

# Response of Standard Eastern Cottonwood and Novel Black Willow Clones to Artificial Lighting

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## Abstract

Dormant hardwood cuttings provide convenient propagation material for various forestry applications. The success of these applications, however, depends on achieving high survival of outplanted rooted cuttings. The type and intensity of artificial lighting during nursery production may affect subsequent field performance of rooted cuttings. Dormant hardwood cuttings of eastern cottonwood (*Populus deltoids* W. Bartram ex Marshall) and black willow (*Salix nigra* Marshall) were exposed to low-intensity wide-spectrum fluorescents, low-intensity LEDs (light-emitting diodes), and high-intensity LEDs for 33 days. Biomass partitioning did not differ among light treatments but root, shoot, and total biomass were higher for black willow compared to eastern cottonwood. For eastern cottonwood, light treatments had no significant effect on net photosynthesis-light response curves, although low-intensity LEDs tended to have the highest shoot-root ratios and maximum photosynthesis. Photosynthetic parameters were not measured on black willow. LEDs' specific light spectrum capabilities and efficient energy production may be a practical, cost-effective tool for improving outplanted seedling quality. Additional research is warranted.

## Introduction

Poplars (*Populus* spp.) and willows (*Salix* spp.) grow rapidly, propagate readily, and are relatively easy to use in tree breeding and biotechnology programs. Because of these characteristics, these species are attractive candidates for short-rotation plantations around the world (Isebrands and Richardson 2014). These plantations can be cultivated to provide fuelwood, pulpwood, bioenergy, and biomass on marginal

lands that are generally undesirable for agricultural use (Isebrands and Karnosky 2001, Rousseau et al. 2012, Volk et al. 2011, Zalesny et al. 2011). The common production system for these trees is through unrooted shoots from a stoolbed nursery, where stools (i.e., stumps) are maintained to produce fresh shoots (FAO 1980, Stanturf et al. 2001). These shoots are excised (i.e., cuttings) and directly outplanted or further developed in outdoor nursery beds or indoor greenhouse beds/containers for 1 to 2 years to develop rooted dormant sets (Isebrands and Richardson 2014). Rooted cuttings' root and shoot systems are commonly trimmed to ease transportation to planting sites, balance above- and below-ground biomass, and reduce planting stress (DesRochers and Tremblay 2009, Grossnickle 2005). Planted poplar and willow rooted cuttings need to be vigorous to outgrow competing vegetation, since they are shade intolerant and accordingly prone to stress from competition.

Proper site preparation (e.g., herbicide, disking, and ripping) in conjunction with pre-planting trimming is critical to maximize cutting growth and survival after outplanting (Dickmann and Stuart 1983, FAO 1980, Stanturf et al. 2001). Survival and growth of planted cuttings has been associated with planting depth, genetics, shoot-root ratios, and cutting length and diameter, which correspond to the amount of stored nutrients and carbohydrates (Burgess et al. 1990, Farmer 1970, Robison et al. 2006, Schuler and McCarthy 2015, Stanturf et al. 2001, Woolfolk and Friend 2003, Verwijst et al. 2012, Zalesny et al. 2011). However, poor planting site conditions, such as well-drained sand, poorly drained silty clay loam, and poorly drained loam soils can diminish seedling survival (Baker and Broadfoot 1979, Dickmann and Stuart 1983, Stanturf et al. 2001).

Artificial lighting technologies to induce favorable seedling biomass partitioning (e.g., high root biomass) and physiology may enhance competitive potential of rooted cuttings on poor sites, thereby reducing the need for competition control and increasing first-rotation yields (Ceulemans et al. 1996, Kuzokina and Quigley 2005, Rousseau et al. 2012). Supplemental lighting has long been utilized within commercial greenhouse production environments where lower light conditions limit plant production (Heuvelink et al. 2006). Currently, high pressure sodium (HPS) lamps are the most common source for supplemental lighting because of their ability to efficiently produce light (Ieperen and Trouwborst 2007). White fluorescents have also been used successfully as supplemental lighting, especially for inducing rooting of cuttings (Cavusoglu et al. 2011). Light emitting diodes (LEDs) were originally investigated in the late 20th century to determine their potential as lighting systems for space-based plant growing systems (Bula et al. 1991). Early work focused on several food crop species such as wheat, radish, spinach, and lettuce (Goins et al. 1997, Yorio et al. 2001). As LED research developed, other applications, such as plant tissue culture and horticultural, were quickly realized (Tennessen et al. 1994). The high intensity and specific light spectrum that LEDs offer may help stimulate favorable stock material characteristics, such as adequate leaf area and root formation (Morrow 2008).

