GROWTH AND MORPHOLOGICAL ACCLIMATION OF SEEDLINGS TO BLUE, GREEN, AND RED LIGHT FROM LIGHT-EMITTING DIODES

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ABSTRACT

GROWTH AND MORPHOLOGICAL ACCLIMATION OF SEEDLINGS TO BLUE, GREEN, AND RED LIGHT FROM LIGHT-EMITTING DIODES

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Several experiments were performed with impatiens, marigold, petunia, salvia, and tomato seedlings to quantify how different ratios of blue (B, peak=446 nm), green (G, peak=516 nm), orange (O, peak=596 nm), red (R, peak=634 nm), and hyper red (HR, peak=664 nm) from light-emitting diodes (LEDs) regulated plant growth while maintaining similar cultural and environmental conditions. Seedlings grown under O, R, and/or HR LEDs with background B and G light developed similar plant growth attributes including leaf size, stem length, and biomass accumulation. Therefore, selection of LEDs for horticultural lighting could be based on other factors such as economics. In another experiment, plants grown under ≥25% B light were 41 to 51% shorter and had 35 to 57% less fresh shoot weight than those grown under only R light at the same total photosynthetic photon flux. In a third experiment, plants grown under as little as 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light were 23 to 50% shorter, had 37 to 50% less fresh weight, up to 43% thinner leaves, and up to 49% less leaf area than plants grown under only R light. Seedlings under 50% G+50% R light were shorter than plants under only R light but taller than plants under only B light, suggesting that G light stimulated blue-light receptors (e.g., cryptochrome), but to a lesser extent than treatments with B light. Therefore, we postulate that a minimal quantity of B light (and to a lesser extent, G light) stimulates one or more B-light receptors that suppresses leaf and stem extension growth, which subsequently limits photon capture and constrains biomass accumulation of seedlings.

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LITERATURE REVIEW

Literature Review: Light Emitting Diodes for the Horticultural Industry

Supplemental lighting is used to increase the photosynthetic daily light integral (DLI; total amount of photosynthetically active light received in a day) in greenhouse production in temperate climates and to extend the photoperiod to increase growth and hasten development of horticultural crops. Supplemental lighting increases photosynthesis and therefore crop growth in greenhouse and nursery environments (Suzuki et al., 2011). In tissue culture propagation, energy for lighting accounts for as much as 40 to 50% of operational costs (Yang et al., 2011). Decreasing lighting costs could increase profit margins and environmental sustainability. Therefore, documenting and comparing the benefits and drawbacks of different lighting technologies, and the morphological and physiological responses of plants grown under them, could improve profitability and production of horticultural crops.

Lighting Technologies Used in Horticulture

Incandescent (INC) lights have been used to grow plants since the early 1900's (Arthur and Harvill, 1937) and are most commonly used today for photoperiodic lighting in greenhouses. In practicality, the installation of INC lamps is not as complex as other lighting technologies (Withrow and Withrow, 1947). INC bulbs have lifetimes between 700 and 4,000 running hours but typically average 1,000 hours (Sager and McFarlane, 1997). These lamps emit a relatively large amount of far-red (FR, 700-800 nm) light relative to red (R, 600-700 nm) light, which promotes stem elongation in plants more than lamps that emit less FR relative to R light (Wheeler, 2008).

Fluorescent lamps are commonly used in entirely enclosed growing environments because they emit a broad spectrum of light (Hemming, 2011). They emit radiation between 300 and 750 nm, and the peak wavebands vary with lamp type (Sager and McFarlane, 1997). Fluorescent lamps have a longer lifetime than INC lamps, with over 6,000 running hours at or above 70% brightness, but their longevity depends on the number of on/off cycles (Sager and McFarlane, 1997). In early studies performed by Withrow and Withrow (1947), plants under sole-source fluorescent lamps were the most vigorous in comparison to plants grown under mercury arc or INC lamps when under an irradiance of 116 $\mu mol \cdot m^{-2} \cdot s^{-1}$. According to Yang et al. (2011), fluorescent lamps have lower efficiencies in eliciting plant carbon accumulation due to less efficient photosynthetic wavelengths than those of newer lighting options, such as light-emitting diodes (LEDs). In a study performed by Yang et al. (2011) where all tissue culture treatments received 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of irradiance (total radiation per surface area), sweet potato *(Ipomoea batatas*) plants receiving the fluorescent lamp treatments developed root biomass, shoot biomass, and root-to-shoot ratio similar to those of plants grown under blue (B, peak=450 nm) and R (peak=660 nm) LEDs.

High-pressure sodium (HPS) lamps are the most common source of supplemental lighting in greenhouses because of their relatively high efficiency in converting energy into photosynthetic light (van Ieperen and Trouwborst, 2008). HPS lamps emit the most radiation between 550 and 650 nm, little in the B region (400 to 500 nm), and have an approximate lifetime of 20,000 hours (Sager and McFarlane, 1997; De Groot and van Vliet, 1986). They are highly energy efficient, producing 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR; 400-700 nm) per watt of energy input (Hemming, 2011). On a relative basis, HPS lamps emit

half of the B spectrum of sunlight, a quarter more R light, and twice the yellow light (Y, 550-600 nm) compared to sunlight (Krizek et al., 1998).

High-pressure mercury lamps have been used in commercial horticulture for supplemental lighting and emit a large portion of B light and relatively little R light (Withrow and Withrow, 1947). The spectrum emitted by mercury lamps is similar to that of HPS lamps, although they emit slightly more B light. However, mercury lamps are potentially more hazardous since they contain toxic and teratogenic mercury (Hemming, 2011). They have a long average lifetime of around 24,000 hours (Sager and McFarlane, 1997). Horticulturally, spinach plants (*Spinacia oleracea* 'Nobel') grown under mercury lamps were spindly, and less than a quarter produced flower buds, while those grown under fluorescent lamps all produced buds. Furthermore, the plants were shorter and fresh and dry weights were lower (Withrow and Withrow, 1947).

Microwave and plasma lamps have been developed for industrial use but their costs and unreliability make them less suitable for many horticultural applications. Microwave-driven sulphur plasma lamps have been marketed for plant production in controlled environments. Compared with sunlight at the same intensity, these microwave lamps emit similar proportions of R and B light but 60% more Y light, which is less effective in eliciting photosynthesis (Krizek et al., 1998). Both et al. (1997) examined a microwave lamp and reported that it emits a PAR spectrum similar to that of a water-cooled HPS lamp, but with less infrared radiation. However, light quality (the spectral distribution of light) changed with intensity – from a majority of R light at high intensities to a majority of B light at low intensities – making the microwave lamps inconsistent (Krizek et al., 1998). The lower heat production attracted researchers for its energy-saving potential, but the light intensity was often too high when lamps were placed in a

greenhouse environment. The microwave lamps also needed extensive air circulation and were a challenge to install in growth chambers (Both et al., 1997). Hogewoning et al. (2010) grew cucumber plants (*Cucumis sativus* 'Hoffmann's Giganta') under plasma lamps and compared them to those grown under fluorescent or HPS lamps at the same irradiance of 100 µmol·m^{-2}·s^{-1}. The plants grown under the plasma lamp had increased elongation, leaf unfolding rates, and almost 2 times the dry weight compared with those under HPS or fluorescent lamps. Because plants have adapted to the solar spectrum in their natural environment, it has been suggested that all tested species of plants under broad-spectrum photosynthetic lighting grow and develop more normally and uniformly, and without morphological abnormalities (e.g., epinasty), than plants grown under sole-source narrow-band LED lighting (Hogewoning et al., 2010).

LEDs are semiconductors where electrons flow from anode to cathode, causing the emission of a narrow waveband (e.g., 30 to 50 nm) of light, which depends on the elements used in the circuit (Bourget, 2008). For example, the earliest LEDs were made of a combination of gallium, aluminum, and arsenide, which caused the emission of R light (Bula et al., 1991); varying the amount of aluminum and gallium changed the peak emittance to between 630 and 940 nm, which led to a marketable FR LED (Barta et al., 1992). LEDs are emerging in the horticultural industry as the technology continues to improve and prices decrease (Mitchell et al., 2012). LEDs possess advantages to conventional lighting technologies in that they have no decreased lifetime with frequent on/off cycles, which is in contrast to fluorescent or HPS lamps (Bourget, 2008). LEDs emit relatively narrow waveband radiation and have an approximate lifetime of 50,000 hours (Bourget, 2008; Morrow, 2008; Philips Lumileds Lighting Company, 2007). LEDs are well suited for commercial plant production due to their high energy efficiency and their spectral specificity. For example, R and B LED arrays used by Yang et al. (2011)

emitted 98.5% of the spectrum in the R and B wavebands, which are considered the most photosynthetically efficient, while fluorescent lamps only emitted 52.9% in those wavebands. Furthermore, these relatively narrow wavebands of light emitted by LEDs make it possible to create a spectrum that elicits desired plant responses (i.e., shoot elongation, leaf size and thickness, leaf sensitivity to light, germination, pigmentation, and flower induction) (Barta et al., 1992; Hemming, 2011). In a study performed by Kato et al. (2011), tomato plants (*Lycopersicon esculentum*) grown under three white LED treatments had 4 to 11% more biomass than plants under fluorescent lights at the same irradiance of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The LEDs tested by Kato et al. (2011) differed in the R:B, which ranged from 0.68 to 1.00, illustrating the variation of LEDs currently on the market.

Many LEDs that emit photosynthetic light are at least as energy efficient as the horticultural industry lighting standard, the HPS lamp. The energy efficiencies (photosynthetic light output per energy input) of conventional lamp types are 6-7% (INC), 22-27% (fluorescent), 22-27% (HPS), and 20-21% (metal halide) (Runkle, 2007). LEDs have photosynthetic efficiencies of ($\mu mol \cdot W^{-1} \cdot s^{-1}$): 2.1 (B, peak=450 nm), 0.83 (R-O, peak=593 nm), 2.5 (R, peak=624 nm), 2.5 (R, peak=634 nm) and 2.9 (R, peak=660 nm) (R. Swamy, Osram Opto Semiconductor, personal correspondence). The efficiency depends on the manufacturer, the components in the LED, the current moving through the semiconductor, and its light intensity (Philips Luxeon Rebel Product Brief, 2011). Other desirable attributes of LEDs are the absence of glass components (as in INC and fluorescent lamps) and trace amounts of mercury (as in fluorescent and metal halide lamps) (Bourget, 2008).

Plant Pigments and the Mechanisms of Photosynthesis and Respiration

Light has both wave and particle properties. The individual particles, or photons, contain a specified amount of energy – a quantum. Photons excite molecules in plants, such as chlorophyll, to briefly jump to higher states of energy, which stimulates photosynthesis through a cyclic series of chemical reactions. The amount of light that is useful for photosysnthesis is the photosynthetic photon flux (*PPF)*, which is the number of micromoles of photons within the 400 to 700 nm waveband, per square meter and second ($\mu mol \cdot m^{-2} \cdot s^{-1}$) (Moe, 1997). Hopkins and Hüner (2004) provide an overview of photosynthesis and pigments in plants. Multiple compounds have the ability to undergo this photosynthetic process including chlorophyll *a*, *b*, *c*, and *d* (only *a* and *b* are present in higher plants), the phycobilins, carotenes, xanthophylls, and anthocyanins. The visible color of a pigment depends on the absorbed and reflected wavelengths of light. The absorption spectrum varies among pigments and among plant species. The chlorophylls are the primary photosynthetic pigments and primarily absorb B and R light, but their peaks of absorption differ: the absorption of *a* peaks at 420 and 670 nm, while *b* peaks at 450 and 640 nm.

Additional accessory pigments that predominantly absorb B light include anthocyanins and carotenoids, such as xanthophylls and carotenes. Carotenoids primarily harvest light but also protect the photosynthetic organs from damage by the oxygen-rich atmosphere (photooxidation). Xanthophylls protect chloroplasts from damage by high light intensities (Hopkins and Hüner, 2004). Anthocyanins primarily absorb light between 475 nm and 560 nm. They are categorized as flavonoid compounds that commonly influence pigmentation of floral organs (to attract pollinators) and protect leaves from ultraviolet (UV; 250-400 nm) radiation (Holton and Cornish, 1995; Hopkins and Hüner, 2004).

Knowledge of the absorption spectra of plant pigments aids in understanding the process of photosynthesis. While accessory plant pigments absorb light, chlorophyll is the primary photosynthetic pigment. Anatomically, photosynthesis occurs in the chloroplasts in the mesophyll (cells between the epidermal layers) of the leaf. A photon with a favorable wavelength (e.g., 400 to 700 nm) enters the epidermal cells and is reflected into the mesophyll tissue. Chloroplasts – the key organelles of photosynthesis – scatter excess light into other chloroplasts, which then can be absorbed. These chloroplasts perform the oxidation-reduction reaction defined by the equation:

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6H_20$$

This reaction proceeds in two steps: the light reaction, which results in the formation of NADPH and ATP; and the dark reaction, in which ATP provides the energy and NADPH provides the electrons needed to reduce CO_2 and convert it to organic molecules (glucose) (Hopkins and Hüner, 2004).

In the chloroplast, stacked structures called thylakoids are collectively called a granum. Imbedded in the thylakoid membrane is a series of multi-protein structures that perform the chemical reactions generating ATP, the usable form of energy. These protein structures include photosystem I, the cytrochrome complex, and photosystem II. When photosystem II accepts a photon, it causes a series of reactions producing the end products of oxygen, hydrogen ions, and additional free electrons from water molecules as represented by the equation:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

The electrons are accepted by a transport molecule, a plastoquinone, and passed to the protein ferredoxin. Finally, $NADP^+$ is formed as a result of the series of electron transfers. These series of reactions are known as the light-dependent reactions because of the need for an input of

energy (via photons) at two locations in the chain for product formation. The light-independent reactions (dark reactions) utilize CO_2 and NADPH to form 3-phosophogylcerate that is used in the central glycolysis pathway. The necessity for external energy to stimulate the formation of intermediates, ultimately leading to glucose production, demonstrates the importance of light for the growth and survival of plants. Thus, lighting used in greenhouses and especially in completely controlled environments such as growth chambers is the most critical factor for plant growth (Hopkins and Hüner, 2004).

Research as early as 1884 showed that absorbed light quantity, the most critical factor influencing plant growth, is directly proportional to total photosynthetic activity (Burns, 1937). After reaching the light compensation point (point at which respiration rate equals photosynthetic rate), photosynthesis increases with increased light intensity until a maximum threshold when the rate of photosynthesis asymptotes (light saturation point). Malayeri et al. (2011) reported that Japanese mint (*Menta arvensis*) had increased photosynthetic rates when grown under white fluorescent lamps at *PPF* of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Biomass accumulation increases with increased rates of photosynthesis (Shirley, 1929). Increased photosynthesis with increasing light intensity until the light saturation point is reached is universal among plants (Smith, 1936).

Carbon dioxide concentration has been shown to be the primary regulator of the physiological process of gas exchange. Raschke (1975) concluded that CO_2 concentrations were crucial to stomatal opening and closing – which allows photosynthesis and respiration processes to occur. Furthermore, increased concentrations of CO_2 allow for plants to photosynthesize at increased rates compared to plants exposed to lower concentrations of CO_2 (Malayeri et al., 2011; Wheeler et al., 1991). On a global scale, the current atmospheric concentration of CO_2 is

approximately 400 ppm, however it is increasing along with other greenhouse gases that could allow for increased photosynthesis rates (NOAA Global Monitoring Division, 2013).

Photoreceptors Regulate Plant Growth and Development

The morphology of plant growth is mediated by multiple photoreceptors: phytochromes, cryptochromes, and phototropins. In contrast to pigments that absorb light energy to drive photosynthesis, photoreceptors such as phytochrome are proteins containing a chromophore that receive light signals, which can alter gene expression to drive developmental or physiological processes. Phytochromes are a family of photoreceptors, each of which has absorption peaks at 660 nm and 735 nm (R and FR light, respectively), and mediates morphological and developmental responses (Hopkins and Hüner, 2004). Phytochromes also absorb a lesser amount of deep B and UV-A (315–400 nm; Atlasz et al., 2009) radiation. They mediate stem elongation, leaf expansion, chloroplast development, flowering, and signal the transcription of other genes (Horwitz et al., 1988; Folta and Childers, 2008; Parks et al., 2001; Valverde et al., 2004). Phytochromes influence the regulation of photoperiodic responses. In *Arabidopsis*, long days stimulate phytochrome *a* to stabilize the CO protein, which induces transcription of the FT gene and induces flowering (Valverde et al., 2004). Phytochromes also plays a role in chlorophyll accumulation; a high R:FR and increasing intensities of R light increase chlorophyll accumulation per leaf area (Hortwitz et al. 1988).

Cryptochromes are photoreceptors that absorb B light and UV-A radiation (Hopkins and Hüner, 2004). In at least some plants, particularly those in the Brassicaceae, cryptochromes influence branching, genetic regulation of stem elongation, and the conversion of a vegetative to a reproductive meristem (via crytochrome2 in some plants) (Folta and Childers, 2008; Imaizumi

et al., 2002; Valverde et al., 2004). Cryptochromes are the photoreceptors that regulate the genetic pathways (via increased CHS and DFR gene expression) of anthocyanin pigmentation (Li and Kubota, 2009; Meng et al., 2004; Ninu et al., 1999). Cryptochrome also mediates green (G, 510 to 610 nm) light responses, including decreased plastid transcription. G light can antagonize some B light responses (e.g., gas exchange, water regulation, phototropism, chlorophyll synthesis, and stem elongation) and could decrease the functionality of cryptochromes (Banerjee et al., 2007; Blaauw and Blaauw-Jansen, 1970; Cosgrove, 1981; Folta and Childers, 2008; Massa et al., 2008; Schwartz and Zeijer, 1984). However, plants could have an independent G light photoreceptor that has not yet been isolated (Folta and Childers, 2008).

The phototropins are photoreceptors that act to synchronize developmental events by meditating B-light stimulated phototropism responses, such as growing towards light (Folta and Childers, 2008). These receptors and their receptor family, associated with the LOV domain, have peak absorption at 450 nm. Assmann et al. (1985) reported that the B photoreceptor, synthesized during the biosynthesis of xanthophyll pigments, was located in the guard cells of stomata. Zeaxanthin, a precursor to xanthophyll pigments that absorbs B light, is regulated by both light and CO_2 concentration. It is at its highest concentration under high light intensities and its lowest in darkness, when it is converted to other intermediates. Low concentrations of CO_2 that lower the pH of the intracellular space also stimulate the production of zeaxanthin (Zeiger and Zhu, 1998). This family of receptors regulates endogenous circadian rhythms and the transition to a reproductive meristem (Folta and Childers, 2008; Imaizumi et al., 2003; Somers et al., 2000). Imaizumi et al. (2003) isolated the FKF1 protein in *Arabidopsis*, which increases after exposure to B light and increases transcription of CO and FT (flowering) genes that control circadian rhythm and photoperiodic flowering.

In most plants, B light is necessary for normal plant morphology; in its absence, leaves curled downward and became deformed (Goins et al., 1998). In geranium (*Pelargonium zonale*), when 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B LED (peak=460 nm) light was irradiated locally to the adaxial surface of the leaf with 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of R LED (peak=660 nm) light, leaf epinasty decreased by 20% compared to leaves irradiated with only R light. The decreased epinasty was dependent on the B light intensity; when leaves were irradiated with 100 or 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light, epinasty decreased by 30 and 40% compared to leaves under only R light, respectively. Leaf angle is regulated by phototropins, but could also be regulated by the differential concentration of auxin (Fukuda et al., 2008; Christopher and Volkenburgh, 1997).

