ (*Pelargonium ×hortorum*), pepper ‘Long Red Slim Cayenne’ (*Capsicum annuum*), petunia ‘Single Dreams White’ and ‘Wave Misty Lilac’, snapdragon ‘Montego Yellow’ (*Antirrhinum majus*), and tomato ‘Supersweet’ (*Solanum lycopersicum*) under ambient solar light supplemented with HPS lamps or LEDs emitting B, R, and/or G light. All lighting treatments provided a PPFD of 90 µmol·m^{-2}·s^{-1} and the supplemental DLI accounted for ≈16 to 40% of the total DLI, depending on solar light levels. Each crop had a similar shoot dry mass (SDM), and except for pepper, all crops had a similar leaf number, leaf area, and plant height across all lighting treatments. After transplant, no consistent variations in crop morphology or successive flowering between lighting treatments were reported.

Although commercial utilization of LEDs is continuing to increase, only approximately 2% of greenhouse SL fixtures in the U.S. were LEDs in 2019 (Lee et al., 2020). While past findings have reported little variance in crop response and quality between those propagated under HPS lamps or LEDs, commercial greenhouse growers delivering a relatively high-light intensity (e.g., PPFD >90 µmol·m^{-2}·s^{-1}) from LEDs have reported undesirable and abnormal purple foliage developing during propagation of some species and cultivars, which has decreased their marketability (Veazie and Whipker, 2024). One crop exhibiting this purpling is petunia, which had a wholesale value of $160 million in 2020 (USDA, 2020; Veazie and Whipker, 2024).

Additionally, low temperatures accompany the seasonally low ambient light levels during peak bedding propagation months in Northern latitudes. These low temperatures are not conducive for the propagation of high-quality young plants and, as such, greenhouse growers must provide supplemental heating to raise the average air daily temperature (ADT) and root-zone temperatures (RZT) of their production environment. The primary goal of heating is to raise plant temperatures towards their species-specific optimum temperature (T_{opt}), which is the

temperature at which rates of growth and development are the highest (Heins et al., 2000). In petunia, the rates of some morphological processes, such as stem elongation, increase as temperatures are raised toward T_{opt}, which is suggested to be at least 26 to 30°C, leading to plants grown at temperatures closer to T_{opt} having longer internode lengths and stems (Kaczperski et al., 1991; Warner, 2010) which may increase the need for plant growth regulator applications. Furthermore, the root-zone can be heated directly with root-zone heating (RZH) systems, which may increase rates of callus and adventitious root formation during propagation from stem-tip cuttings (Hartmann et al., 2011). However, while general propagation ADT and RZT guidelines exist, little work has been done to quantify their interaction with each other and to develop specific genera, species, and cultivar propagation ADT and RZT recommendations (Kohler and Lopez, 2021).

To our knowledge, no published research has evaluated the interactions of SL source and intensity, ADT, and RZT during plant propagation. The objective of this part of the study was to quantify the effects of, and interactions between, ADT, RZT, and SL sources and intensities on the growth and morphology of petunia cuttings. The anticipated outcome and impact of this work is to identify the cause of the leaf purpling during propagation under LED SL and to develop mitigation strategies to prevent or reduce the onset of this physiological disorder without negatively impacting the morphology or quality of rooted cuttings.

Materials and methods

Plant materials

Unrooted and vegetative 3-cm long stem-tip cuttings of two petunia cultivars from the SureShot series, ‘Dark Blue’ and ‘White’, were received on 12 Jan. 2023 and 08 Feb. 2023 (Ball FloraPlant, Las Limas, NIC). These cultivars were selected based on input from commercial

greenhouse growers and a breeding company that reported mild to severe symptom severity across cultivars, with ‘Dark Blue’ reportedly developing more severe purpling than ‘White’. On day 0, 864 cuttings of each cultivar were inserted into 5.1-cm deep, 72-cell trays (PTT72-STD-BLK; East Jordan Plastics, Inc., Beaverton, MI) filled with, by volume, 50% soilless media (containing 70% peat moss, 21% perlite, and 9% vermiculite; Suremix; Michigan Grower Products Inc., Galesburg, MI) and 50% medium-grade perlite (Horticultural Medium Perlite; Perlite Vermiculite Packaging Industries Inc., North Bloomfield, OH). Trays of cuttings were then placed in each of 24 unique treatments consisting of two ADT, two RZT, and three SL sources, with or without shading during callusing.

Greenhouse environmental conditions

Trays were placed on propagation benches in glass-glazed sections of the Plant Science Research Greenhouses at Michigan State University [(MSU), East Lansing, MI (lat. 43° N)]. A vapor-pressure deficit (VPD) of 0.3 kPa was maintained by injecting stream as necessary, which was controlled by a connected datalogger (CR1000; Campbell Scientific Inc., Logan, UT). Reverse-osmosis water was applied as mist to maintain cutting turgidity; with water temperature maintained at 21 °C using a 500-W heater with a submersible thermometer (Hygger Aquarium Heater; Hygger, Shenzhen, CN). A quantum sensor (LI-190R; LI-COR, Lincoln, NE) connected to an environmental computer recorded the PPFD every 30 s, and when the integrated PPFD reached 0.20 $mol \cdot m^{-2} \cdot h^{-1}$, or after 60 min (whichever first occurred), the mist turned on for 5 s which increased misting frequency with increasing ambient radiation intensity. As cuttings callused and developed roots, misting frequency decreased, and was discontinued at day 12. Cuttings were manually irrigated from day 13 to the end of propagation. Both the solution applied through mist and by manual irrigation delivered the following nutrients (in $mg \cdot L^{-1}$): 60

N, 23 P, 60 K, 28 Ca, 4.6 Mg, 1.3 Fe, 0.6 Mn, 0.6 Zn, 0.6 Cu, 0.4 B, and 0.1 Mo (MSU Plug Special; Greencare Fertilizers, Inc., Kankakee, IL).

Temperature treatments

Liner trays were placed into one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C (ADT of 21 °C) or 24/21 °C (ADT of 23 °C). Within each greenhouse compartment, cuttings were divided across three benches such that each bench had four trays of each cultivar. Air temperature was measured by a thermocouple (dry bulb) connected to a data logger (CR1000; Campbell Scientific Inc.). Inside each greenhouse compartment, benches had a bench-top micro-tube RZH system circulating heated water (Biotherm Benchwarmer Kit; TrueLeaf Technologies, Petaluma, CA) to maintain a setpoint of 25 °C. Tubes were insulated with boards of cellofoam-expanded polystyrene and covered by a 2-mm thick sheet of galvanized metal to evenly distribute the heat across the bench. Half of the trays of each cultivar were placed onto the bench with RZH and half were placed on a portion of the bench without RZH. RZT of trays receiving RZH were measured by two thermistors (ST-100; Apogee Instruments, Logan, UT) inserted into two adjacent cells of one of the trays. The RZT of unheated trays was measured by a thermocouple (Type E Thermocouple; Omega Engineering, Stamford, CT) inserted into an individual cell of one of the trays.

Lighting treatments

Each cultivar was propagated at each ADT and RZT and under one of three SL treatments. SL was delivered by HPS lamps [B:G:R:FR ratio of 5:47:40:8 (GE 85379 400-Watt LUCALOX; General Electric, Boston, MA)], low blue (LB) LEDs [B:G:R:FR ratio of 6:1:92:1 (Philips Green Power TopLighting Linear DRWLB; Philips, Eindhoven, NL)] or moderate blue (MB) LEDs [B:G:R:FR ratio of 10:6:82:2 (Philips Green Power TopLighting Linear DRWMB;

Philips)]. All SL sources delivered a PPFD (400-700 nm) of 117 ± 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. A 16-h photoperiod (0600 to 2200 HR) and an average DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ was maintained throughout the study through a combination of sunlight and SL from either LEDs or HPS lamps. SL was delivered throughout the photoperiod whenever the solar PPFD was below ≈440 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The PPFD was measured by a quantum sensor at plant height (LI-190R; LI-COR) and measurements were recorded every 30 s by a datalogger (Campbell Scientific), which averaged these measurements each hour, and the DLI was subsequently calculated. The fixtures were controlled by an environmental control system (Integro 725 3030; Priva North America, Vineland Station, ON, Canada) integrated with a weather station that measured ambient radiation with a quantum sensor. SL emission spectra were measured by a spectrometer (LI-180 Spectrometer; LI-COR Biosciences) at plant height at night before each repetition of the experiment to establish consistent lighting treatments between repetitions. Half of the trays under each SL treatment were placed under a polyvinyl chloride frame covered with 50% shade cloth (SOLARO 5220 D O; Ludvig Svensson, Kinna, Sweden) for the first 6 d of propagation and thus received an average DLI of 5 to 6 $mol \cdot m^{-2} \cdot d^{-1}$. The other half of the cuttings did not receive shading. Plant temperature was measured by an infrared sensor (Type T, OS36-01-T-80F; Omega Engineering) placed ≈2.5 cm above the plant canopy at a downward angle of ≈45°.

Data collection

Average root-zone, plant temperature, PPFD, and VPD data were recorded throughout both replications by an environmental data logger (CR1000; Campbell Scientific Inc.) and are provided in Table II-1 for the first and second replications, respectively.

On day 22 of propagation, 10 cuttings per cultivar and treatment were removed from their trays and the roots were washed to remove the growing substrate. Samples were then blotted dry

with paper towel and separated into root and shoot tissue. 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). Root and shoot tissue were then dried at 70 °C for 3 d and dry masses were recorded. The total (root and shoot) dry mass (TDM), sturdiness quotient (SQ), root-to-shoot ratio (R:S), and quality index (QI) of each cutting was then calculated. The SQ was calculated by dividing stem length by stem caliper. The QI was calculated as TDM × (R:S + SQ).

Experimental design and statistical analysis

The experiment was conducted under a complete block design and data were analyzed using SAS (version 9.2; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance (ANOVA). Means were separated by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Data across replications was combined.

Results

Stem length and caliper

There were main effects of SL, RZH, ADT, and callusing treatments on the stem length of petunia 'Dark Blue', but there were also several interactive effects (Table II-2). Stem length of petunia 'White' was individually influenced by SL, RZH, and ADT and there were fewer interactions. Across all treatments, petunia 'Dark Blue' and 'White' cuttings rooted under HPS lamps were 1.1 and 1.2 cm (29–32%) and 1.6 and 0.7 cm (13–36%) taller than those propagated under LB or MB LEDs, respectively (Figs. II-1 and II-2). Furthermore, stems of petunia 'Dark Blue' grown under 50% shade for 6 d (i.e., for callusing) were similar to or slightly taller than cuttings propagated without the callusing treatment. Stem length of both cultivars often increased when RZH was provided, but responses depended on SL and callusing treatments. Overall, stem

length of ‘Dark Blue’ and ‘White’ increased by 1.9 and 3.2 cm (63% and 91%), respectively, as air ADT increased from 21 to 23 °C.

In general, cuttings of petunia ‘Dark Blue’ grown at an ADT of 23 °C with RZH under HPS lamps or with a callusing treatment were taller than those grown at an ADT of 21 °C without RZH under LEDs or without a callusing treatment. Stem length of petunia ‘Dark Blue’ propagated under HPS lamps at an ADT of 23 °C with RZH was 3.6 cm (135%) greater than that of cuttings grown under LB LEDs at an ADT of 21 °C without RZH (Fig. II-1). Moreover, when combining SL treatments, stem length of petunia ‘Dark Blue’ grown under 50% shade for 6 d of callusing at an ADT of 23 °C and with RZH was 3.2 cm (112%) greater than those propagated without a callusing treatment at an ADT of 21 °C and without RZH.

The stem caliper of petunia ‘Dark Blue’ was influenced by ADT; cuttings propagated at an ADT of 23 °C were generally thicker than those propagated at an ADT of 21 °C (Table II-2, data not presented). There was no effect of SL, RZH, or callus treatment on stem caliper. There were no main treatment effects on stem caliper of petunia ‘White’ (data not presented).

Shoot dry mass

The SDM of petunia ‘Dark Blue’ was individually and interactively influenced by SL and ADT as well as many treatment interactions, whereas the SDM of petunia ‘White’ was only individually influenced by ADT as well as several treatment interactions (Table II-2). The SDM of petunia ‘Dark Blue’ cuttings propagated under HPS lamps was generally greater than those propagated under LB and MB LEDs at each ADT. The SDM of both cultivars was typically greater at an ADT of 23 °C than at 21 °C (Figs. II-3 and II-4). The SDM of ‘Dark Blue’ and ‘White’ increased by 49.0 (73%) and 35.1 mg (33%) as the ADT increased from 21 to 23 °C, respectively. Additionally, SDM of petunia ‘Dark Blue’ propagated under HPS lamps at an ADT

of 23 °C was 72.9 (123%) and 65.8 mg (99%) greater than those under LB and MB LEDs at an ADT of 21 °C. In general, the SDM of petunia 'White' cuttings grown under HPS lamps at an ADT of 23 °C was greater than that of those propagated under LB or MB LEDs at an ADT of 21 °C. The SDM of 'Dark Blue' was generally unaffected by RZH or callusing treatment while that of 'White' was not influenced by SL, RZH, nor callusing.

Root dry mass

The root dry mass (RDM) of petunia 'Dark Blue' was influenced individually by SL, RZH, ADT, and callusing treatments, along with numerous treatment interactions (Table II-2). The RDM of petunia 'White' was individually affected by SL and ADT, as well as many treatment interactions. The RDM of 'Dark Blue' and 'White' cuttings propagated under LB and MB LEDs were generally greater than or similar to those rooted under HPS lamps, when comparing the RDM of cuttings grown at the same ADT (Fig. II-5 and II-6). Additionally, cuttings of 'Dark Blue' grown without a 6 d 50% shade callusing treatment had an RDM that was slightly greater than or similar to that of cuttings grown with a callusing treatment. The RDM of petunia 'Dark Blue' cuttings was usually greater without RZH than with RZH. Furthermore, the RDM of both 'Dark Blue' and 'White' was generally greater at an ADT of 21 °C than at 23 °C, but individual treatment responses varied (Figs. II-5 and II-6). RDM of 'Dark Blue' cuttings propagated under HPS lamps without RZH at an ADT of 21 °C were 15.2 (48%), 21.3 (83%), and 21.5 mg (84%) greater than those grown under LB LEDs, MB LEDs, and HPS lamps with RZH at an ADT of 23 °C, respectively. The RDM of petunia 'White' was generally unaffected by RZH or a callusing treatment.

Quality index

The QI of petunia 'Dark Blue' was individually affected by SL, RZH, ADT and callusing

treatment along with many treatment interactions, while that of petunia ‘White’ was individually influenced by SL, RZH, and ADT as well as the interaction of RZH×ADT×callusing treatment (Table II-2). The QI of both cultivars propagated under LB and MB LEDs were, in most cases, higher than those grown under HPS lamps (Figs. II-7 and II-8). Additionally, the QI of petunia ‘Dark Blue’ propagated without a 6 d 50% shade callusing was, typically, slightly higher than or similar to that grown with a callusing treatment. Cuttings of both cultivars propagated without RZH typically had a QI greater than that of those grown with RZH. Moreover, cuttings of both cultivars grown at an ADT of 21 °C had QIs that were similar to or greater than those propagated at an ADT of 23 °C. In addition, the QI of petunia ‘Dark Blue’ cultivated under LB and MB LEDs without RZH at an ADT of 21 °C were, respectively, 75% and 71% greater than those grown under HPS lamps with RZH at an ADT of 23 °C. The QI of petunia ‘White’ was not influenced by the callusing treatment.

Discussion

The individual effects of SL source and intensity, ADT, and RZT on the growth and development of young plants are generally well documented (Blanchard et al, 2006; Dole and Hamrick, 2006; Blanchard et al., 2011; Hartmann et al., 2011; Lopez et al., 2017; Craver et al., 2019). Furthermore, the effects of SL source and intensity, and ADT on young plant quality have also been reported (Pramuk and Runkle, 2005; Currey and Lopez, 2012; Randall and Lopez, 2015; Kohler and Lopez, 2021). While exploratory research investigating how the morphology and quality of bedding plants are affected by the interaction of ADT and RZT, little research has been published examining how such characteristics are influenced by the interactions of SL source, ADT, and RZT (Kohler and Lopez, 2021). Additionally, limited research has been conducted to investigate how lower light intensity during the callusing phase of asexual

propagation influences young plant growth, morphology, and quality.

Under sole-source lighting, stem elongation of crops is generally inhibited with increasing fractions or flux densities of B light (Wollaeger and Runkle, 2013). However, inside a greenhouse environment, the impact of SL emission spectra on crop morphology and quality depends on the solar DLI (Randall and Lopez, 2014; Poel and Runkle, 2017; Craver et al., 2019). For example, Poel and Runkle (2017) reported little to no differences in biomass accumulation or morphology of geranium, pepper, petunia, snapdragon, or tomato seedlings grown under a PPFD of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from either HPS or LED supplemental lighting providing 10 to 20% B light when they provided 20 to 40% of the total DLI. This aligns with Craver et al. (2019) who reported cuttings had minimal responses to HPS lamps, providing ≈2% B light, or LEDs, providing ≈10% B light, when the supplemental DLI accounted for <33% of the total DLI. However, Randall and Lopez (2014) reported that various bedding plant seedlings were shorter under LEDs emitting 15 to 30% B light compared to those grown under HPS lamps when the supplemental DLI accounted for >43% of the total DLI. Similarly, in the present study where 57 to 76% of the total DLI was provided by SL, stems of petunia 'Dark Blue' and 'White' were shorter under LEDs providing 6 or 10% B light than under HPS lamps (Figs. II-1 and II-2). Furthermore, the SDM of petunia 'Dark Blue' was lower under the LEDs and than under HPS lamps (Fig. II-3). This can likely be attributed to plant temperature, which was 0.9 to 2.8 °C higher under HPS lamps than under LEDs (Table II-1). The response is likely cultivar dependent as the SDM of petunia 'White' was not affected by SL source.

In the present study, the R:FR ratio (or the FR fraction, which is the photon flux density of FR light relative to R+ FR light) of the LEDs was greater than that of the HPS lamps. As a low R:FR ratio (or higher FR fraction) promotes stem elongation in petunia, this may have

contributed to the greater stem length of cuttings grown under HPS lamps (FR fraction of 17%) compared to those propagated under LEDs (FR fraction of 1–2%) (Percival and Carver, 2024). Additionally, cuttings of 'Dark Blue' propagated under the 6-d callusing treatment were probably taller than those grown without the callusing treatment due to the reduced DLI that likely was emitted onto the cuttings grown under the callusing treatment. It has been well documented that petunia cuttings propagated under lower DLIs tend to grow longer shoots as DLI decreases from ≈10 towards 1 $mol \cdot m^{-2} \cdot d^{-1}$ (Lopez and Runkle, 2005; Lopez and Runkle, 2008). While we did not quantify and record the DLI underneath the 50% shade callusing treatments throughout the experiment, it can be assumed that the DLI being received by cuttings grown underneath the shade cloth was likely lower than those grown without shading, leading to the greater stem lengths exhibited by cuttings grown under the callusing treatment.

ADT was the most influential factor affecting the morphological parameters of stem length and SDM of both petunia 'Dark Blue' and 'White' (Figs. II-1–II-4; Table II-2). The stem lengths and SDMs of both cultivars were generally greatest when grown at an ADT of 23 °C rather than 21 °C (Figs. II-1–II-4). This aligns with previous work that indicated greater ADTs increased stem length of petunia 'Sanguna Patio Blue', calibrachoa 'Callie Coral' (*Calibrachoa ×hybrida)*, nemesia 'Aromatica Royal Blue' (*Nemesia fruticans)*, nepeta 'Junior Walker' (*Nepeta ×faassenii*), phlox 'Glamour Girl' (*Phlox paniculata*), and rosemary 'Arp' (*Rosmarinus officinali*), as well as increased SDM of calibrachoa, nemesia, and nepeta (Kohler and Lopez, 2021). However, while the SDM of both cultivars in the present study generally increased as ADT increased from 21 to 23 °C, the SDM of petunia was not significantly influenced by ADT in Kohler and Lopez, 2021. This suggests that the influence of ADT on SDM may be cultivar dependent.

Kohler and Lopez (2021) reported that ADT did not influence the RDM of petunia. However, in the present study, ADT was the most influential factor affecting the RDM of petunia 'White' and one of the most influential factors affecting the RDM of petunia 'Dark Blue' (Table II-2; Figs. II-5 and II-6). Overall, the RDM of both cultivars was greater at an ADT of 21 °C than 23 °C. However, the effect of ADT on RDM varied across treatments and between cultivars. These differences in ADT effect on RDM within and between studies suggests that the response may be cultivar dependent. Additionally, as petunia is a cold-tolerant crop (i.e., has a low base temperature), increases to ADT, regardless of RZH, may have raised both the ADT and RZT beyond the T_{opt}, resulting in a lower RDM at a higher ADT for both cultivars in the present study.

Generally, when RZT is raised during propagation, root growth and development increase up to the T_{opt}, after which, further increases negatively affect rooting (Gislerød, 1983; Wilkerson et al., 2005; Hartmann et al., 2011). However, the influence of RZT on the accumulation of root mass is genus-, species-, and even cultivar-dependent (Cooper, 1973). For example, Owen (2017) observed that the RDM of coral bells 'Black Beauty' (*Heuchera hybrida*) decreased as the RZT increased from 20 to 28 °C. Additionally, regardless of ADT, the RDM of calibrachoa and nemesia decreased as RZT was raised from 21 to 27 °C and, at an ADT of 21 °C, the RDM of petunia also decreased with an increase in RZT (Kohler and Lopez, 2021). Similarly, in the present study, the RDM of petunia 'Dark Blue' of treatments that did not receive RZH was generally greater than or equal to that to those that did (Fig. II-5). This may indicate that the root-zone T_{opt} was surpassed for 'Dark Blue' when RZH was applied. However, as the RDM of petunia 'White' was not affected by the application of RZH, it is possible that the root-zone T_{opt} varied between the two cultivars, further suggesting that root mass accumulation

in response to RZT is cultivar dependent.

The development of a thick stem diameter is also desirable during young plant production to better tolerate shipping and transplanting. In general, stem diameter increases with DLI (Currey et al., 2012). As all cuttings were provided with a DLI of ≈10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ after 6 d, it is no surprise that SL source or callusing treatment did not influence stem caliper. However, ADT did influence the stem caliper of petunia 'Dark Blue'; those grown at an ADT of 23 °C had similar or slightly larger stem diameter than those grown at an ADT of 21 °C (Table II-2). While ADT significantly impacted the stem diameter of petunia 'Dark Blue', stem diameter at an ADT of 23 °C was only 6% (0.1 mm) wider than those at 21 °C, which is negligible when evaluating crop quality (data not presented).

Young plant quality can be objectively evaluated by the QI, which is a quantitative measurement derived from morphological characteristics related to stem length, stem caliper, and RDM. Larger QI values generally indicate greater plant quality and survivability during shipping and mechanical transplanting (Pramuk and Runkle, 2005; Currey et al., 2013). In the present study, SL source and ADT were the two most significant individual factors influencing the QI of both petunia cultivars. Petunias propagated under either LED type generally had higher QIs than those under HPS lamps, and petunias propagated at an ADT of 21 °C generally had higher QIs than those grown at 23 °C (Table II-2; Figs. II-7 and II-8). This is likely due to the large influence that these factors had on stem length and TDM, which highly influence the calculation of the QI. This aligns with previous work that reported the QI of petunia 'Plush Blue' grown under SL provided by LEDs emitting either 70:30 or 85:15 R:B light was either similar to or greater than the QI under HPS lamps (Randall and Lopez, 2014). However, Randall and Lopez (2015) reported that the QI of petunia 'Dreams Midnight' grown under SL provided by

HPS lamps or LEDs providing 87:13 R:B light were similar. The SL sources used by Randall and Lopez (2015) only ≈23 to 36% of the total DLI whereas those of Randall and Lopez (2014) provided ≈33 to 90% of the total DLI. In the present study, we provided ≈70% of the DLI through SL. As SL made up a larger portion of the total DLI in the present study and Randall and Lopez (2014), the influence SL had on overall crop morphology, and thereby QI, was likely greater than in Randall and Lopez (2015), leading to the differences in SL impact on the QI between the studies.

In the present study, the QI of petunia 'Dark Blue' and 'White' were influenced by ADT, whereas Kohler et al. (2021) reported that ADT did not influence the QI of petunia 'Sanguna Patio Blue'. However, due to differences in expected and desired crop morphology across genera, species, and even cultivars, differences in data collection materials and methods between studies, along with the QI being a unitless measurement, direct comparison of QI between crops and across studies is difficult and may be inappropriate. Additionally, the QI does not consider marketability factors such as foliage pigmentation, in its calculations. It should be noted that foliage pigmentation varied drastically across treatments and between the two cultivars such that many cuttings grown would be considered unmarketable. As such, the QI may not be the most suitable tool to evaluate cutting quality in this scenario.