The objective of this study was to examine the effect of different artificial lighting treatments on growth and physiological performance of two species of dormant hardwood clonal cuttings. An established standard eastern cottonwood (*Populus deltoides* W. Bartram ex Marshall) clone (ST-66) was selected to contrast with the novel black willow (*Salix nigra* Marshall) clone (BRZ 3-4). We hypothesized that, while both species show fast growth characteristics, black willow's exceptional rooting capacity (Rousseau et al. 2012) would produce higher total biomass with lower shoot-root ratios in the short run compared with the eastern cottonwood. We also hypothesized that use of LEDs would result in differential biomass partitioning of cuttings as compared to that observed under wide spectrum fluorescents.

## Methods

### Study Species

Eastern cottonwood clone ST-66 was established as a superior clone during late 1960s clonal trials performed by the U.S. Department of Agriculture, Forest Service's Stoneville, MS, office (Mohn et al. 1970). ST-66 was a male clone originally collected from Issaquena County, MS, that exhibited below-average straightness, relatively large branch formation, and relatively late leaf-off dates. In further testing, ST-66 cuttings had a first-year survival of 93 percent, a 5-year height of 17.9 m (58.7 ft) on a silt loam site and 9.9 m (32.5 ft) on a sharkey clay site, and an average 5-year diameter-at-breast-height of 20.6 cm (8.1 in) on the silt loam site and 10.2 cm (4 in) on the sharkey clay site (Mohn et al. 1970). Black willow BRZ 3-4 is a novel clone that is being examined as a component of a new initiative towards black willow biomass production within the lower Mississippi River Alluvial Valley region (Rousseau et al. 2012). The BRZ clone's origin is within the Brazos Rivers collection site in eastern Texas (Rousseau 2016).

### Cuttings Preparation and Light Treatments

Cuttings of eastern cottonwood clone ST-66 and black willow clone BRZ 3-4 were collected from a 1-year-old, coppiced, stoolbed orchard in Stoneville, MS, during late winters of 2013 and 2014. All cuttings were transferred on ice to the University of Arkansas at Monticello and placed in cold storage (4.0°C [39.2°F]) to prevent premature bud break or adventitious root initiation. In early spring 2015, 27 ramets of each clone were taken out of cold storage, rinsed with tap water, and trimmed to 20 cm (7.9 in) in length. Eastern cottonwood cuttings midpoint diameters averaged  $1.3 \pm 0.04$  cm (mean  $\pm$  SE;  $0.51 \pm 0.02$  in) and black willow diameters averaged  $1.5 \pm 0.05$  cm ( $0.59 \pm 0.02$  in). Each cutting was vertically placed, bud tips pointed up, in  $\sim 8$  cm (3.1 in) of ddH<sub>2</sub>O for  $\sim 48$  hours to promote rooting (Desrochers and Thomas 2003, Schaff et al. 2002). All 54 cuttings were planted individually in 950 ml (1 qt) Mini-Treepots™ (Stuewe and Sons, Inc., Tangent, OR) containing, hard-packed, EarthGro® topsoil (Scotts Miracle-Gro Company, Marysville, OH). The Mini-Treepots were placed in 3.28 L (3.47 qt) growing trays (figure 1). Three Mini-Treepots™ of each species were placed in each of nine growing trays.



**Figure 1.** Hardwood cuttings of ST-66 eastern cottonwood and BRZ 3-4 black willow in 950 ml Mini-Tree pots, randomly distributed within 3.28 L growing trays, and assigned to low-intensity fluorescents, low-intensity light-emitting diodes (LEDs), or high-intensity LEDs for 33 days. (Photo by Alexander Hoffman, 2015)

Growing trays were randomly assigned to one of three light treatments (table 1), for a total of three replicates per light treatment. Water was added to each growing tray daily to ensure adequate water supply to cuttings throughout the observation period. Temperature was maintained at 21°C (70°F) and no fertilizer was added.