Stem elongation inhibition and chlorophyll production are mediated by multiple photoreceptors concurrently including phytochrome and cryptochrome. Cosgrove (1981) reported that stem elongation inhibition by B light seemed to be universal to many species; however, the magnitude of the decrease differed among species. When cucumber and sunflower (*Helianthus annuus*) were grown under two B flood lamps (peak ~480 nm) at 5.0 $w \cdot m^{-2}$ for 5 to 30 minutes, both species showed an exponential decrease in extension growth, by 50%, within a period of minutes upon exposure to the B irradiance. The rapid response to B light suggests that the growth response was not dictated by changes in concentration of plant hormones, such as gibberellins or auxin, which could take up to 30 minutes to change in concentration (Cosgrove, 1981). The growth inhibition was mediated by phototropin 1 in the first 30 minutes while the growth inhibition after 30 minutes of light exposure was mediated by cryptochrome (Folta et al., 2003). The long-term alteration of growth rate by cryptochromes may also be mediated by phytochrome because they both regulate gibberellin synthesis (Tsuchida-Mayama et al., 2010;

van Ieperen, 2012). B light, as regulated by cryptochome, influences chlorophyll production during germination (Tripathy and Brown, 1995).

Light can stimulate flowering by increasing gibberellin biosynthesis, FT gene transcription, and sucrose production in plants, which can all influence flowering responses. Photoperiodic plants can be stimulated to flower by an inducing photoperiod, an increased DLI under light-limiting conditions, or both. An inducing photoperiod for short-day plants (SDP) is a long uninterrupted night and for long-day plants (LDP), a short night. The conversion between the P_{FR} (active form) and the P_R (inactive form) form of phytochrome (in response to FR light) regulates photoperiodic responses by upregulating transcription of intermediates (e.g., GA 20-oxidase) and the FT protein (King et al., 2001). Growers use lighting to extend day length or provide night interruption, which can control flowering when the natural day length is not inductive (King, 2011). In addition to photoperiod, a high DLI stimulates increased rates of sucrose production to cause floral initiation in fuchsia (*Fuchsia ×hybrida*). Under a 10-h photoperiod, fuchsia (LD plant) did not flower when grown under a moderate light intensity (220-230 $\mu mol \cdot m^{-2} \cdot s^{-1}$) but did flower when grown under a higher intensity (500-600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (King and Ben-Tal, 2001).

Interactions of Light Quality and Quantity and Carbon Dioxide on Plant Growth

Light intensity, light quality, temperature, and CO_2 concentration interact to control photosynthesis and thus, plant growth. Carbon assimilation of plants can be represented by a light response curve. Genetic differences in plants can influence the shape of the curve as well as environmental conditions. For example, when a shade-intolerant plant is grown under two light intensities (e.g., 250 and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plants grown under the higher intensity fix carbon at

an increased rate compared to those grown under the lower intensity (Figure 1.1). Increased concentrations of CO_2 can further increase carbon assimilation, but the magnitude of the effect is not as great as that for light intensity. Tennessen et al. (1994) described that the photosynthetic and stomatal conductance rates were similar in leaves of kudzu (*Pueraria lobata*) grown under R (peak=656 nm) LEDs or a xenon arc lamp under high light (*PPF* of 1,000 µmol·m^{-2}·s^{-1}) and high (175 Pa; 1727 µL/L) CO_2 concentration. Under low light (*PPF* of 175 µmol·m^{-2}·s^{-1}), plants had a 10 to 15% greater stomatal conductance when grown under R LED light than white light from the xenon arc lamp (Tennessen et al., 1994). Therefore, stomatal gas exchange at varying light intensities can depend on light quality. Sharkey and Raschke (1981) examined effects of light quality from xenon arc lamps with filters to provide B (430-460 nm), G (510-610 nm), or R light (630-680 nm) on stomatal conductance of individual leaves of cocklebur (*Xanthium strumarium*) at a *PPF* between 1 and 100 µmol·m^{-2}·s^{-1}. A *PPF* of 1 to 2 µmol·m^{-2}·s^{-1} caused minimal stomatal opening. Stomatal conductance (g_s) peaked at ~20 µmol·m^{-2}·s^{-1}under red light, whereas B light was five times more effective, resulting in a g_s of 100 µmol·m^{-2}·s^{-1} under CO_2 concentrations of 120 µl·L^{-1}. However, CO_2 concentration primarily regulated stomatal conductance, and light quality at the same intensity had a lesser effect (Sharkey and Raschke, 1981). Under B or R light, the stomata closed when CO_2 concentration exceeded 500 µL·L^{-1} (Sharkey and Raschke, 1981).

Plant Acclimation to Light Quality and Quantity

The quality and quantity of light that plants receive outdoors are never constant. Plants under a canopy of leaves receive both a lower light quantity and a lower R:FR than plants under unshaded light, because plants overhead absorb the majority of available R light and transmit the majority of FR light (Hogewoning et al., 2010; Morgan and Smith 1981; Smith, 1982). On a

cloudy day, the light spectrum has an increased proportion of B light compared with that of a cloudless day (Holmes and Smith 1977). Also, when the sun is low in the sky, at dusk and dawn, the R:FR decreases, which can promote stem elongation. In lambsquarters (*Chenopodium album*), the light intensity and the low proportion of R light caused inconsistent responses with respect to stem elongation, but plants grown under high proportions of FR light had greater stem elongation than those exposed to higher proportions of R light. Likewise, plants grown in shady habitats had increased stem length, particularly during emergence, but the rate of elongation decreased with maturity. The effect of the low R:FR was more apparent in plants that received higher light intensities (Morgan and Smith, 1981).

Plants acclimating to the low R:FR demonstrate the shade-avoidance response, which is an increase in internode and petiole length, leaf area, chlorophyll content, stem length and a decrease in leaf thickness and photosynthetic metabolites (Blackman and Wilson, 1951; Franklin and Whitelam, 2005; Grime and Jeffery, 1965; Jarvis, 1964). Holmes and Smith (1975) reported that wheat leaves lower in the canopy received less than one tenth of the photosynthetic light available to leaves higher in the canopy. Grime and Jeffery (1965) reported that two grassland species, wavy hairgrass and purple betony (*Deschampsia flexuosa* and *Betonica officinalis*, respectively), had lower seed yields and biomass when grown in shady forest conditions compared to plants exposed to higher light intensities in prairie conditions. Plants adapted to shady habitats became photosynthetically saturated at a quarter of the light intensity of sun-adapted plants (species dependent), changing the shape of their photosynthetic response curve (Bohning and Burnside, 1956).

Plant Growth Responses to Light Quality

Plant growth and morphological changes in response to light quality have been studied for decades. However, light intensity was often uncontrolled (e.g., Shirley, 1929; Downs et al., 1959) when using filters or shades, which potentially confounded responses attributed to light quality. Shirley (1929) grew a variety of plants [e.g., dwarf sunflower (*Helianthus cucumerifolius*) and wandering Jew (*Tradescantia fluminensis*)] under full sunlight or greenhouse shading materials to eliminate specific wavebands (i.e., B, R) and plants grown under a full spectrum had increased biomass. Shirley (1929) also reported that B light was more effective at photosynthesis than R light. Downs et al. (1959) grew wheat under fluorescent and INC lights with either an R or B filter, or with no filter. Plants grown under the INC bulbs had more biomass than those under the fluorescent lamps. The R light increased biomass compared to the B light-treated wheat plants. Furthermore, plants under white and B light had greater seed weight than plants grown under either the B fluorescent or INC lamps (Downs et al., 1959).

In some more recent studies, plant growth responses to light quality have been determined without confounding effects of light intensity. Saebo et al. (1995) examined how light emitted by colored fluorescent lamps influenced silver birch (*Betula pendula*) under different ratios of B (410-510 nm), R (640-680 nm), and FR (700-750) light (*PPF* of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Light treatments included a cool-white (CW), warm-white (WW), B, and R fluorescent lamps; INC lamps; and a prismatic lamp (PL) alone and in the following combinations: CW+WW, CW+INC, CW+R, CW+PL and CW+B. Plants grown under treatments with the greatest proportion of B light (under B light or CW) had ≈50% greater photosynthetic activity (approximately 60-70 $\mu mol\ CO_2 \cdot dm^{-2} \cdot$leaf area h^{-1}) than plants grown under INC or R light alone (approximately 30-40 $\mu mol\ CO_2 \cdot dm^{-2} \cdot$leaf area h^{-1}). Plants grown under B light had ≈25% greater chlorophyll concentration per leaf area than CW and ≈50% more than R. In addition,

leaves under B were ≈10% larger than those under CW and ≈5 times larger than those under R light, with 50-70% more epidermal cells than other light treatments. Similar to the findings of Sharkey and Raschke (1981), Saebo et al. (1995) concluded that light with the greatest proportion of B light yielded plants with larger leaves and greater photosynthetic activity and thus, greater biomass.

Plants grown under only B or only R light typically have distinctively different morphologies. For example, when *Arabidopsis* were grown under R, B, or R+B fluorescent lamps, plants irradiated with light between 25 and 160 µmol·m^{-2}·s^{-1} of B light had decreased leaf area, biomass accumulation, and petiole length compared to plants grown under R or R+B light at the same intensity (Eskins, 1992). Leaf morphology can also differ when plants are grown under only B or R light. Fukuda et al. (2008) observed that *Arabidopsis* leaves were 16% thicker when irradiated with 100 µmol·m^{-2}·s^{-1} of B LED (peak=460 nm) light compared to leaves irradiated with the same intensity of R (peak=660 nm) LED light. Epidermal cells were 7-13% longer on the abaxial leaf surface under B light than R light, thereby causing differential growth and epinasty under R light. Leaves under R light also had a 20% greater epinasty index, a score based on the horizontal or vertical orientation of the leaves after 30 d (Fukuda et al., 1993, 2008).

Plants grown under R or B light, alone or combined, have been compared with plants grown under white fluorescent light. Wheat plants (*Triticum aestivum* 'USU-Super Dwarf') were grown under a *PPF* of 350 µmol·m^{-2}·s^{-1} from R LEDs (peak=660), or B or white fluorescent lamps, and those under the R LEDs had ≈50% lower dry weights and 45% lower instantaneous photosynthetic rates than plants under the white fluorescent tubes (Goins et al., 1997). Plants grown under R LEDs supplemented with B fluorescent light had seed yields similar to plants grown under white light (Goins et al., 1997). These results are in agreement with Li et al. (2011)

who studied the effects of *PPF* and light quality on four cultivars of spinach (*Spinacea oleracea*). By examining the relationship between light quality (R, white, or B fluorescent lamps) and two light intensities (100 and 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$), Li et al. (2011) determined that light intensity contributed most to overall growth. This conclusion is consistent with many other studies using fluorescent light (e.g., Malayeri et al., 2011), because spinach grown under the higher light treatment had 10-80% greater leaf and stem biomass than spinach grown under the lower intensity. For example, 'Manyoh' spinach grown under R and white light at *PPF* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had 10-40% more dry biomass than plants grown under only B light, while plants grown without B light had longer internodes and leaves (Li et al., 2011). Similarly, rice (*Oryza sativa*) plants had more biomass and greater net assimilation rates, concentrations of rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase), and chlorophyll under a combination of R (peak ~660 nm) and B (peak ~460 nm) LEDs compared with R LEDs alone at a *PPF* of 380 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Ohashi-Kaneko et al., 2006). In addition to having a decreased concentration of chlorophyll, plants grown under only R light had lower photosynthetic rates, which was attributed to lower stomatal conductance. A physiological explanation for decreased photosynthesis under R light is that photons emitted in this narrow waveband causes an inequality of photons between photosystems I, II, and the electron transport chain (Tennessen et al., 1994). Since each photosystem has limited absorbance, efficient use of R photons by photosystem II is low, especially at wavelengths ≥680 nm (Tennessen et al., 1994; Zeiger and Hepler, 1977).

The ratio of R and B light for desired plant growth for numerous applications continues to be under investigation. Yang et al. (2011) examined the effects of different R (peak=660 nm) and B (peak=450 nm) ratios in sweet potato grown in tissue culture. The R:B treatments were

4:1, 6:1, 8:1, and 10:1 at a constant *PPF* of 35 µmol·m^{-2}·s^{-1}. Stem elongation decreased by 17% and biomass increased by 27% when the R:B was 10:1 compared to plants grown under the 4:1. The root:shoot was 0.28 for the 8:1 treatment and 0.21 under the 4:1 treatment. Overall, the R:B of 8:1 produced plants with similar a root:shoot, but the 10:1 light treatment produced the tallest plants with the greatest biomass (Yang et al., 2011). In a separate study, growth of tomato (*L. esculentum*) was compared under four light quality treatments at a different *PPF* from three white LEDs and a 3-band fluorescent lamp (Kato et al., 2011). The R:B of 0.87 produced plants with approximately 4 to 11% more biomass than treatments with ratios of 0.68, 1.0, and 1.7. In addition, plants grown under the white LEDs produced plants that had 11% greater dry weight than plants under fluorescent lighting (Kato et al., 2011).

There have been conflicting reports about the merit and utility of G light on photosynthesis. Early studies using spectral filters such as Klein (1964) probably altered the light intensity, which would have confounded treatment results and interpretations (Folta and Maruhnich, 2007). Klein (1964) reported that fresh and dry weight of marigold (Tagetes erecta) and garden balsam (*Impatiens balsamina*) increased by over a third when G light was filtered out of the spectrum. In contrast, Kim et al. (2004) maintained a *PPF* of 150 µmol·m^{-2}·s^{-1} and examined the inclusion of G (500-600 nm) light from G fluorescent lamps to background R (600-700 nm) and B (400-500) LED light, or light from CW fluorescent lamps, on growth of lettuce (*Lactuca sativa*). Photosynthetic rates decreased when the percentage of G light exceeded 50%, but biomass increased by 89% with up to 24% G light. When 15% of the total irradiance was from G light, leaf area and dry mass were 70% and 89% greater, respectively, than under only R and B light (Kim et al., 2004). Similarly, Lee et al. (2011) tested a variety of different light quality treatments, including those with G light, on lady slipper orchid (*Paphiopedilum*

'Hsingying Carlos') grown in tissue culture. The six different light quality treatments were CW fluorescent (5000 K) and WW fluorescent (2700 K) lamps, R LEDs (peak=660 nm), B LEDs (peak=450 nm), R+B LEDs at 9:1, and R+G+B LEDs at 8:1:1 (G peak=525 nm). The CW, WW, and R+G+B treatments yielded plants with 24 to 36% longer leaves than under the B LEDs. Plants under the B LEDs were the most compact and had the least stem elongation and leaf area. Plants under the CW fluorescent lamps had the greatest fresh weight (52% greater than plants grown under B light), whereas the R+G+B plants had 84% greater dry weight than plants under B light.

Liu et al. (2011) examined the effects of yellow (Y), G, R and B light and their combinations on cherry tomato. The seven different light quality treatments included a dysprosium white lamp as a control; B (peak=450 nm), G (peak=520), Y (peak=590 nm), or R (peak=650 nm) LEDs; and R+B (1:1) and R+B+G (3:3:1) at a *PPF* of 320 µmol·m^{-2}·s^{-1}. Plants grown under only G, Y, or R light were 89%, 102%, 126% taller, respectively, than plants grown under R+G+B LEDs. Plants had greater fresh weight with a root:shoot of 0.36 under R+B+G LEDs, whereas plants under B or R+B had root:shoot of 0.49 and 0.47, respectively. The plants grown under the G, Y, or R LEDs had the lowest root mass, a root:shoot= 0.28, 0.17, and 0.16, respectively, and the lowest "health index" (factor of root:shoot and chlorophyll content). Plants grown under Y light had a 69% lower shoot dry mass than those grown under B light, which had the greatest dry mass. Chlorophyll content per leaf area was similar among treatments, while instantaneous net photosynthesis was greatest in plants grown under the R+B or R+B+G LEDs, which was more than triple of that of plants under the G or Y LEDs. A mixture of light wavebands, particularly including B light, yielded plants with desirable characteristics for commercial floricultural production such as compact growth (Liu et al., 2011).

UV Effects on Plant Growth and Protective Compound Accumulation

Plants grown at high altitudes are exposed to greater UV radiation, due to a thinner atmosphere, than plants grown at lower altitudes (Sullivan and Teramura, 1992). Ecological studies examining native plants their habitats have sparked a series of controlled environment studies testing the impacts of UV radiation on plant physiology. Studies that have examined the effects of UV radiation on crop quality attributes have reported conflicting results. Excluding UV radiation from the spectrum does not decrease photosynthesis (Popp, 1926). However, UV can inhibit stem elongation, particularly in environments with a low R:FR (Weinig, 2004). Impatiens (*Impatiens capensis*) pre-treated with a low R:FR were about 5% taller when UV radiation was filtered out by UV-opaque panels than when grown under UV-transparent plastic panels in the field, with a similar *PPF* between treatments (Weinig, 2004). UV radiation can damage essential photosynthetic intermediates, the photosystems, and proteins (Fernandes de Oliveira and Nieddu, 2011). Protective mechanisms against excessive irradiances of UV radiation include accumulating phenolic compounds in tissues to absorb UV radiation (Lafontaine et al., 2005). UV radiation can have a desirable effect on crops such as red grape (*Vitis vinifera* 'Cannonau' and 'Bonvale'), in which UV radiation increased the production of polyphenols, an antioxidant beneficial to humans. UV radiation can also have desirable effects on crops such as lettuce by potentially increasing the nutritive value (Watanabe, 2011). For example, lettuce 'Natividad,' 'Dark,' 'Aruba,' and 'New Red Fire' grown outdoors had more red coloration and anthocyanin accumulation than lettuce grown under a polycarbonate-acrylic cover that did not transmit UV light under the same DLI (Shioshita et al., 2007). In a separate study, lettuce 'Red Cross' grown under CW fluorescent (*PPF* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) with either 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of LED UV-A or 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B (peak=476 nm) light had 11 to 16% or 26 to 31% greater concentration of

anthocyanins, respectively, than plants under CW fluorescent lamps alone (Li and Kubota, 2009). Polyphenol and anthocyanin production demonstrate possible benefits of UV radiation in production of food crops, which could limit the prevalence of sole-source lighting without UV (Fernandes de Oliveira and Nieddu, 2011).

Advantages and Barriers to LED Implementation in Horticulture

In temperate climates at higher latitudes, supplemental lighting in greenhouse production can be economical in the production of some crops such as in propagaules, high-value crops, or in high-wire vegetable production. Conventional supplemental lighting is from above the crop, but the crop's upper canopy can shade lower leaves, which reduces photosynthetic activity of the lower leaves. Because LEDs emit less infrared radiation than HPS or fluorescent lamps, LEDs can be used as supplemental inter-canopy lighting (Hemming, 2011). According to a simulation study, the addition of lower-canopy lighting to traditional overhead lighting could increase photosynthetic activity by 10% compared to plants under lighting from only above the crop (van Ieperen and Trouwborst, 2008). However, tomato fruit yields with supplemental inter-canopy lighting of 9 $mol \cdot m^{-2} \cdot d^{-1}$ from LED panels with 95% R (peak=627 nm) and 5% B (peak=450 nm) were similar to those of plants irritated with HPS lamps with the same DLI (Gómez et al., 2013). Therefore, the theoretical increase in photosynthesis from LED inter-lighting may be less than that predicted.