The quality and morphology of bedding plants propagated under LEDs can differ from those propagated under HPS lamps when the supplemental DLI provided by the SL fixtures accounts for a large portion (e.g., >50%) of the total DLI. Most notably, stems may be longer when grown under HPS lamps than under LEDs, leading to lower quality plants (Figs. II-1, II-2, II-7, and II-8). However, growers have options to increase the quality of cuttings propagated under HPS lamps. For example, stem lengths can be suppressed by delivering a relatively high

DLI, relatively low ADT, and moderately high RZT to retard stem elongation and promote root growth. Additionally, while there were several significant interactions between the various environmental parameters, the number and magnitude varied between cultivars (Table II-2). For example, while RZH and ADT interacted to influence the RDM of 'Dark Blue', they did not for 'White'. Furthermore, RZH and ADT had an interactive effect on the RDM of 'Dark Blue' but depended on the SL source and callusing treatment (Fig. II-5).

We conclude that cuttings of similar quality can be produced under HPS lamps or LEDs when other environmental parameters are also considered. For example, cuttings of 'Dark Blue' petunia propagated under MB LEDs at an RZT of 25 °C, an ADT of 21 °C, and without a callusing treatment were of equivalent quality to those propagated under HPS lamps with an RZT of 21 °C, an ADT of 21 °C, and with a callusing treatment. Further research is needed to investigate how environment factors interactively influence the morphology and quality of propagated cuttings, providing growers with strategies to produce high-quality crops under both HPS lamps and LEDs, regardless of the time of year and ambient light conditions.

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APPENDIX

Table II-1. Daily light integral (DLI), root-zone, plant, and air temperatures, and vapor-pressure deficit (VPD) for callusing and post-callusing during replication (Rep.) 1 and 2. SL = supplemental lighting; HPS = high-pressure sodium; LB = low blue; MB = medium blue; LED = light-emitting diode; ADT = average daily temperature; RZH = root-zone heating.

SL	ADT	RZH	Callusing treatment	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)			VPD (kPa)
					Root-zone	Plant	Air	
Rep. 1								
HPS	21 °C	On	Yes	10.7 ± 0.4	23.7 ± 1.6	27.7 ± 2.2	21.2 ± 1.6	0.25 ± 0.03
			No					
		Off	Yes		21.8 ± 2.7			
			No					
	23 °C	On	Yes	11.6 ± 0.5	25.1 ± 2.5	28.8 ± 3.3	23.3 ± 1.7	0.27 ± 0.05
			No					
		Off	Yes		24.1 ± 2.7			
			No					
LB LED	21 °C	On	Yes	9.3 ± 0.3	23.8 ± 1.9	26.8 ± 2.3	21.2 ± 1.6	0.25 ± 0.03
			No					
		Off	Yes		20.6 ± 2.3			
			No					
	23 °C	On	Yes	10.1 ± 0.4	24.5 ± 1.8	28.5 ± 2.2	23.3 ± 1.7	0.27 ± 0.05
			No					
		Off	Yes		22.3 ± 2.3			
			No					
MB LED	21 °C	On	Yes	11.0 ± 0.5	22.8 ± 2.3	24.9 ± 2.1	21.2 ± 1.6	0.25 ± 0.03
			No					
		Off	Yes		20.6 ± 2.1			
			No					
	23 °C	On	Yes	11.3 ± 0.4	25.0 ± 1.6	27.9 ± 2.1	23.3 ± 1.7	0.27 ± 0.05
			No					
		Off	Yes		19.6 ± 1.7			
			No					

Table II-1 (cont'd).

Rep. 2								
HPS	21 °C	On	Yes	10.3 ± 0.4	23.5 ± 2.0	27.3 ± 2.5	21.4 ± 1.8	0.24 ± 0.11
			No					
		Off	Yes		22.4 ± 2.9			
			No					
	23 °C	On	Yes	12.1 ± 0.6	25.2 ± 3.3	28.7 ± 3.8	23.4 ± 2.2	0.30 ± 0.10
			No					
		Off	Yes		24.3 ± 2.9			
			No					
LB LED	21 °C	On	Yes	9.1 ± 0.4	23.8 ± 1.9	26.7 ± 2.2	21.4 ± 1.8	0.24 ± 0.11
			No					
		Off	Yes		21.1 ± 2.6			
			No					
	23 °C	On	Yes	10.6 ± 0.5	24.5 ± 2.6	28.7 ± 3.8	23.4 ± 2.2	0.30 ± 0.10
			No					
		Off	Yes		22.9 ± 2.6			
			No					
MB LED	21 °C	On	Yes	9.6 ± 0.4	24.4 ± 1.4	25.5 ± 2.7	21.4 ± 1.8	0.24 ± 0.11
			No					
		Off	Yes		21.1 ± 2.3			
			No					
	23 °C	On	Yes	11.9 ± 0.5	25.1 ±1.7	28.1 ± 2.4	23.4 ± 2.2	0.30 ± 0.10
			No					
		Off	Yes		19.6 ± 1.7			
			No					

Table II-2. Analyses of variance for the effects of supplemental lighting (SL), root-zone heating (RZH), air average daily temperature (ADT), a callusing treatment, and their interactions on stem length and caliper, root and shoot dry mass, and quality index of petunia ‘Dark Blue’ and ‘White’.

Treatment	Stem length	Stem caliper	Shoot dry mass	Root dry mass	Quality index
‘Dark Blue’					
SL	***[z]	NS	***	**	***
RZH	***	NS	NS	***	***
ADT	***	*	***	***	***
Callus	***	NS	NS	***	***
SL×RZH	NS	NS	NS	*	NS
SL×ADT	***	NS	***	NS	NS
SL×Callus	***	NS	NS	NS	NS
RZH×ADT	NS	NS	NS	**	NS
RZH×Callus	***	NS	**	*	**
ADT×Callus	NS	NS	NS	NS	NS
SL×RZH×ADT	***	NS	NS	***	***
SL×RZH×Callus	***	NS	*	*	*
SL×ADT×Callus	NS	NS	NS	NS	NS
RZH×ADT×Callus	*	NS	*	NS	NS
SL×RZH×ADT×Callus	NS	NS	NS	NS	NS
‘White’					
SL	***[z]	NS	NS	**	***
RZH	***	NS	NS	NS	*
ADT	***	NS	***	***	***
Callus	NS	NS	NS	NS	NS
SL×RZH	NS	NS	*	*	NS
SL×ADT	**	NS	***	NS	NS
SL×Callus	**	NS	NS	NS	NS
RZH×ADT	NS	**	**	NS	NS
RZH×Callus	NS	NS	NS	**	NS
ADT×Callus	NS	NS	NS	NS	NS
SL×RZH×ADT	NS	NS	NS	*	NS
SL×RZH×Callus	NS	NS	NS	NS	NS
SL×ADT×Callus	NS	*	NS	**	*
RZH×ADT×Callus	NS	NS	NS	NS	NS
SL×RZH×ADT×Callus	NS	NS	NS	NS	NS

[z]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table II-3. Analyses of variance for the effects of supplemental lighting (SL), root-zone heating (RZH), air average daily temperature (ADT), a callusing treatment, and their interactions on stem length and caliper, root and shoot dry mass, and quality index of petunia ‘White’.

Treatment	Stem length (cm)	Stem caliper (mm)	Root dry mass (mg)	Shoot dry mass (mg)	Quality index
SL	***[z]	NS	**	NS	***
RZH	***	NS	NS	NS	*
ADT	***	NS	***	***	***
Callus	NS	NS	NS	NS	NS
SL×RZH	NS	NS	*	*	NS
SL×ADT	**	NS	NS	***	NS
SL×Callus	**	NS	NS	NS	NS
RZH×ADT	NS	**	NS	**	NS
RZH×Callus	NS	NS	**	NS	NS
ADT×Callus	NS	NS	NS	NS	NS
SL×RZH×ADT	NS	NS	*	NS	NS
SL×RZH×Callus	NS	NS	NS	NS	NS
SL×ADT×Callus	NS	*	**	NS	*
RZH×ADT×Callus	NS	NS	NS	NS	NS
SL×RZH×ADT×Callus	NS	NS	NS	NS	NS

[z]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

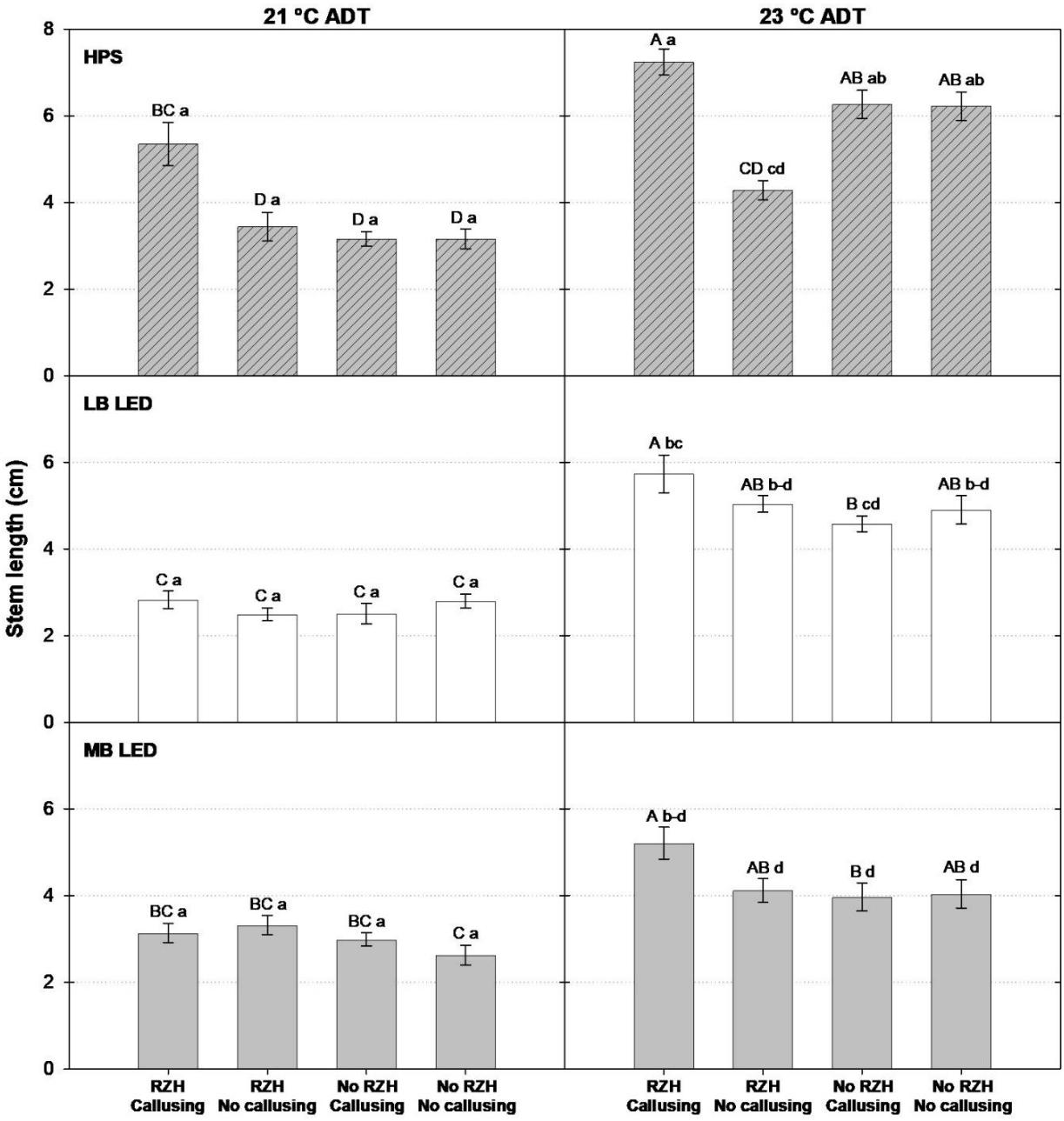

Figure II-1: Average stem length of petunia 'Dark Blue' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) (6:1:91:1 blue:green:red:far-red light), or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

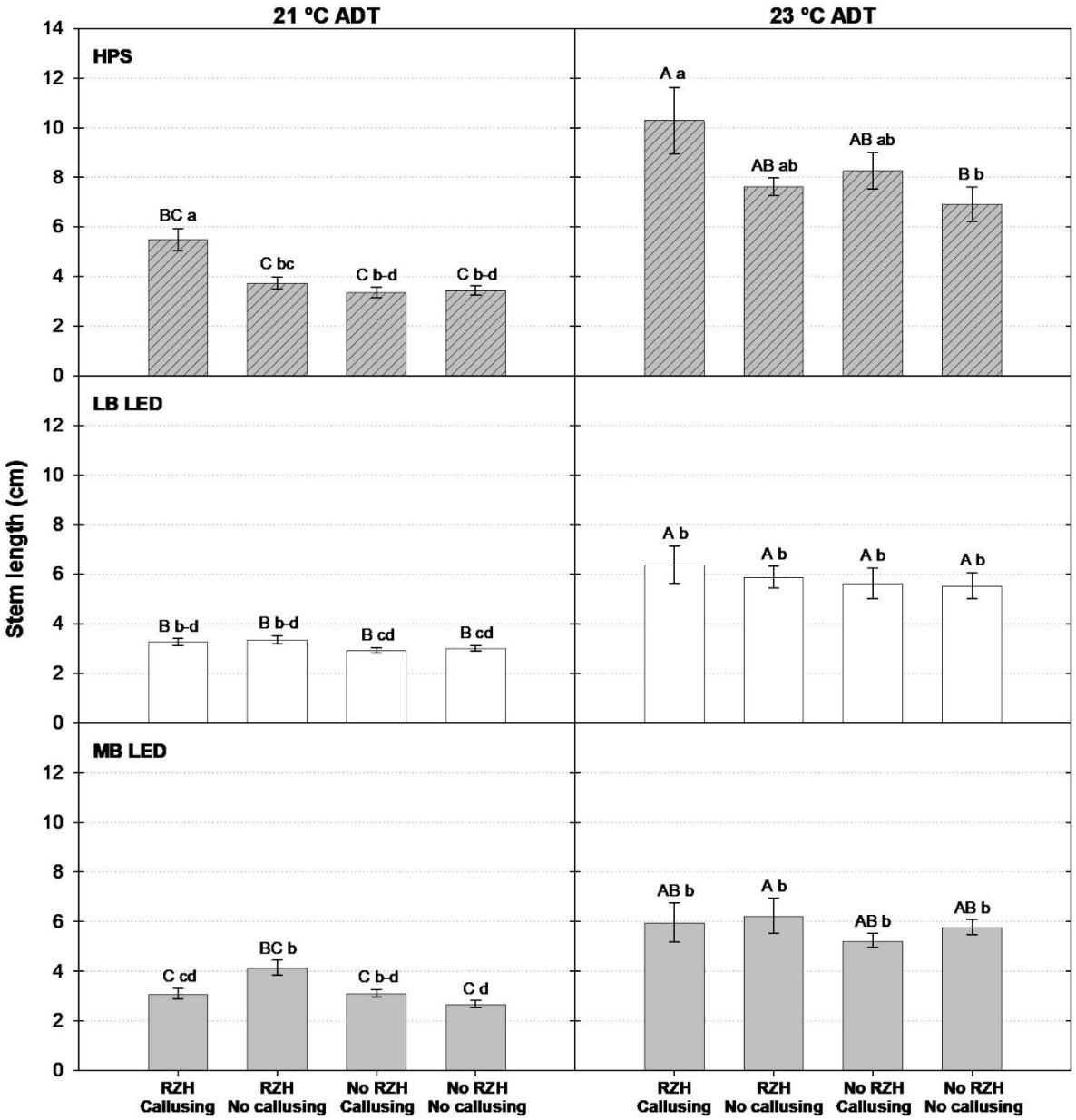

Figure II-2: Average stem length of petunia 'White' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

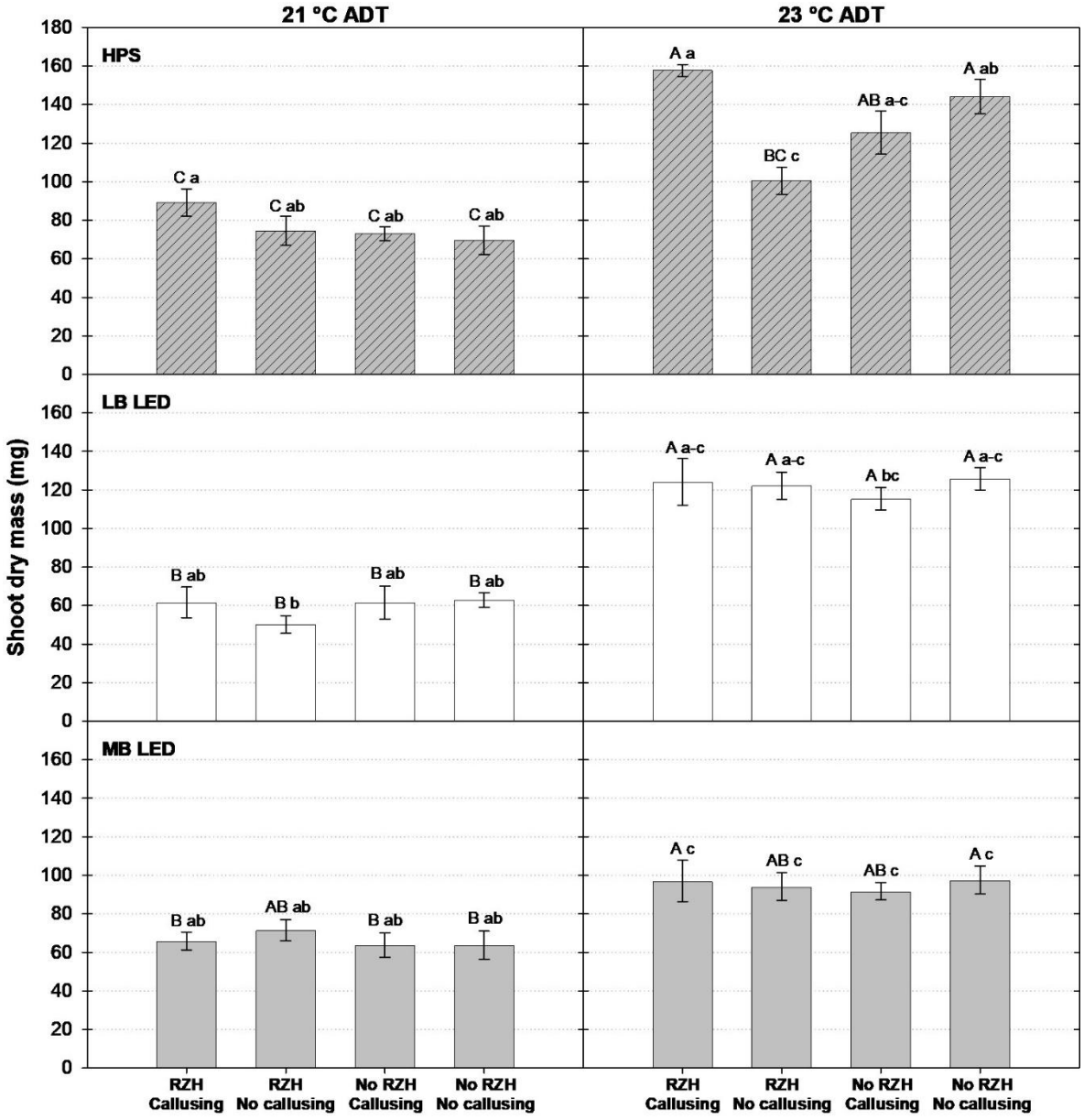

Figure II-3: Average shoot dry mass of petunia 'Dark Blue' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

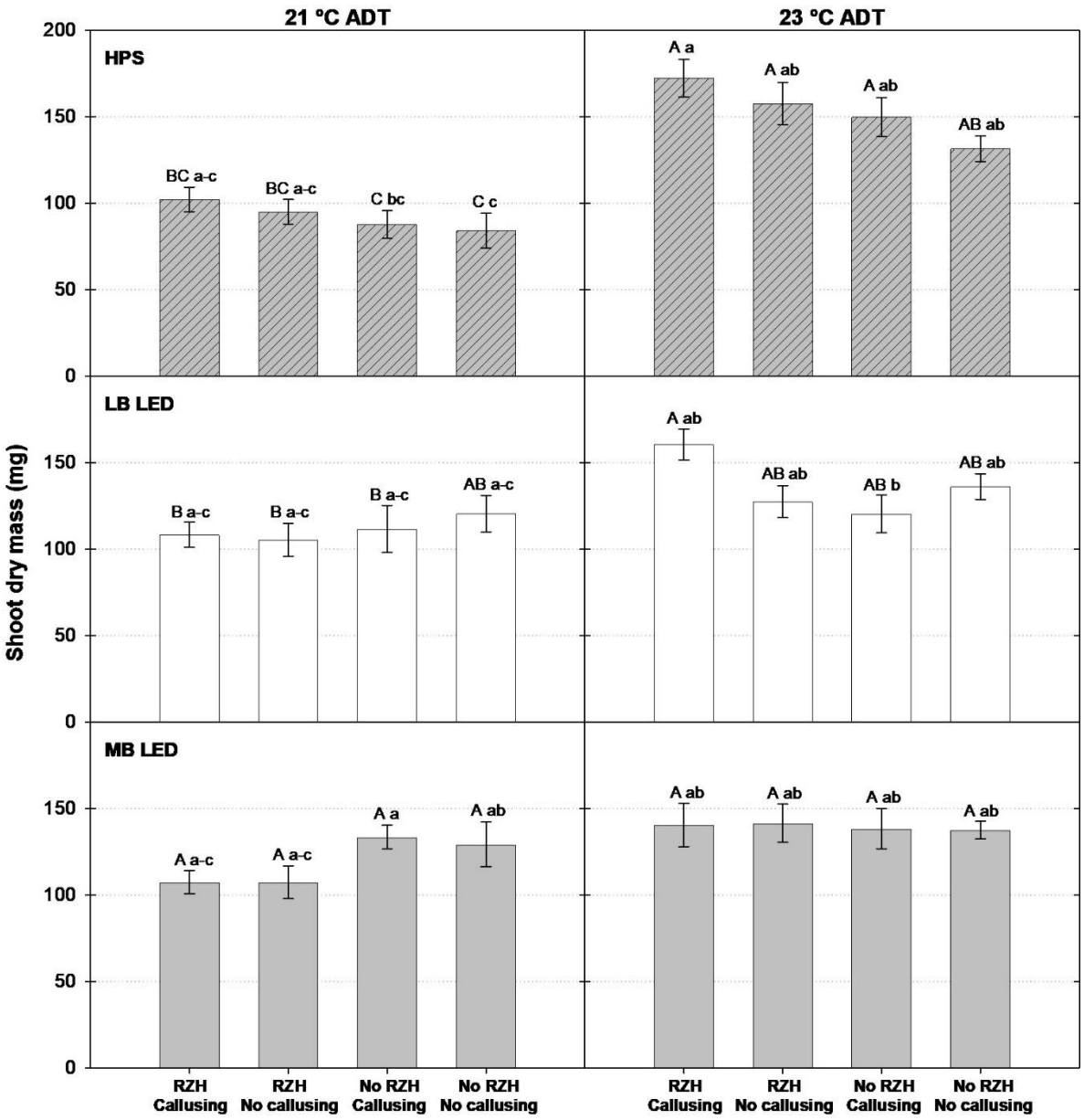

Figure II-4: Average shoot dry mass of petunia 'White' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