Light treatments were selected to compare high- and low-light intensity LEDs with a reference low-intensity white fluorescent. Higher light intensity was achieved by shortening the distance between the cuttings and the LED light source. At the start of the experiment, light intensity was measured above each cutting using a quantum flux meter (Apogee Instruments, Inc., Logan, UT) and checked regularly thereafter to ensure desired light intensities were present within each light rack. Three independent light racks were arranged to prevent overlap among treatments and were randomly assigned to one of the light treatments (table 1). Light photoperiod was 16 hours per day.

## Measurements

The experiment was conducted for 33 days. Cuttings grown under fluorescents and high-intensity LEDs grew tall enough that they came into direct contact with their respective light sources after approximately 21 days. After 33 days, lights were turned off and racks were covered with breathable black mesh to bring all sprouted cuttings to a photosynthetic steady state before measuring. Cuttings were kept in this state for ~18 hours, and then a portable photosynthetic system (LI-6400 XT, LI-COR, Inc., Logan, NE) coupled with a leaf chamber

**Table 1.** Mean photosynthetically active radiation (PAR) intensity and standard deviation (SD) for each light treatment

Light rack	Light source	Light intensity ( $\mu\text{mol m}^{-2} \text{s}^{-1} \pm \text{SD}$ )
1	Six Osram Sylvania GRO-LUX wide spectrum 40 W tubes.	$83.78 \pm 10.87$
2	Two Tesler 120 W 4:1 red (630 nm) to blue (430 nm) diode rectangular indoor LED grow lights.	$161.67 \pm 33.74$
3	Two Tesler 120 W 4:1 red (630 nm) to blue (430 nm) diode rectangular indoor LED grow lights	$87.5 \pm 12.18$

fluorometer was used to measure photosynthetic parameters of each plant's highest positioned, fully mature leaf that produced an adequate amount of leaf surface area (at least 2 cm<sup>2</sup>, [0.31 in<sup>2</sup>]). Black willow cuttings within all light racks produced ample aboveground biomass but inadequate leaf surface area and were therefore not measured for photosynthetic parameters.

Photosynthesis measurements were obtained through a LightCurve auto program (6400-01, LI-COR Inc., Logan, NE), which exposed each leaf to a series of declining photosynthetically active radiation (PAR) intensities (2,000, 1,500, 1,000, 800, 500, 250, 100, 50, 25, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). At each PAR intensity, the cuttings' net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was recorded to construct a net photosynthetic light-response curve (rate of photosynthesis per irradiance level;  $P_N/I$ ) for each plant. Measurement intervals were 120 to 200 seconds. A matching infrared gas analyzer parameter of 50  $\mu\text{mol CO}_2$  was used in conjunction with a CO<sub>2</sub> mixer that kept an internal chamber CO<sub>2</sub> concentration of 400 mol(CO<sub>2</sub>).mol(air)<sup>-1</sup> (6400-01, LI-COR Inc., Logan, NE).

Recorded photosynthesis values were analyzed using the Lobo et al. (2013) Microsoft Excel Macro. The macro utilized a solver function to perform  $P_N/I$  curve construction using nine of the most frequently employed  $P_N/I$  curve models, including versions of the rectangular hyperbola Michaelis-Menten, nonrectangular hyperbola, exponential, and Ye models. All nine models were fit to the net photosynthetic light response datasets and compared in terms of goodness of fit, which showed the exponential model as an optimal model. Further exploration showed that the exponential model's fit to net photosynthetic light curves at a maximum PAR value of 1,000  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  produced the highest  $r^2$ , and lowest sum of square error (SSE) values.



The maximum net photosynthetic rate ( $P_{N(I_{max})}$ ), 95 percent light saturation point ( $I_{sat(95)}$ ), light compensation point ( $I_{comp}$ ), and photosynthetic efficiency ( $\Phi_{(I_{comp}-I_{200})}$ ) were calculated from the exponential macro model for each cutting.