Many characteristics of LEDs, such as their energy efficiency, decreased environmental impact, and the ability to deliver specific wavebands of light make them well suited for

widespread utilization in the horticultural industry (Bourget, 2008; Runkle et al., 2011; Sager and McFarlane, 1997). Plant factories, which increase land use efficiency by implementing multi-tier shelving systems, have used fluorescent lighting for photosynthesis. Since LEDs produce minimal infrared radiation, they can be placed closer to plants without increases in plant temperature (Watanabe, 2011). However, a few barriers are hampering LED technology adoption for commercial plant production (Bourget, 2008). First, the initial high cost of LED lighting is an obstacle to their direct implementation in the horticultural industry; a life-cycle assessment should be performed in each lighting situation. Second, LEDs require large heat sinks to dissipate heat produced by their control boards. While their technology has improved considerably in the last few years, LED intensity needs to continue to increase or large densities of LEDs are needed to make them practical for some plant production applications (Runkle et al., 2011). Also, non-white lighting, common in LED fixtures intended for plant growth in sole-source environments, can make it difficult - to determine how plants are growing and to assess for pathogens since leaves may not appear as green. Some plants grown under sole-source LED lighting deficient in UV, B, and/or FR light can develop morphological abnormalities such as epinasty and edema (Massa et al., 2006, 2008). Unless these abnormalities can be understood and mitigated, growing marketable crops of certain varieties may be a challenge. Finally, LED lighting systems require their own unique fixtures which prevent growers from simply retrofitting existing lamps.

APPENDIX

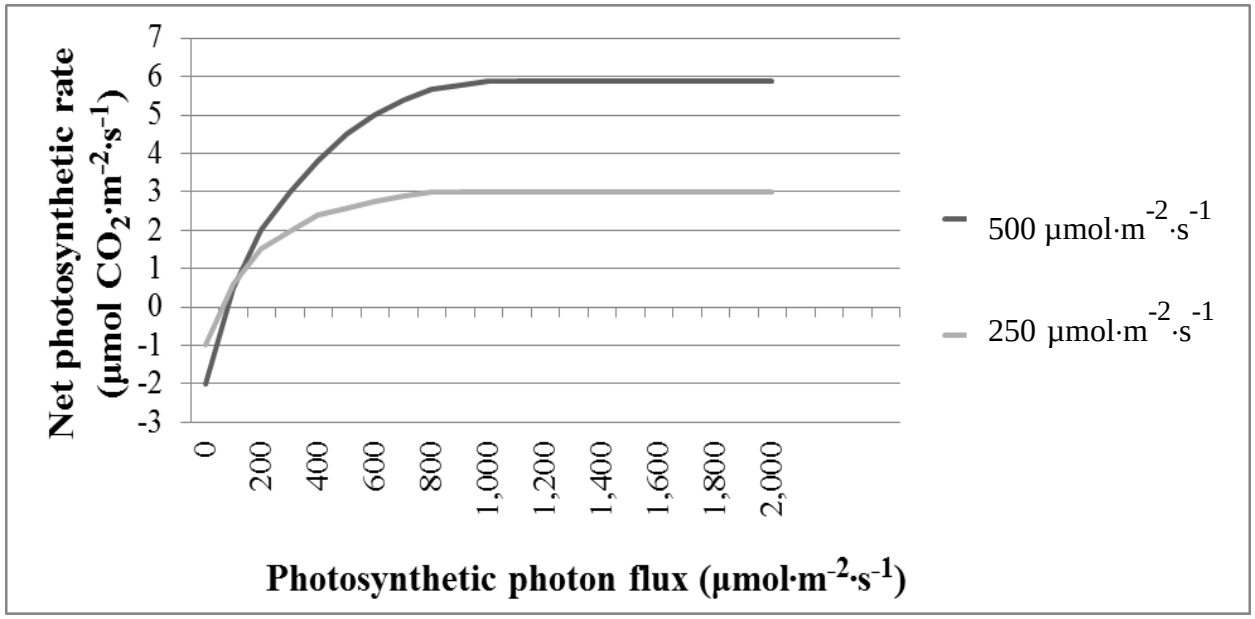

Figure 1.1. Example of a photosynthetic light response curve of the same species grown at two irradiances.

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SECTION II

GROWTH RESPONSES OF ORNAMENTAL ANNUAL SEEDLINGS UNDER DIFFERENT WAVELENGTHS OF RED LIGHT PROVIDED BY LIGHT-EMITTING DIODES

Growth Responses of Ornamental Annual Seedlings under Different Wavelengths of Red Light Provided by Light-emitting Diodes

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Abstract

Light-emitting diodes (LEDs) are of increasing interest in controlled environment plant production due to their increasing energy efficiency, long lifetime, and narrow bandwidth capability. Red light (600 to 700 nm) is generally the most efficient wavelength for photosynthesis, but little research has been published comparing growth responses under specific wavelengths of red, especially on ornamental plants. We grew seedlings of four popular bedding plants at 20 °C under six sole-source LED lighting treatments in two different experiments. In Expt. 1, a *PPF* of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was provided for 18 $h \cdot d^{-1}$ by 10% blue (B; peak=446 nm) and 10% green (G; peak=516 nm) light, with the remaining percentages consisting of orange (O; peak=596 nm) - red (R; peak=634 nm) - hyper red (HR; peak=664 nm) of 20-30-30, 0-80-0, 0-60-20, 0-40-40, 0-20-60, and 0-0-80, respectively. In Expt. 2, two light intensities (low, 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and high, 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were delivered by 10% B and 10% G light and the following percentages of R-HR: 0-80, 80-0, and 40-40. Seedlings of impatiens (*Impatiens walleriana*), marigold (*Tagetes patula*), petunia (*Petunia ×hybrida*), and tomato (*Lycopersicon esculentum*) were grown for 31 to 45 d in Expt. 1 and impatiens, petunia, tomato and salvia (*Salvia splendens*) were grown for 32 to 39 d in Expt. 2. There were few consistent effects of lighting treatment on growth parameters. However, plants grown under the 0-40-40 treatment in Expt 1. were usually relatively short while those under the 0-80-0 treatment were the tallest. In Expt. 2, impatiens had greater shoot dry weight under all treatments at the high light intensity compared to the low light intensity, while the chlorophyll concentration of all plant species was greater at 80-0 at 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than for plants grown under 40-40 at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. We conclude that O, R, and HR light have similar effects on plant growth at the intensities tested when background levels of G and B light are provided. For producers, the choice of R LEDs

used for plant growth could therefore depend on other factors, such as cost, electrical efficiency, and longevity.

Introduction

Radiation within the 400 to 700 nm waveband drives photosynthesis and is referred to as the photosynthetically active radiation (PAR). By definition, all wavelengths within this range are considered to stimulate photosynthesis equally. However, McCree (1972) produced a relative quantum efficiency (RQE) curve between 350 and 750 nm based on the photosynthetic activity of 22 crop species. The RQE curve has a primary peak at 620 nm and a secondary peak at 440 nm, which establishes that red (R; 600 to 700 nm) and blue (B; 400 to 500 nm) wavebands are more efficient in eliciting a photosynthetic response than wavelengths between 500 and 600 (green and yellow light). The peak RQE of R light is 30% higher than the B peak, and R light from 600 to 640 nm has the highest quantum yield (Evans, 1987; Inada, 1976; McCree, 1972).

Physiologically the different quantum efficiencies of PAR are due to the absorption spectra of plant pigments and the over-excitation of photosystem I (PSI), compared to photosystem II (PSII). Photosynthetic photons stimulate the excitation of PSI and PSII photosystems and the ratio of absorbed photons (≥580 nm) between the photosystems influences the RQE (Hogewoning et al., 2012). Hogewoning et al. (2012) grew cucumber (*Cucumis sativus*) plants under an artificial sunlight spectrum, an artificial shade spectrum [greater far-red (>680 nm) light] and under B LED (peak=445 nm) light (*PPF*=100 µmol·m^{-2}·s^{-1}; 16-h photoperiod). The highest quantum efficiency recorded was between 620 and 640 nm. Similar to McCree (1972), the quantum yield was 70% of the maximum between 427 and 560 nm because of the lower absorbance of these wavelengths and the lower quantum efficiencies. The over-excitation

of PSI and greater quantum efficiency occurred in cucumber under artificial shade, whereas PSII was overexcited with greater quantum efficiency in plants grown under artificial sunlight and B light (Hogewoning et al., 2012). In contrast to Emerson et al. (1957), Hogewoning et al. (2012) also concluded that a combination of wavelengths within PAR could increase quantum yield and thus, plant growth.

Although R light can be the most effective in stimulating carbon fixation in photosynthesis, plants accumulate biomass faster and have a normal morphology with the addition of B light, G light, or both (Eskins, 1992; Kim et al., 2004). Sweet potato *(Ipomoea batatas*) produced similar proportions of roots and shoots when grown under a R:B ratio of 8:1 (R LED peak=660 nm and B LED peak=450 nm) at a *PPF* of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Yang et al., 2011). Tomato plants (*Lycopersicon esculentum*) also had a similar root:shoot ratio when the R:B was 9:1 at a *PPF* of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kato et al., 2011). The addition of green light can also increase biomass accumulation in plant production. Lettuce (*Lactuca sativa*) plants accumulated more biomass with the addition of up to 24% green light (510 to 610 nm) from green fluorescent bulbs or LEDs when the *PPF* was 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kim et al., 2004). Plants grown under R light alone can develop abnormal morphological traits, such as in lettuce, where hypocotyls were elongated (Hoenecke et al., 1992). In addition, pepper plants (*Capsicum annuum*) developed severe edema (development of tumors as a result of a greater rate of water uptake than transpiration) when grown under sole R light or less than 10 to 15 % B light (Massa et al., 2008).

The emission of narrow-waveband light by LEDs provides the opportunity to test the effects of specific wavebands of light on plant growth and development. LEDs used for sole-source photosynthetic lighting can enable commercial growers to produce plants with desired

characteristics and to optimize the spectra for each crop and stage of development (Folta and Childers, 2008; Stutte, 2009). LEDs are well suited for commercial plant production due to their improving energy efficiency, spectral specificity, and longer lifetimes than the current industry standard lamps (e.g., fluorescent and high-pressure sodium) (Bourget, 2008; Morrow, 2008). LEDs emitting photons with greater RQEs could increase photosynthesis (Stutte, 2009) and potentially decrease commercial plant production time and costs compared to less efficient wavelengths of light.

To our knowledge, no studies have been published that compared the effect of different wavebands of R light on plant growth. We grew seedlings of ornamental plants under different ratios of R (peak=634 nm) and HR (peak=664 nm) light, as well as under orange light (peak=596 nm), to determine whether a particular wavelength or a combination of wavelengths of R light increased plant growth. We postulated that growth attributes of young plants would be similar under the same *PPF* as long as equal amounts of background B and G were provided in all treatments.

Materials and Methods

Expt. 1. The effect of red light wavelengths on plant growth. Four popular bedding plant species, vegetable and floral crops, with varying shade tolerances, were chosen for study: tomato (*Lycopersicum esculentum* 'Early Girl'), marigold (*Tagetes patula* 'Deep Orange'), impatiens (*Impatiens walleriana* 'SuperElfin XP Red'), and petunia (*Petunia ×hybrida* 'Wave Pink').Seeds were sown in 128-cell (2.7 × 2.7 cm; 12.0-mL volume) plug trays at a commercial greenhouse (C. Raker and Sons, Inc., Litchfield, MI) and transferred to research greenhouses at Michigan State Univ. (East Lansing) within 2 d. Seeds were kept in a propagation greenhouse at 23°C until

>70% germinated, which was 2 d (replication 1) or 7 d (replication 2) after seed sow. Each plug tray was then cut into six sections each with ≥20 seedlings, thinned to one plant per cell, and placed in the LED modules.

Light environments. Six LED modules were custom-designed and constructed for experimentation (Osram OptoSemiconductors, Northville, MI; Figure 2.1). The white rigid plastic modules had four sides and were 80 cm deep, 27 cm wide, and 52 cm tall. The top of each module contained blue (B, peak=446 nm), green (G, peak=516 nm), orange (O, peak=596 nm), red (R, peak=634 nm), and hyper red (HR, peak=664 nm) LEDs (Osram OptoSemiconductors) that were uniformly distributed, facing downwards inside the module. Eighty LEDs of each color were mounted on fan-cooled driver boards that were open to the environment to allow for adequate cooling. The light output of each color of LED could be adjusted manually by a dimmer switch. The LEDs were mounted 25 to 33 cm from the foliage canopy. To improve air circulation within the module, 33 holes (diameter=4 cm) were cut in the bottom. The light modules were placed on open, metal mesh benches inside a refrigerated walk-in growth chamber.

Six light treatments were randomly allocated to the light modules for each replication and the light quality treatments were set to the desired ratios using a portable spectroradiometer (StellarNet Inc., model PS-200, Apogee Instruments, Inc., Logan, UT) with a *PPF* constant at 160 μmol·m^{-2}·s^{-1}. All treatments delivered 10% B and 10% G light, with the remaining light quality percentages consisting of O-R-HR of 20-30-30, 0-80-0, 0-60-20, 0-40-40, 0-20-60, and 0-0-80. Predicted phytochrome photoequilibrium (P_{FR}/P, where $P = P_R+P_{FR}$) values were similar among all light treatments (0.88 to 0.89). To increase uniformity of light intensity within each module, wire mesh was placed in the middle half of the chamber, just below the LEDs. The

plant trays were randomly rearranged daily to reduce spatial variability inside each module. The spectral qualities of the light treatments were evaluated at six positions inside each LED module with the spectroradiometer (Figure 2.2 A).

Plants were grown under an 18-h photoperiod (0500 to 2300 HR) as controlled by a data logger (CR10; Campbell Scientific, Logan, UT). Temperature was set to 20°C and was monitored by infrared sensors (Type K, OS36-01; Omega Engineering) positioned 17 cm from the module bottom and pointing towards the canopy of the closest plant tray, as well as shielded thermocouples (0.13-mm type E; Omega Engineering, Stamford, CT) inside each module at plant level. Light intensity was measured continuously in each module by quantum sensors (LI-COR, Lincoln, NE) placed in the middle of each module at plug tray level. Environmental parameters were measured every 10 s and data were recorded by the data logger every 10 minutes throughout the duration of the experiments (Table 2.1). Plants were irrigated as needed, once or twice daily, through subsurface irrigation with deionized water supplemented with a water-soluble fertilizer providing (in $mg \cdot L^{-1}$) 50 N, 19 P, 50 K, 23 Ca, 4 Mg, 1 Fe, 0.5 Mn, Zn, and Cu, 0.3 B, and 0.1 Mo (MSU Plug Special; GreenCare Fertilizers, Inc., Kankakee, IL).

Data collection and analysis. The experiment was performed twice and 10 plants of each species and treatment were selected at random and harvested the following number of days after seed sow (rep 1, 2): tomato (33, 31), marigold (34, 33), impatiens (43, 38), and petunia (45, 39). The following data were collected on plants in each treatment: leaf number (total leaf number including axillary branches on impatiens and petunia), leaf area [using a leaf area meter (LI-3000; LI-COR)], stem height (from media level to apical meristem), shoot fresh weight, shoot dry weight (dried in a NAPCO 630 oven at ≥66 °C for ≥5 d), number of visible flower buds (if present) and flower bud fresh weight (if applicable). Effects of species and light treatments were

compared by analysis of variance using SAS (SAS Institute, Cary, NC) PROC MIXED or PROC GLIMMIX (Poisson distribution for count data), with an additional program (Arnold M. Saxton, Univ. of Tennessee) that provided pairwise comparisons between treatments using Tukey honestly significant test at $P \leq 0.05$.

Expt. 2. The effect of R light ratios at two intensities. Experimental procedures were followed as reported in Expt. 1 unless otherwise noted. One 128-cell tray of the same tomato, impatiens, and petunia varieties in addition to salvia (*Salvia splendens* 'Vista Red') were obtained from a commercial greenhouse (C. Raker and Sons, Inc.). Two light intensities [125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (low) or 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (high)] were delivered with three light quality treatments. All treatments delivered 10% B and 10% G light, with the remaining light quality percentages consisting of R-HR: 0-80, 40-40, and 80-0 (Figure 2.2B). Ten randomly-selected plants were harvested the following number of days after germination (replication 1, 2): tomato (32, 33), impatiens (35, 34), petunia (37, 35), and salvia (39, 36). The total leaf number, including axillary branches, was counted on impatiens.

Chlorophyll assay. Chlorophyll concentrations were measured using the procedure described by Richardson et al. (2002) 28 d after seed sow. Leaf samples of 0.100 g ± .002, measured using a Denver Instrument APX-320 scale (Bohemia, NY), were placed in disposable culture glass tubes (16 × 100 mm; WMR International, West Chester, PA) and 7 mL of dimethylsulfoxide (DMSO; EMD Millipore, Billerica, MA) was added using an Eppendorf Easypet electronic pipette (Hamburg, Germany) and heated in a deionized water bath (Isotemp 210, Fisher Scientific, Pittsburg, PA) to 65°C for 40 minutes. Three mL of DMSO was added to each sample tube and the electronic pipette was used to place 1.5 mL of the extraction solution into foil-wrapped 1.7 mL Posi-Click tubes (Denville Scientific Inc., South Plainfield, NJ) to

prevent photo- or thermo-degradation. Each sample was poured into a 1.5 mL Semimicro polystyrene cuvette (Generation Biotech, Lawrenceville, NJ) and the absorbance of each sample was measured against a blank standard (DMSO) at 645 and 663 nm using a spectrophotometer (BioSpec 1601, Shimadzu, Kyoto, Japan). Chlorophyll *a*, *b*, and total chlorophyll concentrations were determined using the equations by Arnon (1949).

Results

Expt. 1. Leaf area and number. There was a significant light treatment and replication interaction on leaf area and therefore, replications were analyzed separately (Figure 2.3). Impatiens leaf area was similar among treatments in replication 1, but it was largest under the 20-30-30 (%O-%R-%HR) treatment in replication 2. Marigold had the largest leaves under the 0-20-60 treatment in replicate 1. In contrast, in the second replicate, marigold grown under the 0-40-40 and 20-30-30 treatments had larger leaves than plants under 0-80-0 or 0-20-60 treatments. Marigold developed dark purple spotting on leaves in all treatments and in both replications (Figure 2.4). Similar to marigold, in the second replicate, tomato grown under the 0-60-20 treatment had larger leaves than the two treatments with at least 60% HR light. Tomato seedlings in all treatments developed edema, a purple leaf coloration particularly on the abaxial surface, and interveinal chlorosis. Petunia seedlings grown under 60% HR light had larger leaves than seedlings grown without any HR light in both replicates. The mean leaf number was similar among treatments for all species and was 25.4, 9.9, 7.7, and 29.0 for impatiens, marigold, tomato, and petunia, respectively (data not shown).

Seedling height. Plant height of impatiens was similar under the light quality treatments, whereas marigold and tomato were generally shortest under the 0-40-40 treatment and tallest

under the 0-80-0 treatment. Impatiens grown under 80% R light were taller than plants grown under the 20-30-30 treatment in the first replicate, while plants grown under 80% HR light were shorter than all other treatments in the second replicate. Marigold grown under 80% R light in the first replicate was taller than all other treatments except plants grown under the 0-20-60 treatment. Marigold height was similar in the second replicate except that those under the 0-60-20 treatment which were relatively tall. Tomato grown under the 80% R treatment was taller than under the 0-40-40 treatment in both replicates.

