Radiation Intensity and Quality Affect Indoor Acclimation of Blueberry Transplants

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Abstract. The objectives were to characterize and compare shrinkage (i.e., transplant loss) and growth of tissue-cultured blueberry (*Vaccinium corymbosum*) transplants acclimated in greenhouses or indoors under 1) different photosynthetic photon flux densities (PPFDs) (Expt. 1); or 2) spectral changes over time using broad-spectrum white (W; 400 to 700 nm) light-emitting diodes (LEDs) without or with red or far-red (FR) radiation (Expt. 2). In Expt. 1, ‘Emerald’ and ‘Snowchaser’ transplants were acclimated for 8 weeks under PPFDs of 35, 70, 105, or $140 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by W LED fixtures for $20 \text{ h}\cdot\text{d}^{-1}$. In another treatment, PPFD was increased over time by moving transplants from treatment compartments providing 70 to $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the end of week 4. Transplants were also acclimated in either a research or a commercial greenhouse (RGH or CGH, respectively). Shrinkage was unaffected by PPFD, but all transplants acclimated indoors had lower shrinkage ($\leq 4\%$) than those in the greenhouse (15% and 17% in RGH and CGH, respectively), and generally produced more shoot and root biomass, regardless of PPFD. Growth responses to increasing PPFD were linear in most cases, although treatment effects after finishing were generally not significant among PPFD treatments. In Expt. 2, ‘Emerald’ transplants were acclimated for 8 weeks under constant W, W + red (WR), or W + FR (WFR) radiation, all of which provided a PPFD of $70 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for $20 \text{ h}\cdot\text{d}^{-1}$. At the end of week 4, a group of transplants from WR and WFR were moved to treatment compartments with W (WR_W or WFR_W, respectively) or from W to a research greenhouse (W_{GH}), where another group of transplants were also acclimated for 8 weeks (GH). Shrinkage of transplants acclimated indoors was also low in Expt. 2, ranging from 1% to 4%. In contrast, shrinkage of transplants acclimated in GH or under W_{GH} was 37% or 14%, respectively. Growth of indoor-acclimated transplants was generally greater than that in GH or under W_{GH}. Although growth responses were generally similar indoors, plants acclimated under WFR had a higher root dry mass (DM) and longer roots compared with GH and W_{GH}.

Most specialty crops produced in the United States are propagated in greenhouses. Problems with daily and seasonal environmental changes during greenhouse propagation can lead to slow rooting, inconsistent

growth, poor transplant quality, and mortality, all of which result in significant loss of profits (commonly known as “shrinkage”). Although growers tend to alleviate some of these challenges with cooling and heating systems, supplemental lighting, and shade curtains, greenhouse conditions cannot be sufficiently controlled in a predictable and consistent manner (Runkle, 2020). For that reason, several greenhouse growers in the United States are considering the adoption of sole-source lighting for indoor propagation (Greenhouse Grower, 2020). Gibson et al. (2020) found that industry stakeholders are particularly interested in acclimating tissue culture (TC) transplants indoors. That is because the ex vitro acclimation process requires TC transplants to adjust to rapid changes in environmental conditions, which can be challenging to achieve in a greenhouse for some species and cultivars that require long periods of adjustment.

Blueberries, a specialty crop with an estimated U.S. value of \$822M (USDA, 2018), are typically propagated through TC in

a laboratory, followed by an acclimation phase under mist irrigation in a greenhouse. Although protocols have been developed to optimize in vitro micropropagation (Debnath and Goyali, 2020; Guo et al., 2019; Zimmerman and Broome, 1980), few published studies have evaluated methods to improve acclimation and reduce shrinkage after TC blueberries are transplanted ex vitro (Hung et al., 2016; Isutsa et al., 1994; Zobayed, 2020). The delicate nature of these transplants makes them highly susceptible to dehydration during greenhouse acclimation; therefore, up to 70% of shrinkage is common when blueberry transplants are moved to a greenhouse, particularly during the summer (T. Strode, personal communication).

Understanding growth limits of TC transplants like blueberry in response to radiation intensity (often measured as PPFD, 400 to 700 nm) is necessary, particularly considering that they are susceptible to various stresses before roots fully develop. Furthermore, rooting responses ultimately determine the capacity of most transplants to benefit from PPFDs that are conducive to faster growth indoors (He, 2020). Radiation quality (i.e., color, spectra) also has great potential to affect developmental processes that can lead to faster rooting and growth. For example, red (600 to 700 nm) and FR (700 to 800 nm) radiation may stimulate root formation of transplants due to phytochrome-mediated responses that regulate the development of root primordia (Christiaens et al., 2019). However, FR radiation tends to increase stem elongation and, thus, may not be desirable throughout the entire propagation period.

The objectives of this study were to characterize and compare shrinkage and growth of TC blueberry transplants acclimated in greenhouses or indoors under 1) different PPFDs (Expt. 1); or 2) spectral changes over time using broad-spectrum W (400 to 700 nm) LEDs without or with red or FR radiation (Expt. 2). We hypothesized that intermediate-to-high PPFDs used indoors would accelerate and increase growth compared with a low PPFD or greenhouse propagation. We further hypothesized that FR radiation would promote early root formation, resulting in lower shrinkage and more growth compared with indoor acclimation under W without or with red radiation or greenhouse propagation.