Immediately following each cutting's net photosynthetic rapid light curve measurements, all aboveground biomass (i.e., shoot) was clipped, bagged, dried at 65 °C (149 °F) for 72 hours, and weighed. All root material was trimmed, bagged, labeled, and placed into cold storage until all samples were collected. To ensure finer root samples were collected, each pot's residual soil mixture was washed in a GVF Hydro-pneumatic Elutriation System (Gillison's Variety Fabrication, Inc., Benzonia, MI) which utilized air and water to float the roots and other organic matter out of the soil samples and onto a mesh screen. A 540-micron mesh was used to separate the roots from water exiting the extraction system. All the collected washed root segments were then combined with their respective sample bags, which were then dried at 65°C (149°F) for 72 hours. The dried root material was weighed to obtain a dry root biomass. Shoot-root ratio was then calculated for each cutting.

## Statistical Analysis

The experiment was designed as a completely randomized split-plot design. Light treatment was the whole-plot factor and species was the within-plot factor. Due to the use of one light rack per treatment, growing trays were considered replicates. The effects of grouping three growing trays within a light rack and grouping three Mini-Treepots™ per species within a light rack were treated as random effects. A mixed effects linear regression model was fit for each biomass component and photosynthesis metric with light, species, and their interaction as fixed effects. One cottonwood cutting had a negligible amount of root biomass, which was considered a measurement outlier, and thus removed from analysis. Photosynthesis analysis was restricted to cottonwood cuttings due to insufficient leaf area of black willow. Statistical significance was recognized at  $\alpha = 0.05$  for all models.

## Results

Across light treatments, black willow produced more biomass ( $p < 0.01$  for all biomass parameters) than

eastern cottonwood (figure 2). Although not statistically significant ( $p = 0.07$ ), eastern cottonwood tended to have a higher shoot-root dry biomass ratio than black willow (figure 2). No significant light by species interactions were found for any biomass parameters. Likewise, light treatments did not significantly affect biomass components although cuttings grown in the low-intensity LED treatment tended to have the highest mean shoot-root ratio and those grown in the high-intensity LED treatment tended to have the most total biomass (figure 2). For eastern cottonwood ST-66, no significant differences were found among light treatments for maximum net photosynthesis ( $p = 0.95$ ), 95 percent light saturation point ( $p = 0.92$ ), light compensation point ( $p = 0.94$ ), or photosynthetic efficiency ( $p = 0.84$ ) (figure 3).