Fresh shoot weight. There were no consistent trends on the effect of light quality treatments on fresh weight of seedling shoots. Impatiens shoot fresh weight was similar among treatments in the first replicate, but it was greatest under the 20-30-30 treatment in the second replicate. In the first replicate, marigold grown under 80% R light had greater fresh weight than those grown under other treatments except under the 20-30-30 treatment or 80% HR light. In contrast, in the second replicate, marigold had a similar fresh weight except for those grown under 80% R or the 0-40-40 treatment. Tomato grown under the 0-60-20 treatment had a greater fresh weight than plants under other treatments with less R and more HR light. Fresh weight of petunia was consistently relatively high under the 0-60-20 and 20-30-30 treatments and relatively low under the 0-80-0 treatment (Figure 2.3).

Dry shoot weight. The mean shoot dry weight was generally similar among treatments for all species, and was 0.20, 0.21, 0.29, and 0.14 g for impatiens, marigold, tomato, and petunia, respectively (data not shown). The mean shoot dry weight for impatiens in the first replicate was relatively low in plants grown under the 0-60-20 treatment. In contrast, the shoot dry weight of impatiens in the second replicate was relatively high under the 0-40-40 treatment and relatively low under the two treatments with the most HR light. The shoot dry weight of tomato was

relatively high under treatment 0-60-20 and likewise, relatively low under the two treatments with the most HR light. Petunia had relatively high dry biomass when grown under 80% R light, while they had the least biomass when grown under the 20-30-30 treatment.

Expt 2. Leaf area and number. There was a significant light treatment and replication interaction on leaf area and therefore, replications were analyzed separately (Figure 2.5). There were few discernible leaf area trends in the first replicate, but in the second, all species under the low 80%R-0%HR (0-80_{low}) treatment had a greater leaf area than in any other treatment. Impatiens grown under the two 0-80 treatments had greater leaf areas than plants under the 80-0_{high} treatment in the first replicate. Similarly, leaves of impatiens under the treatment 80-0_{high} were relatively small compared to other treatments in the second replicate. In the first replicate, salvia seedlings under the 40-40_{low} treatment had larger leaves than plants grown under either treatment 0-80_{high} or 80-0_{high}. Similarly, plants under the latter two treatments were relatively small in the second replicate. Tomato seedlings had larger leaves under the 0-80_{low} and 40-40_{low} treatments compared to under the 0-80_{high} treatment in both replicates. Tomato developed edema, chlorosis, necrotic leaf margins, and purple pigmentation in all treatments in both replicates. Petunia grown under the 0-80_{high} and 80-0_{high} treatments had relatively small leaves in both replicates. The mean leaf number was similar among treatments and was 20.2, 5.2, 11.0, and 11.3 for impatiens, tomato, salvia, and petunia, respectively (data not shown).

Seedling height. Impatiens grown under treatment 0-80_{low} were taller than plants of all other treatments in the first replicate, but plants grown under treatments 40-40_{low} and 80-0_{low} were the tallest in the second replicate. Salvia under the low-intensity treatments were taller than those under the high-intensity treatments in both replicates with the exception of those in treatment 0-80_{low} in the second replicate. In the first replicate, tomato grown under treatment 0-

80_{low} were taller than all other treatments whereas in the second replicate, plants were tallest under treatment 80-0_{low}.

Fresh shoot weight. There were no consistent effects of light quality treatments on fresh shoot weight among species and between replications (Figure 2.6). Impatiens grown under treatments 0-80_{low}, 0-80_{high} and 40-40_{high} had greater fresh shoot weight than plants under treatments 40-40_{low} or 80-0_{low} in the first replicate but not in the second replicate. Salvia grown under treatment 40-40_{high} had a greater fresh weight compared with all other treatments except 40-40_{low} in the first replicate and all but 80-0_{low} in the second replicate. Impatiens, salvia, and petunia grown under treatment 80-0_{low} had the greatest fresh weight in the second replicate. Tomato had a relatively high biomass when grown under treatments 0-80_{low} and 80-0_{low} in the second replicate. Petunia grown under treatment 40-40_{high} had greater fresh weight than plants grown under treatment 40-40_{low} in both replicates.

Dry shoot weight. With the exception of tomato under the 0-80 treatments, shoot dry weight was similar to or greater under the high light environments than the low light treatments within a specific light quality ratio. Impatiens had greater dry weight under all high-intensity treatments compared to those grown under low-intensity in the first replicate. In the second replicate, impatiens grown under treatment 80-0_{high} had relatively high dry weights. In contrast, salvia had the greatest dry weight under the 40-40_{high} treatment in both replicates. Similarly, tomato had relatively high dry weight when grown under the 40-40_{high} treatment. Petunia grown under treatments 40-40_{high} and 80-0_{high} had relatively high dry weight in both replicates.

Chlorophyll concentration. Chlorophyll concentration was greatest for impatiens, tomato, and petunia under the 80-0_{low} treatment (83.8, 119, and 90.5 mg Chl·g^{-1} fresh tissue, respectively) and was the greatest for salvia under the 40-40_{low} treatment (138 mg Chl·g^{-1} fresh

tissue). Chlorophyll concentration under these treatments was set to 100% and chlorophyll concentration for the other treatments was calculated relative to these values. Chlorophyll concentration was relatively high in plants grown under 80-0_{low} for all species (Figure 2.7). In addition, chlorophyll was similar to or reduced under high light within each light quality treatment, especially in petunia. Chlorophyll concentration was statistically similar within each crop under the high light treatments, whereas in the low light treatments, impatiens and petunia had a relatively low amount of chlorophyll under the 0-80 treatments.

Discussion

LEDs emit a wide range of wavelengths, including those within the photosynthetic active waveband. Our objective was to determine whether young plants grew differently under one, two, or three different peaks of O or R light. In two different experiments, plants grew similarly and there were few consistent treatment effects between replicates and among species. There were a few consistent treatment effects, for example plants under the 40% R + 40% HR treatment were usually relatively short, but differences were extremely minor. When three ratios of R and HR were delivered at two intensities in Expt. 2, plants grown under twice the light intensity were similar to or shorter than plants at the lower intensity. Since an increase in extension growth is common in shade-intolerant species under low light intensities, it is not surprising that the magnitude of the difference tended to be greater for sun-adapted species such as salvia and tomato than in the shade-tolerant impatiens (Runkle and Heins, 2006; Smith, 1994).

The relative photosynthetic quantum efficiency of the treatments was calculated according to McCree (1972) and Sager et al. (1988), and was greatest for the 80% R light treatment (0.89) and least for the 80% HR light treatment (0.88). Therefore, at an intensity of

160 µmol·m^{-2}·s^{-1}, the effective irradiance of the R and HR light treatments only differed by 1% (141 vs. 142 µmol·m^{-2}·s^{-1}). Not surprisingly, biomass accumulation was often similar under the light quality treatments at the same *PPF*. Exposure to LEDs with peak emissions of 634 nm (R) and 664 nm (HR) likely resulted in similar stomatal conductances and photosynthetic rates because their peak wavelengths are below the critical threshold of 680 nm, above which decreased growth rates have been previously reported due to an inequality of photons between photosystems I, II, and the electron transport chain (Tennessen et al., 1994; Zeiger and Hepler, 1977). When salvia, ageratum (*Ageratum houstonianum*), and marigold were grown under 90 µmol·m^{-2}·s^{-1} from R (peak=650 nm) + B (peak=470 nm), B + FR (peak=720 nm), or R + FR LEDs, those under B or R with the addition of FR light had approximately 30-60% less dry weight than those grown without wavebands ≥680 nm (Heo et al., 2006). Consistent with McCree (1972), Heo et al., (2006) reported that plants grown with 720 nm light had lower photosynthetic rates and, therefore, decreased dry weights than those only grown with light between 400 and 700 nm.

The well-established paradigm is that increasing light intensity increases photosynthesis, biomass accumulation, and harvestable yield. For example, Japanese mint (*Menta arvensis*) had up to a 50% increase in instantaneous photosynthetic rates when grown under white fluorescent lamps at a *PPF* of 200 µmol·m^{-2}·s^{-1} compared to 100 µmol·m^{-2}·s^{-1} (Malayeri et al., 2011). Spinach (*Spinacea oleracea*) grown under B, R, or white fluorescent lamps at 300 µmol·m^{-2}·s^{-1} had 10 to 80% greater leaf and stem biomass than spinach grown under 100 µmol·m^{-2}·s^{-1}, depending on the cultivar, when the light quality was kept consistent (Li et al., 2011). However, our study and related studies depart from this trend. We found that fresh and dry weights of seedlings under a *PPF* of 125 µmol·m^{-2}·s^{-1} were similar to those grown at 250 µmol·m^{-2}·s^{-1}.

Similarly, strawberry was grown under different combinations of R (peak=660 nm) and B (peak=450 nm) LED lighting at a *PPF* of 45, 60, or 75 µmol·m^{-2}·s^{-1} for 45 d. Plants grown under 60 µmol·m^{-2}·s^{-1} had 7% greater shoot fresh weight than those grown under 75 µmol·m^{-2}·s^{-1} (Nhut et al., 2003). These counter-intuitive results may result from plant acclimation responses to low light. One acclimation response to low light intensity is an increase in leaf area, such as that observed in strawberry (Jurik et al., 1979). In our study, salvia, tomato, and petunia grown under 80% HR at 125 µmol·m^{-2}·s^{-1} in Expt. 2 had relatively low leaf areas while all species had comparatively high leaf areas when grown under 80% R at a *PPF* of 250 µmol·m^{-2}·s^{-1}.

Another way that plants respond to light intensity is by changing their chlorophyll concentration. By using a SPAD measurement, Nhut et al. (2003) reported that strawberry plants had the greatest chlorophyll content index when irradiated with a *PPF* of 60 µmol·m^{-2}·s^{-1}, the second greatest under 75 µmol·m^{-2}·s^{-1}, and the least under 45 µmol·m^{-2}·s^{-1} (Nhut et al., 2003). Similarly, in Expt. 2, plants under the low-intensity light treatment had a greater leaf chlorophyll concentration than the high light treatment, enabling plants to better harvest photons and accumulate similar biomass as plants grown under twice the light intensity. Leaves of plants grown under the low-intensity light treatments had up to 40% more chlorophyll than leaves of the high-intensity light treatment, especially under the 80% R treatment, which could explain the increased fresh and dry weight observed for that treatment. Relatively little is known about the effects of different wavelengths of R light on chlorophyll concentration, although other wavebands are known to affect chlorophyll. For example, Saebo et al. (1995) examined how a *PPF* = 30 µmol·m^{-2}·s^{-1} emitted by colored fluorescent lamps influenced growth of silver birch (*Betula pendula*) under different ratios of B (410-510 nm), R (640-680 nm), and FR (700-750 nm) light. Plants grown under B light had ≈25% greater chlorophyll concentration per leaf area

than plants grown under cool-white fluorescent light and ≈50% more than R. In addition, the chlorophyll concentration of leaves in cucumber (*Cucumis sativus*), was approximately 36% greater under 50% B LED (peak=450 nm) light than without B light [remaining percentage of light provided by R LED (peak=638 nm)], while the leaf photosynthetic capacity was three times greater, respectively, at a *PPF*=100 µmol·m^{-2}·s^{-1} (Hogewoning et al., 2010).

With the exception of tomato, all plants developed normally under the lighting treatments that all had background B and G light. The purple pigmentation present on the abaxial leaf surface was likely not nutrient related since media pH was within the normal range (5.5 to 6.2; Nau, 2011) and plants received complete fertigation throughout the duration of experiments. Environments without UV radiation, specifically UV-B (280 to 315 nm; Jenkins, 2009) (e.g., Lang and Tibbitts, 1983; Jones and Burgess, 1977; Nilsen, 1971), without B light (Massa et al., 2008), without FR light (Morrow and Tibbitts, 1988), or with high humidity (e.g., Balge et al., 1969; Warrington, 1980) and have been associated with edema in some crops, especially those in the Solanaceae. Massa et al. (2006) reported that edema or intumescence developed on cowpea plants (*Vigna unguiculata*) when grown under <10 to 15 percent B light (peak=440 nm) when in an R dominant (peak=660 nm) environment. Similarly, pepper plants (*Capsicum annuum*) developed severe edema on the leaves and fruit, which negatively affected their fruit productivity. However, tomato ‘Persimmon’ did not exhibit edema under the same environmental conditions. Thus, edema seems to be cultivar- and species-specific (Massa et al., 2008). Morrow and Tibbitts (1988) reported that wild tomato (*L. hirsutum*) developed edema on 63% of the sampled leaf area surface when under R fluorescent lamps whereas it was absent under B fluorescent lamps at a *PPF* of 25 µmol·m^{-2}·s^{-1}. The development of edema on tomato in all R-dominant treatments is consistent with those of Morrow and Tibbitts (1988), but is not

consistent with Massa et al. (2006) who suggests that 10% B light (present in all our treatments) should have been sufficient to prevent edema (Massa et al., 2008).

Since there were few consistent differences in plant growth between different wavelengths of orange-red light, R LEDs could be chosen based on other factors such as electrical efficiency. We measured the energy consumed by our modules with a wattage meter (Kill a Watt meter, Arbor Scientific, Ann Arbor, MI) with the LEDs off and again with each color emitting 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The B, G, O, R, and HR LEDs in our modules had the following efficiencies ($\mu mol \cdot W^{-1}$): 2.39, 0.84, 0.72, 2.29, and 2.46. This analysis indicated that the HR LEDs were 7% more efficient than the R LEDs, while the O LEDs were less than one-third as efficient as the R or HR LEDs. The B LEDs were also relatively efficient whereas the G LEDs had a low efficiency. Therefore, horticultural lighting should utilize the B, R, and/or HR LEDs for maximum energy efficiency. In addition, other factors such as cost, longevity, and reliability should be considered when choosing LEDs for horticultural lighting.

Summary

In two different experiments, testing the effects of the ratio of R and HR light on young plant growth, very few consistent treatment effects existed between replicates and among species. In Expt 1., plants grown at a *PPF* of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ grew similarly under R and HR LEDs likely because of similar relative photosynthetic quantum efficiencies of the treatments. When three ratios of R and HR were delivered at two intensities in Expt. 2, plants grown under twice the light intensity were similar to or shorter than plants at the lower intensity and had relatively similar fresh weights. The relatively high fresh and dry weight of plants under some of the lower intensity treatments can be attributed plant adaptations such as greater chlorophyll

concentrations in leaves of plants grown under the low-intensity or increased leaf expansion. Since there were few consistent differences in plant growth between different wavelengths of O or R light, R LEDs could be chosen based on other factors such as electrical efficiency, cost, longevity, and reliability.

APPENDIX

Table 2.1. Actual air temperatures (°C, measured by thermocouples) and canopy temperatures (°C, measured by infrared sensors) during Expt. 1 and 2 for all light quality treatments (reported in percentages of orange (O), red (R), and hyper red (HR) light. All treatments also received 10% blue and 10% green light. All temperatures had a standard error ±0.1°C.

	Light quality			Light intensity	Replication 1		Replication 2	
Expt.	O	R	HR	($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Air	Canopy	Air	Canopy
1	0	80	0	160	21.3	20.8	20.9	20.2
	0	60	20	160	21.1	20.7	20.8	20.4
	0	40	40	160	21.1	20.5	20.5	20.1
	20	30	30	160	21.0	20.3	21.5	20.7
	0	20	60	160	21.3	20.7	21.0	20.4
	0	0	80	160	20.9	20.2	20.4	19.8
2	0	0	80	125	20.6	20.4	20.5	20.6
	0	40	40	125	20.5	20.5	20.5	20.4
	0	80	0	125	20.5	20.4	20.6	20.4
	0	0	80	250	21.7	21.2	21.1	21.0
	0	40	40	250	21.6	20.8	21.6	20.8
	0	80	0	250	21.1	21.0	21.7	21.2

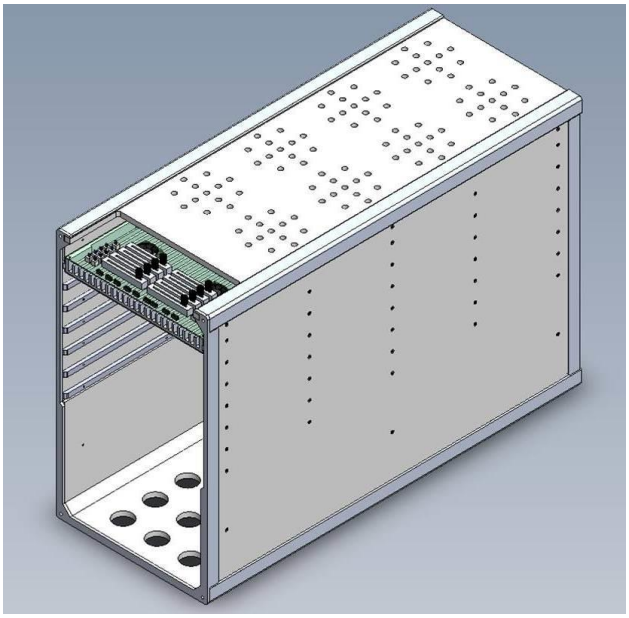

Figure 2.1. Diagram of custom-built chambers that delivered light from light-emitting diodes (LEDs) courtesy of OSRAM OptoSemiconductors. Dimmer switches located on the fan-cooled driver boards enabled the light intensity of each of the five colors of LEDs to be independently adjusted to the desired output. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

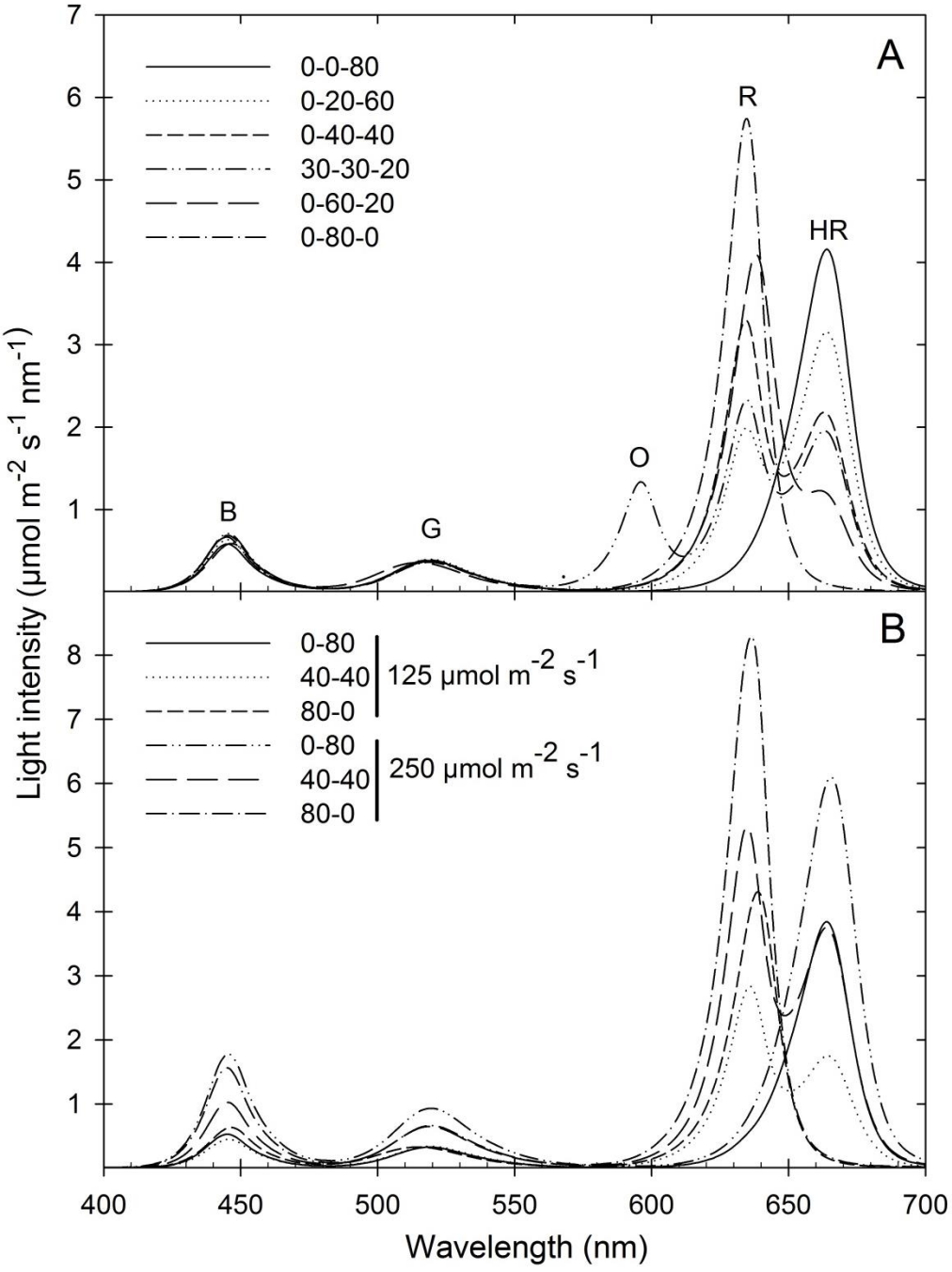

Figure 2.2. Spectral distribution of light quality treatments consisting of blue (B), green (G), orange (0), red (R), and hyper red (HR) at *PPF*=160 µmol·m^{-2}·s^{-1} (Expt. 1; A) or 125 and 250 µmol·m^{-2}·s^{-1} (Expt. 2; B). All treatments also received 10% B and 10% G light with the remaining percentages in the format of O-R-HR (Expt. 1) or R-HR (Expt. 2).