Materials and Methods

Plant material and early culture. In Expt. 1, TC ‘Emerald’ and ‘Snowchaser’ highbush blueberries were received from a commercial transplant supplier (AgriStarts, Apopka, FL) on 14 Feb. 2019. Industry standard, 288-cell propagation trays (5 mL individual cell volume; Blackmore Co., Belleville, MI) were cut into 20-cell (4×5) partial trays, filled with 100% unlimed blond Canadian *Sphagnum* peat (Premier Tech, Quebec, Canada), and sub-irrigated with $0.14 \text{ L}\cdot\text{m}^{-3}$ wetting agent (AquaGro 2000 M; Aquatrols, West Deptford, NJ). For each cultivar, 20 uniform

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unrooted transplants with a single 2- to 3-cm-tall stem were removed from their vessel and planted one plant per cell onto each partial tray, which were then placed inside a walk-in growth chamber (C6 Control System with EcoSys Software; Environmental Growth Chambers, Chagrin Falls, OH) on 15 Feb. 2019. Transplants were acclimated for 48 h under a PPFD of $35 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by broadband W LED fixtures (RAY66 PhysioSpec Indoor; Fluence Bioengineering, Austin, TX) used for 20 h·d⁻¹ (0500 to 0100 HR) and controlled by dimmers (Solunar, Fluence Bioengineering) connected to a backup battery (BE425M-LM; APC, West Kingston, RI). Average ambient temperature, relative humidity (RH), and carbon dioxide (CO₂) concentration were set at constant 20 °C, 95%, and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively.

In Expt. 2, TC 'Emerald' blueberries were received from the same transplant supplier (AgriStarts) on 16 June 2020. Industry standard, 128-cell propagation trays (10 mL individual cell volume, Blackmore Co.) were cut into nine-cell (3 × 3) partial trays filled with a commercial peat-based substrate composed of (v/v) 79% to 87% peatmoss, 10% to 14% perlite, and 3% to 7% vermiculite (PRO-MIX BX Mycorrhizae; Premier Tech Horticulture, Quebec, Canada). On 22 June 2020, nine uniform transplants were planted one plant per cell onto each partial tray and acclimated following the same procedures previously described.

Treatments. In Expt. 1, transplants were moved inside two walk-in growth chambers (C6 Control System) on 17 Feb. 2019. Each chamber was equipped with two opposite shelving units that served as blocks, each with four treatment compartments (61-cm width × 183-cm length × 47-cm height) that provided different PPFDs from broadband W LED fixtures (RAY66 PhysioSpec Indoor) to six partial trays per cultivar. The PPFD treatments were 35, 70, 105, and $140 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided for 20 h·d⁻¹ (0500 to 0100 HR), which resulted in daily light integrals (DLIs) of 2.5, 5.0, 7.6, or 10.1 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, respectively. Lamp output was controlled with dimmers (Solunar, Fluence Bioengineering) based on radiation maps generated using a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT) placed at midcanopy height. A fifth treatment consisted of increasing the PPFD

over time, which was achieved by moving six additional partial trays from treatment compartments providing 70 to 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the end of week 4 (INCR). In two additional treatments, four groups of three partial trays were placed on separate benches in either an RGH in Gainesville, FL, or a CGH in Apopka, FL (AgriStarts), both polycarbonate structures with computerized environmental control systems.

Similar to the setup previously described, each treatment in Expt. 2 was replicated in four individual treatment compartments (61-cm width × 183-cm length × 47-cm height) inside two walk-in growth chambers (C6 Control System), each with two opposite shelving units that served as blocks. Each compartment initially held six partial trays placed on 24 June 2020. The back and sides of all compartments were covered with a 0.3-mm thick black and white polyethylene film to minimize radiation pollution ($\leq 1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) within the experimental area. The radiation-quality treatments were constant W, WR, or WFR (detailed in Tables 1 and 2). All treatments provided a PPFD of $70 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Transplants under the WFR treatment also received 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR radiation, which resulted in a total photon flux density (PPFD + FR) of $140 \pm 2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Treatments were delivered by two broadband W LED fixtures (RAY66 PhysioSpec Indoor, Fluence Bioengineering) without or with a single red or FR LED fixture (RAY66, Fluence Bioengineering) with peak wavelengths of 664 and 730 nm, respectively, provided for 20 h·d⁻¹ (0500 to 0100 HR). At the end of week 4, three partial trays from WR and WFR were moved to additional treatment compartments with W (WR_W or WFR_W, respectively) or from W to a research greenhouse (W_{GH}), where they were grown for another 4 weeks to evaluate the effect of a spectral change over time. An additional treatment was included in which four groups of three partial trays were acclimated for 8 weeks in an RGH.