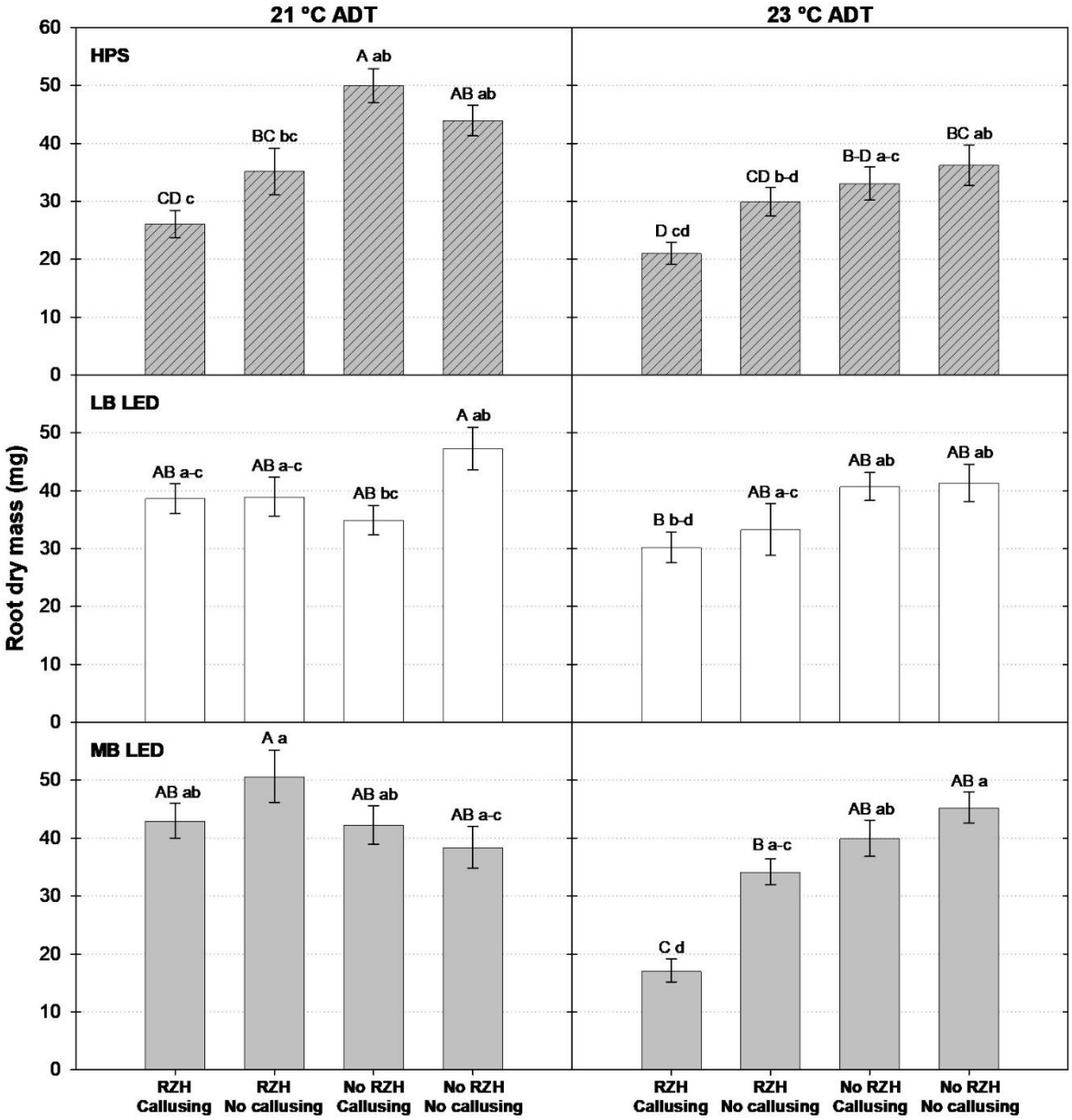

Figure II-5: Average root dry mass of petunia 'Dark Blue' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

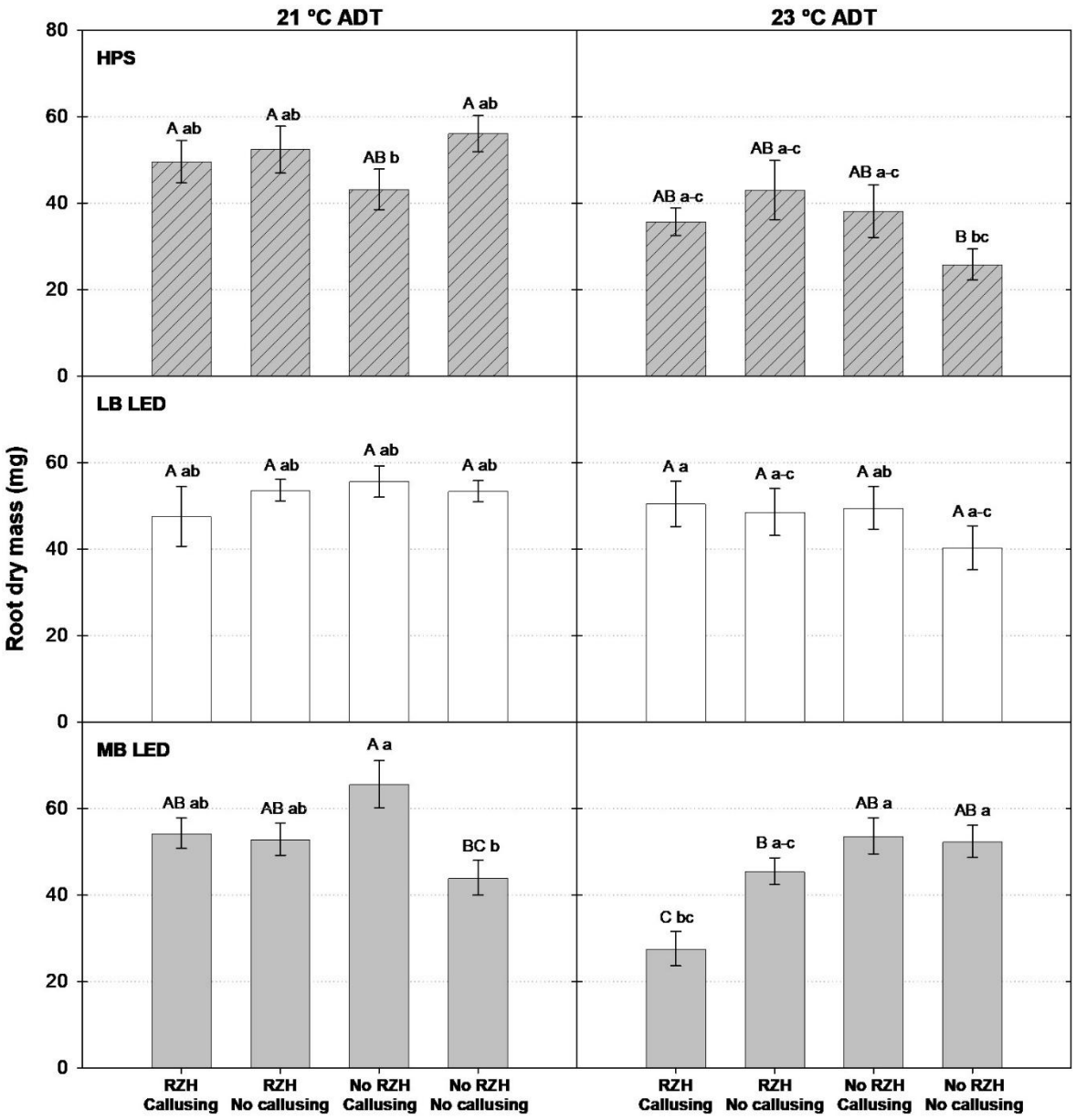

Figure II-6: Average root dry mass of petunia 'White' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

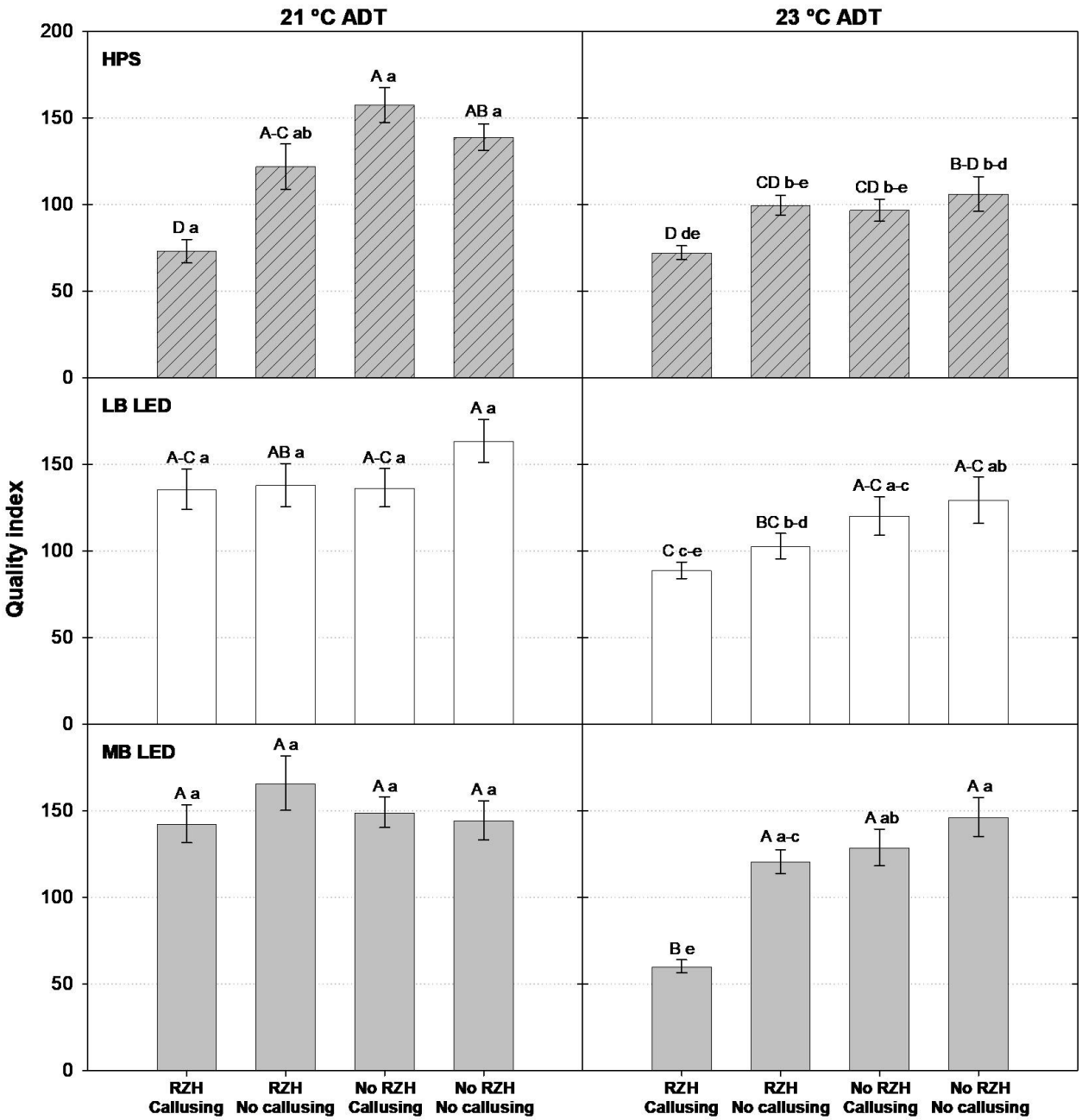

Figure II-7: Average quality index of petunia 'Dark Blue' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

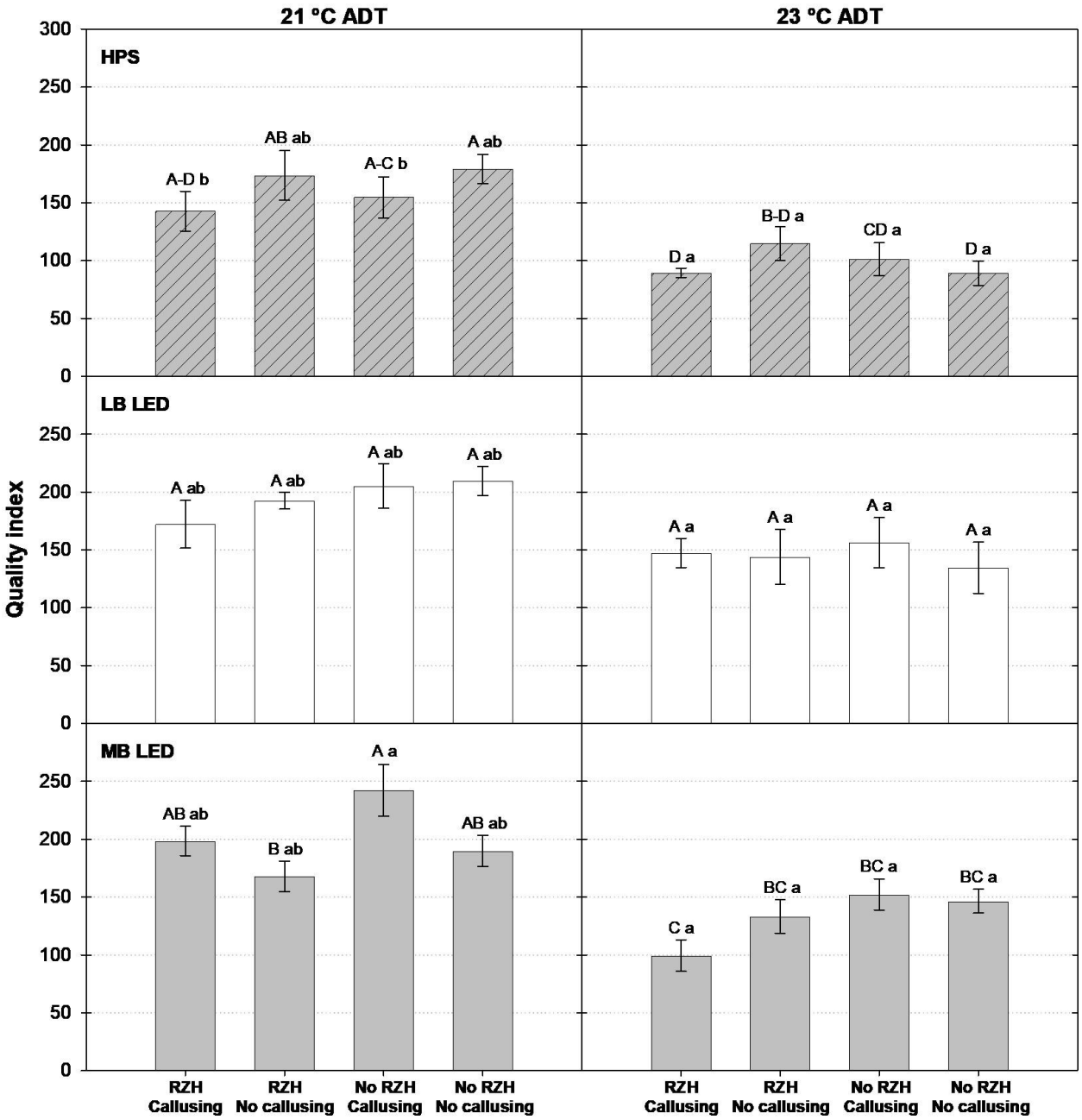

Figure II-8: Average quality index of petunia 'White' at the end of propagation (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a supplemental lighting (SL) treatment while different lowercase letters within columns are significantly different within an average daily temperature (ADT) according to Tukey's honestly significant difference (HSD) test ($P < 0.05$). SL treatments were delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diodes (LEDs) [6:1:91:1 (%) blue:green:red:far-red light], or medium blue (MB) LEDs [10:6:82:2 (%) blue:green:red:far-red light] RZH = root-zone heating. Cal = callusing treatment.

SECTION III

TEMPERATURE MANAGEMENT AND SUPPLEMENTAL LIGHTING STRATEGY EFFECTS ON THE PROPAGATION OF *Petunia ×hybrida* PART 2: FOILAGE COLOR AND ANTHOCYANIN AND NUTRIENT CONTENT

Temperature management and supplemental lighting strategy effects on the propagation of *Petunia* ×*hybrida* part 2: Foliage color and anthocyanin and nutrient content

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Abstract

In high latitudes, commercial greenhouse growers apply supplemental lighting (SL) and heating to compensate for low ambient radiation, air average daily temperatures (ADTs), and root-zone temperatures (RZTs) during peak bedding plant propagation. Growers have traditionally employed high-pressure sodium (HPS) lamps to deliver SL, but are adopting light-emitting diode (LED) fixtures due to their improved energy efficacy. However, propagators have reported changes in crop morphology and coloration when cuttings of some species, including petunia (*Petunia ×hybrida*), are grown under LEDs, suggesting possible nutrient deficiencies. The objective of this study was to quantify how temperature (ADT and RZT), light (SL sources and light intensity during callusing) influences the nutrient content and coloration of petunia. Shoot-tip cuttings of petunia SureShot 'Dark Blue' and 'White' were inserted into 72-cell trays and propagated inside a greenhouse at an air ADT of 21 or 23 °C and with an RZT of 21 or 25 °C. Cuttings were grown under SL delivered by HPS lamps or two types of LED fixtures proving different light qualities (low blue or moderate blue) each at a photosynthetic photon flux density (PPFD) of 60 or 120 μmol·m^{-2}·s^{-1} for the first 6 d, then 120 μmol·m^{-2}·s^{-1} for the remaining 16 d. In general, cuttings of 'Dark Blue' grown under LEDs emitting a moderate amount of blue light had lower phosphorus content than those grown under HPS lamps. Cuttings of both cultivars grown under HPS lamps generally had lower zinc content in their leaves than those grown under either LED fixture. Overall, cuttings of both 'Dark Blue' propagated under LEDs had higher anthocyanin content in their leaves and were more red and blue than those grown under HPS lamps. The coloration and anthocyanin content of 'White' were generally unaffected.

Introduction

The emission spectrum of supplemental lighting (SL) fixtures can influence crop

morphology, foliage color, and quality, especially when SL accounts for a majority of the total daily light integral (DLI) (Randall and Lopez, 2014, 2015; Craver et al., 2019). However, the influence of these differences on crop responses, such as pigmentation, vary across studies, with some crops exhibiting little to no influence of SL spectrum. For example, Randall and Lopez (2015) found no differences in chlorophyll content in vinca 'Titan Red Dark' (*Catharanthus roseus*), impatiens 'Super Elfin XP Blue Pearl' (*Impatiens walleriana*), geranium 'Bullseye Red' (*Pelargonium ×hortorum*), and petunia 'Dreams Midnight' (*Petunia ×hybrida*) when grown under ambient light supplemented by either high-pressure sodium (HPS) lamps or light-emitting diodes (LEDs) emitting the same red:blue (R:B) light ratio 87:13 at 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, other bedding plant seedlings grown under HPS lamps were generally taller than those grown under LEDs. In contrast, Poel and Runkle (2017) found little to no effect on plant height when propagating seedlings of geranium 'Pinto Premium Salmon', petunia 'Single Dreams White' and 'Wave Misty Lilac', snapdragon 'Montego Yellow' (*Antirrhinum majus*), and tomato 'Supersweet' (*Solanum lycopersicum*) under sunlight supplemented by either LEDs emitting B (waveband of 400 to 499 nm), R (waveband of 600 to 699 nm), and/or green (G) (waveband of 500 to 599 nm) light or HPS lamps. Moreover, all crops yielded comparable shoot dry masses (SDM), leaf area, and leaf number across lighting treatments. Additionally, no consistent differences in crop quality or flowering were reported during the finishing stage of these crops.

While the influence of SL on crop responses and quality vary, commercial greenhouse growers employing LEDs at a relatively high photosynthetic photon flux density (PPFD) (e.g., >80 $\mu mol \cdot m^{-2} \cdot s^{-1}$) have reported the development of purple foliage and chlorosis during propagation of cuttings on some crops (Veazie and Whipker, 2024). This purpling has the potential to make young plants unmarketable, depending on its severity. A possible reason for

this undesirable purpling under LEDs, and not under HPS lamps, may be due to the increased proportion of B light emitted by most commercial LED fixtures (Thimijan and Heins, 1982; Bourget, 2008; Veazie and Whipker, 2024). B light induces the synthesis of anthocyanins in various crops, such as soybeans (*Glycine max*), lettuce (*Lactuca sativa*), and petunia (Hu et al., 2021). Anthocyanins are pigments that, when accumulated, lead to the development of red or purple tissue (Alvarez-Suarez et al., 2021). These pigments have free radical scavenging activity that neutralize and/or stabilize reactive oxygen species, and when they accumulate in leaf tissue, can increase plant tolerance to oxidative stress (Gould et al., 2002; Ding et al., 2020). SL emitting higher proportions of B light may lead to the upregulation of the expression of genes involved in anthocyanin synthesis and the subsequent accumulation of these pigments in various tissues (Craver et al., 2020; Jia et al., 2024).

Purple foliage can also develop in cuttings exposed to high-light intensities. During high-light stress, anthocyanins may accumulate to prevent photoinhibition by absorbing B and G light, thereby limiting the amount of light reaching chloroplasts (Neill and Gould, 2003). Throughout the vegetative growth stage, exposure to high-light conditions may yield darker or purple-pigmented tissues. For example, petunia grown under PPDFs between 50 and 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ accumulated significantly less anthocyanins than plants grown at 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which displayed visibly purple tissue (Albert et al., 2009). Furthermore, during the propagation of butterhead lettuce ‘Teodore’, the anthocyanin concentration in seedlings increased by 1782% as light intensity was increased from 60 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Das et al., 2024).

Another potential cause of the foliage purpling is nutrient deficiencies. Specifically, phosphorous (P) deficiency can cause anthocyanin accumulation, leading to the purpling of shoots and particularly lower (more mature) leaves (de Mallo Prado, 2021; Shi et al., 2023).

Stock plants from which cuttings are harvested typically receive low amounts of P to minimize internode length of stems to facilitate shipping (Calkins, 2022). Unrooted cuttings also lack root structures needed to uptake P upon insertion into the rooting substrate (Armstrong, 1999). Fertilizer low in nitrogen (N) may also lead to the development of purple foliage in some species. For example, alternanthera 'Purple Prince' (*Alternanthera brasiliana*) displayed red and purple foliage at a higher intensity, along with higher leaf concentration of anthocyanin, when grown with lower rates of N (Henry, 2017). Additionally, deficiencies in nutrients required for callusing and root formation during propagation can lead to the onset of P deficiency. For example, zinc (Zn) is necessary for the synthesis of auxin, a hormone involved heavily in the development of adventitious roots. Therefore, Zn deficiency may inhibit rooting and contribute to the development of P deficiency (Gibson, 2006; Lambers et al., 2008).

Additionally, low temperature can increase anthocyanin biosynthesis and accumulation in stem and leaf tissues (Lo Piero et al., 2005; Naing et al., 2018). This accumulation allows photochemical reactions to continue at suboptimal temperatures. For example, bittervine (*Mikania micrantha*) grown at 20 °C photosynthesized at higher rates when leaf anthocyanin concentration increased following exposure to a 4 °C chilling treatment (Zhang et al., 2019). In addition, plant temperature under LEDs is typically lower than that of crops grown under HPS lamps. LEDs emit much less radiant heat than HPS lamps, which may emit up to 73% of radiation as thermal radiation. A lower plant temperature could increase oxidative stress and reduce rates of biochemical reactions, such as chlorophyll synthesis, within the crop, which may lead to the accumulation of anthocyanins (Bubenheim et al.,1988; Heins et al., 2000).

Furthermore, low root-zone temperatures (RZT) can also contribute to the accumulation of anthocyanins. Sakamoto and Suzuki (2015) reported that red-leaf lettuce 'Red Wave' grown at

an RZT of 10 °C accumulated significantly more anthocyanins in leaves compared with RZTs of 20, 25, and 30 °C. As callusing, root formation, and the uptake of some nutrients all feature active processes that require energy, a higher RZT provides more energy to drive these processes. If RZT is suboptimal, these processes may be retarded, which can lead to the accumulation of reactive oxygen species, the onset of oxidative stress, and the development of nutrient deficiencies and, thereby, increased foliage purpling (Roussos, 2023).

The goal of this research was to identify the main cause(s) of foliage purpling during propagation of bedding plants grown under LED SL so that this physiological disorder could be mitigated by commercial greenhouse growers. We performed experiments to determine the interactive effects of SL source, intensity, average daily temperature (ADT), and RZT on the coloration and nutrient uptake of petunia during asexual propagation. The specific objectives were to 1) quantify the effects of ADT, RZT, and SL source and intensity on the coloration, anthocyanin content, and nutrient concentration of petunia cuttings; 2) determine cultivar-specific variation in the development of purple pigmentation; and 3) determine if changes in leaf color could be attributed to the accumulation of anthocyanins, nutrient deficiencies in foliar tissue, or both. We postulated that the combination of reduced energy output and lower R:B ratio would lower plant temperature, subsequently decreasing absorption of nutrients (including P) and increase oxidative stress (from increased B light fraction), which would cause the accumulation of anthocyanins as a plant-protective stress response, leading to purpling of foliage.

Materials and methods

Plant materials, greenhouse environmental conditions, and temperature and lighting treatments

Plant materials, greenhouse environmental conditions, and temperature and lighting

treatments were exactly as described in Temperature management and supplemental lighting strategy effects on the propagation of *Petunia ×hybrida* part I: Morphology and quality. SL emission spectra were measured by a spectrometer (LI-180 Spectrometer; LI-COR Biosciences) at plant height without ambient photosynthetically active radiation (at night) before each replication of the experiment to establish consistent lighting treatments between repetitions (Fig. III-1).

Data collection

On cutting arrival, 25 cuttings of each cultivar were shipped and analyzed to quantify baseline anthocyanin content in mg/g (Ball Horticultural Company, West Chicago, IL). Anthocyanin content was determined based on a protocol adapted from Albert et al. (2010) and Liu et al. (2012). A range of 0.2 to 0.4 g of fresh leaf tissue was homogenized in liquid nitrogen prior to being mixed with 10 mL of acidic methanol (1% HCl weight in volume). After, the mixtures were incubated at 40 °C overnight in an unilluminated environment. The following day, samples were centrifuged at 14,000 rpm for 5 minutes. Next, 0.3 mL of supernatant was mixed with 0.3 mL of distilled water. Anthocyanins were then extracted from chlorophylls through the addition of 0.5 mL chloroform. Following a brief centrifugation, a spectrophotometer (NanoDrop One Spectrophotometer; Thermo Fisher Scientific, Waltham, MA) was used to measure the total anthocyanin content in the aqueous phase (wavelength of 530 nm). The amount of anthocyanin per sample was then calculated through use of the molar extinction coefficient of anthocyanin and the average molecular weights of delphinidin and petunidin. Upon cutting arrival, an additional 25 cuttings of each cultivar were sent to a commercial laboratory to quantify baseline macro- and micro-nutrient tissue concentrations for each cultivar (Quality Analytical Laboratories, Panama City, FL). Leaf color (average of 3 leaves per cutting) of 15 cuttings per

cultivar was measured upon cutting arrival by 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 again 12 days after sticking on 3 leaves of each of 5 cuttings per cultivar and treatment. Baseline chlorophyll fluorescence (CF) was also measured on the day of cutting arrival using a fluorimeter (Handy PEA; Hansatech Instrument Ltd., Norfolk UK) to quantify plant stress. Leaves were dark-adapted for 15 min before CF was measured on 1 leaf of each of 10 cuttings per cultivar. CF was repeatedly measured (5 cuttings per cultivar and treatment) on days 2, 4, 6, 8, and 10 after sticking.