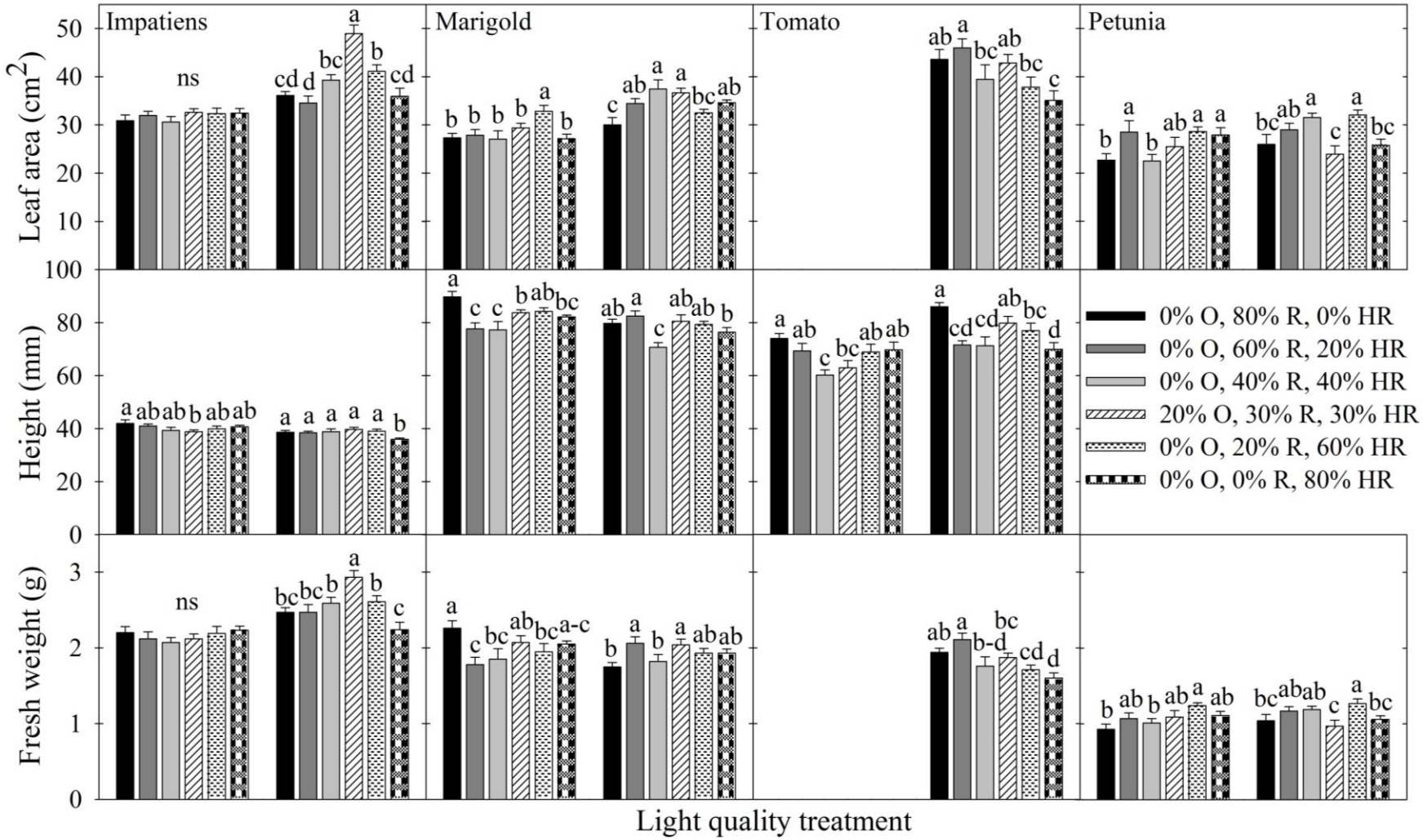

Figure 2.3. Mean leaf area, height, and fresh shoot weight for impatiens, marigold, tomato and petunia for six light quality treatments (O: orange, R: red, HR: hyper red) in Expt. 1 where all treatments received 10% blue and 10% green light and a *PPF* = 160 µmol·m^{-2}·s^{-1}. Replication 1 is shown on the left and replication 2 is shown on right. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error. Tomato leaf area and fresh shoot weight data for replication 1 were not included due to desiccation near the end of the experiment.

Figure 2.4. Spotting on the adaxial surface of marigold leaves (left) and edema and purple coloration of tomato (right). Symptoms were present in all light quality treatments in Expt. 1.

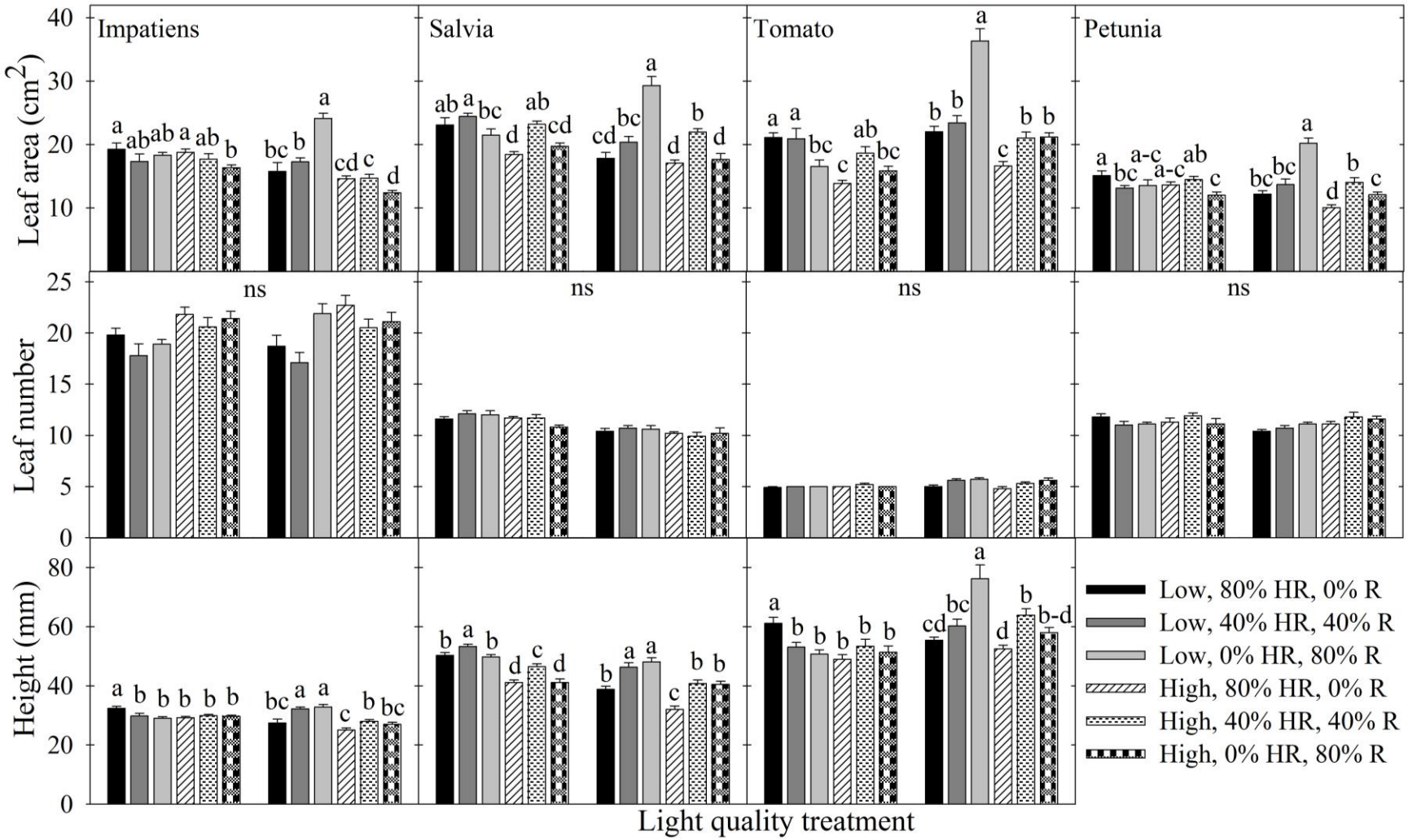

Figure 2.5. Mean leaf area, leaf number, and height for impatiens, marigold, tomato, and petunia for six light quality treatments (R: red, HR: hyper red) where all treatments received 10% blue and 10% green light. The *PPF* was 125 or 250 µmol·m^{-2}·s^{-1} (low or high, respectively). Replication 1 is shown on the left and replication 2 is shown on right. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

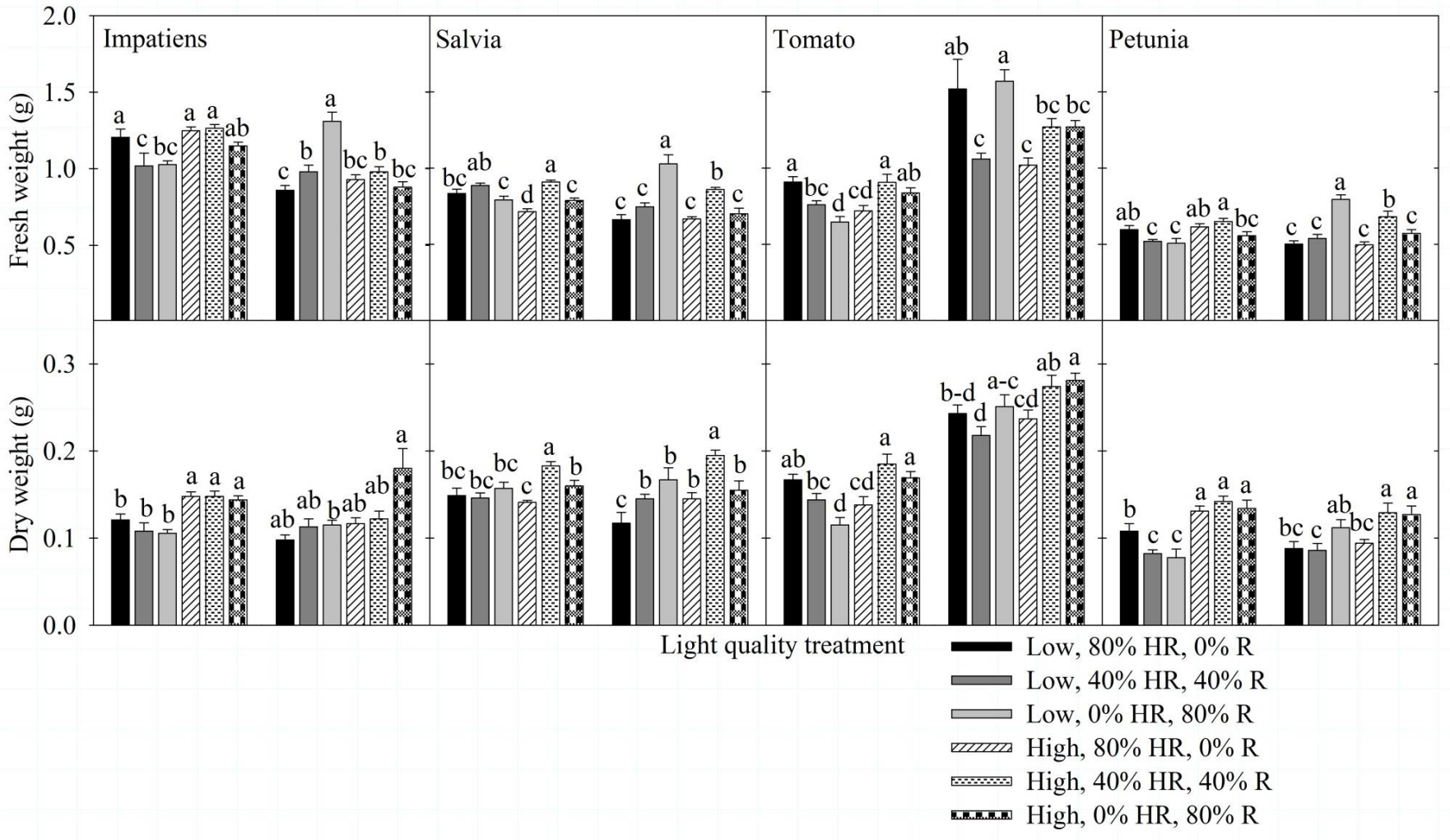

Figure 2.6. Mean fresh and dry shoot weights for impatiens, marigold, tomato and petunia for six light quality treatments (R: red, HR: hyper red) in Expt. 2 where all treatments received 10% blue and 10% green light. The *PPF* was 125 or 250 µmol·m^{-2}·s^{-1} (low or high, respectively). Replication 1 is shown on the left and replication 2 is shown on right. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

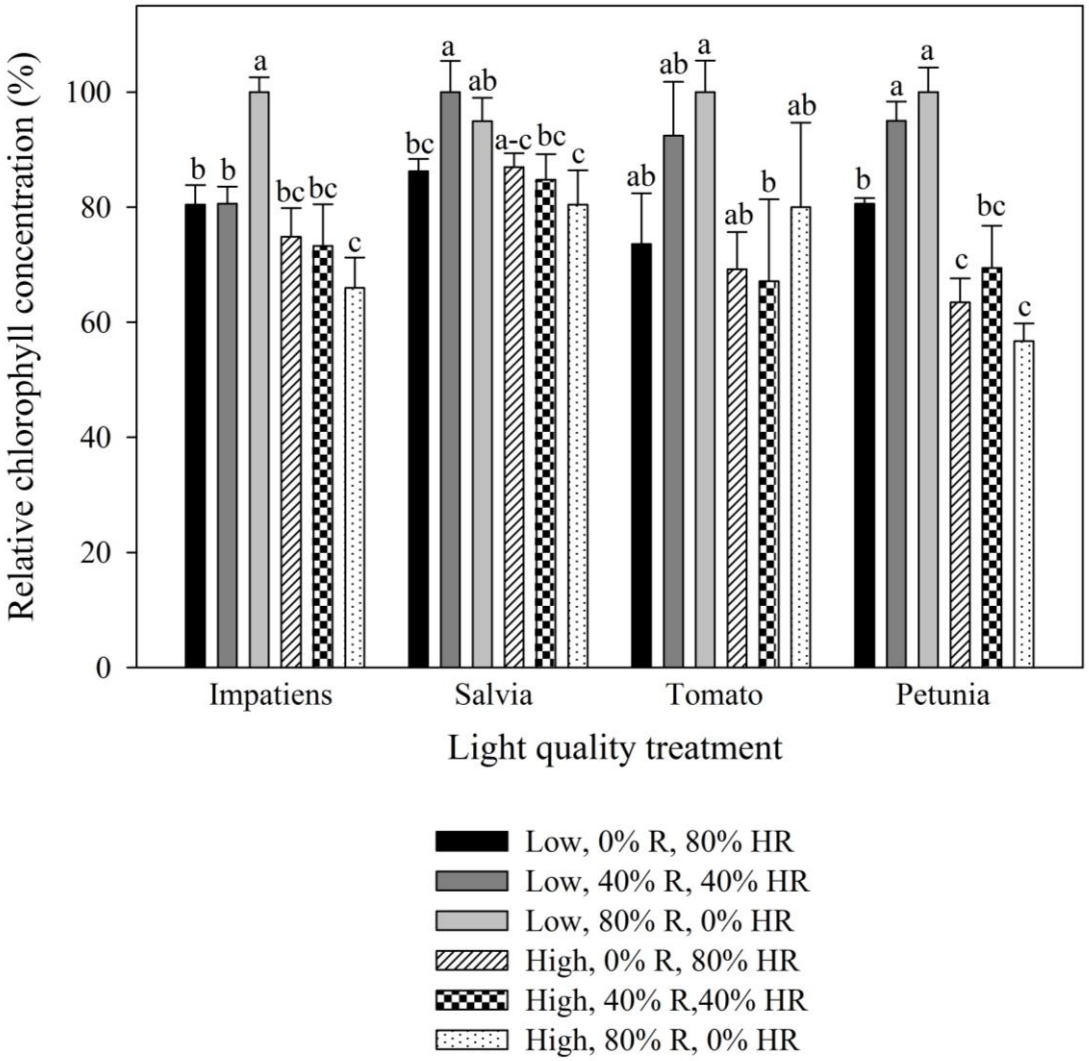

Figure 2.7. Relative chlorophyll concentration for replicate two for impatiens, tomato, petunia, and salvia for six light treatments (R: red, HR: hyper red) in Expt. 2. All treatments received 10% blue and 10% green light and the *PPF* was 125 or 250 µmol·m^{-2}·s^{-1} (low or high, respectively). Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

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SECTION III

GROWTH ACCLIMATION OF SEEDLINGS TO BLUE, GREEN, AND RED LIGHT FROM LIGHT-EMITTING DIODES AT A FIXED IRRADIANCE

Growth Acclimation of Seedlings to Blue, Green, and Red Light from Light-emitting Diodes at a Fixed Irradiance

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Additional index words. Bedding plants, controlled environments, LEDs, light quality, transplants

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Abstract

Many studies have examined the effects of blue and green light on plant growth, but little research has been published comparing growth responses under ratios of blue and green light with background levels of red light under stringent environmental conditions, especially on ornamental plants. We grew seedlings of four annuals under six sole-source LED lighting treatments or one cool-white fluorescent treatment that each delivered a *PPF* of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with an 18-h photoperiod. The following treatments were provided using blue (B, peak=446 nm), green (G, peak=516 nm), red (R, peak=634 nm), and hyper red (HR, peak=664 nm) LEDs: $B_{25}+G_{25}+R_{25}+HR_{25}$ (25% light from each), $B_{50}+G_{50}$, $B_{50}+R_{25}+HR_{25}$, $G_{50}+R_{25}+HR_{25}$, $R_{50}+HR_{50}$, and B_{100}. Seedlings of impatiens (*Impatiens walleriana*), salvia (*Salvia splendens*), petunia (*Petunia ×hybrida*), and tomato (*Lycopersicum esculentum*) were grown for 31 to 36 d at a constant 20 °C. Leaf number was similar among all treatments but plants grown under ≥25% B light were 41 to 57% shorter than those under $R_{50}+HR_{50}$. Plants under $R_{50}+HR_{50}$ had 47 to 130% greater leaf area and 48 to 112% greater fresh shoot weight than plants grown under treatments with ≥25% B. Plants grown under $R_{50}+HR_{50}$ had a similar fresh shoot weight to those grown under fluorescent light for all species except tomato. Edema was severe in tomato grown under the $R_{50}+HR_{50}$ treatment but was absent when grown under the $B_{50}+G_{50}$ treatment. We conclude that high quality seedlings can be produced under LED lighting that includes at least a minimal (e.g., 25%) quantity of blue or green light, and their compactness could eliminate the need for other height control strategies.