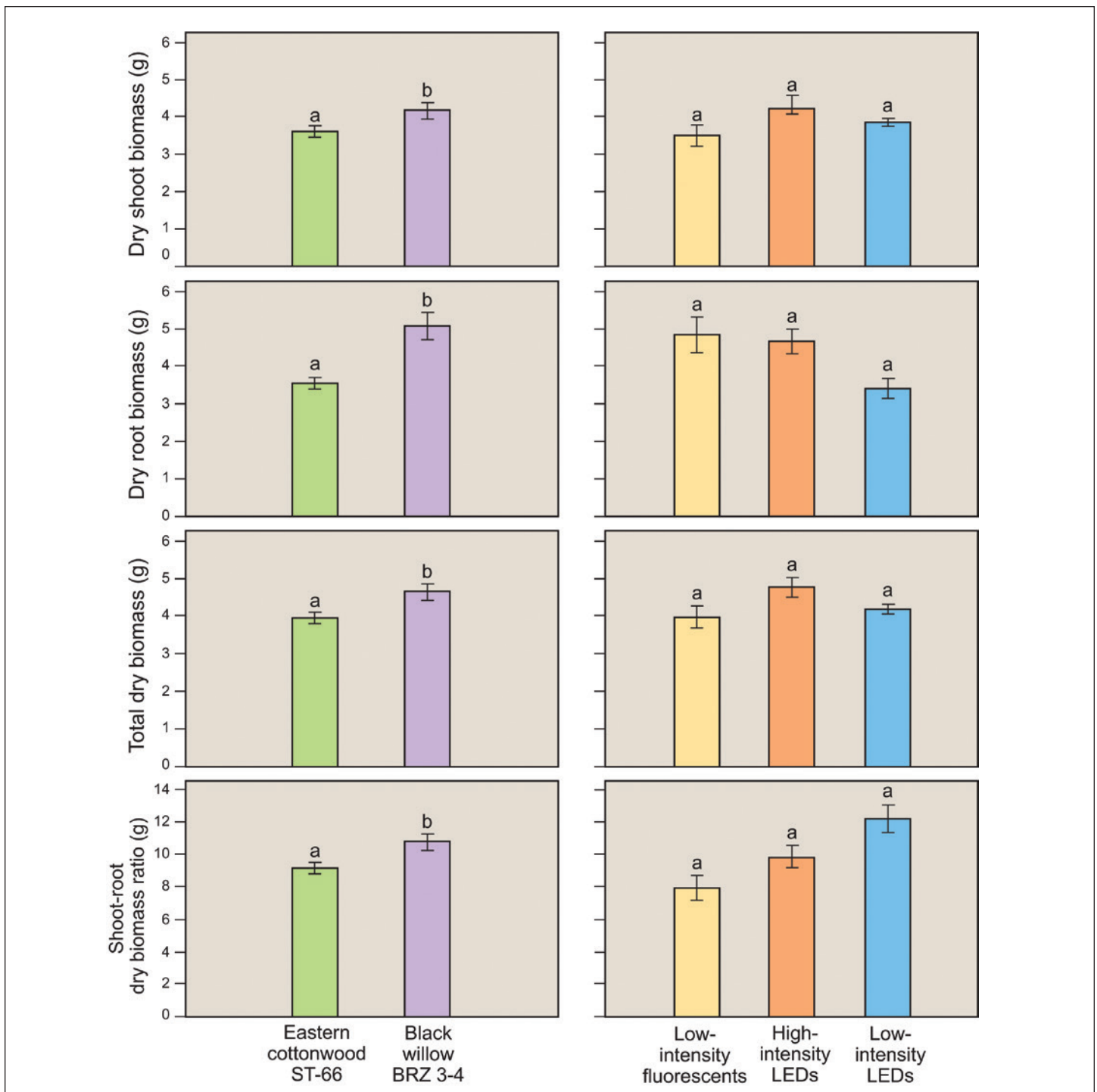
## Discussion

### Biomass Components

Given the exceptional rooting capacity of black willow (Rousseau et al. 2012), we hypothesized that black willow would produce higher total biomass with lower shoot-root ratios in the short run compared to eastern cottonwood. Our results partially support this hypothesis with greater biomass for black willow, although shoot-root did not differ significantly from that of eastern cottonwood (figure 2). Black willow higher total biomass suggested a faster growth rate of this clone and consequently greater potential as a candidate biomass species.

Light treatments did not result in differential biomass partitioning of cuttings under the light intensities tested in this study. This lack of significant differences within biomass components suggests that the contrasting light intensities achieved were less than optimal. Furthermore, the LED lights used in this study produced narrow, red and blue wavelength bands that constitute a distinct light environment as compared to the Gro-Lux wide-spectrum fluorescents, which produced a narrow blue band, a broader red band, and a substantial far-red light emission. The interaction of light quality and intensity may have diminished the statistical significance among light treatments.

Greater root biomass under the fluorescents and high-intensity LED, albeit not significantly different from low-intensity LED, suggested higher potential



**Figure 2.** Mean and standard error (SE) of shoot biomass, root biomass, total biomass, and shoot-root biomass ratio for species and light treatments. Different letters denote statistical significance at  $\alpha = 0.05$  within each block. LEDs = light-emitting diodes.

growth under these treatments. Cuttings grown under these treatments, however, came into direct contact with their light sources, which restricted further stem elongation. Samuoliene et al. (2010) documented increased frigo strawberry (*Fragaria x ananassa* Duch.) sprout stem elongation and shoot-root ratios when exposed to red LEDs; when a 13-percent blue light component was introduced in conjunction with the red LEDs, stem elon-

gation decreased. Brown et al. (1995) investigated dry matter partitioning and physiology of Hungarian wax peppers (*Capsicum annum* L.) exposed to red (~660 nm) LEDs only, supplemented with blue fluorescent, or supplemented with far-red (~735 nm) LEDs at  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. Peppers exposed to red LEDs only incurred reduced biomass production and fewer leaves than when blue fluorescents were added, whereas the addition of