On day 22 of propagation, the leaf tissue of 30 cuttings per cultivar and treatment was harvested and dried in a drying oven at 70 °C for 3 d. After drying, samples were sent to a commercial lab to quantify macro- and micro-nutrient concentrations (Quality Analytical Laboratories). On the same day, seven whole cuttings per cultivar per treatment were harvested and sent to Ball Helix for anthocyanin content analysis following the aforementioned protocol (Ball Horticultural Company, West Chicago, IL).

Experimental design and statistical analysis

The experiment was conducted under a complete block design and data were analyzed using SAS (version 9.2; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance (ANOVA). Means were separated by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Data across replications was combined.

Results

Macronutrient content

The percent dry mass of N, P, potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) of 'Dark Blue' were all affected by the interactions and/or main treatment effects of SL,

ADT, RZT, and callusing treatment (Table III-1). Generally, the percentage of N, K, Ca, and Mg of 'Dark Blue' grown under HPS lamps were greater than or equal to those cuttings propagated under LB or MB LEDs (Fig. III-2). Additionally, the percentages of P in 'Dark Blue' cuttings grown under MB LEDs were typically lower than or equal to those grown under HPS lamps or LB LEDs (Fig. III-3). The percentages of N, P, K, Ca, and Mg of 'Dark Blue' propagated at a warmer air ADT and RZT were commonly greater than or equal to those grown at the cooler air ADT and RZT. Additionally, the percentages of N, P, and K of 'Dark Blue' grown at an ADT of 23 °C with a 6 d 50% callusing treatment were 41%, 41%, and 46% greater than those grown at an ADT of 21 °C without a callusing treatment.

For 'White', there were varied main treatment effects on the macronutrient content along with various treatment interactions (Table III-1). The percentages of N, K, Ca, and S in 'White' grown under HPS lamps were generally greater or equal to those grown under LB or MB LEDs (Fig. III-4). The percentages of N, P, K, Ca, Mg, and S of 'White' grown with a RZT of 25 °C were typically greater than or equal to those grown with a RZT of 21 °C (Fig. III-5). The percentages of N, P, K, Ca, and Mg of 'White' grown at an ADT of 23 °C were generally greater than or equal to those grown at an ADT of 21 °C. The percentages of N, P, K, Ca, and S of 'White' grown with a 6 d 50% shade callusing treatment were generally greater than or equal to those grown without a callusing treatment. Furthermore, the N, K, Ca, and S values of 'White' grown at an ADT of 23 °C with an RZT of 25 °C were 127%, 138%, 60%, and 28% higher than those grown at an ADT of 21 °C with an RZT of 21 °C. The P and Mg content of 'White' were unaffected by SL and the S content was not influenced by ADT.

Additional nutrient content

The influence of SL, RZT, ADT, along with their interactions, varied on the

micronutrient content of ‘Dark Blue’, expressed as the ppm of dry mass, of iron (Fe), boron (B), copper (Cu), zinc, (Zn), molybdenum (Mo), sodium (Na), and aluminum (Al) (Table III-2). The ppm of Fe and Zn in leaves of ‘Dark Blue’ cuttings grown under either LB or MB LEDs were typically greater than or equal to that of those grown under HPS lamps, while the ppm of B of cuttings grown under HPS lamps was usually greater than or equal to those grown under either LED (Fig. III-6). The ppm of B and Cu of leaves of ‘Dark Blue grown at an ADT of 21 °C were generally greater than or equal to that of those grown at an ADT of 23 °C. Additionally, the ppm of B of ‘Dark Blue’ grown at an ADT of 21 °C with a RZT of 25 °C was 17% greater than in those grown at an ADT of 23 °C at a RZT of 21 °C. The content of all measured micronutrients in ‘Dark Blue’ were generally unaffected by the 6 d 50% shade callusing treatment. The content of manganese (Mn) was not affected by any treatment.

The influence of SL, RZH, ADT, and their interactions, varied across the micronutrient content (Fe, B, Cu, Zn, Mo, Na, and Al) of ‘White’ (Table III-2). The ppm of Fe and Zn of ‘White’ grown under LB LEDs was generally greater than or equal to those grown under HPS lamps and MB LEDs (data not presented). The ppm of B and Cu of cuttings of ‘White’ grown under HPS lamps were typically greater than or equal to those grown under MB LEDs. The ppm of Fe, B, and Cu of cuttings of ‘White’ grown with a RZT of 25 °C were generally greater than or equal to those grown with a RZT of 21 °C. The ppm of B of ‘White’ grown at an ADT of 21 °C was generally greater than or equal to those grown at an ADT of 23 °C. The ppm of Zn, Mo, and Al of ‘White’ grown at an ADT of 23 °C were generally greater than those grown at an ADT of 21 °C. Furthermore, the ppm of B and Cu of ‘White’ grown at an ADT of 21 °C with a RZT of 25 °C were 69% and 29% greater than in those grown at an ADT of 23 °C with a RZT of 21 °C, respectively. In addition, the ppm of Zn in ‘White’ grown under LB LEDs at an ADT of 23

°C was 144% and 149% greater than that of those grown under HPS lamps or MB LEDs at an ADT of 21 °C.

Anthocyanin content

There were main effects of SL, RZT, and ADT on the anthocyanin content of 'Dark Blue' leaves as well as an interactive effect of RZT×ADT (Table III-3). The anthocyanin content of 'Dark Blue' grown under LB or MB LEDs was generally greater than or equal to those grown under HPS lamps (Fig. III-7). For example, when grown at an ADT of 21 °C with a RZT of 25 °C and a 6 d 50% shade callusing treatment, the anthocyanin content of 'Dark Blue' cuttings grown under LB and MB LEDs was 192% and 279% greater than that of those grown under HPS lamps, respectively. The anthocyanin content of 'Dark Blue' grown at an ADT of 21 °C or with a RZT of 21 °C was generally greater than or equal to that of those grown at an ADT of 23 °C or with a RZT of 25 °C. In addition, the anthocyanin content of 'Dark Blue' grown at an ADT of 21 °C with a RZT of 21 °C was 233% and 303% greater than those grown at an ADT of 23 °C with a RZT of 25 °C or 21 °C, respectively.

Coloration

The green to red color spectrum (Chromatic a*) of Dark Blue' foliage was individually influenced by SL, RZH, ADT, and callusing treatment, as well as interactively by various treatment combinations (Table III-3). The a* of 'Dark Blue' propagated under MB LEDs was generally more negative (more green in color) than or equal to those grown under LB LEDs or HPS lamps (Fig. III-8). The a* of 'Dark Blue' propagated at an ADT of 23 °C or RZT of 21 °C was typically more negative than or equal to those grown at an ADT of 21 °C or RZT of 25 °C. The a* of 'Dark Blue' cuttings grown under a 50% shade callusing treatment for 6 d was generally more negative than or equal to that of those grown without the callusing treatment.

Additionally, the a* of cuttings grown under HPS lamps at an ADT of 23 °C was 40 or 62% more negative (more green and less red) than those grown under either MB or LB LEDs, respectively, at an ADT of 21 °C. The a* of foliage of 'White' was generally unaffected by main treatments or their interactions (Table III-3; Fig. III-9).

The blue to yellow color spectrum (Chromatic b*) of 'Dark Blue' foliage was mainly influenced by placement under 50% shade for 6 d of callusing, while that of 'White' was influenced by SL, RZH, ADT, and a callusing treatment, as well as some interactions (Table III-3). The b* of 'Dark Blue' grown under 50% shade was generally greater (more yellow in color) than or equal to those cuttings not under shade, while the opposite trend was observed in 'White' (Figs. III-10 and III-11). The b* of 'White' propagated under LB LEDs was generally greater than or equal to that of those grown under MB LEDs or HPS lamps while the b* of 'White' grown under MB LEDs was typically greater than or equal to that of those grown under HPS lamps. The average b* of 'White' grown across treatments with either an ADT of 21 °C or an RZT of 21 °C was generally greater than that of those grown at an ADT of 23 °C or an RZT of 25 °C. Furthermore, the b* of 'White' propagated at an ADT of 21 °C and with an RZT of 21 °C was 26%, 26%, and 28% greater than those grown at an ADT of 23 °C with an RZT of 21 or 25 °C and those grown at an ADT of 21 °C with an RZT of 25 °C, respectively.

There were main effects of ADT and callusing treatment on the foliage lightness (L*) of 'Dark Blue', while that of 'White' was individually influenced by SL and RZT (Table 5). Across all treatments, the L* of 'Dark Blue' propagated at an ADT of 23 °C was on average 2% greater (more white and less black) than those grown at an ADT of 21 °C (data not presented). Additionally, the L* of 'Dark Blue' grown under a 50% shade callusing treatment was, across treatments, 2% greater than those grown without a callusing treatment. The L* of 'White' grown

under MB and LB LEDs was 4% and 3% greater, respectively, than those grown under HPS lamps. Moreover, the L* of 'White' grown with a RZT of 25 °C was, across treatments, 3% greater than that of those grown with an RZT of 21 °C. The L* of 'Dark Blue' was unaffected by SL and RZT while that of 'White' was unaffected by ADT or callusing treatment.

Discussion

Spectral differences in lighting fixtures have been shown to induce differences in nutrient uptake and macro and micronutrient content of a variety of species (Samuolienė et al., 2019). For example, Boldt and Altland (2022) grew basil 'Genovese Emily' (*Ocimum basilicum*), pepper 'California Wonder' (*Capsicum annum*), vinca 'Cora Burgundy' (*Catharanthus roseus*), zinnia 'Zahara Cherry' (*Zinnia marlyandica*), geranium 'Maverick Red' (*Pelargonium ×hortorum*), pansy 'Delta Premium Blue blotch' (*Viola ×wittrockiana*), spinach 'Whale' (*Spinacea oleracea*), and tomato 'Early Girl' (*Solanum lycopersicum*) under SL provided by HPS lamps emitting a B:R:FR light ratio (%) of 6:41:10 and LEDs emitting a B:R:FR ratio of 51:50:22 and a PFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. While some crops accumulated higher amounts of specific nutrients under LEDs, such as zinnia accumulating higher P levels, generally, plants grown under LEDs accumulated lower nutrient concentrations than those grown under HPS lamps. Craver et al. (2019) reported that the concentration of N, P, Ca, Mg, Fe, and Mn in petunia 'Single Dreams White' grown under HPS lamps was greater than that of those grown under LEDs emitting a R:B ratio of 9:1. Similarly, in the present study, the N, K, Ca, Mg, and B content of 'Dark Blue', as well as the N, K, Ca, S, B, and Cu content of 'White', were generally greater in cuttings grown under HPS lamps than in those grown under LEDs (Figs. III-2 and III-4). This may be attributed to the increased plant temperature observed in cuttings grown under HPS lamps that could have led to a higher rate of stomatal opening and transpiration and, from that, nutrient uptake and

accumulation (Lambers et al., 2008; Blanchard et al., 2006; Urban et al., 2017). Further work must be conducted to determine the validity of this hypothesis.

Furthermore, Kopsell and Sams (2013) reported that the P, K, Mg, Ca, S, B, Cu, Fe, Mn, Mo, Na, and Zn content of broccoli (*Brassica oleacea* var. *italica*) microgreens, was greater in seedlings grown under sole-source lighting from LEDs emitting 41 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light (peak wavelength 470 nm) when compared to those grown under 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of combined R and B at a R:B ratio of 88:12 increased. The authors attributed the increase in nutrient content to the increased stomatal opening and flux of ions into and out of guard cell membranes attributed to B light exposure (Sharkey and Raschke, 1981; Babourina et al., 2002; Shimazaki et al., 2007). In the present study, the Fe and Zn content of both 'Dark Blue' and 'White' grown under LEDs was generally greater than those grown under HPS lamps (Fig. III-6). This may be due to the larger percentage of B light, which could have thereby led to increased stomatal opening and membrane transport of various nutrients, emitted by the LEDs used when compared to HPS lamps (Fig. III-1) (Bourget, 2008). Future investigations on the influence of SL spectrum on stomatal opening and mass flow of nutrients is required to confirm this hypothesis.

Little work has been done to determine how SL light intensity influences the nutrient content of bedding plants. In the present study, the N, K, and S content of both cultivars, as well as the Ca content of 'White', grown with a 6 d 50% shade callusing treatment was generally greater than or equal to that of those without the treatment (Figs. III-2 and III-4). While light measurements were not taken underneath the shade of the callusing treatment, it may be assumed that the light level was lower underneath the shade. From this, it is possible that the lower light intensity, which, during asexual propagation, is associated with reduced light stress and water loss and increased stomatal opening, resulted in an increase in nutrient accumulation (Hartmann

et al., 2011; Lopez et al., 2017). Nonetheless, additional work must be conducted to validate this hypothesis.

The temperature of the root-zone influences the uptake macronutrients, with different nutrients having different optimal temperatures across crops at which point their content within a leaf is maximized. For example Tindall et al. (1990) grew seedlings of tomato 'Burpee's Big Boy Hybrid' at an ADT of 21.1 °C and RZTs of 10, 15.6, 21.1, 26.7, 32.2, and 37.8 °C. They observed that the uptake of N, P, K, Ca, and Mg, as well as the rate of stem elongation and plant height, peaked around a RZT of 25 °C, with increased temperatures reducing uptake and nutrient content levels. Similarly, in the present study, the concentration of N, P, K, Ca, and Mg of both petunia 'Dark Blue' and 'White' was generally greater at a RZT of 25 °C than 21 °C (Figs. III-2, III-3, III-4, and III-5). While previous research has suggested that the optimum temperature for vegetative processes, such as stem elongation or biomass accumulation, of petunia is near 25 °C, little work has been done to determine the temperature at which macronutrient uptake and/or content peaks (Merritt and Kohl, 1982; Olberg and Lopez, 2016). While further work to determine the species specific RZT at which macronutrient uptake and content peaks in petunia should be conducted, our results suggest that it is at least 25 °C.

Similarly to macronutrient content, micronutrient content may also be affected directly by RZT. In the previously described study, Tindall et al. (1990) observed that the uptake of Cu, Mn, and Zn peaked at a RZT of 25 °C while the uptake and content of B, Fe, and Mo was unaffected by temperature. In the present study, the content of Cu of petunia 'White' was higher at a RZT of 25 °C than at 21 °C and the content of Mo did not vary across temperature treatments (data not presented). Contrastingly, the content of Mn and Zn in both 'Dark Blue' or 'White' was generally unaffected by RZT. Furthermore, the content of Fe and B in 'White' was influenced by

temperature, with greater content occurring at a RZT of 25 °C. This suggests that temperature optimums for micronutrient uptake and content may be species and even cultivar dependent.

Likewise to RZT, ADT also influences the uptake and foliar content of both macro and micronutrients. For example, Lahav and Turner (1984) observed that the Fe content of leaves in Williams banana 'Giant Cavendish' (*Musa acuminata*) suckers increased as day/night temperatures were raised from 17/10°C to 37/30°C. Generally, when nutrient supply is not limited, ADTs resulting in greater biomass accumulation will lead to higher uptake and accumulation of nutrients (Turner and Lahav, 1995). Similarly, in the present study where higher shoot dry masses were accumulated at an ADT of 23 °C for both petunia 'Dark Blue' and 'White', the dry mass percentage of N, P, K, Ca, and Mg were greater at a higher ADT (Figs. III-2–III-5). Additionally, the ppm of Zn and Al in 'White' were higher under a higher ADT (data not presented). At higher ADTs, plant temperature increases along with air temperature. This leads to higher rates of physiological processes and biochemical reactions, such as photosynthesis and transpiration, within the plant (Heins et al., 2000). From that, it is understandable that the accumulation of various macro and micronutrients would increase at higher ADT. However, the ppm of B in both 'Dark Blue' and 'White' was notably higher in plants grown at an ADT of 21 °C rather than 23 °C. While B uptake and accumulation has been observed to increase at higher temperatures, as B primarily enters the plant through mass flow which increases at higher rates of transpiration, it has also been noted to decrease as relative humidity increases (Bowen, 1972; Bulut, 2019). In the present study, as the VPD was maintained at 0.3 kPa across all treatments, it is possible that cuttings grown under an ADT of 23 °C were under a higher relative humidity, thereby stunting the rate of B uptake and accumulation.

Generally, it is recommended that the percentage of P obtained from a leaf tissue analysis

of petunia should be within 0.47 to 0.93% to avoid deficiency or toxicity symptoms (Bryson and Mills, 2014). However, across all treatments and both cultivars, no sampled tissues ever reached this threshold, with all falling at or below 0.44% (Figs. III-3 and III-5). This being said, the incidence rate and severity levels of purpling varied drastically across treatments and between cultivars, with the purpling of some cuttings of the same cultivar being more extreme than those with a greater percentage of P in their leaves. Additionally, while P deficiency in petunia typically manifests as the development of dark green or purple foliage in older leaves, the purpling observed in this study occurred on both old and new leaves. From this, while it is possible that the development of purple foliage was exacerbated by P deficiency, it is unlikely that it is solely due to a lack of P uptake. From this, further research to determine if lower rates of P nutrition can induce, or if higher rates can prevent, purpling should be conducted.

The influence of light quality on anthocyanin content and leaf coloration has been documented in a variety of species. Notably, lights emitting a higher amount of high energy light, such as UV-B or B light, may increase the synthesis and accumulation of anthocyanins in foliage, resulting in the development of purple leaves (Alvarez-Suarez et al., 2021; Hu et al., 2021). For example, red coloration in lettuce has been reported to increase as the fraction of B light being emitted onto them increases (Meng and Runkle, 2023). Additionally, increased levels of B light have been shown to increase anthocyanin accumulation in petunia grown under LEDs (Fu et al., 2020). In the present study, the anthocyanin content of ‘Dark Blue’ was greatest in plants grown under either LB or MB LEDs (Fig. III-7). This is likely due to the increased B light fraction being emitted by the LEDs used when compared to HPS lamps, resulting in an increased synthesis and thereby accumulation of anthocyanins in the leaves. The tendency for cuttings of both cultivars propagated under LEDs to be more red (i.e., higher a*) or more blue (i.e., lower

b*) is likely due to this accumulation of anthocyanins. Light intensity has been shown to influence coloration of petunia, with plants grown at higher light intensities becoming more red (Albert et al., 2009). Results of the present study support this as cuttings of ‘Dark Blue’ grown without a 6 d 50% shade treatment, which likely were exposed to higher light intensities over that period, were generally as red or redder than those grown with the treatment (Fig. III-8).

Low ADT and RZT have been shown to result in the accumulation of anthocyanins in tissues of various crops (Tokuhisa et al., 1997; Sakamoto and Suzuki, 2015). In the present study, the anthocyanin content of ‘Dark Blue’ was generally higher under an ADT of 21 °C or a RZT of 21 °C, with an ADT of 23 °C or a RZT of 25 °C resulting in a lower anthocyanin content (Table III-3; Fig. III-7). As the leaves of ‘Dark Blue’ grown at lower temperatures were often as red or redder than those grown at warmer temperatures, this is likely attributed to the increase in anthocyanin content (Fig. III-8). The lower plant temperatures experienced by cuttings grown under LEDs, when compared to those grown under HPS lamps, may also have led to an increase in anthocyanin content.

To conclude, cuttings of ‘Dark Blue’ were generally more red and blue than those of ‘White’. Differences in nutritional content across treatment likely occurred due to differences in transpiration rate and stomatal opening, as well as ion transport, caused by variation in SL spectra and temperature, yet further research is required to confirm these hypotheses. However, as cuttings of both cultivars displayed purpling of various severity and at different incidence rates, irrespective as to if nutrient content was in recommended ranges, it is unlikely that the reported purpling during asexual propagation under LEDs is due to a nutrient deficiency (Santos et al., 2011; Bryson and Mills, 2014). Furthermore, the purpling observed is uncharacteristic of common nutrient deficiencies as it occurs in tissue regardless of age. Suboptimal temperatures

lead to greater accumulation of anthocyanins which could lead to an increase in foliage purpling. This purpling can be exacerbated further when cuttings are propagated under LEDs, or SL, emitting a relatively moderate fraction of B light. From this, growers interested in utilizing LED SL may expect to have to raise ADT and/or RZT to maintain a desirable crop coloration.

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APPENDIX

Table III-1. Analyses of variance for the effects of supplemental lighting (SL), root-zone heating (RZH), air average daily temperature (ADT), a callusing treatment (CAL), and their interactions on the mean macronutrient content (% fresh mass) of petunia 'Dark Blue' and 'White'.

	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sulfur (%)
			'Dark Blue'			
SL	***[z]	*	***	***	*	**
RZH	***	***	***	***	***	NS
ADT	***	***	***	**	**	***
Cal	***	**	***	**	*	*
SL×RZH	NS	NS	***	NS	NS	***
SL×ADT	NS	NS	*	*	NS	*
SL×Cal	NS	NS	NS	NS	NS	NS
RZH×ADT	NS	NS	***	***	NS	***
RZH×Cal	NS	NS	***	NS	NS	**
ADT×Cal	***	*	**	NS	NS	NS
SL×RZH×ADT	NS	NS	NS	*	NS	NS
SL×RZH×Cal	NS	NS	NS	NS	NS	NS
SL×ADT×Cal	NS	NS	NS	NS	NS	*
RZH×ADT×Cal	NS	NS	NS	NS	NS	NS
SL×RZH×ADT×Cal	NS	NS	**	NS	NS	**
			'White'			
SL	***	NS	***	***	NS	*
RZH	***	***	***	***	**	***
ADT	***	***	***	***	***	NS
Cal	***	***	***	**	*	***
SL×RZH	NS	NS	NS	NS	NS	*
SL×ADT	NS	NS	NS	***	NS	**
SL×Cal	NS	NS	NS	NS	NS	NS
RZH×ADT	***	NS	***	***	NS	***
RZH×Cal	**	NS	*	NS	NS	NS
ADT×Cal	NS	NS	NS	NS	NS	NS
SL×RZH×ADT	NS	NS	NS	*	NS	NS
SL×RZH×Cal	NS	NS	NS	NS	NS	NS
SL×ADT×Cal	NS	NS	NS	NS	NS	NS
RZH×ADT×Cal	NS	NS	NS	NS	NS	NS
SL×RZH×ADT×Cal	NS	NS	NS	NS	NS	NS

[z] NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

Table III-2. Analyses of variance for the effects of supplemental lighting (SL), root-zone heating (RZH), air average daily temperature (ADT), a callusing treatment, and their interactions on the mean micronutrient, sodium, and aluminum content (ppm fresh mass) of petunia 'Dark Blue' and 'White'.

	Iron (ppm)	Manganese (ppm)	Boron (ppm)	Copper (ppm)	Zinc (ppm)	Molybdenum (ppm)	Sodium (ppm)	Aluminum (ppm)
				'Dark Blue'				
SL	*[z]	NS	***	NS	***	NS	NS	**
RZH	NS	NS	*	NS	NS	***	***	*
ADT	NS	NS	***	***	NS	NS	NS	NS
Cal	NS	NS	NS	*	NS	NS	NS	NS
SL×RZH	NS	NS	***	NS	NS	NS	*	NS
SL×ADT	NS	NS	NS	NS	NS	*	*	NS
SL×Cal	NS	NS	NS	NS	NS	NS	NS	NS
RZH×ADT	**	NS	***	NS	NS	NS	*	NS
RZH×Cal	NS	NS	*	NS	NS	*	NS	NS
ADT×Cal	NS	NS	NS	NS	NS	NS	NS	NS
SL×RZH×ADT	**	NS	***	**	*	NS	*	NS
SL×RZH×Cal	NS	NS	NS	NS	NS	NS	NS	NS
SL×ADT×Cal	NS	NS	NS	NS	NS	*	NS	NS
RZH×ADT×Cal	NS	NS	*	NS	NS	NS	NS	NS
SL×RZH×ADT×Cal	NS	NS	NS	NS	NS	NS	NS	NS
				'White'				
SL	***	NS	**	**	***	NS	NS	NS
RZH	***	*	***	***	NS	NS	**	NS
ADT	NS	*	***	**	***	***	NS	***
Cal	NS	NS	NS	**	***	*	*	NS
SL×RZH	NS	NS	NS	NS	NS	NS	NS	NS
SL×ADT	*	NS	*	NS	***	NS	NS	NS
SL×Cal	NS	NS	NS	NS	**	NS	NS	NS
RZH×ADT	***	NS	***	**	NS	NS	NS	NS
RZH×Cal	NS	NS	NS	NS	NS	NS	NS	NS
ADT×Cal	NS	NS	NS	NS	***	NS	NS	NS
SL×RZH×ADT	NS	NS	NS	NS	NS	NS	NS	NS
SL×RZH×Cal	*	NS	NS	NS	NS	NS	NS	NS
SL×ADT×Cal	*	NS	NS	NS	**	NS	NS	NS
RZH×ADT×Cal	*	NS	NS	NS	NS	NS	NS	NS
SL×RZH×ADT×Cal	NS	NS	NS	NS	NS	NS	NS	NS

[z] NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

Table III-3. Analyses of variance for the effects of supplemental lighting (SL), root-zone heating (RZH), air average daily temperature (ADT), a callusing treatment, and their interactions on the foliage anthocyanin content (g/g), redness and greenness (Chromametric a*),blueness and yellowness (Chromametric b*), and lightness (L*) values of petunia 'Dark Blue' and 'White'. Cal = callusing treatment.