Introduction

Light-emitting diodes (LEDs) are well suited for commercial plant production due to their high energy efficiency and spectral specificity (Mitchell et al., 2012). When operated at favorable temperatures, well-constructed LEDs have an operating lifetime of 50,000 hours or more, which is at least two times longer than conventional high-pressure sodium (HPS) or fluorescent lamps (Bourget, 2008; Morrow, 2008). LEDs also have potential to be used as inter-canopy lighting, to increase nutrient concentration in edible crops, and to decrease pesticide and plant growth regulator application (Cosgrove, 1981; Doukas and Payne, 2007; Hemming, 2011; van Ieperen and Trouwborst, 2008; Kumar and Poehling, 2006; Li and Kubota, 2009; Rapisarda et al., 2006; Watanabe, 2011; Weinig, 2004). Using LEDs for sole-source photosynthetic lighting is of commercial interest because high-density shelving systems could allow for multiple-tiered growing, which can increase land use efficiency (Watanabe, 2011). LEDs also have the potential to replace HPS lamps in greenhouse production of ornamental and food crops.

Sole-source lighting with blue (B, 400-500 nm) or red (R, 600-700 nm) LEDs or fluorescent lamps has been studied primarily on food crops. For example, wheat (*Triticum aestivum*) grown under a *PPF* of 350 µmol·m^{-2}·s^{-1} from R LEDs (peak=660 nm) had ~50% less dry weight than under B or white fluorescent lamps, and the instantaneous photosynthetic rate was 45% less than plants under white light (Goins et al., 1997). Similarly, when four cultivars of spinach (*Spinacea oleracea*) were irradiated at a *PPF* of 300 µmol·m^{-2}·s^{-1}, those grown under R or white fluorescent light had 10 to 80% more biomass than plants grown under only B fluorescent light (Li et al., 2011). Spinach and several other species had thinner leaves with less chlorophyll and thinner, longer stems, when irradiated with only R light, while plants grown under only B light had reduced biomass accumulation (Eskins, 1992; Fukuda et al., 1993, 2008; Li et al., 2011; Saebo et al., 1995). For example, *Arabidopsis* grown at a *PPF* of 50 µmol·m^{-2}·s^{-1}

had less biomass under B fluorescent light and up to a third shorter petioles compared to plants grown under R fluorescent light (Eskins, 1992). Furthermore, geranium (*Pelargonium zonale*) leaves were ≈16% thicker, particularly the palisade layers, when irradiated with 100 μmol·m^{-2}·s^{-1} of B LED (peak=460 nm) light compared to plants irradiated with the same intensity of R (peak=660 nm) LED light (Fukuda et al., 2008).

In the production of ornamentals, quality parameters such as internode length and plant biomass can influence their marketability. Plants grown under a mixture of wavelengths could possess more desirable, marketable traits than those grown under a single light waveband. Multiple waveband research with LEDs has focused on R and B light, and specific R:B responses have varied among studies (Goins et al., 1997; Kato et al., 2011; Yang et al., 2011). Yang et al. (2011) reported that an R (peak=660 nm) to B (peak=450 nm) ratio of 8:1 produced sweet potato (*Ipomoea batatas*) plants with the greatest root to shoot ratio, while plants grown under the highest ratio of R light (10:1) had the least. Providing B light in an R-dominant environment can also increase seed yield, increase chlorophyll content, and promote flowering in some crops (Goins et al., 1997; Imaizumi et al., 2003; Li et al., 2011; Ohashi-Kaneko et al., 2006; Saebo et al., 1995; Tennessen et al., 1994). For example, wheat plants grown under R LEDs (peak=660 nm) had approximately half of the number of tillers as plants grown under 90% R LED light and 10% B fluorescent light or white fluorescent light (Goins et al., 1997). The chlorophyll content of lady slipper orchid (*Paphiopedilum* 'Hsingying Carlos') was 24% greater under R+B than R LED light (Lee et al., 2011).

The addition of green (G, 500-600 nm) light to R and B light can increase biomass accumulation in some crops. For example, lettuce (*Lactuca sativa*) was grown at a *PPF* of 150 μmol·m^{-2}·s^{-1} under R and B LED light with and without G fluorescent lamps (Kim et al., 2004).

Lettuce had lower rates of photosynthesis when the percentage of G light exceeded 50, but lettuce had 89% more biomass when the light spectrum included up to 24% G light. Similarly, when lady slipper orchid was grown in tissue culture, plants under R, G, and B LEDs (ratio of 8:1:1; peak=660, 525, and 450 nm, respectively) had 66 to 84% greater shoot dry weight than plants irradiated with only B, R, or a combination of R and B (9:1) at the same *PPF* (Lee et al., 2011). However, growth under G light alone can be poor: the net photosynthetic rate of cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*) was approximately three times lower when grown under only G light (peak=520 nm; *PPF* of 320 µmol·m^{-2}·s^{-1}) than under only B (peak=450 nm), only R (peak=650 nm), or B+R light (Liu et al., 2011a).

Relatively little research has been published that compares the ratio of B to R light, with and without G light, on plant growth parameters in carefully controlled environments. We grew seedlings of ornamental plants under ratios of B (peak=446 nm), G (peak=516 nm), R (peak=634 nm) and HR (peak=664 nm) light to quantify how the light spectrum regulated plant growth and morphology. Our objective was to quantify how seedlings grew and acclimated to B, G, and R light and to help facilitate the production of young ornamental crops under solid-state lighting with desirable quality characteristics.

Materials and Methods

Seeds of tomato (*Lycopersicum esculentum* 'Early Girl'), salvia (*Salvia splendens* 'Vista Red'), impatiens (*Impatiens walleriana* 'SuperElfin XP Red'), and petunia (*Petunia ×hybrida* 'Wave Pink') were sown in 128-cell (2.7 × 2.7 cm; 12.0-mL volume) plug trays at a commercial greenhouse (C. Raker and Sons, Inc., Litchfield, MI). Upon arrival at Michigan State University

East Lansing, MI) a seedling tray of each species was then cut into sections each with ≥20 seedlings, thinned to one plant per cell, and immediately placed in the lighting treatments.

Light treatments and environment. Six LED light modules were custom-designed and constructed for experimentation as described in Chapter 2. The top of each module contained B (peak=446 nm), G (peak=516 nm), R (peak=634 nm), and HR (peak=664 nm) LEDs that were uniformly distributed, facing downward inside the module. The LED modules were placed on open, metal mesh benches inside a refrigerated walk-in growth chamber. Six light quality treatments that delivered a *PPF* of 160 μmol·m^{-2}·s^{-1} emitted the following light quality treatments: B_{25}+G_{25}+R_{25}+HR_{25} (25% light from each), B_{50}+G_{50}, B_{50}+R_{25}+HR_{25}, G_{50}+R_{25}+HR_{25}, R_{50}+HR_{50}, and B_{100}. The intensities of these LED were adjusted based on an average of six measurements from a spectroradiometer (PS-200, Apogee Instruments, Inc., Logan, UT) made at seedling tray level at different horizontal positions inside each module. To provide a more uniform light intensity within each module, wire mesh was placed in the middle half of the chamber, just below the LEDs. In a separate growth chamber, plants were grown under cool-white fluorescent lamps (F96T12; Philips, Amsterdam, Netherlands) with the same *PPF* and temperature setpoints, which served as a control. Plant trays were randomly rearranged daily to reduce any positional effects inside each module or chamber. The spectral quality of the light treatments was measured at six positions in each treatment with the spectroradiometer (Figure 3.1). Plants were grown under an 18-h photoperiod (0500 to 2300 HR) as controlled by data loggers (CR10; Campbell Scientific, Logan, UT) and the growth chambers were set at 20 °C. In each treatment, infrared sensors (Type K, OS36-01, Omega Engineering; Stamford, CT) that pointed at a downward angle towards the closest tray of plants measured canopy temperature and shielded thermocouples (0.13-mm type E; Omega Engineering) at plant level measured air

temperature. In addition, quantum sensors (LI-COR, Lincoln, NE) placed in the middle of each treatment at plug tray level measured light intensity. The infrared sensors, thermocouples, and quantum sensors were connected to the same data loggers and recorded data every 10 s. The dataloggers recorded means every 10 minutes throughout the duration of the experimental replications (Table 3.1). Plants were irrigated as needed by subsurface irrigation with distilled water supplemented with a water-soluble fertilizer providing (in $mg \cdot L^{-1}$) 50 N, 19 P, 50 K, 23 Ca, 4 Mg, 1 Fe, 0.5 Mn, Zn, and Cu, 0.3 B, and 0.1 Mo (MSU Plug Special; GreenCare Fertilizers, Inc., Kankakee, IL).

Data collection. The experiment was performed three times. Ten random plants of each species and treatment were harvested per replication the following number of days after seed sow (rep 1, 2, 3): tomato (32, 31, 31), impatiens (33, 32, 32), petunia (34, 33, 35), and salvia (36, 34, 36). During the second experimental replicate, the LEDs never turned off due to a malfunction with the datalogger, so those data were excluded from the dataset. Data from the first replication of the control treatment was also excluded because the fluorescent lamps delivered less directional light than that under the LEDs, which allowed light to better penetrate the canopy than under the LED treatments. The fluorescent lamps were adjusted for the second and third replicates of the experiment so that lighting was only overhead (similar to the LED treatments). The following data were collected at harvest: leaf number on the primary stem, total leaf area [measured using a leaf area meter (LI-3000; LI-COR)], fresh shoot weight (using a Mettler Toledo PG5002 scale, Columbus, OH), dry shoot weight (after plants were dried in an oven at ≥66 °C for ≥5 d and using the same scale), and macroscopic flower bud number. A visible leaf that was ≥25% unfolded was counted in leaf node number. Stem length was measured by a ruler (from the media surface to the apical meristem) on all plants except for petunia, which grew as a

rosette. The number of edemic leaflets was counted on tomato; there was no edema on the other plants. Tomato was also subjectively evaluated for chlorosis by assigning a score from 1 (most severe, 100% yellow) to 5 (no chlorosis, 100% green). Chlorophyll concentration was determined as reported in Chapter 2 on the following days after seed sow (rep 1, 2, 3): 29, 25, and 30 d. The absorbance of each sample was measured against the blank (DMSO) at 645 and 663 nm with a spectrophotometer (Hitachi U-3000, Tokyo, Japan).

Statistical analysis. Data were analyzed with SAS (SAS Institute, Cary, NC) means procedure (PROC MEANS), mixed model procedure (PROC MIXED), general linear mixed model procedure (PROC GLIMMIX; Poisson distribution for count data), with the pdmix800 program (Arnold M. Saxton, University of Tennessee) that provided pairwise comparisons between treatments using Tukey's honestly significant test at $P \leq 0.05$.

Results

Leaf number and relative leaf area. In all species, the mean leaf number was similar among treatments and was 8.6, 9.6, 5.3, and 10.1 for impatiens, salvia, tomato, and petunia, respectively (Figure 3.2). Total leaf area was greatest in impatiens, tomato, and petunia under the fluorescent lamps (19.8, 24.4, and 31.0 cm^2, respectively) and in salvia under the R_{50}+HR_{50} treatment (31.1 cm^2). Leaf area under these treatments was set to 100% and leaf area for the other treatments was calculated relative to these values. Plants grown under R_{50}+HR_{50} had 55 to 114%, 47 to 88%, 49 to 101%, and 57 to 130% greater leaf area for impatiens, salvia, tomato and petunia, respectively, compared to those grown with ≥25% B light. Leaf area of plants grown under fluorescent lighting was similar to that of plants grown under R_{50}+HR_{50} for all species except tomato, in which the leaf area was 8% less. Tomato grown under B_{50}+G_{50} had a greater

leaf area than plants under treatments B_{25}+G_{25}+R_{25}+HR_{25} and B_{50}+R_{25}+HR_{25} and had relatively small leaves when grown under treatment B_{50}+R_{25}+HR_{25}.

Seedling height. Height was greatest for impatiens, tomato and salvia under the R_{50}+HR_{50} treatment (48, 99, and 63 mm, respectively). Height under these treatments was set to 100% and then the height for the other treatments was calculated relative to these values. Impatiens, tomato and salvia grown under ≥25% B light were 47 to 53%, 46 to 50%, or 41 to 57% shorter, respectively, than all plants grown under R_{50}+HR_{50}. Plants grown under treatment G_{50}+R_{25}+HR_{25} were of similar height to those grown under fluorescent light, but were 23%, 21%, or 27% shorter in impatiens, tomato, and salvia, respectively than those grown under R_{50}+HR_{50}. Impatiens, tomato, and salvia grown under treatment G_{50}+R_{25}+HR_{25} were 45 to 64%, 42 to 56%, or 24 to 72% taller, respectively, than all plants grown with ≥25% B light. Salvia grown under treatments B_{25}+G_{25}+R_{25}+HR_{25} and B_{50}+R_{25}+HR_{25} were the shortest.

Fresh shoot weight. Fresh shoot weight was greatest for impatiens, tomato and salvia under the R_{50}+HR_{50} treatment (1.16, 1.38, and 1.19 g, respectively), while it was greatest under the fluorescent lamps for petunia (0.81 g) (Figure 3.3). Fresh weight under these treatments was set to 100% and then the fresh weight for all other treatments was calculated relative to these values. Plants grown under R_{50}+HR_{50} had a similar fresh weight as those grown under fluorescent light for all species except tomato. Tomato, salvia and petunia grown under R_{50}+HR_{50} had 54 to 83%, 48 to 87%, or 58 to 112% greater fresh weight, respectively, than plants grown under treatment G_{50}+R_{25}+HR_{25} or those with ≥25% B light. Impatiens grown under R_{50}+HR_{50} had 43 to 83% greater fresh weight than plants grown with ≥25% B light. Petunia and salvia grown under all other treatments had similar fresh weights. Tomato grown under fluorescent light had 18% less fresh weight than those grown under R_{50}+HR_{50} but 31 to

51% greater fresh weight than plants grown with ≥25% B light. Fresh weight of salvia and petunia under 25 or 50% G light was similar to that of plants grown under treatments that contained B light.

Dry shoot weight. Dry shoot weight was greatest for impatiens, tomato, salvia and petunia under the R_{50}+HR_{50} treatment (100, 210, 210, and 110 mg respectively). Dry weight under these treatments was set to 100% and then dry weight for all other treatments was calculated relative to these values. Tomato and salvia grown under the R_{50}+HR_{50} treatment had 54 to 86%, or 61 to 109% greater dry weight, respectively, than plants grown under all other treatments. Impatiens grown under B_{100} light had 44% less dry weight compared to those grown under R_{50}+HR_{50}. Compared to the G_{50}+R_{25}+HR_{25} treatment, dry weight of petunia was 45% greater under the R_{50}+HR_{50} treatment and 63 to 71% lower under the B_{50}+R_{25}+HR_{25} or B_{100} treatments.

Flower bud number and edema. Impatiens was the only plant that had visible flower buds at the end of the treatments. Impatiens grown under ≥50% B light had 63 to 106% more flower buds on average than those grown under treatment B_{25}+G_{25}+R_{25}+HR_{25}. Plants grown under fluorescent light developed 250% fewer flower buds on average than those grown under treatment B_{25}+G_{25}+R_{25}+HR_{25}. Impatiens grown without B light had the least number of flower buds. Tomato developed the most leaflets with edema on plants grown under R_{50}+HR_{50} light, while edema was absent or nearly absent when grown under the B_{50}+G_{50} or the fluorescent lighting treatment. Plants under the treatment G_{50}+R_{25}+HR_{25} developed 40% fewer leaflets with edema than those under R_{50}+HR_{50}, but 68% more leaflets with edema than plants under the B_{25}+G_{25}+R_{25}+HR_{25} treatment.

Chlorophyll concentration and tomato chlorosis score. The concentration of chlorophyll was similar among all treatments for impatiens and petunia (Figure 3.4). However, in salvia and tomato, it was relatively high under fluorescent light and relatively low under all other treatments except salvia under treatments R_{50}+HR_{50} and B_{50}+G_{50} and tomato under the B_{50}+G_{50} treatment. Tomato grown without B light developed more chlorosis (2 to 3 points lower on the chlorosis scale) than all other treatments.

Discussion

The primary objective of our experiment was to quantify growth characteristics of seedlings grown under different ratios of B, G, and R light while all other environmental parameters, including *PPF*, were constant. In our study, leaf area was 47 to 130% greater in plants grown under the R_{50}+HR_{50} treatment compared to those grown with ≥25% B light. Several studies including Eskins (1992), Ohashi-Kaneko et al. (2007), and Lee et al. (2011) reported similar results: plants grown under R light had a greater leaf area than when grown under a majority of, or only, B light. For example, leaf area of *Arabidopsis* irradiated at a *PPF* of 50 µmol·m^{-2}·s^{-1} was approximately two times greater when the spectrum included 60% B light compared with only R fluorescent light (Eskins, 1992). In a separate study, lettuce, spinach, and komatsuna (*Brassica campestris*) were grown at a *PPF* of 300 µmol·m^{-2}·s^{-1} from R, B, and white fluorescent lamps (Ohashi-Kaneko et al., 2007). Lettuce had a 44% greater leaf area under R light than under B light but it was similar under R, R+B, or white light. Spinach, in contrast, had a 192% greater leaf area under R+B compared to only B light. Similarly, lady slipper orchid grown under cool- or warm-white fluorescent lamps or under R+G+B (8:1:1) LEDs yielded plants with 24 to 36% longer leaves than under only B LEDs (Lee et al., 2011). In contrast to

results reported for tomato, leaf area of marigold (*Tagetes erecta*) grown at a *PPF* of 90 µmol·m^{-2}·s^{-1} was 20% greater under R+B LEDs than under white fluorescent light (Heo et al., 2006).