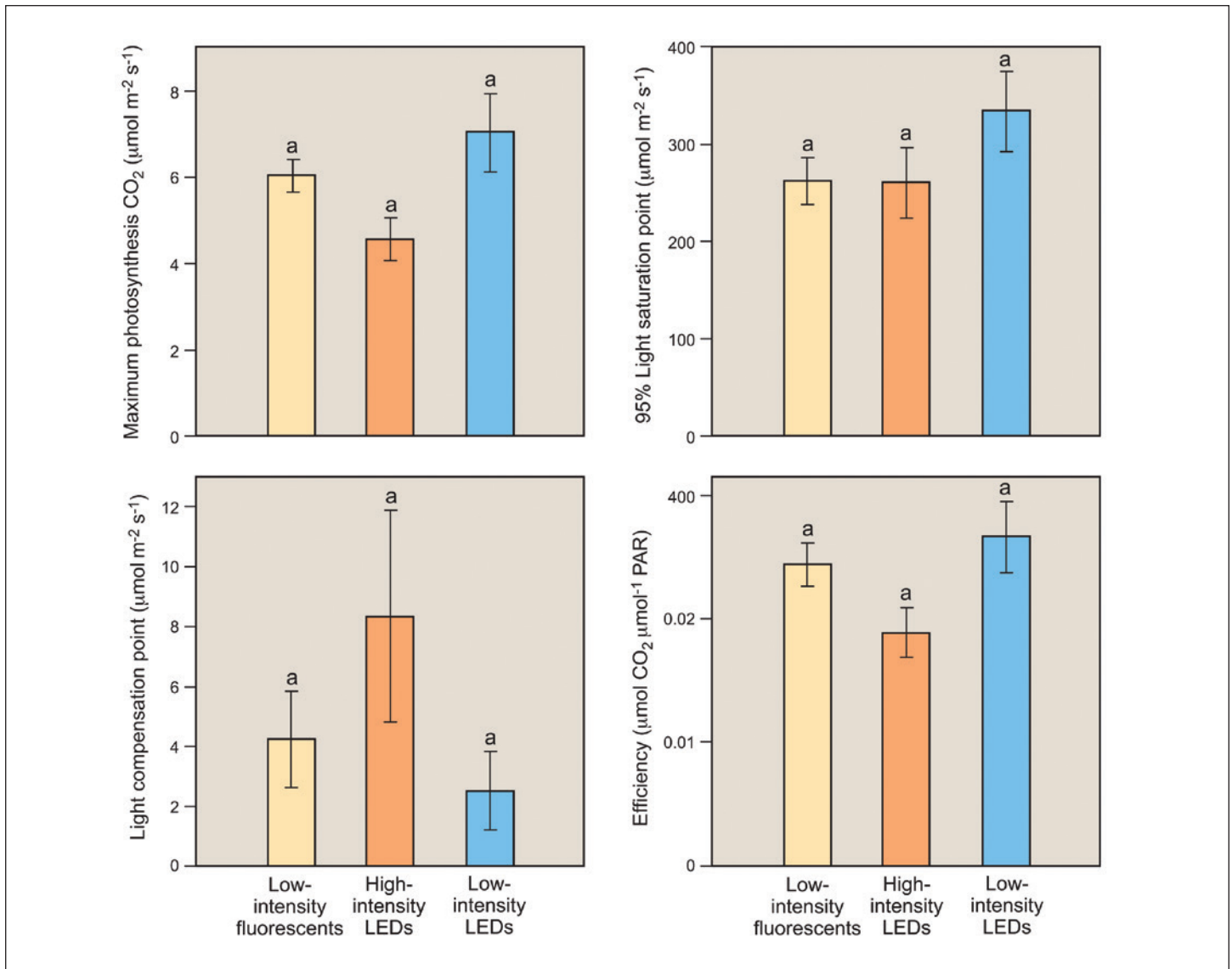
far-red LEDs resulted in taller plants and overall greater stem biomass. Additionally, the ratio of far-red:red light stimulates activity of plant phytochrome receptors, which have ecological value for shade avoidance, with a greater proportion of far-red light associated with accelerated stem elongation through the perception of shaded conditions (Schmitt et al. 2003). This is particularly prominent in shade-intolerant species, such as cottonwood and willow species.