	Anthocyanin content (g/g)	a*	b*	L*
	'Dark Blue'			
SL	***	***	NS	NS
RZH	***	***	NS	NS
ADT	***	***	NS	*
Cal	NS	***	***	*
SL×RZH	NS	**	NS	NS
SL×ADT	NS	***	NS	NS
SL×Cal	NS	NS	NS	NS
RZH×ADT	***	***	NS	NS
RZH×Cal	NS	NS	NS	NS
ADT×Cal	NS	**	NS	NS
SL×RZH×ADT	NS	NS	NS	NS
SL×RZH×Cal	NS	*	NS	NS
SL×ADT×Cal	NS	*	NS	NS
RZH×ADT×Cal	NS	NS	NS	NS
SL×RZH×ADT×Cal	NS	NS	NS	NS
	'White'			
SL	—[y]	**	***	**
RZH	—	NS	***	**
ADT	—	NS	***	NS
Cal	—	NS	***	NS
SL×RZH	—	NS	NS	NS
SL×ADT	—	**	NS	NS
SL×Cal	—	NS	NS	NS
RZH×ADT	—	*	***	NS
RZH×Cal	—	NS	NS	NS
ADT×Cal	—	NS	**	NS
SL×RZH×ADT	—	NS	NS	NS
SL×RZH×Cal	—	**	NS	NS
SL×ADT×Cal	—	NS	NS	NS
RZH×ADT×Cal	—	NS	NS	NS
SL×RZH×ADT×Cal	—	NS	NS	NS

[z] NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

[y] —, data not taken

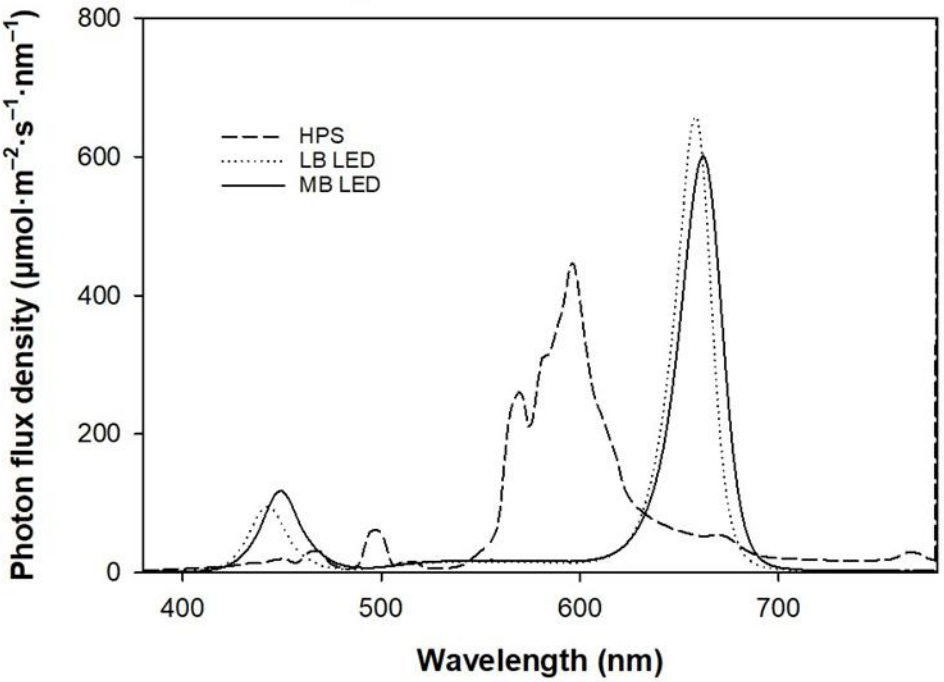

Figure III-1. Spectral distributions of the supplemental lighting treatments delivered by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light.

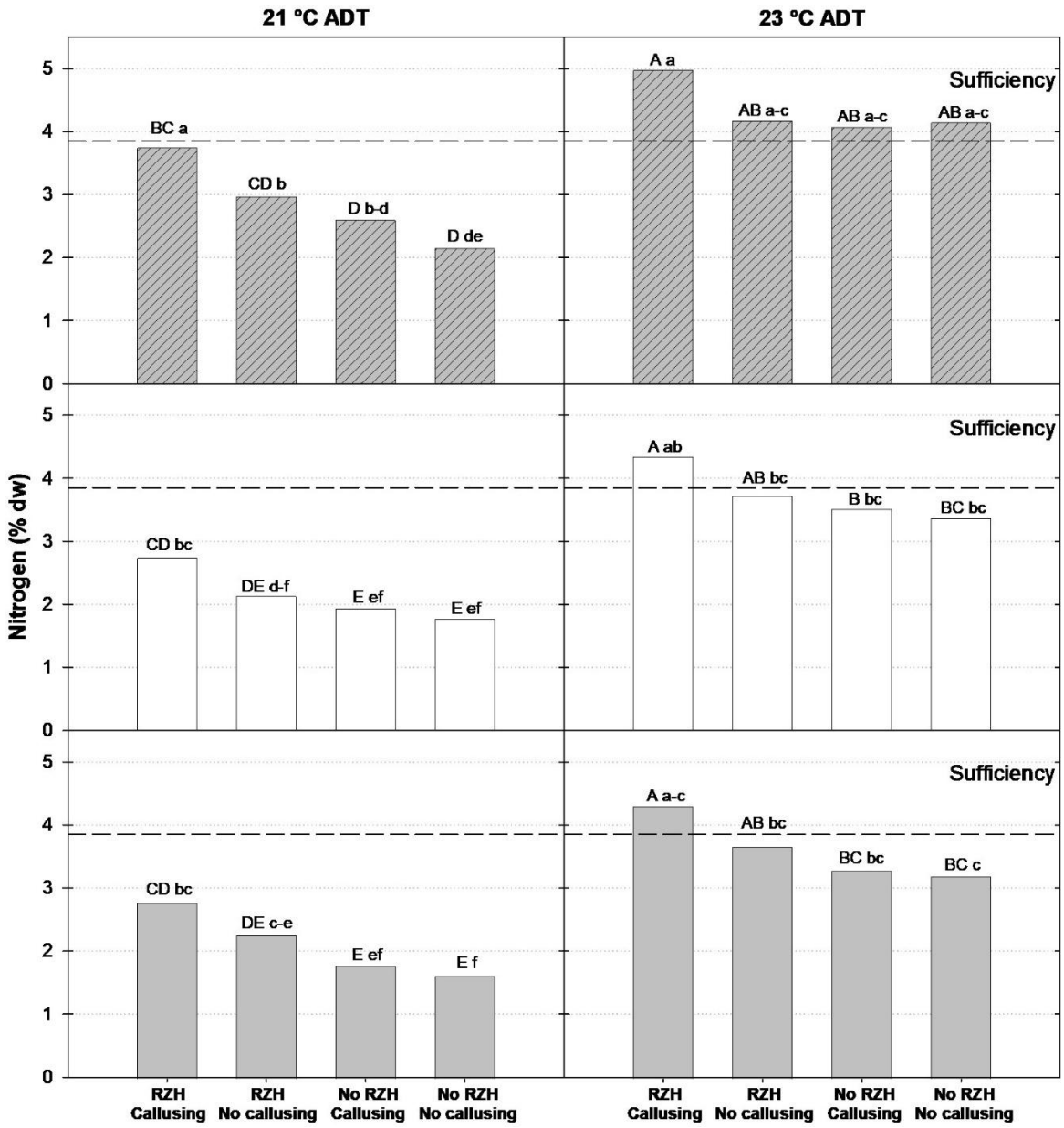

Figure III-2: Average nitrogen content (% dry weight) of petunia 'Dark Blue' cuttings rooted in a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

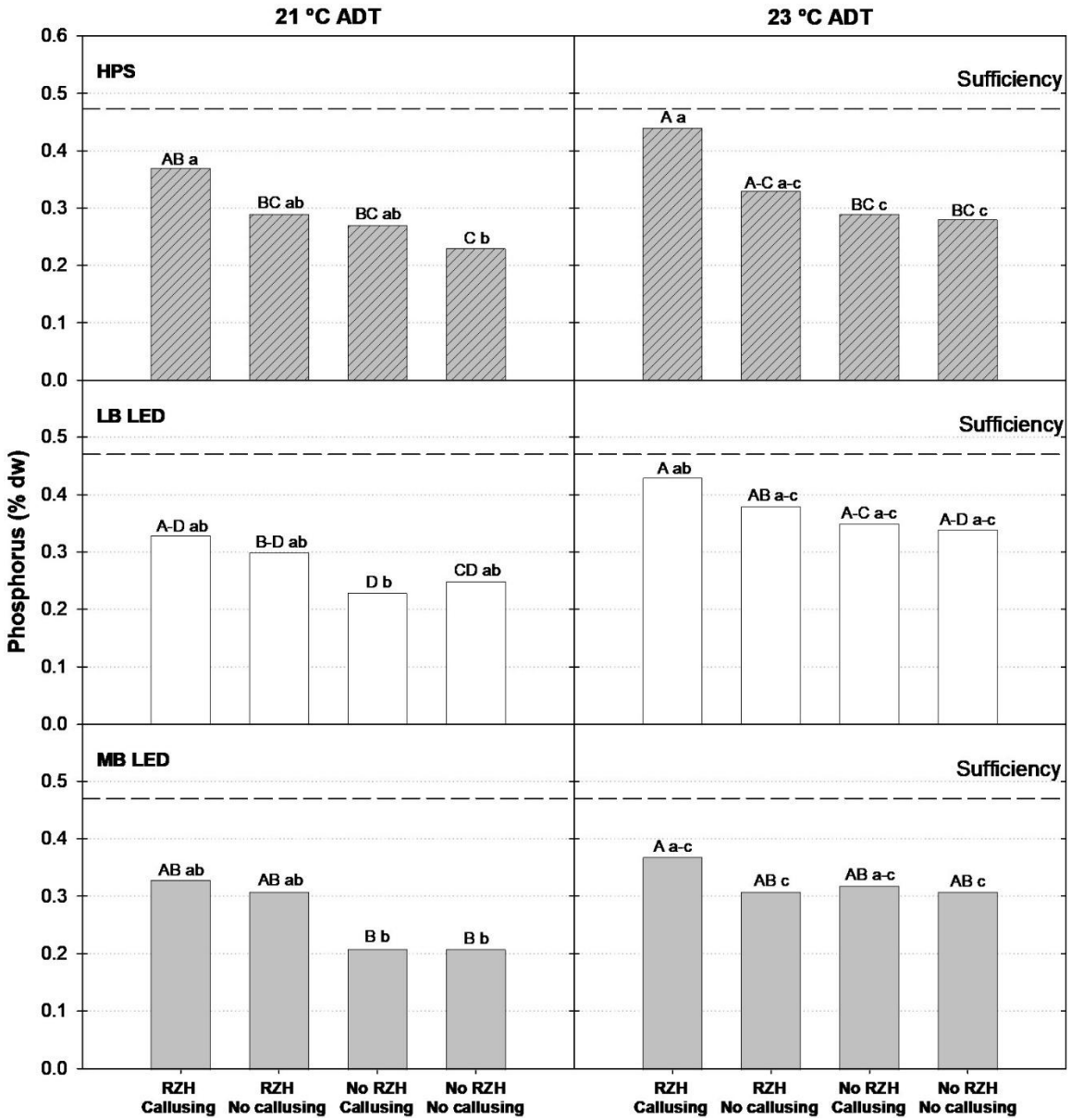

Figure III-3: Average phosphorus content (% dry weight) of petunia 'Dark Blue' cuttings rooted in a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

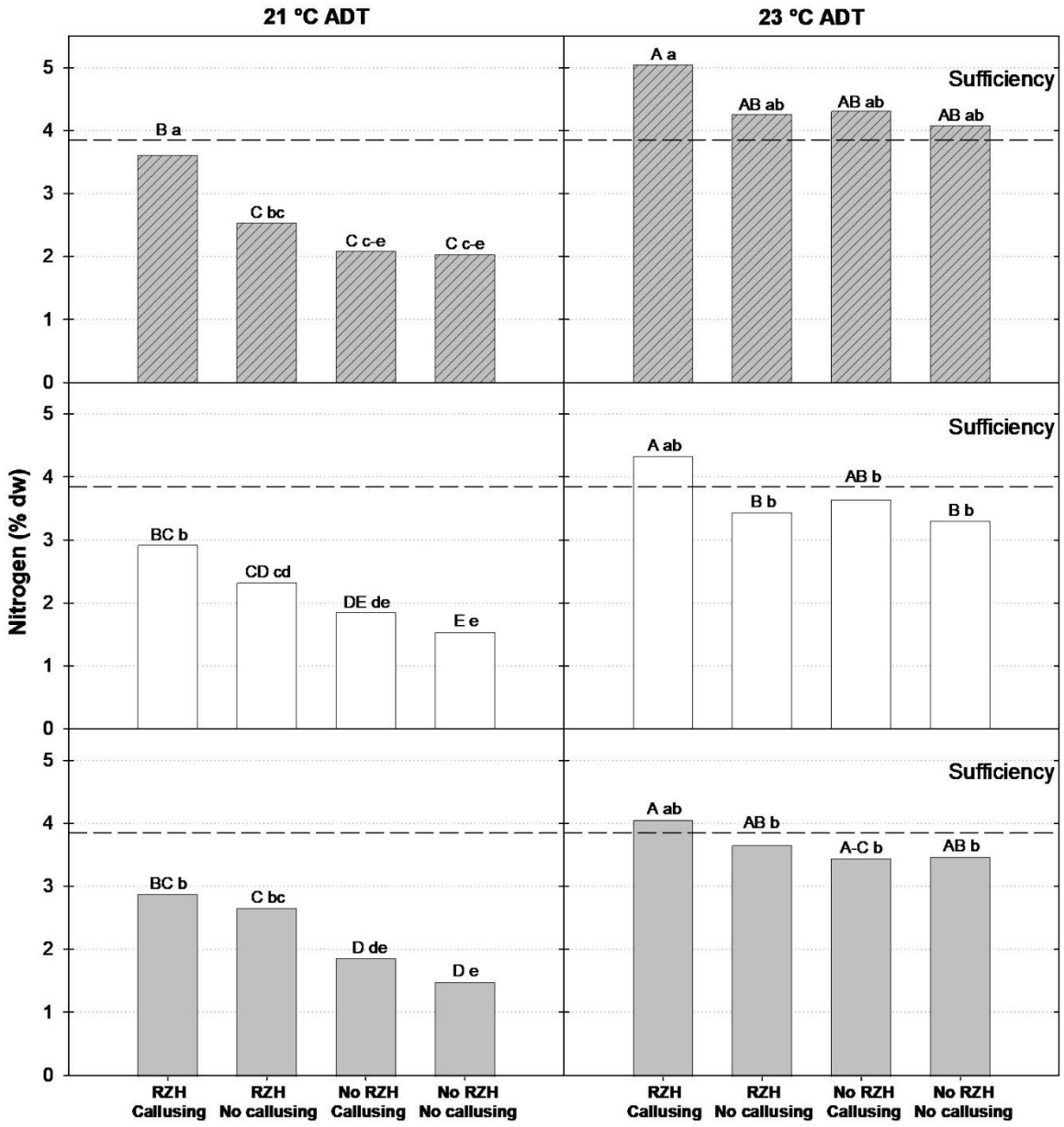

Figure III-4: Average nitrogen content (% dry weight) of petunia 'White' cuttings rooted in a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

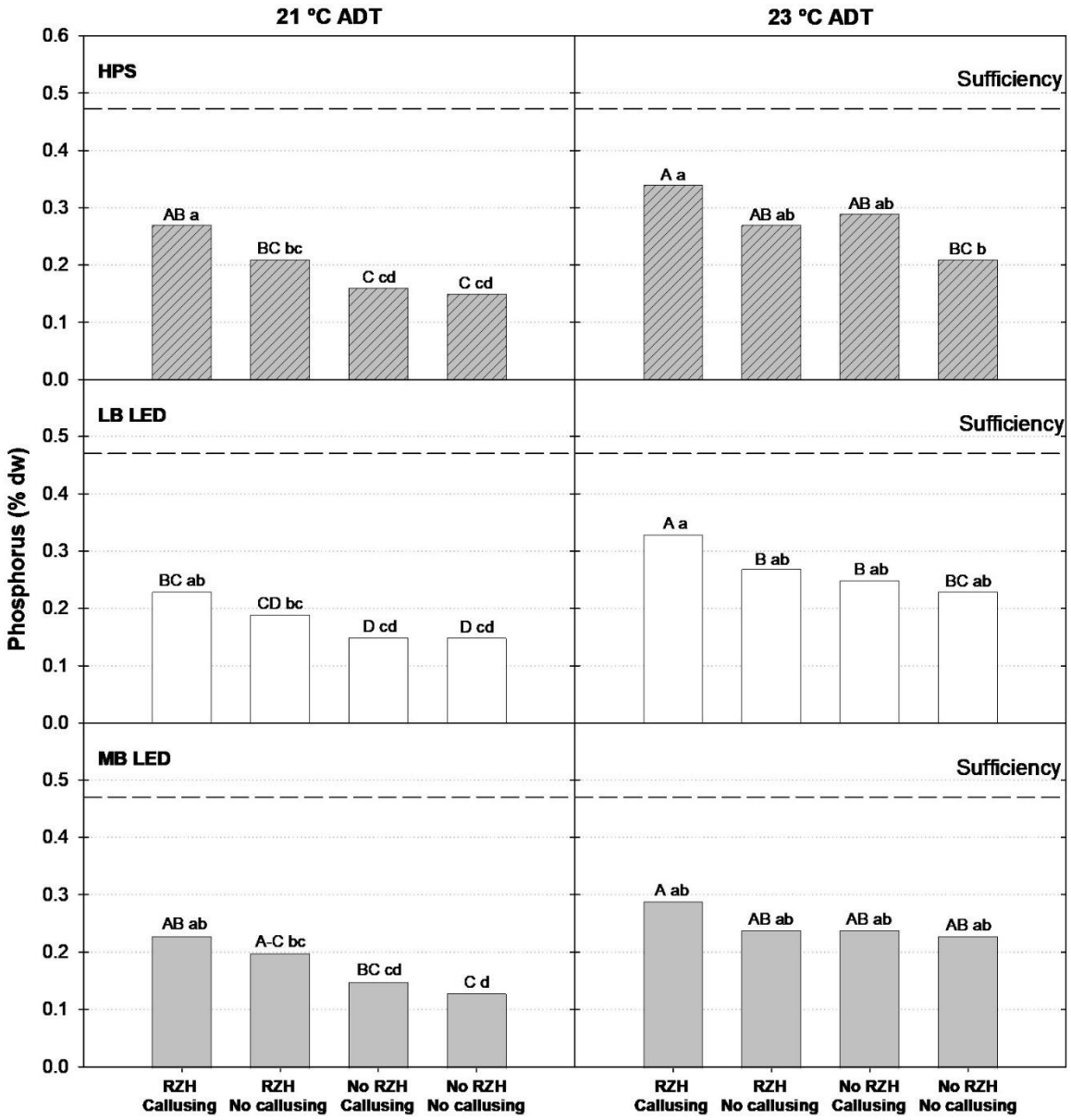

Figure III-5: Average phosphorus content (% dry weight) of petunia 'White' cuttings rooted in a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

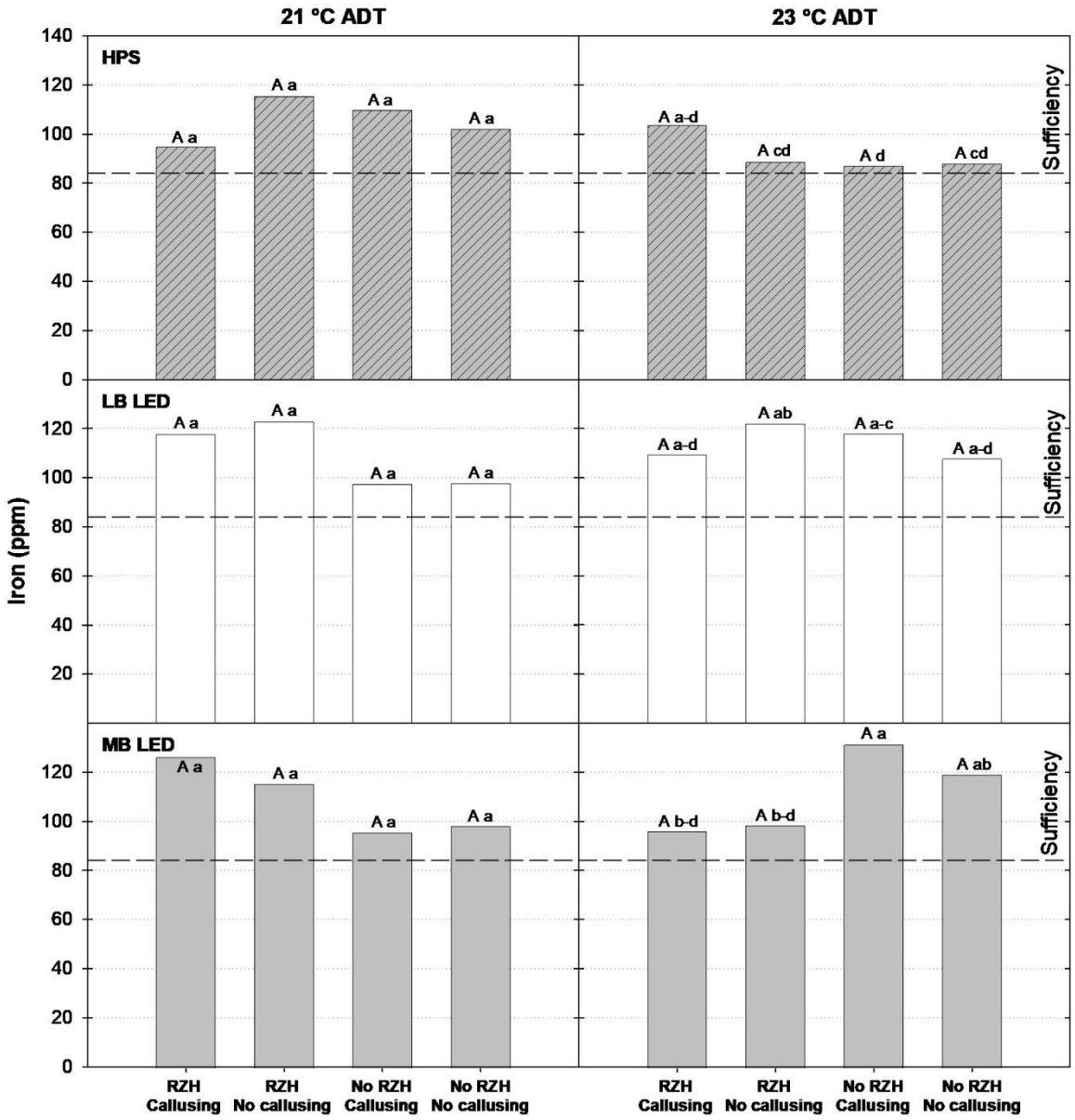

Figure III-6: Average iron content (ppm dry weight) of petunia 'Dark Blue cuttings rooted in a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