Environmental conditions and cultural practices during acclimation. In both experiments, the ambient setpoints for air temperature and CO₂ concentration of the chambers were set at day/night 22/18 °C and 800 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively. The RH was maintained at 95% during the first 2 weeks using ultrasonic foggers (12 Disc Mist Maker; The House of Hydro, Ft.

Myers, FL) and progressively reduced until levels reached 70%. Within each compartment, air temperature, RH, and leaf temperature were monitored with temperature and RH probes (HMP60-L; Campbell Scientific, Logan, UT) and fixed-mounted infrared radiometers (SI-131-SS, Apogee Instruments) interfaced to a datalogger (CR1000, Campbell Scientific). Data were measured every 15 s and the average was logged every 10 min. In each growth chamber, average CO₂ concentration was logged every 15 min by a built-in datalogger (DL1 Datalogger; Environmental Growth Chambers). In addition, until the end of week 4 in Expt. 2, all partial trays were covered with clear plastic, vented humidity domes (54 cm × 28 cm × 15 cm; Acro Dome, Acro Plastics, LTD, Edmonton, Alberta, Canada) to maximize RH during root formation. The average daily air temperature, leaf temperature, RH, and CO₂ concentration were (mean ± sd) 22.6 ± 0.1 °C, 23.7 ± 0.4 °C, 89.2% ± 5.6%, and 789 ± 37 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively, during Expt. 1, and 21.4 ± 0.1 °C, 21.8 ± 0.2 °C, 94.1% ± 2.0%, and 839 ± 97 $\mu\text{mol}\cdot\text{mol}^{-1}$, respectively, during Expt. 2.

In both experiments, transplants acclimated indoors were initially sub-irrigated with tap water [electrical conductivity of 0.4 dS·m⁻¹, pH of 8.3, and 40 mg·L⁻¹ of calcium carbonate (CaCO₃)]. Fertilizer application began at week 3, once root growth was visible. A water-soluble fertilizer (17N-4P-17K; Blackmore Company, Belleville, MI) was used with each sub-irrigation event, providing (in mg·L⁻¹) 19 ammonium (NH₄)-N, 56 nitrate (NO₃)-N, 10 P, 62 K, 15 Ca, 8 Mg, 0.38 Fe, 0.19 Mn, 0.19 Zn, 0.04 Cu, 0.08 B, and 0.04 Mo.

Transplants acclimated in the research greenhouse (RGH and GH for Expts. 1 and 2, respectively) were shaded during the first 4 weeks of the experiments using fixed shade curtains (56% shade; Solaro 5620 O-R-FR; Ludvig Svensson, Inc., Charlotte, NC). Aluminized 49% shade curtains (OLS 50, Ludvig Svensson, Inc.) controlled by an environmental control system (Gemlink; Hortimax, Rancho Santa Margarita, CA) were also retracted when the outdoor PPFD reached 750 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Environmental conditions were monitored with a portable datalogger (HOBO MicroStation; Onset Computer Corporation, Bourne, MA)

Table 1. Spectral characteristics of sole-source lighting treatments used to evaluate indoor acclimation of blueberry transplants in Expts. 1 and 2.

	Blue (401–500 nm)	Green (501–600 nm)	Red (601–700 nm)	Far-red (701–800 nm)	TPFD ^y (401–800 nm)	PPFD ^x (401–700 nm)	YPFD ^w	
Treatment ^z	(μmol·m ⁻² ·s ⁻¹)							R:FR ^v
W	14 (19%) ^u	29 (41%)	28 (39%)	1 (1%)	72	71	62	12
WR	8 (11%)	16 (24%)	46 (64%)	1 (1%)	71	70	65	64
WFR	12 (9%)	27 (19%)	30 (22%)	71 (50%)	140	70	75	0.2

^zW = white; R = red; FR = far-red light-emitting diodes; all treatments evaluated in Expt. 1 used W.

^yTPFD = total photon flux density (photon flux integral between 401 and 800 nm).

^xPPFD = photosynthetic photon flux density (photon flux integral between 401 and 700 nm).

^wYPFD = yield photon flux density, which is the product of TPFD and relative quantum efficiency, as calculated with a spectroradiometer (SS-110; Apogee Instruments Inc., Logan, UT).

^vR:FR = ratio of photon flux integral of R and FR radiation, as calculated with a spectroradiometer (SS-110, Apogee Instruments Inc.).

^aNumbers in parentheses represent the percentage of each waveband from TPFD.

Table 2. Mean shrinkage, leaf number, and leaf area of 'Emerald' blueberry transplants acclimated for 8 weeks indoors under sole-source lighting or in a greenhouse in Expt. 2.

Treatment code ²	Weeks 0 to 4	Weeks 5 to 8	Shrinkage (%)	Leaves (no.)	Leaf area (cm ²)
W	W	W	1.4 c ³	15.8 a	36.8 a
WR	W+R	W+R	1.4 c	14.5 ab	31.8 ab
WFR	W+FR	W+FR	1.4 c	15.4 ab	40.9 a
GH	GH	GH	36.8 a	14.7 ab	17.1 b
W _{GH}	W	GH	13.9 b	13.4 b	17.3 b
WR _W	W+R	W	1.4 c	15.9 a	36.7 a
WFR _W	W+FR	W	4.2 c	15.3 ab	30.8 ab

²W = white; R = red; FR = far-red light-emitting diodes; GH = greenhouse. Treatment codes with a subscript indicate that transplants were moved to another environment at week 4 (represented by the subscript).