Plants grown under only R light typically accumulate more biomass than when grown under additional wavebands of photosynthetic light. In our study, the shade-intolerant plants tomato, salvia and petunia grown without B or G light had 48 to 112% greater fresh shoot weight than plants grown under treatments with ≥25% B or 50% G light. Similar results occurred in the shade-tolerant impatiens. Tomato grown under fluorescent light had 18% less fresh shoot weight than those grown under only R light, while all other plant species in our study had similar fresh shoot weights between the two treatments. Our results were consistent with those reported for four cultivars of spinach and *Arabidopsis* (Eskins, 1992; Li et al., 2011). Likewise, komatsuna had 43% greater dry weight under R light than white light (Ohashi-Kaneko et al., 2007). In contrast, rice (*Oryza sativa*) plants had ≈25% greater biomass under a combination of R (peak ~660 nm) and B (peak ~460 nm; R:B = 4:1) LEDs compared with R LEDs alone at a *PPF* of 380 µmol·m^{-2}·s^{-1} (Ohashi-Kaneko et al., 2006). The conflicting results could be attributed to the differences in peak wavelength of the B LEDs, which differed by 14 nm from that of ours, and the *PPF* was over twice that used in this study. Also in contrast to our results, lettuce had 28% greater shoot dry weight under white light compared to only R light at a *PPF* that was 88% greater than ours (Ohashi-Kaneko et al., 2007). These differences in results suggest that the effect of light quality on plant growth is highly species specific and could depend on light intensity.

The utility of G light in biomass accumulation varies with crop species and depends on its relative proportion to the total spectrum. In contrast to results with lady slipper orchid (Lee et al., 2011) and lettuce (Kim et al., 2004), plants in our study had similar fresh weights under 25%

or 50% G light compared to plants under only B light. Consistent with results reported for cherry tomato (Liu et al., 2011a), plants under different combinations of R and B light with or without the addition of G light had similar shoot fresh or dry weights. Therefore, our results indicate that 25% G light can substitute for 25% B light without influencing biomass accumulation, at least for the crops grown and at the intensity provided.

Compact growth is often a desirable characteristic in young plant production. Including B light in photosynthetic lighting could be used to produce relatively short plants. In this study, impatiens, tomato, and salvia grown under ≥25% B light were 41 to 57% shorter than plants grown under treatment R_{50}+HR_{50}. Plants grown under treatment G_{50}+R_{25}+HR_{25} were of similar height to those grown under fluorescent light. Phytochrome and cryptochrome, which are photoreceptors that detect and respond to the R to far-red (FR) ratio and or B light, respectively, mediate extension growth of plants (Cashmore et al., 1999; Lin, 2000; Sellaro et al., 2010; Smith, 2000). When both a high R:FR ratio and B light are present in the light environment, they synergistically suppress stem elongation to a greater extent than if only one or the other were present (Casal and Mazzella, 1998; Hennig et al., 1999; Sellaro et al., 2010; Smith, 2000). Because very few photons ≥700 nm were present in our light treatments, the R:FR did not change and so extension growth responses cannot be readily attributed to phytochrome. While extremely low-fluence B light (0.1 µmol·m^{-2}·s^{-1}) can stimulate phototropins and cause an increase in extension growth, our treatments had a minimum of 10 µmol·m^{-2}·s^{-1} of B light, so phototropins were likely not involved. Therefore, stem elongation inhibition can likely be attributed to the B-light-stimulated cryptochrome receptors (Liu et al., 2011b; Takemiya et al., 2005; Wang et al., 2013). Maximal cryptochrome activity is stimulated by wavebands from 390 to 480 nm, however cryptochrome-like responses can still be activated by wavelengths up to 550

nm (Ahmad et al., 2002). In *Arabidopsis*, *CRY1* genes regulate the de-etiolation of seedlings by altering gene expression and transcription of those downstream, such as *COP1* and *HY5*, respectively (Jiao et al., 2007; Liu et al., 2011b; Yang et al., 2005). These genes subsequently regulate the signaling of phytohormones such as auxin, brassinosteroid, and gibberellic acid (GA) (Liu et al., 2011b). For example, sorghum (*Sorghum bicolor*) mutant *har1* had increased DELLA (GA repressor) expression and lower bioactive concentrations of GA when irradiated with 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B LED (peak = 470 nm) light compared to those under 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of FR (peak = 740 nm) LED light (Gao et al., 2012). Upon external GA application, cell elongation and stem elongation was restored, which suggested that B light up-regulates DELLA proteins, which in turn decreases GA biosynthesis (Gao et al., 2012). Numerous studies with whole plants have reported B-light inhibition of extension growth (Liu et al., 2011a; Runkle and Heins, 2001). For example, cherry tomatoes grown under B or R+G+B LEDs were 33 or 49% shorter, respectively, than plants grown under R LEDs (Liu et al., 2011a). Likewise, petioles of strawberry (*Fragaria ×ananassa*) grown under R+B (1:1; peak=660, 450 nm) or B LED light at a *PPF* of 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were 10% or 22% shorter, respectively, than plants grown under only R light (Nhut et al., 2003).

Cryptochrome-mediated stem extension suppression is stimulated by wavebands up to 550 nm, whereas longer waveband radiation (550 to 600 nm) can have the opposite effect (Ahmad et al., 2002; Sellaro et al., 2010). Longer waveband G light can act similar to FR light and even cause shade-avoidance symptoms (e.g., elongated petioles, leaf hyponasty, early flowering) via *cry* and also via a cryptochrome-independent receptor not yet identified (Folta and Maruhnich, 2007; Wang and Folta, 2013; Zhang et al., 2011). Studies have suggested a new class of photoreceptors, such as cyanochromes (which are in cyanobacteria), however their presence or

the presence of another unidentified receptor remains unclear (Wang and Folta, 2013; Ulijasz et al., 2009). In addition, the duration, peak wavelength, and intensity of G light, as well as its intensity relative to other wavebands, affect plant physiological responses (Bouly et al., 2007; Folta and Maruhnich, 2007; Lin et al., 1996; Wang and Folta, 2013). A short pulse of G light can reverse the B-light inhibition of stem elongation by stimulating the *cry* receptors (Bouly et al., 2007; Folta and Maruhnich, 2007). For example, *Arabidopsis* had up to a 30% increase in stem elongation when 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of G LED (peak = 525 nm) light was delivered as a pulse to low-fluence (<4 $\mu mol \cdot m^{-2} \cdot s^{-1}$) R (peak = 630 nm) and B (peak = 470 nm) LED light (Folta, 2004). However, all of our treatments that included G light also delivered a relatively high intensity of B or R light (≥25 $\mu mol \cdot m^{-2} \cdot s^{-1}$), which could have overcome any independent effects of G light (Lin et al., 1996; Wang and Folta, 2013). Therefore, in our study, seedling stems elongated similarly with or without G light when B light was also provided.

The peak wavelength of G light influences the relative amount of cryptochrome stimulation. For example, lettuce grown under 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of G LED light with peak = 510 nm had 55 to 57% greater dry mass than plants grown under peak wavelengths at 520 or 530 nm (Johkan et al., 2012). Wang et al. (2013) reported that any fluence rate of G light over 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had an inhibitory effect on stem elongation. Consistent with that paradigm, all four species in our study under treatment 50% G light (80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with peak = 516 nm) with 25% R and 25% HR light had a stem height shorter than plants under only R light and taller than plants under treatments with B light. This suggests that G light stimulated cryptochrome responses, but to a lesser extent than treatments with B light, because the peak of our G LEDs was greater than wavelengths that elicit maximal cryptochrome activity (390 to 480 nm) (Ahmad et al., 2002).

In some situations, early flowering can be desirable in young plant production while in other cases it is not. Altering the spectra of B, R, or FR light can influence flower induction (Cerdán and Chory, 2004; Lin, 2000). Impatiens in our study produced 82% fewer flower buds under fluorescent lighting than under treatment B_{50}+R_{25}+HR_{25}. Furthermore, impatiens grown under 100% B light had 71 times more flower buds than those grown under treatment R_{50}+HR_{50}. Similar to our results, marigold and salvia produced 43 or 100% more flower buds, respectively, under B+R (peaks = 440 and 650 nm) LED light compared to those grown under fluorescent light at a *PPF* of 90 µmol·m^{-2}·s^{-1} (Heo et al., 2006). However, ageratum (*Ageratum houstonianum*) produced 50% more flower buds under fluorescent light than the R+B light treatment. In a similar study, marigold and salvia grown under B or R LEDs developed a similar number of flower buds, but 77 to 86% fewer than those under fluorescent lamps (Heo et al., 2002). An increase in flower bud number with increasing B light could be attributed to *CRY2* cryptochrome activity. *CRY2* degradation can stimulate the photoperiodic flowering response and act on downstream genes including *CO* and *FT* (El-Assal et al., 2003; Chaves et al., 2011). Most *CRY2* mutants were developed in photoperiodic (e.g., *Arabidopsis*) species (Lin, 2000), therefore it is not known if *CRY2* also influences flowering in day-neutral plants like impatiens.

Edema has been associated with plants grown in R-dominant environments with little or no B or FR light, UV-B (280 to 315 nm; Jenkins, 2009) radiation, or a high humidity, especially those in the Solanaceae (Lang and Tibbitts, 1983; Massa et al., 2008; Morrow and Tibbitts, 1988; Nilsen, 1971). In our study, tomato developed the most leaflets with edema on plants grown under R_{50}+HR_{50} light, while edema was absent or nearly absent when grown under the B_{50}+G_{50} or fluorescent lighting treatment. The fluorescent treatment had 31 µmol·m^{-2}·s^{-1} of B light and <5 µmol·m^{-2}·s^{-1} of UV-A radiation. Plants under the treatment G_{50}+R_{25}+HR_{25} developed 40%

fewer leaflets with edema than those under R_{50}+HR_{50}. Similarly, wild tomato (*L. hirsutum*) developed edema on 63 or 3% of the sampled leaf area surface when under R or G fluorescent lamps, respectively, whereas it was absent under B fluorescent lamps at a *PPF* of 25 µmol·m^{-2}·s^{-1} (Morrow and Tibbitts, 1988).

Numerous studies have reported greater chlorophyll concentrations in leaves under B light compared to those under R light (e.g., Hogewoning et al., 2010). However, chlorophyll concentration of impatiens and petunia in our study were similar under the lighting treatments, which was similar to that reported in cherry tomato and lettuce (Li and Kubota, 2009; Liu et al., 2011a). Salvia and tomato chlorophyll concentration was relatively high under fluorescent light, while it was relatively low under all other treatments except plants grown under treatments R_{50}+HR_{50} and B_{50}+G_{50} light for salvia and B_{50}+G_{50} for tomato. Similarly, rice had greater concentrations of chlorophyll under a combination of R and B LEDs compared with R LEDs alone (Ohashi-Kaneko et al., 2006). Likewise, cucumber (*Cucumis sativus*) under ratios of R (peak=638 nm) and B (peak=450 nm) LEDs at a *PPF* of 100 µmol·m^{-2}·s^{-1} had increasing chlorophyll content per unit leaf area with increasing percentages of B light (Hogewoning et al., 2010). However, percentage of chlorophyll per gram of dry mass was similar among plants that received ≥15% B light. Similar to our results with impatiens and petunia, chlorophyll content per leaf area of cherry tomato was similar among treatments delivering B, G, and R light, alone or combined (Liu et al., 2011a). Chlorophyll content of lettuce was also similar among combinations of cool-white fluorescent and R (peak=658 nm) or B (peak=476 nm) LED light at a *PPF* of 300 µmol·m^{-2}·s^{-1} (Li and Kubota, 2009). These results collectively indicate that in some plants, light quality influences chlorophyll biosynthesis, degradation, or both, whereas in other plants, such responses are limited or do not occur.

APPENDIX

Table 3.1. Actual air and canopy temperatures (°C) as measured by thermocouples and infrared sensors for the six LED-lighting treatments (B: blue, G: green, R: red, HR: hyper red) and one fluorescent lighting treatment. The values after each LED type represent its percentage of the total *PPF*. All temperatures had a standard error ±0.1 °C.

	Replication 1		Replication 2		Replication 3	
Light quality treatment	Air	Canopy	Air	Canopy	Air	Canopy
$R_{50}+HR_{50}$	20.6	20.7	21.0	20.6	21.6	20.6
$G_{50}+R_{25}+HR_{25}$	20.9	21.1	22.0	21.4	21.6	20.9
$B_{25}+G_{25}+R_{25}+HR_{25}$	20.9	20.6	20.8	21.2	21.2	20.6
$B_{50}+G_{50}$	21.0	20.8	21.4	21.8	21.3	21.3
$B_{50}+R_{25}+HR_{25}$	20.9	20.9	21.3	21.2	20.5	20.8
B_{100}	21.4	20.8	21.3	20.8	20.9	20.6
Fluorescent	21.4	21.2	22.4	21.7	22.2	22.0

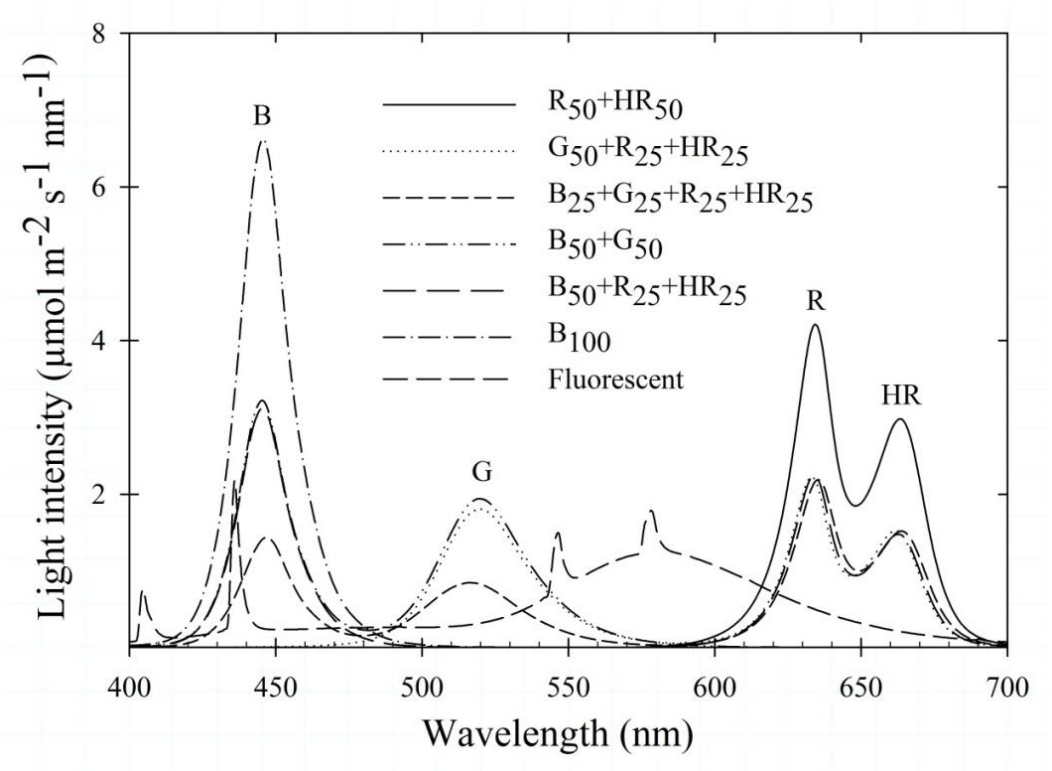

Figure 3.1. The spectral distribution of six light quality treatments delivered by blue (B), green (G), red (R), and hyper red (HR) LEDs and one treatment delivered by cool-white fluorescent lamps, each delivering a *PPF* of 160 µmol·m^{-2}·s^{-1}. The values after each LED type represent its percentage of the total *PPF*.

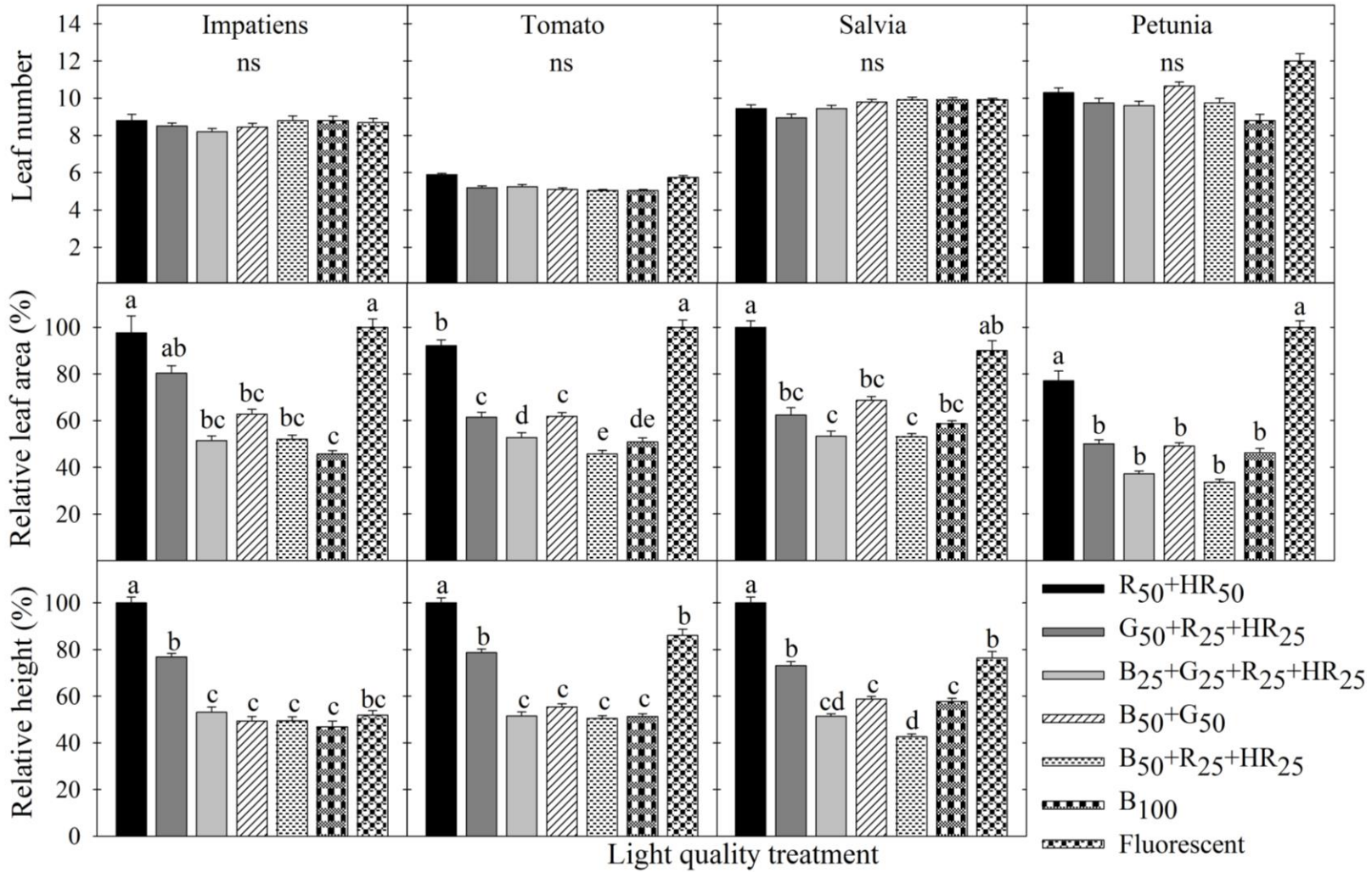

Figure 3.2. Pooled mean leaf number, leaf area, and height of four seedling crops grown under six light quality treatments delivered by LEDs (B: blue, G: green, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The values after each LED type represent their percentages of the total *PPF*. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