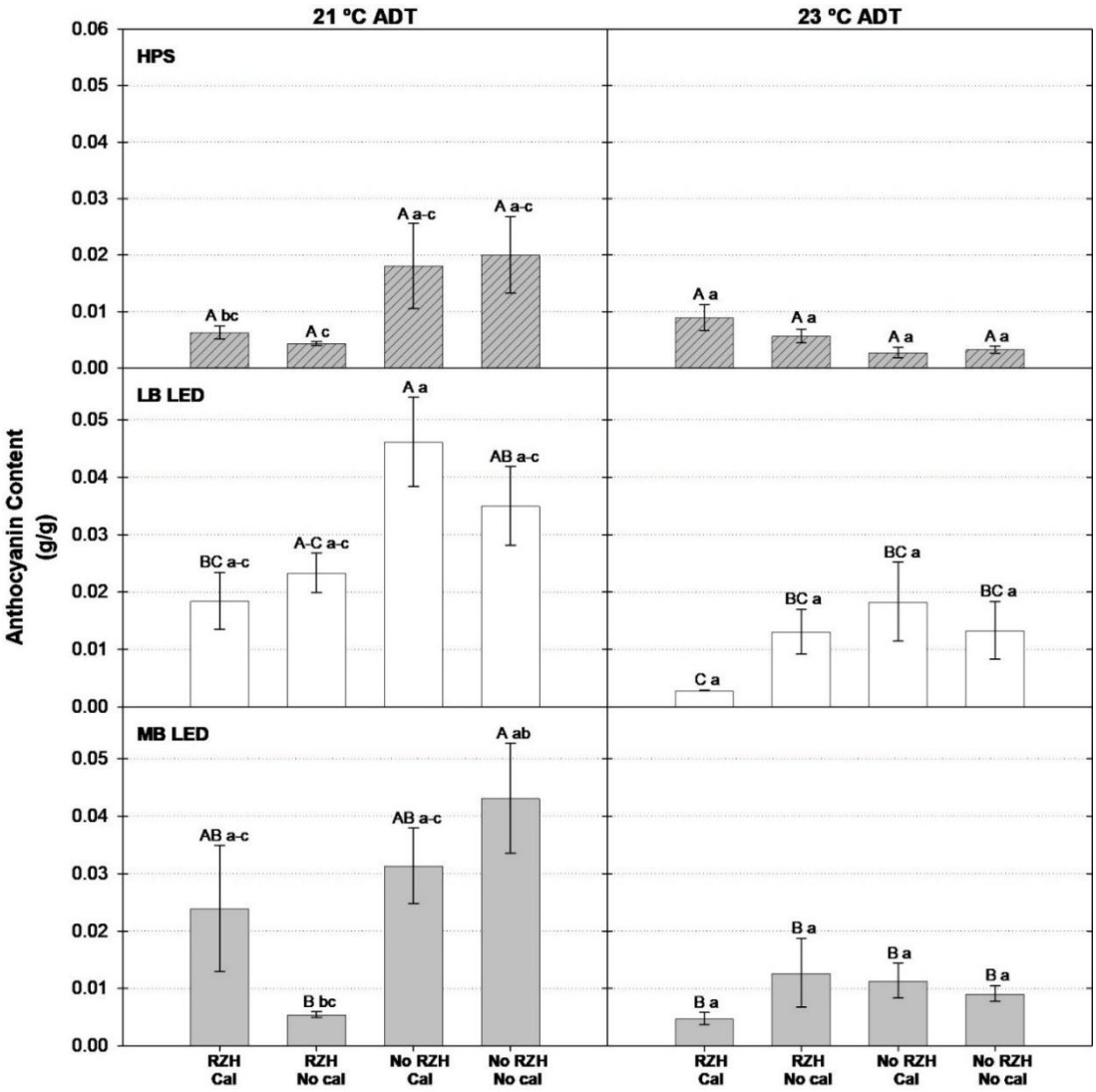

Figure III-7. Average anthocyanin content (g/g) of petunia 'Dark Blue' cuttings rooted in one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light. Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a SL treatment while different lowercase letters within columns are significantly different within an ADT according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

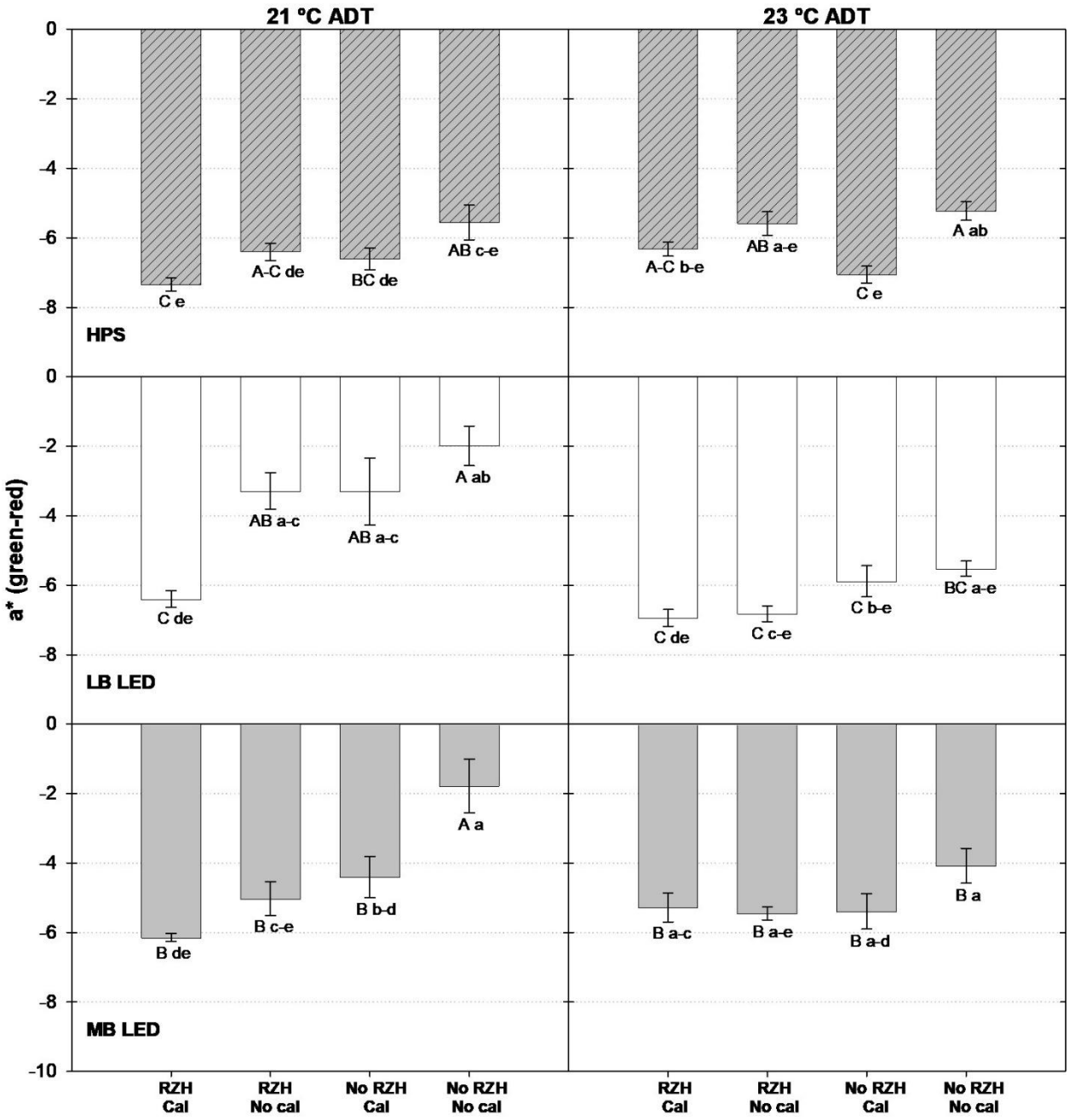

Figure III-8. Average a* value of petunia 'Dark Blue' cuttings rooted in one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light.

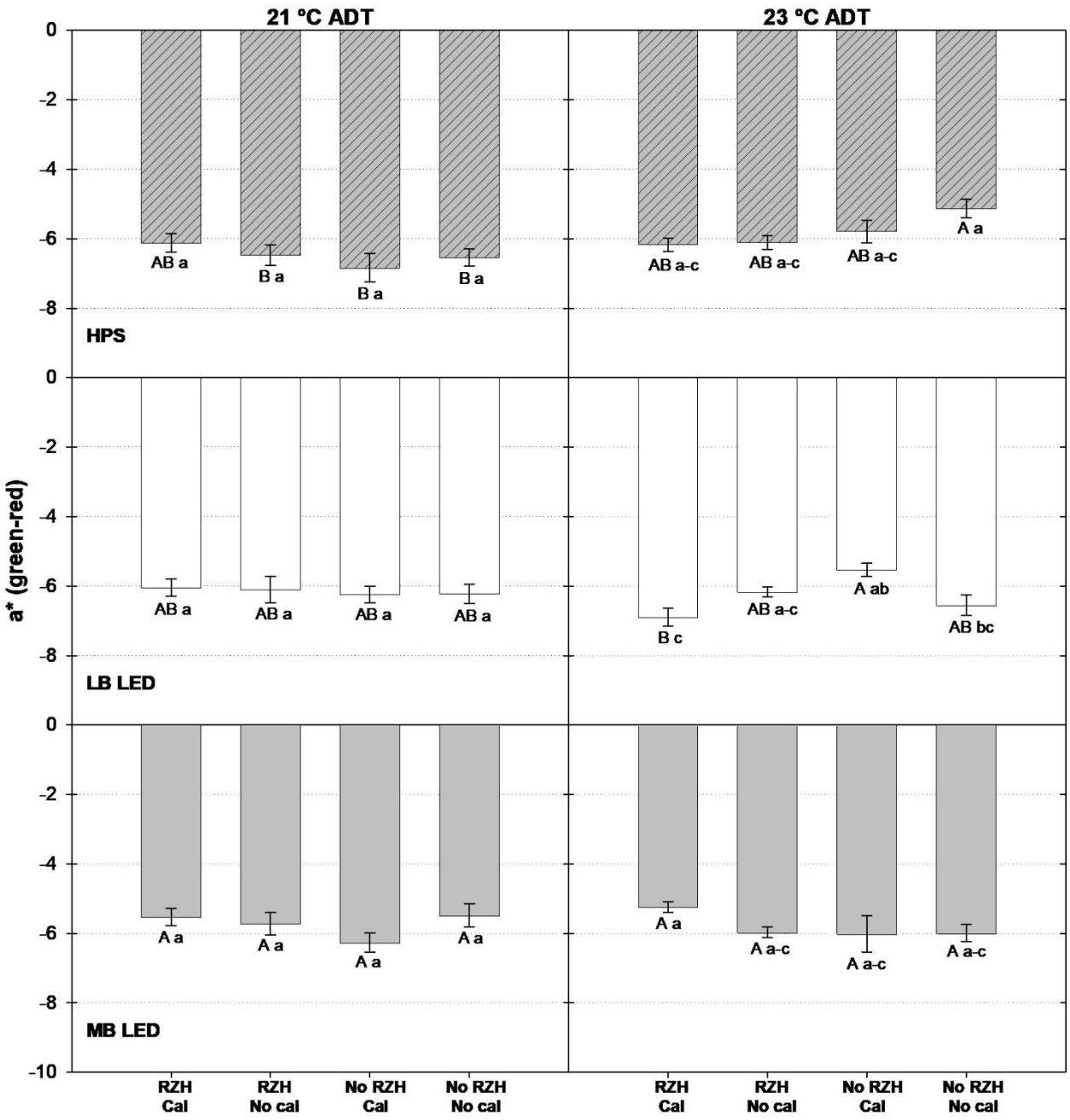

Figure III-9. Average a* value of petunia 'White' cuttings rooted in one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light.

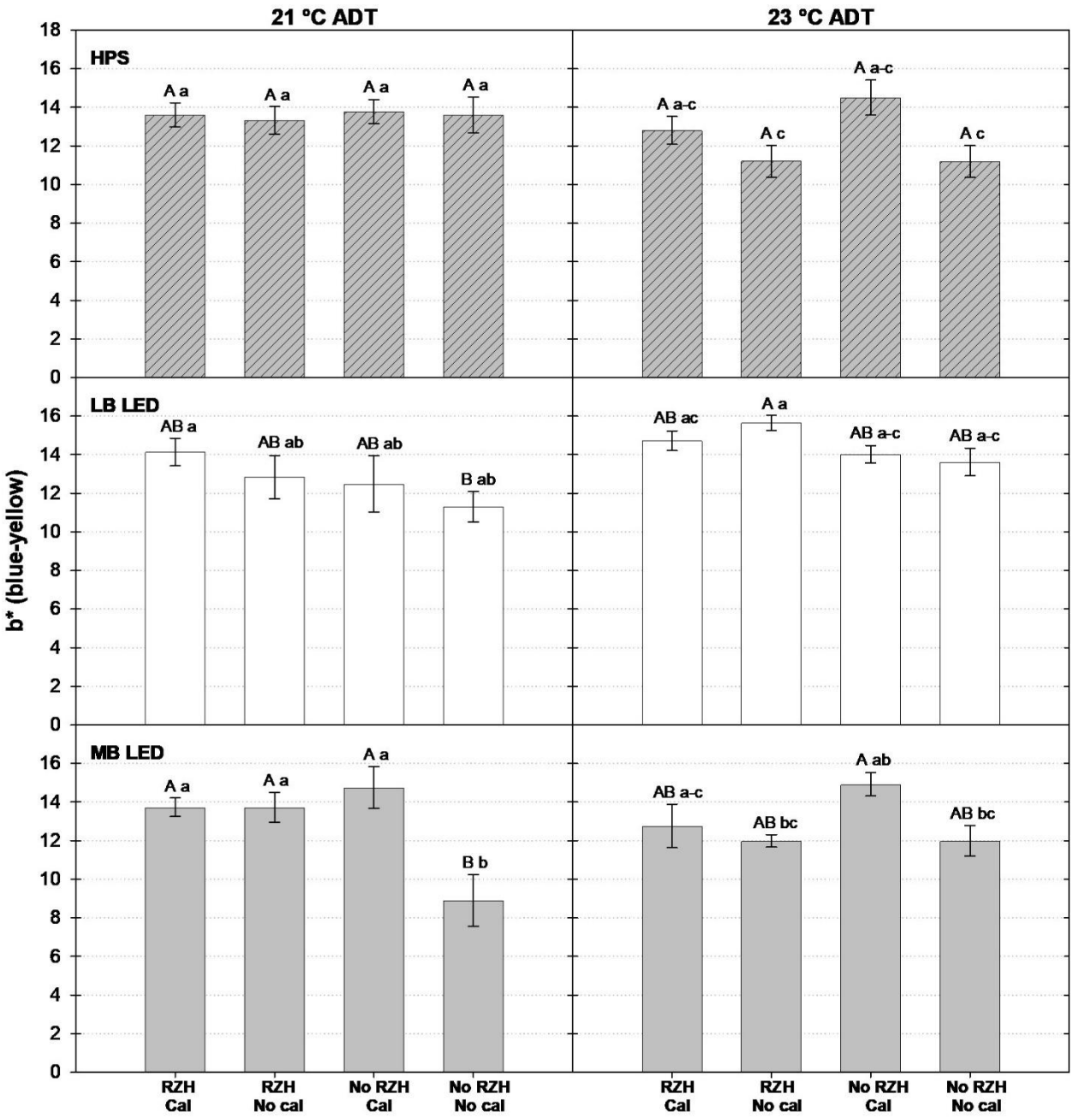

Figure III-10. Average b* value of petunia 'Dark Blue' cuttings rooted in one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light.

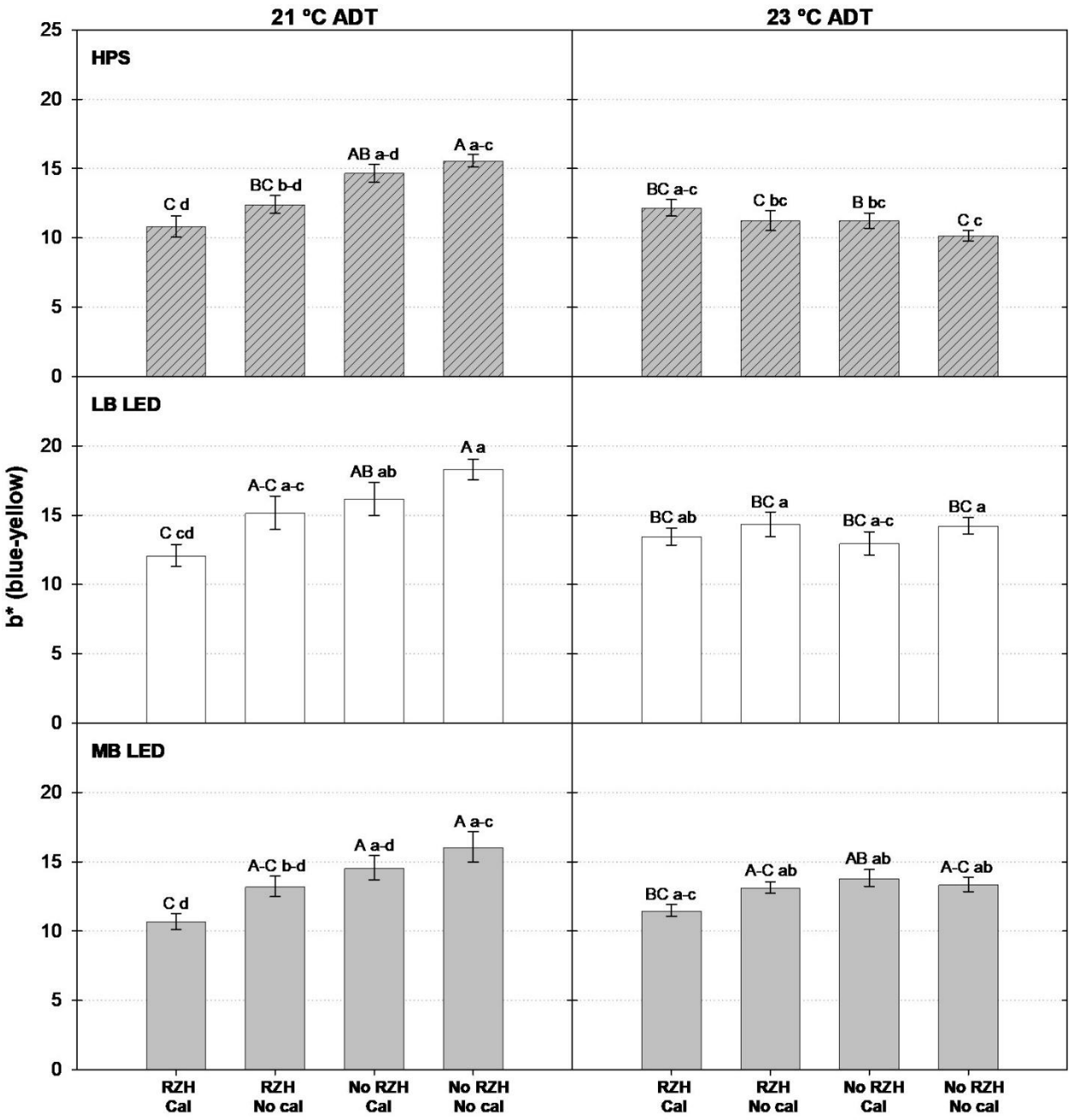

Figure III-11. Average b* value of petunia 'White' cuttings rooted in one of two greenhouse compartments with either a 12-h day/12-h night air temperature setpoint of 22/19 °C [average daily temperature (ADT) of 21 °C] or 24/21 °C (ADT of 23 °C) and on benches with root-zone heating (RZH) or without (no RZH). Cutting either received a 6-day callusing treatment where they were placed under a 50% shade cloth or no callusing treatment where they were placed directly under supplemental lighting (SL) provided by high-pressure sodium (HPS) lamps, low blue (LB) light-emitting diode (LED) fixtures providing a light ratio (%) of 6:1:91:1 blue:green:red:far-red light or medium blue (MB) LEDs providing a light ratio (%) of 10:6:82:2 blue:green:red:far-red light.

SECTION IV

LED SUPPLEMENTAL LIGHT INTENSITY AND ROOT-ZONE HEATING, BUT NOT FAR-RED LIGHT, INFLUENCE MORPHOLOGY, LEAF COLOR, AND ANTHOCYANIN CONTENT OF *Petunia ×hybrida* DURING ROOTING

LED supplemental light intensity and root-zone heating, but not far-red light, influence morphology, leaf color, and anthocyanin content of *Petunia* ×*hybrida* during rooting

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Abstract

In high latitude regions (≥40 °, commercial greenhouse growers utilize supplemental lighting (SL) and root-zone heating to decrease rooting time and increase the quality of young plants during periods of low solar irradiance. Growers increasingly utilize light-emitting diode (LED) fixtures but some have reported the development of purple foliage of some crops, including petunia (*Petunia ×hybrida*), during rooting. The objectives of this study were to determine 1) the influence of LED emission spectra on the morphology and coloration of cuttings; 2) if cuttings grown under higher intensity SL developed more severe purpling; and 3) to develop strategies to reduce or prevent the purpling. Shoot-tip cuttings of petunia SureShot 'Dark Blue' and 'White' 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 (low G and FR) or 10:18:59:13 (moderate G and FR) at a total photon flux density (TPFD) of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Cuttings of both cultivars grown under LEDs emitting a higher R:FR ratio were generally more compact. Additionally, cuttings of both cultivars grown under a supplemental PFD of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were typically taller with thinner stem diameters than those grown under 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Cuttings of 'Dark Blue' grown under a higher PFD were overall more red, more blue, and had higher total anthocyanin concentrations in their leaves than those under the lower PFD. Cuttings of 'White' grown with an RZT of 25 °C were generally taller than those with an RZT of 21 °C, whereas cuttings of 'Dark Blue' grown with higher RZT had overall lower anthocyanin concentrations in their leaves. Furthermore, cuttings of both cultivars had overall higher root-dry masses when grown with a higher RZT. These results indicate that suggest that the severity of foliage purpling developed during propagation under LEDs, while

being cultivar dependent, is not related to the R:FR ratio of SL. In addition, pigment accumulation may be mitigated by root-zone heating or limiting the supplemental PFD (<120 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during periods of low solar irradiance.

Introduction

Traditionally, greenhouse supplemental lighting (SL) has been delivered with high-pressure sodium (HPS) lamps (Ciolkosz et al., 2001). However, due to improvements in light-emitting diode (LED) technologies, the use of high-intensity LED fixtures to provide SL has been increasing (Mitchell et al., 2012). While LEDs are generally more expensive to install, they emit less heat, have longer life spans, and more efficiently convert electricity into photosynthetically active radiation (PAR) than HPS lamps (Haitz et al., 2000; Bourget, 2008; Mitchell et al., 2015; Nowakowska et al., 2023). LEDs can emit a variety of spectra, but for horticultural applications, they typically emit red (R; 600–699 nm) light, blue (B; 400–499 nm) and/or white (W) light, with 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 than most horticultural LED fixtures (Bourget, 2008; Nelson and Bugbee, 2014).

Plants grown under LEDs may display differences in leaf color and pigmentation, plant morphology, rooting, and crop quality 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% (Randall and Lopez, 2014, 2015; Craver et al., 2019). However, the influence of SL spectrum on these quality metrics reportedly varies across studies. Randall and Lopez (2015) found chlorophyll content in vinca ‘Titan Red Dark’ (*Catharanthus roseus*), impatiens ‘Super Elfin XP Blue Pearl’ (*Impatiens walleriana*), geranium ‘Bullseye Red’

(*Pelargonium* ×*hortorum*), and petunia ‘Dreams Midnight’ (*Petunia* ×*hybrida*) was similar when grown under sunlight supplemented by HPS lamps or LEDs emitting a light ratio of 87:13 red:blue light 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 suggest that differences in SL spectrum between HPS lamps and LEDs may not influence crop morphology. For example, Poel and Runkle (2017) reported the height of seedlings of geranium ‘Pinto Premium Salmon’, petunia ‘Single Dreams White’ and ‘Wave Misty Lilac’, snapdragon ‘Montego Yellow’ (*Antirrhinum majus*), and tomato ‘Supersweet’ (*Solanum lycopersicum*) was 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 differences in crop quality or flowering during the finishing stage of any crop.

Although the influence of SL spectrum on crop coloration and morphology is still being researched, commercial plant propagators are continuing to adopt LEDs as their main form of SL (Lee et al., 2020). As more growers utilize LEDs, reports of the development of purple foliage in bedding plants propagated under fixtures emitting high-intensity light (PPFD ≥80 µmol·m^{-2}·s^{-1}) have been 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. One of the most noteworthy crops exhibiting this purple pigmentation is petunia, whose market value accounted for nearly 11% of the $3.56 billion of all floral and foliage crop sales across all container types in 2022 (USDA, 2023; Miller, 2024).

This leaf purpling could possibly be caused by the relatively higher amount of B light, and less G and FR, being radiated by most LED fixture types when compared to HPS lamps

(Runkle, 2024; Veazie and Whipker, 2024). 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 may be triggered by stressors that can cause oxidative damage, as they possess free radical scavenging capabilities that can stabilize or neutralize reactive oxygen species (Gould et al., 2002; Ding et al., 2020). Since high-energy B light can cause oxidative stress and the subsequent upregulation in the expression of genes controlling anthocyanin production, the increased percentage 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 may 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 under 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which exhibited purple tissue (Albert et al., 2009). Additionally, anthocyanin concentration of butterhead lettuce ‘Teodore’ increased by up to 17.8 times during propagation as the PPFD was raised from 60 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Das et al., 2024).