## Photosynthesis

Photosynthetic data on eastern cottonwood, although statistically not significant, provided some trends. It is surprising that the highest light intensity did not result in the highest maximum photosynthetic rates, but it is possible that even the highest light level was below

that necessary to stimulate increased photosynthetic capacity. In fact, the light saturation points were all around  $300 \mu\text{mol m}^{-2} \text{sec}^{-1}$ , which was higher than the “high-intensity” LED treatment (figure 3).

Cuttings in all light treatments were observed to have slight yellowing of juvenile leaves, indicating a possible nutrient deficiency (figure 4). The green veins and greenish-yellow interveinal areas indicate iron or potassium deficiencies (HacsKaylo et al. 1960). Because no fertilization was added during the study period, the cuttings relied on their stored nutrients and nutrients in the growing medium for root, shoot, and leaf growth. These available nutrient levels may have been inadequate for growth and photosynthetic capacity. While these deficiencies may have affected cuttings across light treatments, the low-intensity LEDs seemed to



**Figure 3.** Mean and standard error (SE) of eastern cottonwood ST-66 maximum photosynthesis, 95 percent light saturation point, light compensation point, and photosynthetic efficiency by light treatment. Different letters denote statistical significance at  $\alpha = 0.05$ . LEDs = light-emitting diodes.



**Figure 4.** After 33 days of development within a low-intensity wide-spectrum fluorescent, low-intensity light-emitting diodes (LEDs), and high-intensity LED light racks, the majority of eastern cottonwood ST-66 (left) and black willow BRZ 3-4 (right) cuttings exhibited mild yellowing of leaves. (Photo by Alexander Hoffman, 2015)

produce the most vigorous cuttings. This is counterintuitive for a shade-intolerant plant, but may have been the result of plant growth out-stripping the nutrient supply in the higher light treatments.

The low-intensity LEDs provided high red:blue and red:far-red light ratios, which may have facilitated their marginal increase in photosynthesis performance. Blue light has been documented in regulating phototropism, photomorphogenesis, stomatal opening, and leaf photosynthesis (Whitelam and Haddiday 2007). Additionally, higher biomass production and photosynthetic capacity have been observed when a blue light component is supplied in conjunction with red light (Brown et al. 1995, Bukhov et al. 1995, Hogewoning et al. 2010, Matsuda et al. 2004, Yorio 2001). Favorable photosynthesis performance of the low-intensity LED may have also been facilitated by the cuttings' high shoot-root ratios. Investing the initial stored carbohydrates and newly produced photosynthates towards aboveground biomass may have allowed the cuttings to be more physiologically efficient.

The direct contact of shoot tips and juvenile leaves with the high-intensity LEDs probably induced negative physiological feedbacks, which diminished the potential for an accurate depiction of the red-blue light quality's effects and how applying those at a high intensity may affect cutting physiology. Similar complications were encountered with cuttings exposed to

low-intensity fluorescents, whose high far-red:red light ratios could have facilitated stem elongation. Several studies have documented an advantageous physiological response to high far-red:red light ratios through enhanced stem elongation, increased biomass production, and the assimilation of more photosynthates into leaf area production for better light harvesting (Ballare et al. 1990, Gilbert et al. 1995, Ritchie 1997).

## Conclusion

Artificial light sources (low-intensity fluorescents compared with low- and high-intensity LEDs) did not result in differences in biomass partitioning (shoot:root) in eastern cottonwood ST-66 or black willow BRZ 3-4 clonal cuttings. The black willow clone, however, produced greater shoot, root, and total biomass than the eastern cottonwood clone after 33 days. The specific light spectrum capabilities, especially the blue to red light ratio, and efficient energy production of LEDs warrant further research into their capabilities to influence biomass partitioning and consequently improve the competitive potential of vegetatively propagated clones on poor quality sites.

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