³Means within column followed by the same letter are not different based on Tukey's honestly significant difference test at $P \leq 0.05$ ($n = 4$).

and the average DLI, air temperature, and RH were $3.8 \pm 1.3 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $23.2 \pm 0.4^\circ\text{C}$, and $81.5\% \pm 9.0\%$, respectively, during Expt. 1 and $7.4 \pm 1.5 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $27.4 \pm 1.6^\circ\text{C}$, and $85.6\% \pm 8.6\%$, respectively, during Expt. 2.

In both experiments, transplants in the research greenhouse (RGH and GH for Expts. 1 and 2, respectively) were irrigated using overhead mist with 69 μm -diameter emitters (Coolnet Pro Fogger, Netafim, Israel) spaced 91 cm apart. During the first 2 weeks, mist was provided for 10 s every 15 min or when the radiation level inside the greenhouse accumulated $100 \text{ mmol}\cdot\text{m}^{-2}$ per mist cycle, and every 30 min at night. On weeks 3 to 4, mist was provided for 7 s every 20 min or when the radiation level inside the greenhouse accumulated $120 \text{ mmol}\cdot\text{m}^{-2}$ per mist cycle, and every 2 h at night. Mist frequency was further reduced from weeks 4 to 8 during the daytime to 7 s every hour or when the radiation level inside the greenhouse accumulated $120 \text{ mmol}\cdot\text{m}^{-2}$, with no mist provided during the night period. The same fertilizer previously described was applied as a foliar spray once daily from week 3 onward.

In Expt. 1, transplants in the CGH were acclimated following standard commercial practices. Transplants were moved from the laboratory into the greenhouse immediately after planting into twelve 20-cell (4×5) partial trays (5 mL individual cell volume) arranged in four groups of three. Mist irrigation was provided for 4 s every 12 min throughout the experimental period and transplants were never fertilized. Environmental conditions were monitored with a portable datalogger (HOBO MicroStation) and the average daily DLI, air temperature, and RH during the experiment were $4.3 \pm 1.4 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $22.8 \pm 1.9^\circ\text{C}$, and $79.4\% \pm 8.9\%$, respectively.

Finishing culture and environment. In both experiments, after 8 weeks of treatment exposure, two randomly selected transplants from each partial tray were transplanted into 72-cell trays (30.7 mL individual cell volume) filled with the same commercial substrate used during acclimation. Transplants were grown for 3 weeks in a computer-controlled greenhouse with polycarbonate glazing located in Gainesville, FL. The average DLI, air temperature, and RH were $13.5 \pm 6.3 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $23.7 \pm 0.7^\circ\text{C}$, and $81.2\% \pm 10.4\%$, respectively, during Expt. 1, and $12.4 \pm 4.1 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, $26.8 \pm 1.3^\circ\text{C}$, and

$88.2\% \pm 2.9\%$, respectively, during Expt. 2. Transplants were overhead fertigated as needed using the same fertilizer solution described previously.

Data collection and analyses. In both experiments, shrinkage was quantified as transplant loss percentage per partial tray at week 8. In Expt. 1, five randomly selected transplants per partial tray were destructively harvested at weeks 4 and 8. Stem length was measured with a ruler from the base of the substrate surface to the top of the transplant canopy. Shoot fresh mass (FM) was measured at harvest, and shoots were oven-dried to a constant mass at 70°C for shoot DM determination. After roots were washed from the substrate, they were separated and spread on a $10 \text{ cm} \times 15 \text{ cm}$ polycarbonate container filled with a thin layer of water and laid on a horizontal plane to acquire images of root morphology with a scanner (Perfection 4990 Photo; Epson, Suwa, Japan). Total root length and root area were calculated from scanned images using WinRHIZO (Regent Instrument Inc., Quebec, Canada). Immediately after scanning, roots were oven-dried to a constant mass at 70°C for DM determination.

In Expt. 2, three randomly selected transplants per partial tray were destructively harvested from the four initial treatments (W, WR, WFR, and GH) at week 4. Data collected included stem length, shoot and root DM, length of the longest root (measured with a ruler), and rooting quality based on a subjective scale from 1 to 5, where 1 = no or few roots, not transplantable, 2 = lopsided and/or weakly developed root system, 3 = moderately developed root system, 4 = well-developed root system, and 5 = extensive root system distributed around the tray cell. At week 8, stem length and leaf number per transplant ($>1 \text{ cm}$) were measured from all remaining transplants in the seven final treatments. Leaf area was subsequently measured from up to three randomly selected transplants per partial tray (depending on shrinkage) using a leaf area meter (LI-3100C; LICOR Biosciences, Lincoln, NE). Shoot and root DM, length of the longest root, and rooting quality were measured from those same transplants following the procedures described previously. In both experiments, stem length, number of branches, and shoot and root DM were measured at week 11 from all transplants

acclimated in the greenhouse during the finish stage.