In addition to light stress, low temperatures may also cause oxidative stress. Suboptimal tissue temperature may lead to the synthesis and accumulation of anthocyanins, possibly leading to the development of purple foliage. For example, red-leaf lettuce ‘Red Wave’ grown at a root-zone temperature (RZT) of 10 °C amassed substantially more anthocyanins in its leaves than

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 PPFDs 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 also 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 ≈1.5 °C lower under LEDs emitting a B:R ratio of 1:4 when compared to those grown under HPS lamps. HPS lamps emit more heat than LEDs, and typically radiate the energy downward onto a crop, resulting in warmer plant temperatures compared to 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 red-leaf lettuce ‘Satine’ and green-leaf lettuce ‘Lugano’ under LED SL and found that the anthocyanin content was not affected when FR composed 0.3, 1.4, 1.8, or 2.1% of the SL spectrum. As these were relatively low portions of FR radiation, it may be possible that further increasing the percentage of FR could influence anthocyanin concentrations in leaves. For example, work under sole-source lighting (SSL) has revealed that adding FR light reduced anthocyanin concentration of baby-leaf lettuce ‘Red Cross’ grown under W light or W and ultraviolet (UV) light emitting LEDs (Li and Kubota, 2009). In contrast, anthocyanin concentrations of lettuce ‘Yanzhi’ and ‘Red Butter’ grown under LED SSL emitting various percentages of UV-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 radiation from SL on the coloration and accumulation of anthocyanins in asexually propagated bedding plants has not been established. Therefore, the objectives of this study were to 1) quantify the influence of FR light, light intensity, and RZT, as well as their interactions, on the morphology, coloration, and anthocyanin content of petunia cuttings; 2) to determine if changes in foliage coloration and the development of purple foliage could be attributed to the accumulation of anthocyanins in leaves; and 3) determine cultivar-specific variations in the severity and incidence of foliage purpling developed under LEDs emitting very low or moderate FR percentages.

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, NI), marking the start of the first and second replications, respectively. Commercial greenhouse propagators had previously reported high incidence and severity of foliage purpling in ‘Dark Blue’, but not ‘White’. As such, these cultivars were selected due to their described variation in the development of purple tissues under LED SL. On the day of arrival, 1,152 cuttings of each cultivar were inserted into 5.1-cm deep, 72-cell trays (PTT72-STD-BLK; East Jordan Plastics, Inc., Beaverton, MI) filled with, by volume, 50% soilless media (containing 70% peat moss, 21% perlite, and 9% vermiculite; Suremix; Michigan Grower Products Inc., Galesburg, MI) and 50% medium-grade perlite (Horticultural Medium Perlite; Perlite Vermiculite Packaging Industries Inc., North Bloomfield, OH). Two trays of each cultivar were then 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 [(MSU), East Lansing, MI (lat. 43° N)]. A vapor-pressure deficit (VPD) setpoint of 0.3 kPa was established for the first seven days of propagation by injecting stream as necessary, which was controlled by a datalogger (CR1000; Campbell Scientific Inc., Logan, UT). The VPD setpoint was raised to 0.5 kPa on day 8 of propagation and maintained to day 14, after which 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 recorded every 30 s by an environmental computer connected to a quantum sensor (LI-190R; LI-COR, Lincoln, NE). 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. After this, 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 N, 23 P, 60 K, 28 Ca, 4.6 Mg, 1.3 Fe, 0.6 Mn, 0.6 Zn, 0.6 Cu, 0.4 B, and 0.1 Mo (MSU Plug Special; Greencare Fertilizers, Inc., Kankakee, IL).

Greenhouses maintained a 12-h day/12-h night air temperature setpoint of 22/19 °C (ADT of 21 °C). Air temperature was measured by a thermocouple connected to a data logger (CR1000; Campbell Scientific Inc.) Plant temperature was measured by an infrared sensor (Type T, OS36-01-T-80F; Omega Engineering) placed ≈2.5 cm above the plant canopy at a downward

angle of ≈45°.

Lighting treatments

Both cultivars were propagated under one of two SL treatments at one of two lighting intensities. SL was delivered by medium blue (MB) LEDs [B:G:R:FR ratio % of 10:7:82:1 (Philips Green Power TopLighting Linear DRWMB; Philips, Eindhoven, NL)] or FR LEDs [B:G:R:FR ratio of 10:18:59:13 (Philips Green Power TopLighting Linear DR/W/FR_2 MB; Philips)]. Both SL sources emitted 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 achieve a light intensity treatment of ≈70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, aluminum mesh was used to cover the LED fixtures. Once covered, LEDs emitted a TPFD of 70.3 ± 2.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy height. The average daily light integrals (DLIs) were ≈8 to 10 $mol \cdot m^{-2} \cdot d^{-1}$ and ≈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 (Table IV-1). A 16-h photoperiod (0600 to 2200 HR) was maintained throughout the study through a combination of sunlight and SL from either LED fixture. SL was delivered throughout the photoperiod when the solar PPFD was below ≈440 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and fixtures were controlled by an environmental control system (Integro 725 3030; Priva North America, Vineland Station, ON, Canada) integrated with a weather station that determined ambient radiation via a quantum sensor. The PPFD was measured by a quantum sensor at plant height (LI-190R; LI-COR) every 30 s by a datalogger (Campbell Scientific), hourly averages were recorded, and the DLI was calculated. SL radiation 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. IV-1).

Root-zone temperature treatments

Benches within each greenhouse section were equipped with bench-top micro-tube root-zone heating (RZH) systems that circulated heated water (Biotherm Benchwarmer Kit; TrueLeaf Technologies, Petaluma, CA) 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 onto 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) and thermocouples (Type E Thermocouple; Omega Engineering, Stamford, CT) inserted into individual tray cells.

Data collection

Average root-zone, plant, and air temperatures, DLIs, and weekly VPDs during both replications are provided in Table IV-1.

On day 22 of propagation, 10 cuttings of both cultivars of each treatment were removed from their trays and washed to remove growing media from their roots. Cuttings were then dried via blotting by paper towel and root and shoot tissue were subsequently separated through the use of 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). Shoot and root tissues were then desiccated in a drying oven at 70 °C for 3 d, after which shoot and root dry masses were recorded.

Total anthocyanin content was extracted and measured as described by Darby et al. (2024). Extraction and analysis took place at the University of Tennessee. Foliage tissue of both cultivars and of each treatment were freeze dried and then ground into a powder with a ceramic

mortar and pestle filled with liquid nitrogen. Pigment extraction was performed under R light (peak wavelength: 654 nm). 100 mg of tissue per cultivar and treatment was then 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). Mixtures were then 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. The solution was then 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 then measured by a Biotek PowerWave XS Microplate Reader (Agilent Technologies, Santa Clara CA). Optical density was recorded at 530 nm and total anthocyanin contents were calculated based on the calibration curve of cyanidin-3-o-glucoside chloride (MilliporeSigma, Burlington MA). Additionally, leaf color (average of three leaves per cutting) of 15 cuttings per cultivar was measured by 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 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 carried out with a complete block design and data were analyzed using SAS (version 9.2; SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance (ANOVA). Means were separated by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Data across replications were combined.

Results

Stem length and caliper

The stem length and caliper of both ‘Dark Blue’ and ‘White’ were influenced by SL and light intensity, with that of ‘Dark Blue’ also being influenced by the interaction of SL and light intensity and that of ‘White’ being impacted by RZH (Table IV-2). In general, the stem length of both ‘Dark Blue’ and ‘White’ grown under FR LEDs were greater than or equal to those grown under MB LEDs (Fig. IV-2). The stem calipers of ‘Dark Blue’ and ‘White’ grown under a light intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were typically greater than or equal to those grown at a light intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. IV-3). Stem lengths of ‘Dark Blue’ grown under a light intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were generally greater than or equal to that of those grown under a light intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with the stem lengths of cuttings grown under MB LEDs at an intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ being 25% greater than those grown at a light intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Additionally, the stem lengths of cuttings of ‘Dark Blue’ grown under FR LEDs at an intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 26% greater than that of those grown under MB LEDs at an intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Stem length of ‘Dark Blue’, as well as the stem caliper of both cultivars, was unaffected by RZH.

Shoot and root dry mass

There were no main treatment effects on the SDM of ‘Dark Blue’, while the SDM of ‘White’ was only influenced by light intensity (Table IV-2). In general, the SDM of ‘White’ grown at a light intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was greater than or equal to that of those grown at an intensity of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. IV-4). The RDM of both ‘Dark Blue’ and ‘White’ was influenced by light intensity and RZH, with that of ‘Dark Blue’ being influenced by multiple treatment interactions. The RDM of both ‘Dark Blue’ and ‘White’ grown at a light intensity of

120 µmol·m^{-2}·s^{-1} was generally greater than or equal to that of those grown at a light intensity of 70 µmol·m^{-2}·s^{-1} (Fig. IVI5). The RDM of both ‘Dark Blue’ and ‘White’ grown with an RZT of 25 °C was typically greater than or equal to that of those grown with an RZT of 21 °C. Furthermore, the RDM of ‘Dark Blue’ grown under a light intensity of 120 µmol·m^{-2}·s^{-1} at an RZT of 25 °C was 36% greater than those grown under an intensity 70 µmol·m^{-2}·s^{-}1 of at an RZT of 21 °C.

Anthocyanin content and coloration

The total anthocyanin content of both ‘Dark Blue’ and ‘White’ were influenced by light intensity, with ‘Dark Blue’ also being influenced by RZH and ‘White’ being influenced by SL and various treatment interactions (Table IV-3). The total anthocyanin content of ‘Dark Blue’ grown under a light intensity of 120 µmol·m^{-2}·s^{-1} was generally greater than or equal to that of those grown under an intensity of 70 µmol·m^{-2}·s^{-1} (Fig. IV-6). For example, when cuttings were propagated under MB LEDs at an RZT of 21 °C, the anthocyanin content of cuttings grown under light intensity of 120 µmol·m^{-2}·s^{-1} was 49% greater than that of those grown under an intensity of 70 µmol·m^{-2}·s^{-1}. Additionally, the anthocyanin content of ‘Dark Blue’ grown with an RZT of 21 °C was generally greater than or equal to those grown with an RZT of 25 °C. For example, when grown under MB LEDs at an intensity of 120 µmol·m^{-2}·s^{-1}, the anthocyanin content of cuttings grown at an RZT of 21 °C was 132% greater than those grown at an intensity of 70 µmol·m^{-2}·s^{-1}. The anthocyanin content of ‘White’ grown under FR LEDs was generally greater than or equal to that of those grown under MB LEDs. The anthocyanin content of ‘Dark Blue’ was not influenced by SL and that of ‘White’ was not influenced by RZH.

The foliage lightness (L*) of ‘Dark Blue’ was influenced by SL, light intensity, as well as multiple treatment interactions while that of ‘White’ was influenced by light intensity (Table IV-

3). The lightness of ‘Dark Blue’ grown at a light intensity of 70 µmol·m^{-2}·s^{-1} was generally greater than or equal to those grown at a light intensity of 120 µmol·m^{-2}·s^{-1}. The foliage lightness of ‘Dark Blue’ was not influenced by RZH while the lightness of ‘White’ was unaffected by SL and RZH.

The greenness and redness (chromametric a*) of ‘Dark Blue’ was affected by SL, light intensity, and RZH as well as all treatment interactions while that of ‘White’ was only influenced by light intensity (Table IV-3). The a* value of ‘Dark Blue’ grown under MB LEDs was generally less negative (i.e., more red) than or equal to those grown under FR LEDs (Fig. IV-7). In addition, the a* of leaves of ‘Dark Blue’ grown under a light intensity of 120 µmol·m^{-2}·s^{-1} was typically more positive than or equal to that of those grown under a light intensity of 70 µmol·m^{-2}·s^{-1}. The a* of ‘Dark Blue’ grown at an RZT of 21 °C was generally more positive than or equal to those grown with an RZT of 25 °C. Furthermore, the a* of ‘Dark Blue’ grown under a light intensity of 120 µmol·m^{-2}·s^{-1} at an RZT of 21 °C was usually 46% more positive than or equal to those grown at a light intensity of 70 µmol·m^{-2}·s^{-1} with an RZT of 25 °C. The a* of ‘White’ was not influenced by SL or RZH.

The blueness and yellowness (chromametric b*) of ‘Dark Blue’ was influenced by light intensity while that of ‘White’ was influenced by RZH (Table IV-3). The b* value of ‘Dark Blue’ was also affected by the interaction of SL and light intensity as well as the interaction between light intensity and RZH. In general, the b* of ‘Dark Blue’ grown under a light intensity of 70 µmol·m^{-2}·s^{-1} was generally greater than that of those grown under 120 µmol·m^{-2}·s^{-1} (Fig. IV-8). The b* value of ‘Dark Blue’ was unaffected by SL and RZH while that of ‘White’ was unaffected by SL or light intensity.

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 ratio of R to FR light (R:FR ratio) decreases. For example, Brown et al. (1995) reported pepper (*Capsicum annum*) 'Hungarian Wax' to significantly taller when grown under LEDs emitting a R:FR ratio of 83:17 than when grown under metal halide lamps or LEDs emitting R:FR ratios >100. 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). In the present study, cuttings of both 'Dark Blue' and 'White' grown under LEDs emitting a higher percentage of FR light (or under a lower R:FR) ratio were generally as tall or slightly taller than those grown under LEDs emitting a higher R:FR ratio. As cuttings grown under the FR LEDs were exposed to a lower R:FR ratio than those under the MB LEDs, this response was not surprising. Limited work has been conducted to determine the influence of supplemental FR radiation on the accumulation of anthocyanins and coloration of bedding plants during propagation. In the present study, the anthocyanin concentration of 'White' grown under FR LEDs was greater than or equal to that of those grown under MB LEDs, while that of 'Dark Blue' was unaffected by spectral differences provided by the SL fixtures. This suggests that the impact of the R:FR 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 petunia 'Tiny Tunia Violet Ice' rooted cuttings was reduced by 35% as DLI increased from 1.2 to 3.9 $mol \cdot m^{-2} \cdot d^{-1}$ (Lopez and Runkle 2005). Similarly, in the present study, cuttings grown under a supplemental TPFD of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$, (DLI of 8.7 $mol \cdot m^{-2} \cdot d^{-1}$), tended to exhibit similar or shorter stems

than those grown under a TPFD 70 µmol·m^{-2}·s^{-1} (DLI of 7.1 mol·m^{-2}·d^{-1}; Tables IV-1 and IV-2; Fig. IV-2). For example, cuttings of 'Dark Blue' grown under MB LEDs at an RZT of 21 °C exhibited 13% shorter stems when grown at a TPFD of 120 µmol·m^{-2}·s^{-1} when compared to those grown under a TPFD 70 µmol·m^{-2}·s^{-1}.

Regarding stem caliper, Currey et al. (2012) reported that the stem caliper of Angelonia 'AngelMist White Cloud' (*Angelonia angustifolia*), argyranthemum 'Madeira Cherry Red' (*Argyranthemum frutescens*), diascia 'Wink Coral' (*Diascia barberae*), nemesia 'Aromatica Royal' (*Nemesia frticans*), osteospermum 'Voltage Yellow' (*Osteospermum ecklonis*), and verbena 'Aztec Violet' (*Verbena ×hybrida*) increased as the DLI was raised from 1.2 to 12.3 mol·m^{-2}·d^{-1}. In the present study, light intensity was the most impactful variable influencing stem caliper, with cuttings propagated under a higher DLI, developed larger stem calipers (Fig. IV-3). In addition to stem caliper, the RDM of both petunia cultivars was influenced by light intensity, with cuttings grown under higher DLIs, yielded higher RDMs (Fig. IV-5). These results are consistent with Currey et al. (2012) who also reported the RDM of all previously listed species, in addition to lantana 'Lucky Gold' (*Lantana camara*), scaevola 'Blue Print' (*Scaevola hybrid*), and bacopa 'Abunda Giant White' (*Sutera cordata*), under increasing DLI. These results are also consistent with Lopez and Runkle (2008), who reported the RDM of petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' increased by 680%, 2395%, and 108%, respectively, as DLI is raised from 1.2 to 8.4 mol·m^{-2}·d^{-1}. From this, the application of relatively high PPFDs, may be necessary during cutting propagation to produce compact, fully-rooted cuttings with thick stems suitable to handle the stresses of shipping and transplanting (Pramuk and Runkle, 2005; Currey et al., 2012; Randall and Lopez, 2014).

The SDM of both petunia ‘Dark Blue’ and ‘White’ were generally unaffected by SL, light intensity, or RZH (Table IV-1; Fig. IV-4). This differs from Lopez and Runkle (2008) who reported that the SDM of various petunia cultivars increased as DLI increased from 1.2 to 8.4 $mol \cdot m^{-2} \cdot d^{-1}$. This suggests that the influence of higher DLI on SDM of petunia may be cultivar-dependent. However, as the increase in DLI is relatively smaller in the present study, it is possible that the difference in DLI simply was not large enough to yield an effect on SDM.

It has been described that petunia ‘Rose of Heaven’ (*Petunia axillaris*×*Petunia hybrida*), as well as *Lc* petunia from the 118C seed line, accumulated higher anthocyanins concentrations when grown under metal halide SL providing a PPFD of 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as opposed to PPFDs of 50–350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Albert et. al., 2009). Similarly, in the present study, the anthocyanin concentration of petunia ‘Dark Blue’ was greater in cuttings grown under a TPFD of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than those grown under a TPFD of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Table IV-3; Fig. IV-6). While direct reporting on the influence of supplemental light intensity on the redness, greenness, blueness, and yellowness of petunia is limited, it is likely that increased concentrations of anthocyanin in leaves could lead to 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 suggests 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, the anthocyanin content, a*, and b* values of ‘White’ were generally unaffected by light intensity, the response being cultivar dependent as previously reported by Smith (2025).

The influence of RZT on stem length and RDM is described as being genus and species (Cooper, 1973). For instance, Owen (2017) reported the RDM of coral bells ‘Black Beauty’

(*Heuchera hybrida*) was reduced as the RZT was raised from 20 to 28 °C, while Kohler and Lopez (2021) observed that the RDM of calibrachoa 'Callie Coral'(*Calibrachoa ×hybrida*), nemesia 'Aromatica Royal Blue', and petunia 'Sanguna Patio Blue', grown at an ADT of 21 °C, decreased as RZT was raised from 21 to 27 °C. Contrastingly, petunia 'Dark Blue' and 'White' developed a larger RDM when rooted at an RZT of 25 °C than those rooted with an RZT of 21 °C (Fig. IV-5). This suggests that RZT influence 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 plant temperature, leading to increased stem elongation (Vogelezang, 1989). In the present study, petunia 'White' 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. IV-2). While the differences in plant temperature between cuttings receiving and not receiving RZH were not measured, it is likely that the extended stem growth experienced by 'White' cuttings grown over warmer RZTs is due to an increase in plant temperature caused by higher RZTs. However, as RZT did not influence the stem length of 'Dark Blue', this response is likely cultivar dependent.

These results suggest that the purpling being reported by propagators utilizing LED supplemental lighting on their crops may be in part the result of high light intensities (≥90 $\mu mol \cdot m^{-2} \cdot s^{-1}$) such fixtures emit. These high SL light intensities are likely leading to an increase in the concentration of anthocyanins in the foliage of crops grown under them, leading to an increase in the redness and blueness of leaves. This accumulation of anthocyanins may be mitigated through the application of RZH to raise RZTs by ≈ 4°C or by lowering the SL intensity used during propagation. From this, further work using SL fixtures delivering a wider range of R:FR ratios may be necessary to elucidate any relationships between R:FR ratio and the

parameters measured in the present study.

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APPENDIX

Table IV-1. Daily light integral (DLI), root-zone, plant, and air temperatures, and vapor-pressure deficit (VPD) for callusing and post-callusing of petunia 'Dark Blue' and "White' during replications (Rep.) 1 and 2. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing 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). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) 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 ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)			VPD (kPa)		
				Root-zone	Plant	Air	Week 1	Week 2	Week 3
				Rep. 1					
MB LED	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 LED	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					
				Rep. 2					
MB LED	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 LED	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					

Table IV-2. Analyses of variance for the effects of supplemental lighting (SL), SL intensity, and root-zone heating (RZH), and their interactions on stem length and caliper, root and shoot dry mass of petunia 'Dark Blue' and 'White'.

Treatment	Stem length (cm)	Stem caliper (mm)	Shoot dry mass (mg)	Root dry mass (mg)
		'Dark Blue'		
SL	***[z]	*	NS	NS
Intensity	***	***	NS	***
RZH	NS	NS	NS	*
SL×Intensity	**	NS	NS	NS
SL×RZH	NS	NS	NS	***
Intensity×RZH	NS	NS	NS	*
SL×Intensity×RZH	NS	NS	NS	NS
		'White'		
SL	***	*	NS	NS
Intensity	**	***	**	***
RZH	***	NS	NS	***
SL×Intensity	NS	NS	NS	NS
SL×RZH	NS	NS	NS	NS
Intensity×RZH	NS	NS	NS	NS
SL×Intensity×RZH	NS	NS	NS	NS

[z]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

Table IV-3. Analyses of variance for the effects of supplemental lighting (SL), SL intensity, root-zone heating (RZH), and their interactions on total anthocyanin content, L*, a*, and b* values of petunia ‘Dark Blue’ and ‘White’.

Treatment	Total anthocyanin content (mg/g)	L*	a*	b*
	‘Dark Blue’			
SL	NS [z]	***	*	NS
Intensity	***	***	***	***
RZH	***	NS	***	NS
SL×Intensity	NS	**	**	**
SL×RZH	NS	NS	*	NS
Intensity×RZH	NS	**	**	*
SL×Intensity×RZH	NS	*	*	NS
	‘White’			
SL	**	NS	NS	NS
Intensity	*	**	**	NS
RZH	NS	NS	NS	**
SL×Intensity	*	NS	NS	NS
SL×RZH	NS	NS	NS	NS
Intensity×RZH	**	NS	NS	NS
SL×Intensity×RZH	*	NS	NS	NS

[z]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively

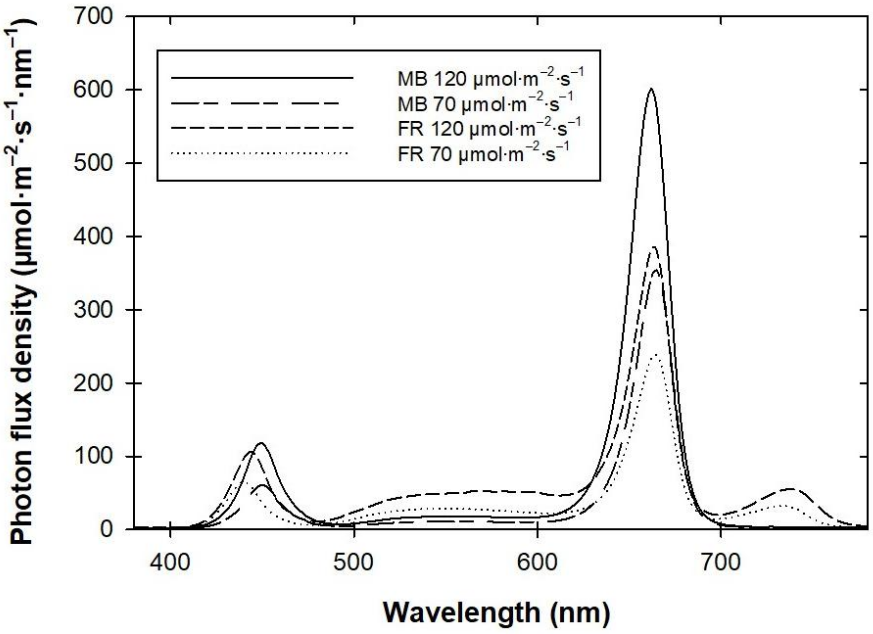

Figure IV-1: Spectral distributions of four supplemental lighting treatments delivered by either medium blue (MB) light-emitting diodes (LEDs) [10:7:82:1 (%) blue:green:red:far-red light] or far-red (FR) LEDs [10:18:59:13 (%) blue:green:red:far-red light] at an intensity of 70 µmol·m^{-2}·s^{-1} or 120 µmol·m^{-2}·s^{-1}.