One transplant per partial tray was used to quantify chlorophyll and anthocyanin concentration in Expt. 2 using 3.1-cm^2 disks collected at week 8 from middle-canopy leaves. Tissue samples were flash-frozen in liquid nitrogen and immediately placed in a freezer at -80°C until the point of extraction. Chlorophyll content was measured following the dimethyl sulfoxide (DMSO) extraction method described in Richardson et al. (2002). Glass vials were wrapped in aluminum foil to protect samples from radiation exposure. The vials contained 7 mL DMSO and were pre-heated in a 65°C water bath. Samples were placed inside vials with DMSO and extracted for 30 min in the dark, after which the extracted liquid was brought to a volume of 10 mL with DMSO, and 2.5 mL of each extract was then transferred to a disposable polystyrene cuvette. Pure DMSO was used as the blank. The absorbance of both blank and samples were measured with a spectrophotometer (SpectraMax Plus 384; Molecular Devices, Sunnyvale, CA) at 480, 649, and 665 nm. Chlorophyll was calculated using the equations from Wellburn (1994). Anthocyanins were measured using the methanol extraction method described in Gould et al. (2000). Samples were agitated gently in the dark for 24 h at 4°C in 1 mL of 3 M hydrogen chloride, water, and methanol (1:3:16 v/v). Samples were then placed in a centrifuge for 15 min. The absorbance of the extracts was measured with a spectrophotometer at 530 (A530) and 653 (A653) nm, with methanol used as the blank solution. Anthocyanin concentration was calculated as $A530 - (0.24 \times A653)$.

Each experiment was performed once and had four treatment replications. In both experiments, transplants acclimated indoors were arranged in a randomized complete block design in which each shelf within a growth chamber was regarded as a block with one treatment compartment as a replication. Greenhouse-acclimated transplants were arranged in a completely randomized design with four replicate group sections. In Expt. 1, data from six or three partial trays in the indoor or greenhouse treatments, respectively, were averaged and treated as a single data point per replication for each cultivar. Because the cultivar $\times$ treatment interaction was not significant ($P > 0.05$), data were

pooled for the main effect treatment means ($n = 8$). A linear regression analysis was then conducted to compare growth trends from transplants acclimated under all constant PPFD treatments (35, 70, 105, and $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) using JMP (Version 15; SAS Institute Inc., Cary, NC). To identify differences among all treatments (including INCR, RGH, and CGH), pairwise comparisons for the main effect treatment means were also completed using Tukey's honestly significant difference (HSD) test ($P \leq 0.05$). During the finish stage, transplants were arranged in a completely randomized design. Regression analyses and treatment mean comparison were completed following the same procedures previously described. In Expt. 2, data collected from the same partial tray were averaged and

treated as a single data point per replication ($n = 4$). Pairwise comparisons for the main effect treatment means were completed using Tukey's HSD test ($P \leq 0.05$).

Results

Expt. 1. Although shrinkage was unaffected by PPFD, transplants acclimated indoors had a significantly lower shrinkage percentage ($<4\%$) than those in the greenhouse, where at week 8, shrinkage of transplants acclimated in RGH or CGH was 15% or 17%, respectively (Fig. 1A). There were no treatment differences in stem length at week 4 (Fig. 1B); however, there was a negative linear relationship between PPFD and stem length at week 8. Transplants acclimated under $105 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, INCR, or those

acclimated in RGH or CGH had 18%, 15%, 17%, or 17% shorter stems, respectively, than those acclimated under a PPFD of $35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Similarly, transplants acclimated under PPFDs of 70 or $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, as well as those under INCR, had shorter stems than those acclimated in CGH at week 8. In both harvest weeks, shoot DM increased in response to PPFD (Fig. 1C). At week 4, transplants acclimated under PPFDs of 105 or $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had a similar shoot DM, which was 47% to 282% higher than that in any other treatment. At week 8, transplants acclimated indoors under a PPFD of $35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or in CGH had lower shoot DM than those in all other treatments, and this shoot growth was also lower than that at week 4 under 105 or $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Fig. 1. Shrinkage and shoot growth of blueberry transplants acclimated indoors under five photosynthetic photon flux density (PPFD) treatments (where INCR = plants moved to an increasing PPFD from 70 to $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at week 4), or in a research (RGH) or commercial (CGH) greenhouse in Expt. 1. Equations, correlation coefficients (r^2), and P values are presented when the linear response to increasing PPFD was statistically significant (solid line) but not when not significant (dashed line). Means followed by the same letter are not different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Data points represent the treatment mean $\pm$ SE ($n = 8$).

Fig. 2. Root growth of blueberry transplants acclimated indoors under five photosynthetic photon flux density (PPFD) treatments (where INCR = plants moved to an increasing PPFD from 70 to 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at week 4), or in a research (RGH) or commercial (CGH) greenhouse in Expt. 1. Equations, correlation coefficients (r^2), and P values are presented when the linear response to increasing PPFD was statistically significant (solid line) but not when not significant (dashed line). Within week, means followed by the same letter are not different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Data points represent the treatment mean $\pm$ SE ($n = 8$).

Total root length increased with increasing PPFD in both harvest weeks (Fig. 2A). However, the positive effects of higher PPFDs were more noticeable at week 8, when transplants acclimated under INCR or under PPFDs of 105 or 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced the largest roots. In contrast, transplants acclimated under a PPFD of 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or in CGH produced the shortest roots. There was a positive linear response to PPFD for root area at week 4 (Fig. 2B); however, the response to PPFD was not significant for root area at week 8, as transplants acclimated under a PPFD of 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced from 27% to 114% more root area than those in any other treatment. Root DM also had a positive linear response to PPFD in both harvest weeks (Fig. 2C), which suggests that the response to root area at week 8 under

70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ could be attributed to human error. At week 4, transplants acclimated under PPFDs of 105 or 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced from 39% to 102% more root DM than those under 35 or 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, or those acclimated in RGH or CGH, but their root DM was similar to that produced under INCR. At week 8, root DM was lowest in transplants acclimated under a PPFD of 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or in CGH. Similar to results for shoot DM, transplants acclimated under 105 or 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ had already produced more root DM at week 4 than that produced under 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or in CGH at week 8.

The linear response to PPFD was not significant during the finish stage for stem length, shoot DM, and root DM (Fig. 3A–C); however, stems of transplants acclimated under 70 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were 9%, 13%, 35%, or 40%

longer than those under 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, INCR, RGH, or CGH, respectively. In addition, stems of greenhouse-acclimated transplants were consistently shorter than those acclimated indoors. There was also a slight decrease in the number of branches of transplants acclimated in CGH compared with those indoors. Further, transplants acclimated under a PPFD of 35 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced up to 41% more branches than those in either greenhouse environment (Fig. 3D). Regardless of PPFD, transplants acclimated indoors produced more root and shoot biomass during the finish stage than those acclimated in RGH or CGH.

Expt. 2. At week 8, shrinkage of transplants acclimated indoors ranged from 1% to 4% (Table 2). In contrast, shrinkage of transplants acclimated in GH was 37%, which was higher than that of those moved from W

Fig. 3. Growth effects after finishing of blueberry transplants acclimated indoors under five photosynthetic photon flux density (PPFD) treatments (where INCR = plants moved to an increasing PPFD from 70 to 140 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at week 4), or in a research (RGH) or commercial (CGH) greenhouse in Expt. 1. Equations, correlation coefficients (r^2), and P values are presented when the linear response to increasing PPFD was statistically significant (solid line) but not when not significant (dashed line). Means followed by the same letter are not different based on Tukey's honestly significant difference test ($\alpha = 0.05$). Data points represent the treatment mean $\pm$ SE ($n = 8$).

to the GH (W_{GH}), which had a shrinkage percentage of 14%. At week 4, transplants acclimated continuously under FR (W_{FR}) had 10% or 8% longer stems than those under W or in GH, respectively, but stem length was similar between transplants acclimated under W_{FR} and W_{R} (Table 3). At week 8, stems of

transplants acclimated under W_{FR} were longer than those under W_{FR} , W_{GH} , or in GH.

At week 4, transplants acclimated in GH produced the lowest shoot DM, and values were similar among all indoor-acclimated transplants (Table 3). At week 8, transplants acclimated under W_{FR} and W_{R} had 87%

higher shoot DM than those under W_{GH} . At week 4, transplants under W_{FR} had 163% or 85% higher root DM than those in GH or under W_{GH} , respectively, but root DM was similar between W_{FR} and W_{R} . Similarly, transplants under W_{FR} had more than twice the root DM of those in GH or under W_{GH}

Table 4. Growth effects after finishing ‘Emerald’ blueberry transplants acclimated indoors under sole-source lighting or in a greenhouse in Expt. 2.

Treatment	Stem length (cm)	Branches (no.)	Shoot dry mass (mg)	Root dry mass (mg)
W	11.0 a	2.2 a	0.136 ab	0.027 ab
WR	10.7 ab	2.3 a	0.138 ab	0.030 ab
WFR	11.1 a	2.5 a	0.160 a	0.032 a
GH	8.9 b	1.9 a	0.072 b	0.018 b
W _{GH}	9.2 ab	1.8 a	0.066 b	0.014 b
WR _W	11.3 a	2.3 a	0.155 a	0.029 ab
WFR _W	10.1 ab	2.3 a	0.112 ab	0.027 ab

²W = white; R = red; FR = far-red light-emitting diodes; GH = greenhouse. Treatment codes with a subscript indicate that transplants were moved to another environment at week 4, represented by the subscript (see Table 2).