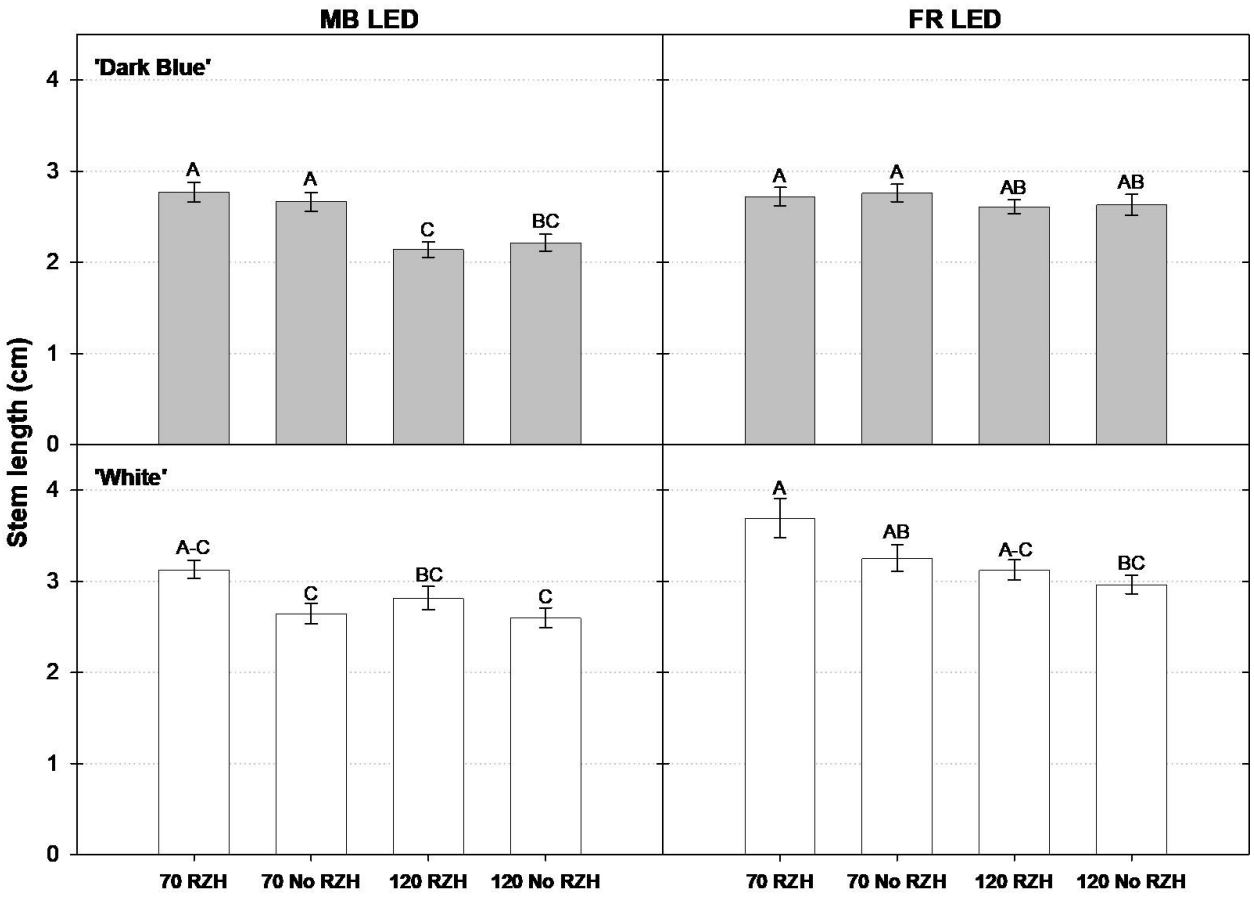

Figure IV-2: Average stem length of petunia 'Dark Blue' and 'White'. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m^{-2}·s^{-1} and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light, respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

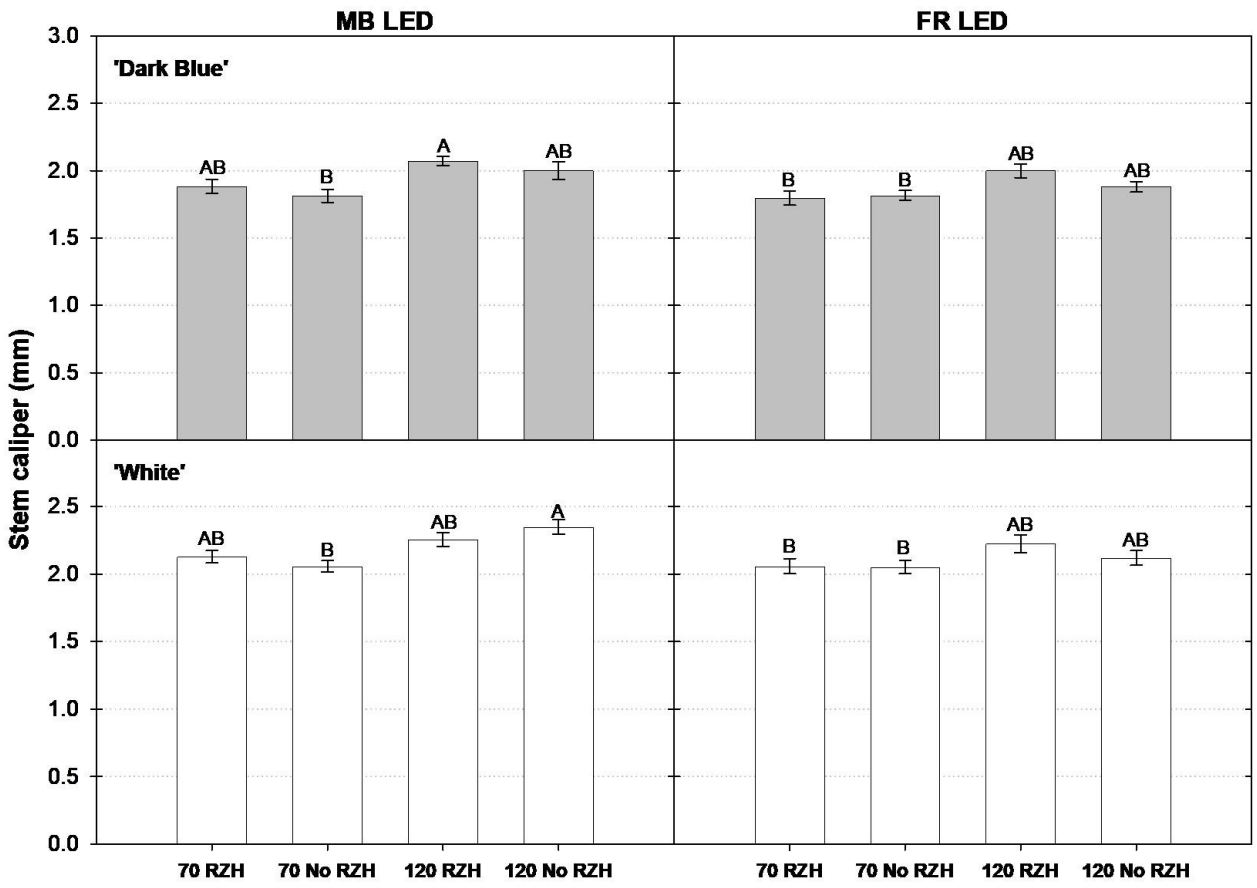

Figure IV-3: Average stem caliper of petunia 'Dark Blue' and 'White'. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light, respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

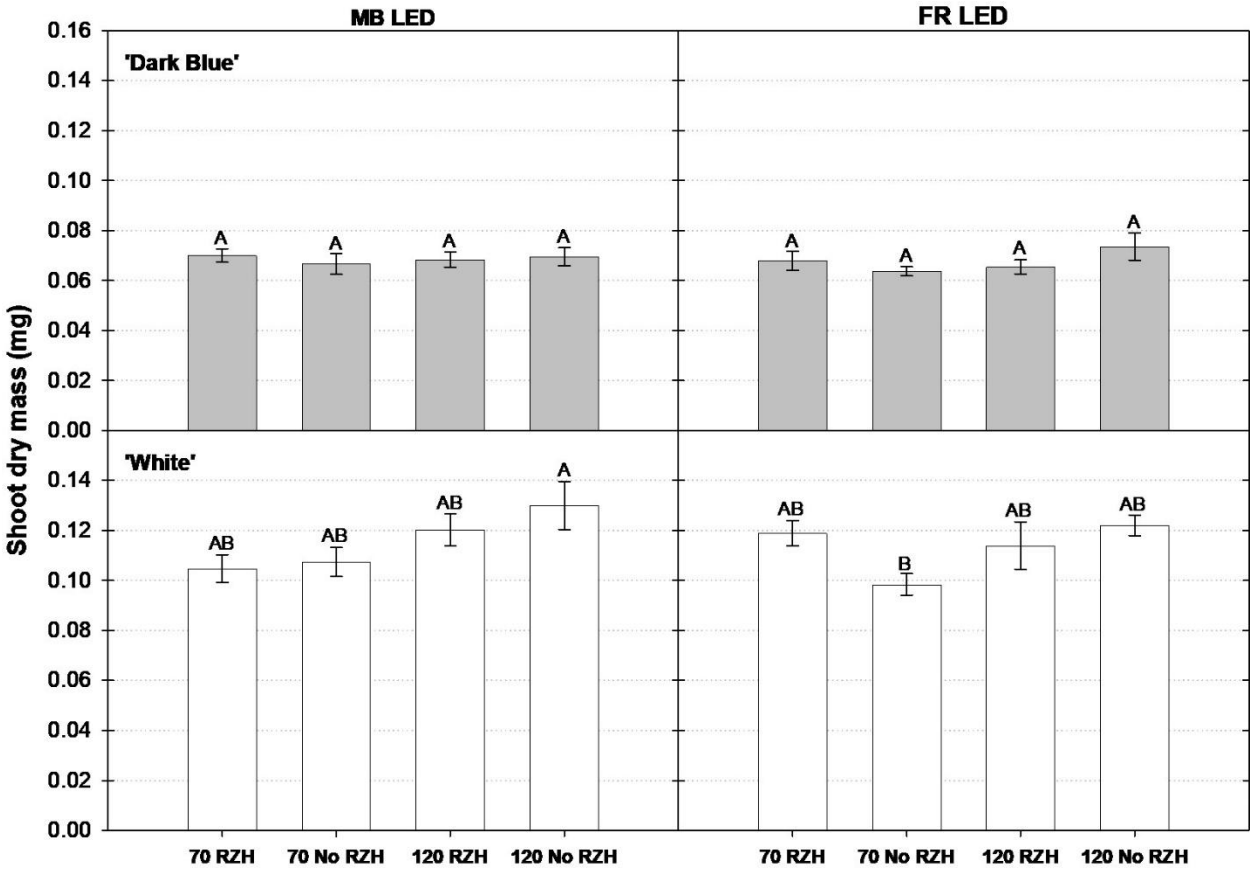

Figure IV-4: Average shoot dry mass of petunia ‘Dark Blue’ and ‘White’. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m–2·s–1 and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light], respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey’s honestly significant difference (HSD) test (P <0.05).

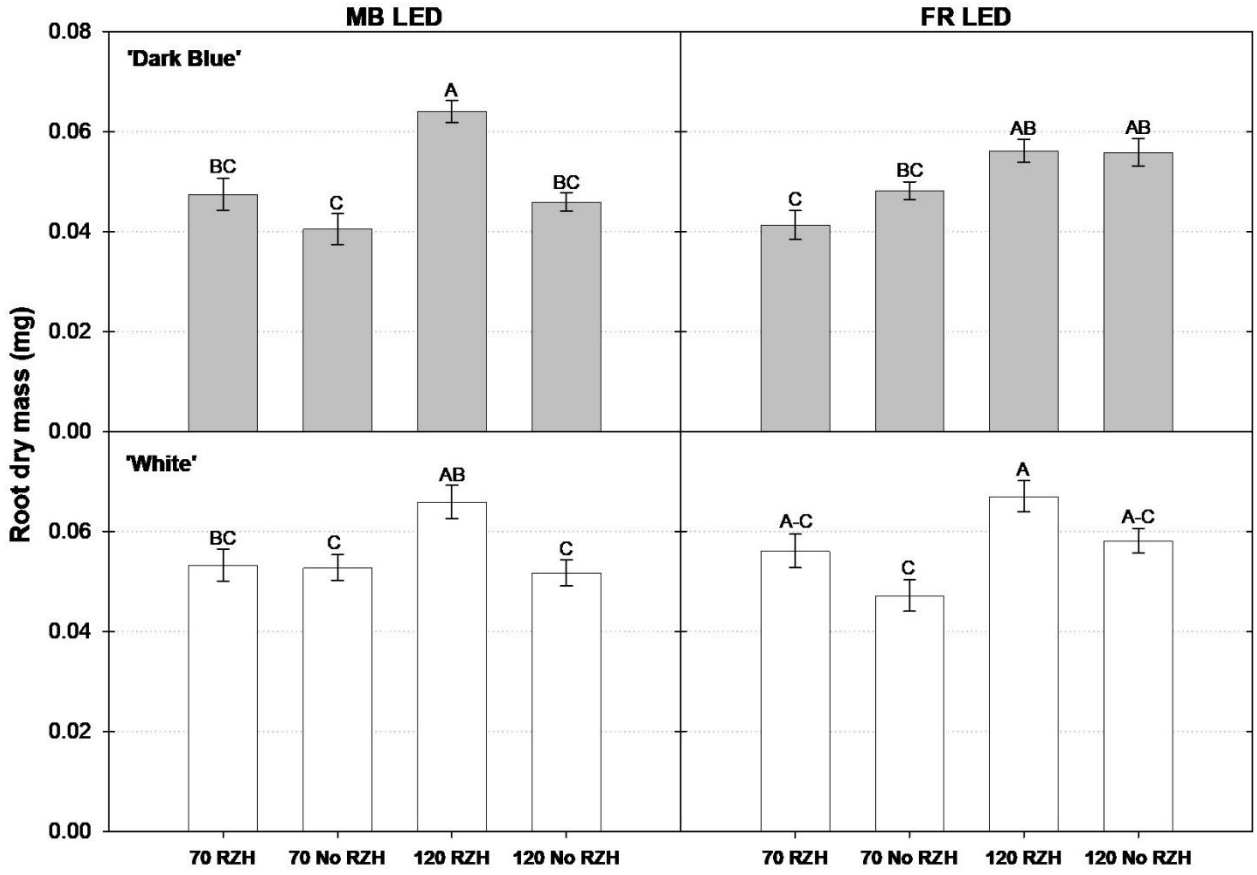

Figure IV-5: Average root dry mass of petunia 'Dark Blue' and 'White'. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m^{-2}·s^{-1} and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light, respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey's honestly significant difference (HSD) test ($P < 0.05$).

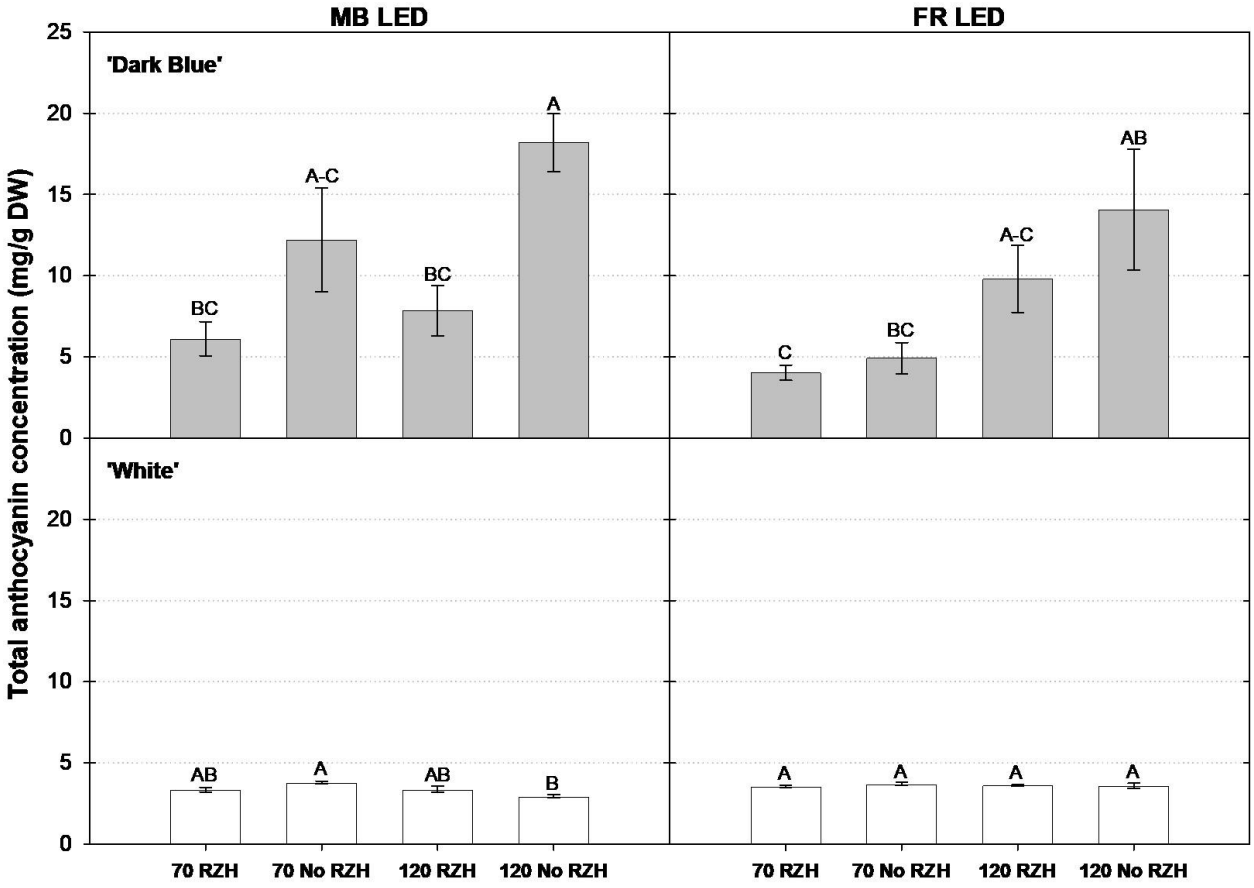

Figure IV-6: Average total anthocyanin content of petunia ‘Dark Blue’ and ‘White’. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m–2·s–1 and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light], respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey’s honestly significant difference (HSD) test ($P < 0.05$).

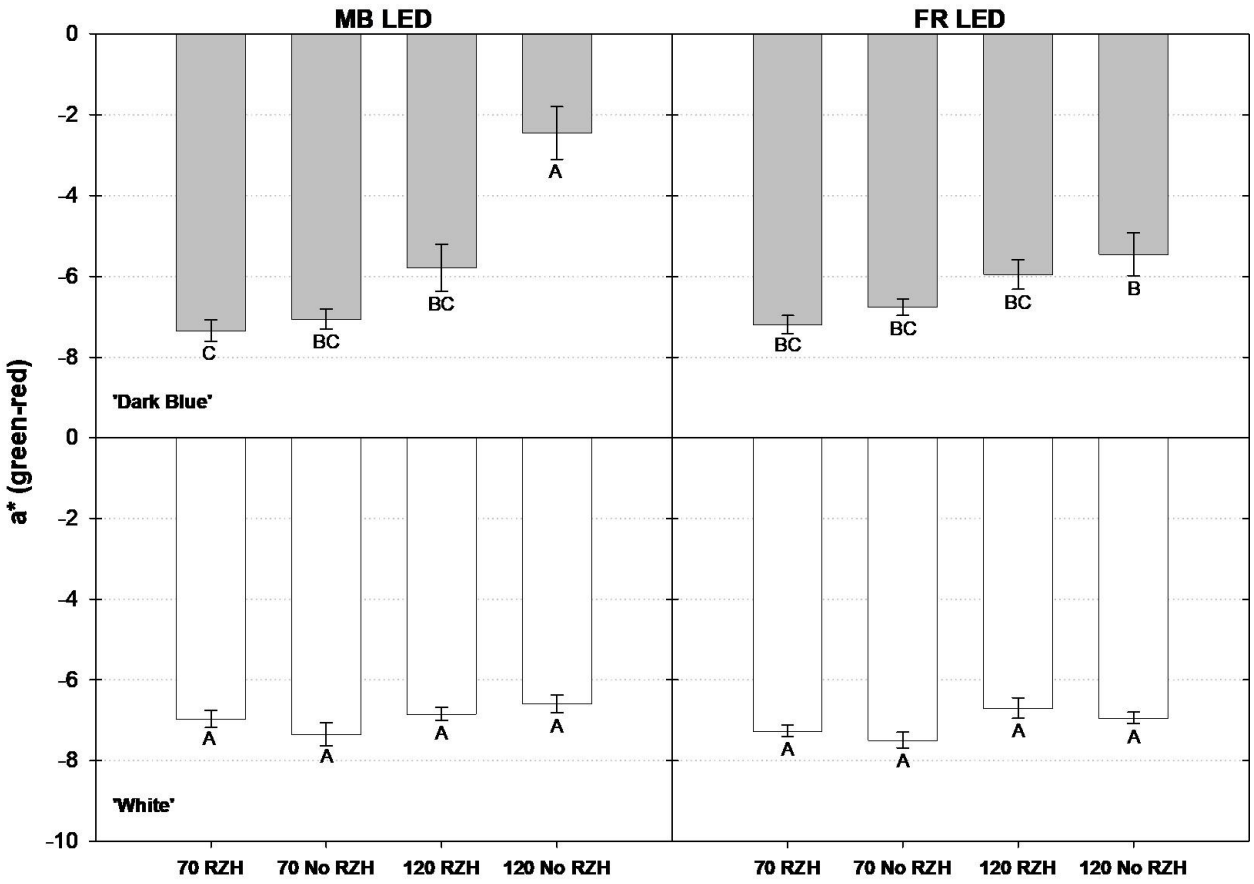

Figure IV-7: Average a* value of petunia ‘Dark Blue’ and ‘White’. More negative values denote a more green coloration while more positive values indicate a more red coloration. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m–2·s–1 and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light], respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey’s honestly significant difference (HSD) test (P <0.05).

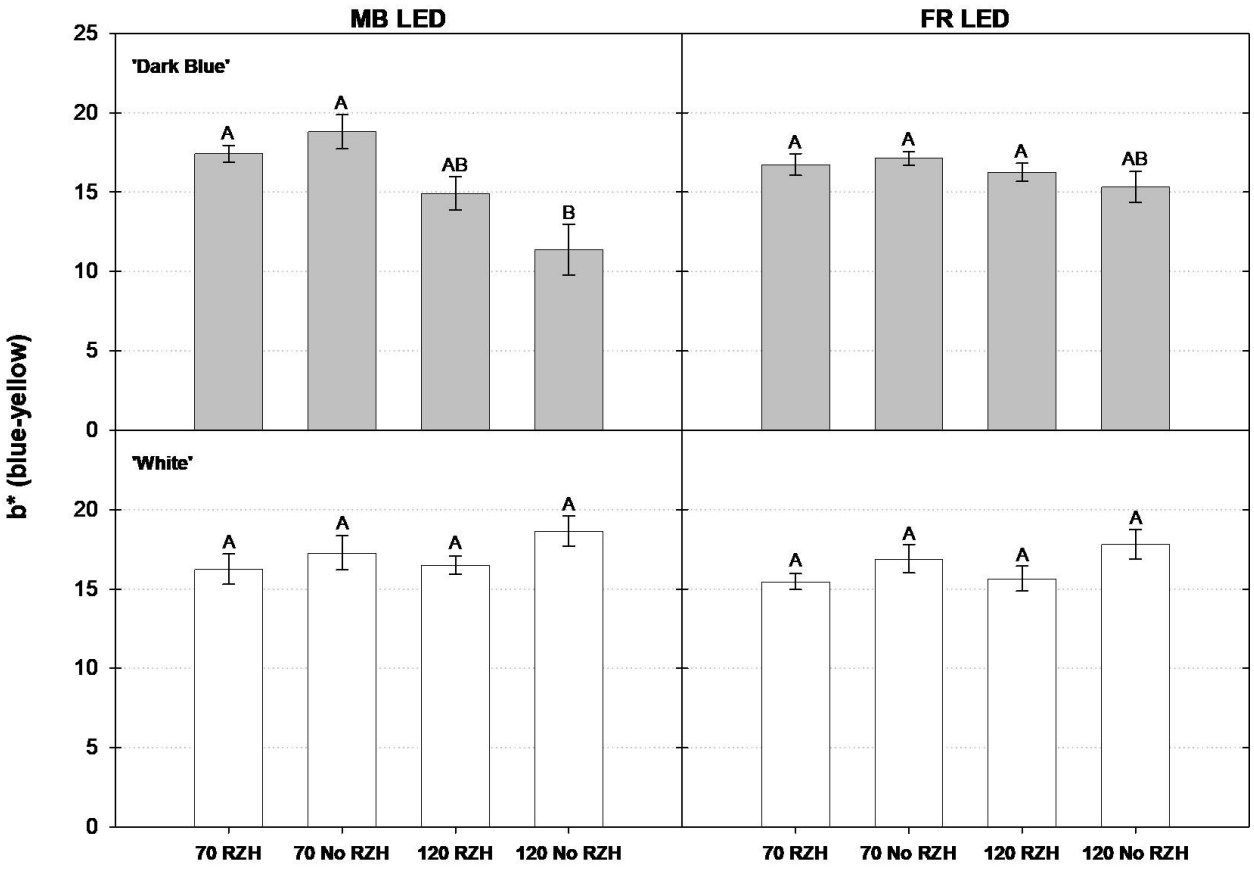

Figure IV-8: Average b* value of petunia ‘Dark Blue’ and ‘White’. More positive values indicate a more yellow coloration while less positive values denote a more blue coloration. Cuttings were rooted under supplemental light-emitting diode (LED) fixtures providing a total photon flux density (TPFD) of 70 or 120 µmol·m–2·s–1 and on benches with or without root-zone heating (RZH). Medium blue (MB) and far-red (FR) fixtures emitted a light ratio (%) of 10:7:82:1 and 10:18:59:13 blue:green:red:far-red light], respectively (combined Reps. 1 and 2). Error bars represent standard errors of the mean. Different uppercase letters across rows are significantly different within a cultivar according to Tukey’s honestly significant difference (HSD) test ($P < 0.05$).