³Means within column followed by the same letter are not different based on Tukey’s honestly significant difference test at $P \leq 0.05$ ($n = 4$).

qualities like those used in our studies can maintain a low shrinkage percentage compared with GH propagation (Table 2, Fig. 1A). Although controlled environments offer opportunities to control environmental conditions in ways that can help minimize dehydration *ex vitro*, further studies are needed to better understand rate-limiting steps during indoor propagation. For example, elucidating how different PPFDs from sole-source lighting affect leaf-energy dynamics and vapor pressure deficit could help identify optimal control strategies that can minimize dehydration and maximize rate of adventitious rooting. In addition, careful quantification of the effects that radiation quality has on transpiration and water loss could prove to be beneficial when selecting LED fixtures for indoor acclimation.

Radiation quantity effects on growth. It is widely known that low PPFDs cause stem elongation as a shade-avoidance response in plants (Franklin and Whitelam, 2005). Accordingly, others have reported elongated stems of transplant grown under low compared with high PPFDs (Hernández and Kubota, 2014; Lopez and Runkle, 2008; Park et al., 2011; Pramuk and Runkle, 2005; Torres and Lopez, 2011), which correspond with our finding for stem length at week 8 (Fig. 1B). A plausible explanation for the lack of treatment differences in stem length at week 4 could be related to the fact that active shoot growth was most likely delayed at the expense of root formation and growth during the first few weeks of acclimation. This delay is not uncommon, as TC transplants must transition from heterotrophic to autotrophic conditions before resuming active plant growth (Pospíšilová et al., 1999; Van Huylenbroeck and Riek, 1995).

In both harvest weeks, higher PPFDs during indoor acclimation linearly increased shoot and root DM of blueberry transplants (Figs. 1C and 2C). This corresponds with the findings of others who have reported a general increase in biomass production of transplants with higher (but not saturating) PPFDs (Currey et al., 2012; Hernández and Kubota, 2014; Isutsa et al., 1994; Loach and Gay, 1979; Lopez and Runkle, 2008; Owen and Lopez, 2018; Pramuk and Runkle, 2005; Tombesi et al., 2015; Torres and Lopez, 2011). Numerous studies have shown that for seedlings, and to an extent for unrooted cuttings, higher DLIs typically provided with higher PPFDs, promote root initiation and formation, and shoot and root growth (Faust

et al., 2017; Randall and Lopez, 2014). These enhancements in growth and development in response to higher DLIs are often associated with an increase in carbohydrate availability by the synthesis of photoassimilates (Rapaka et al., 2005). However, as indicated in some of the studies highlighted previously, transplants have maximum PPFD thresholds where processes can be inhibited. These thresholds are typically lower for TC transplants than for seedlings and unrooted cuttings. For example, the recommended PPFDs for the initial stages of propagation range from ≈ 100 to $250 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for seedlings (He, 2020; Randall and Lopez, 2014) and 120 to $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for unrooted cuttings (Faust et al., 2017). In contrast, PPFDs $> 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ are commonly considered stressful when TC transplants start to become photoautotrophic *ex vitro*. A potential alternative to increase growth of TC transplants using higher DLIs is to extend the photoperiod, provided that long days do not affect subsequent growth and development.

Except for shoot DM at week 4, transplants acclimated under PPFDs of 105 or $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced similar shoot and root growth to those acclimated under INCR (Figs. 1 and 2). In addition, considering that PPFD minimally affected subsequent transplant growth at the finish stage (Fig. 3), initially acclimating TC transplants under $70 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and subsequently providing higher PPFDs could be used as a strategy to reduce energy costs during indoor propagation, as the energy cost from sole-source lighting is expected to be the highest operating cost (Fisher et al., 2020). Nevertheless, only transplants acclimated under 105 or $140 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ produced more shoot and root DM than those in CGH at week 4, illustrating the potential to reduce the propagation cycle by constantly providing higher PPFDs during indoor acclimation.

In general, growth responses to increasing PPFD during indoor acclimation were linear, suggesting that higher PPFDs could further increase subsequent growth of blueberry transplants (Figs. 1 and 2). However, further studies are needed to identify the PPFD threshold that will maximize growth without inducing undesirable stress responses, which could include the production of reactive oxygen species in chloroplasts and the accumulation of antioxidant enzymes, among others (Van Huylenbroeck et al., 2000; Vieira et al., 2019).

Accordingly, although not measured in Expt. 1, we found that plants acclimated under higher PPFDs showed a red leaf pigmentation (Fig. 5). Isutsa et al. (1994) also reported a “red discoloration” in the leaves of TC blueberry transplants acclimated under $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ compared with those under 30 or $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. As described by others, red photoprotective pigments like anthocyanins tend to increase in response to high PPFD to help reduce excess radiation from reaching chloroplasts in leaves (Steyn et al., 2002; Trojak and Skowron, 2017). Interestingly, this red pigmentation was not visible at the end of the finish stage (data not shown). It is unclear if the changes in pigmentation indoors limited growth in response to higher PPFDs. However, studies have shown that plant growth and the accumulation of photoprotective pigments like anthocyanins are competing processes, and thus, the increase of one may result in a decrease of the other (Boldt et al., 2014).

Radiation-quality effects on growth. The differences among radiation-quality treatments were small and spectral changes over time (WR_W or WFR_W) did not provide significant growth advantages compared with constant radiation-quality treatments (W, WR, or WFR) (Tables 2 and 3). However, growth of indoor-acclimated transplants was generally greater

Fig. 5. Representative ‘Snowchaser’ blueberry transplants after 8 weeks of indoor acclimation under different photosynthetic photon flux densities (PPFD) in Expt. 1.

than that in GH or under W_{GH} . Although transplants acclimated under W, WFR, and WR_W produced larger leaves than those in GH or under W_{GH} after 8 weeks (Fig. 4), only those acclimated under WFR produced a higher root DM and longer roots than in GH or under W_{GH} .

Others have shown positive rooting effects in response to FR radiation, which is thought to regulate adventitious root formation by inducing changes in auxin homeostasis and signaling (Christiaens et al., 2016). Christiaens et al. (2019) reported a higher root DM when chrysanthemum (*Chrysanthemum morifolium*) cuttings were grown under a PPFD of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ supplemented with $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of FR compared with monochromatic red or blue + red + FR radiation with a total photon flux density (PPFD + FR) of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The authors suggested that the beneficial rooting effect of FR radiation is associated with a shade-avoidance response that increases auxin biosynthesis (Christiaens et al., 2019). These auxins increase stem elongation and leaf area expansion and improve rooting success of cuttings. Accordingly, we found that transplants acclimated under WFR produced longer stems than those in GH and W_{GH} (Table 3), and the differences were maintained between WFR and GH after 3 weeks of growth during the finish stage (Table 4). Transplants under W and WR_W also produced longer stems than those in GH during acclimation and after the finish stage, suggesting that other factors beyond FR radiation affected stem elongation.

In addition to potential rooting advantages, several studies have reported growth benefits when growing transplants under different proportions of FR radiation. Some of those benefits include increases in shoot DM and early flower induction (Park and Runkle, 2017, 2018, 2019). Elkins and van Iersel (2020) evaluated 18 intensities of supplemental FR radiation on the growth and morphology of foxglove (*Digitalis purpurea*) seedlings. The authors were unable to identify a saturating photon flux density of FR radiation, as the overall growth responses were linear across the range of FR intensities evaluated (Elkins and van Iersel, 2020). Although they reported that FR had little to no effect on specific leaf area and seedling compactness, height increased by 38% as FR increased. This and other studies suggest that FR-induced increases in canopy size can enable plants to capture more radiation to drive photosynthesis and growth (Legendre and van Iersel, 2021). However, it is important to consider that although FR helps increase radiation interception and potentially, biomass accumulation, it typically induces stem elongation, which is generally undesirable in propagation.

Based on our results from Expt. 2 and the findings of others, supplementing PPFD with FR radiation has potential to improve growth and adventitious rooting of transplants (Tables 2–4). However, recommended intensities of FR to maximize rooting while maintaining compact growth are unknown, and those are likely to be species-specific. Further studies are needed to evaluate different

intensities of FR radiation, enabling the differentiation from potential effects on leaf area expansion and stem elongation (Elkins and van Iersel, 2020) vs. hormonal effects on adventitious rooting (Christiaens et al., 2019). In addition, although there were no differences in chlorophyll and anthocyanin concentration, the obvious changes in leaf pigmentation observed in our study could be indicators of differences in the photosynthetic capacity of blueberry transplants in response to FR (Fig. 4), which should be further explored to better understand the potential to maximize productivity of indoor propagation with LEDs.

In conclusion, indoor acclimation offers significant opportunities to help reduce shrinkage, which is particularly beneficial for TC blueberry transplants that are highly susceptible to shrinkage during ex vitro acclimation in greenhouses. Growth responses to increasing PPFD during indoor acclimation were linear in most cases, suggesting that higher PPFDs could further increase subsequent transplant growth. Although treatment effects after finishing were generally similar among the PPFD treatments evaluated in Expt. 1, constantly providing higher PPFDs during indoor acclimation could help reduce the propagation cycle for blueberry transplants. Furthermore, increasing the PPFD over time with INCR had small growth effects but could prove to be an economical alternative for indoor acclimation of TC transplants, as it could help reduce the operational expense of providing sole-source lighting.

Our findings from Expt. 2 show that growth of indoor-acclimated transplants was generally greater than that in GH or under W_{GH} . However, spectral changes over time did not provide major growth advantages compared with constant radiation-quality treatments indoors. Transplants acclimated under WFR generally produced a higher shoot and root DM than those in GH or under W_{GH} , and these differences were maintained after 3 weeks of growth in the finish stage. Our findings suggest that supplementing PPFD with FR radiation has potential to improve growth and adventitious rooting of blueberry transplants. However, recommended intensities of FR to maximize rooting while maintaining compact growth are unknown. Further studies are needed to better understand rate-limiting steps during indoor propagation, particularly those in response to radiation quantity and quality. In addition, more work is needed to explain the relationship between leaf pigmentation and growth of blueberry transplants.

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