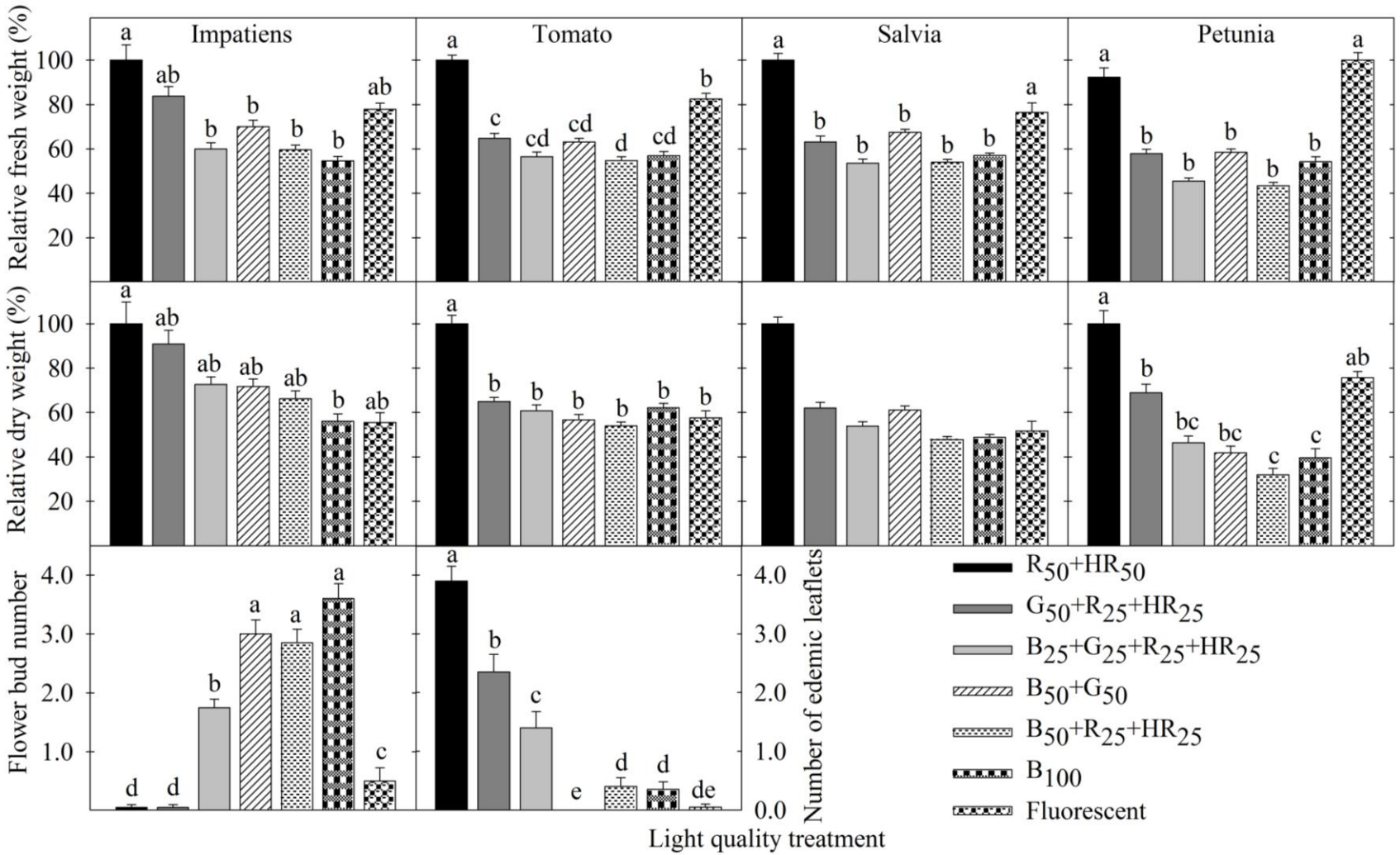

Figure 3.3. Pooled mean fresh and dry shoot weights for four seedling crops, impatiens flower bud number, and number of tomato leaves exhibiting edema under six light quality treatments delivered by LEDs (B: blue, G: green, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The values after each LED type represent their percentages of the total *PPF*. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

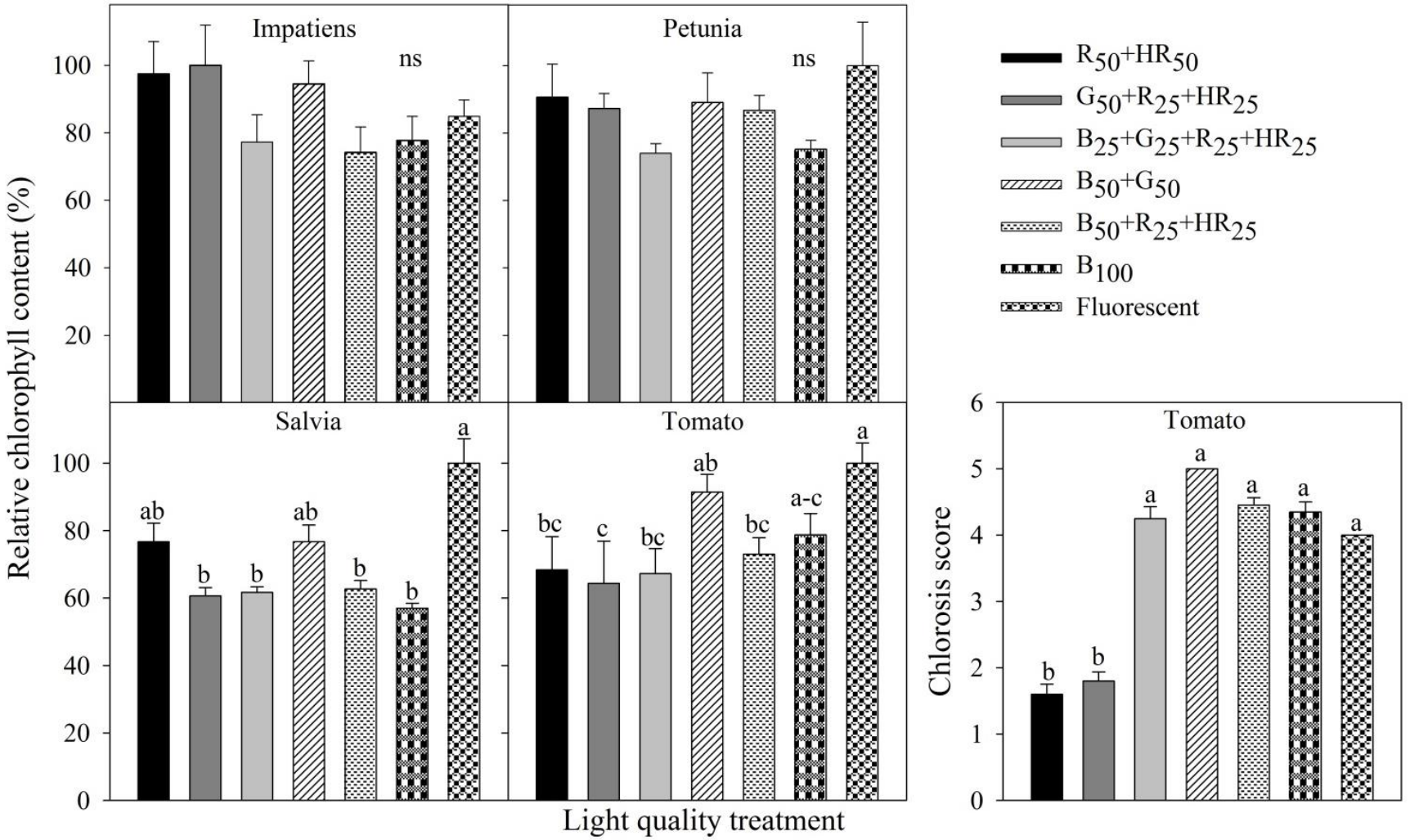

Figure 3.4. Pooled chlorophyll concentrations for impatiens, petunia, salvia, and tomato and chlorosis score (1: most chlorotic, 5: least chlorotic) for tomato grown under six light quality treatments delivered by LEDs (B: blue, G: green, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The values after each LED type represent their percentages of the total *PPF*. Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

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SECTION IV

MORPHOLOGICAL ACCLIMATION OF ORNAMENTAL SEEDLINGS TO RED AND BLUE LIGHT FROM LIGHT-EMITTING DIODES AT A FIXED IRRADIANCE

Morphological Acclimation of Ornamental Seedlings to Red and Blue Light from Light-emitting Diodes at a Fixed Irradiance

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Abstract

Plant growth is plastic and adaptive to the light environment; characteristics such as extension growth, architecture, and leaf morphology change depending on the light spectrum. Although blue (B, 400 to 500 nm) and red (R, 600 to 700 nm) light are generally the most efficient wavelengths for eliciting photosynthesis, both are often required for relatively normal growth. Our objective was to quantify how the B:R influenced plant growth and morphology and understand how plants acclimated to these light environments. We grew seedlings of four ornamental annuals under six sole-source light-emitting diode (LED) lighting treatments or one cool-white fluorescent treatment that each delivered a *PPF* of 160 µmol·m^{-2}·s^{-1} with an 18-h photoperiod. The following treatments were provided using B (peak=446 nm), R (peak=634 nm), and hyper red (HR, peak=664 nm) LEDs: B_{160} (160 µmol·m^{-2}·s^{-1}), B_{80}+R_{40}+HR_{40}, B_{40}+R_{60}+HR_{60}, B_{20}+R_{70}+HR_{70}, B_{10}+R_{75}+HR_{75}, and R_{80}+HR_{80}. Seedlings of impatiens (*Impatiens walleriana*), salvia (*Salvia splendens*), petunia (*Petunia ×hybrida*), and tomato (*Lycopersicum esculentum*) were grown for 31 to 37 d at a constant 20 °C. Plants with as little as 10 µmol·m^{-2}·s^{-1} of B light were 23 to 50% shorter and had 17 to 50% smaller leaves than plants under the R_{80}+HR_{80} treatment. Impatiens and salvia had 53 to 98% greater fresh shoot weight under treatments without B light than with ≥80 µmol·m^{-2}·s^{-1}. Leaves of salvia were 37 to 43% thinner in plants grown without B light or plants grown under fluorescent lamps compared to those grown under only R light, whereas leaves of tomato were 41% thinner under fluorescent lamps than under the treatment with 25% B light. Plants had greater chlorophyll concentrations and thinner leaves under fluorescent lamps than those under the LED treatments, while tomato under a higher B:R had thicker leaves than those under a lower B:R. We conclude that B light

inhibits leaf and stem expansion, which subsequently limits photon capture and constrains biomass accumulation, and increases leaf thickness and chlorophyll concentration.

Introduction

Blue (B, 400-500 nm) and red (R, 600-700 nm) light are generally considered the most efficient wavelengths for eliciting photosynthesis in plants (McCree, 1972; Sager et al., 1988). Therefore, B and R light-emitting diodes (LEDs) with peak light emission that coincide with peaks of the relative quantum efficiency curve (McCree, 1972) make them a logical choice for sole-source commercial plant production (Mitchell et al., 2012). Previous results with tomato (*Lycopersicum esculentum*), salvia (*Salvia splendens*), impatiens (*Impatiens walleriana*), and petunia (*Petunia ×hybrida*) (Chapter 3) and those for lettuce (*Lactuca sativa*; Johkan et al., 2010), cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*; Liu et al., 2011b), rice (*Oryza sativa*; Ohashi-Kaneko et al., 2006) and strawberry (*Fragaria* × *ananassa*; Nhut et al., 2003;) showed that plants grown under a combination of wavebands, particularly including B light, have growth characteristics more similar to those grown under sunlight than those under a single waveband of light. However, the addition of B to R light can decrease shoot biomass, such as in lettuce, which had 25 or 17% less fresh shoot biomass in plants grown under B light alone compared to those under R or R+B light, respectively (Johkan et al., 2010). Similarly, salvia, petunia, and tomato seedlings grown under 50% green (G; 500-600 nm)+50% R light from LEDs or those with ≥25% B light at the same photosynthetic photon flux (*PPF*) had 35 to 57% less fresh shoot weight than plants under only R light (Chapter 3).

Marketable characteristics of young ornamental plants include, but are not limited to, compact growth, presence of a well-developed root system, and adequate branching. Plant

growth retardants and limited watering and fertility are methods commercial growers employ to suppress extension growth (Hendriks and Ueber, 1995). Extension growth can also be inhibited by modifying the light spectrum, especially by B light and the R:far-red (FR, 700 to 800 nm) light ratio (Liu et al., 2011a; Smith, 2000). B-light-stimulated cryptochrome receptors suppress gibberellic acid (GA) biosynthesis which, in turn, inhibits cell elongation and stem extension of plants (Ahmad et al., 2002; Cashmore et al., 1999; Liu et al., 2011a; Sellaro et al., 2010). For example, sweet potato stems were 17% shorter when the B:R was 1:10 compared to plants grown under a 1:4 at a *PPF* of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Yang et al., 2011). In a separate study, cherry tomato grown under B or R+B LEDs were 33 or 49% shorter than plants grown under only R LEDs at a *PPF* of 320 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Liu et al., 2011b). Phytochromes are a family of photoreceptors with absorption peaks at 660 nm and 735 nm and also mediate stem elongation as well as leaf expansion, chloroplast development, and flowering (Horwitz et al., 1988; Folta and Childers, 2008; Parks et al., 2001; Valverde et al., 2004).

In addition to extension growth, plants acclimate to a high B:R by increasing chlorophyll concentration (Lichtenthaler et al., 1981). B light stimulates cryptochrome (*CRY1*), which upregulates the transcription of genes for chlorophyll synthesis (Li et al., 2009). High chlorophyll content in plants, which causes a dark green coloration of leaves, is also desirable in commercial production of young plants such as microgreens, herbs, and ornamental propagules. Growing plants under sole-source solid-state lighting that includes B light in an R-dominant background can yield this characteristic (Goins et al., 1997; Li et al., 2011; Ohashi-Kaneko et al., 2006; Saebo et al., 1995; Tennessen et al., 1994). For example, lettuce grown under B or B+R (1:1) LEDs had ≈11% greater chlorophyll per unit of dry mass than plants grown under R LEDs at the same intensity (Johkan et al., 2010). In a separate study, cucumber (*Cucumis sativus*) had

increasing chlorophyll content per unit leaf area with increasing ratios of B:R light at the same intensity (Hogewoning et al., 2010).

Plants also acclimate to a low R:FR or B-deficient environment by increasing leaf thickness (Fan et al., 2013; Fukuda et al., 2008; Schuerger et al., 1997). Thicker leaves have not been directly attributed to cryptochrome or phototropin photoreceptors (Ohashi-Kaneko et al., 2006). However, the *CRY1* cryptochrome receptor downregulate GA biosynthesis and therefore suppresses leaf expansion, which in turn results in thicker leaves (Ahmad et al., 2002; Liu et al., 2011a; Sellaro et al., 2010). Therefore, plants grown under solely R light typically have larger, thinner leaves than those of plants grown under light that includes B. For example, pepper plants (*Capsicum annuum*) grown at a *PPF* of 330 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had 24%, 37%, or 29% greater overall leaf thickness, palisade parenchyma, or spongy parenchyma layers, respectively, under R LEDs and B fluorescent lamps than plants grown under R LEDs alone (Schuerger et al., 1997). Similarly, geranium (*Pelargonium zonale*) irradiated with 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B LED light had ≈16% thicker leaves compared to plants under R LED light at the same intensity (Fukuda et al., 2008).

Our objective was to quantify how plants acclimate to light environments with different B:R ratios to facilitate the commercial production of young plants with desirable morphological characteristics. We grew seedlings of four common annuals under ratios of B (peak=446 nm), R (peak=634 nm), and HR (peak=664 nm) light from LEDs to enumerate how light quality regulated plant growth characteristics including stem length, leaf morphology, chlorophyll content, and shoot biomass accumulation.

Materials and Methods

Seeds of tomato (*Lycopersicum esculentum* 'Early Girl'), salvia (*Salvia splendens* 'Vista Red'), impatiens (*Impatiens walleriana* 'SuperElfin XP Red'), and petunia (*Petunia ×hybrida* 'Wave Pink') were sown in 128-cell (2.7 × 2.7 cm; 12.0-mL volume) seedling trays by a commercial young plant producer (C. Raker and Sons, Inc., Litchfield, MI). Trays were moved to Michigan State University (East Lansing, MI) within 2 d and each seedling tray of each species was then cut into sections that each contained ≥20 seedlings, thinned to one plant per cell, and immediately placed in the lighting treatments.

Light treatments and environment. Six modules that were described in Chapter 2 contained dimmable B (peak=446 nm), R (peak=634 nm), and HR (peak=664 nm) LEDs. The intensities of these three LED types were adjusted to create six light quality treatments based on an average of six measurements from a spectroradiometer (PS-200, Apogee Instruments, Inc., Logan, UT) made at seedling tray level at different horizontal positions inside each module. Each module delivered a *PPF* of 160 μmol·m^{-2}·s^{-1} that consisted of the following light treatments: B_{160} (160 μmol·m^{-2}·s^{-1} of B and no R or HR light), B_{80}+R_{40}+HR_{40}, B_{40}+R_{60}+HR_{60}, B_{20}+R_{70}+HR_{70}, B_{10}+R_{75}+HR_{75}, and R_{80}+HR_{80} (Figure 4.1). The experiment was performed three times. In a separate growth chamber, plants were grown under cool-white fluorescent lamps (F96T12; Philips, Amsterdam, Netherlands) with the same *PPF* and temperature setpoints, which served as a control. The fluence of photons in the B, R, and FR (700 to 750 nm) wavebands was calculated for the fluorescent lamps and was 33, 43, and 2.5 μmol·m^{-2}·s^{-1}, respectively. Plants were grown under an 18-h photoperiod (0500 to 2300 HR) as controlled by a data logger (CR10; Campbell Scientific, Logan, UT) and the growth chambers were set at 20 °C. In each treatment, air and plant canopy temperature and light intensity were continuously measured as described in Chapter 3 and means are presented in Table 4.1. Plants were uniformly

irrigated as needed by subsurface irrigation with a water-soluble fertilizer as described in Chapter 3.

Data collection. Ten random plants of each species and treatment were harvested per replication the following number of days after seed sow (rep. 1, 2, 3): tomato (32, 31, 33), impatiens (33, 33, 34), petunia (34, 35, 35), and salvia (36, 34, 37). The following data were collected at harvest: leaf (at node) number; total leaf area [measured using a leaf area meter (LI-3000; LI-COR)]; fresh shoot, leaf (without petiole), and petiole weight; shoot dry weight (after plants were dried at ≥66 °C for ≥5 d and using the same scale), and macroscopic flower bud number. A visible leaf that was ≥25% unfolded was counted in leaf number. Stem length was measured by a ruler (from the media surface to the apical meristem) on all plants except for petunia, which grew as a rosette. The number of edemic leaflets was counted on tomato; there was no edema on the other plants. Tomato was also subjectively evaluated for chlorosis by assigning a score from 1 (most severe, 100% yellow) to 5 (no chlorosis, 100% green). Chlorophyll concentration was determined as reported in Chapter 2 on the following days after seed sow (rep 1, 2, 3): 29, 31, and 30. The absorbance of each sample was measured against the blank (DMSO) at 645 and 663 nm with a spectrophotometer (Hitachi U-3000, Tokyo, Japan).

Leaf thickness of tomato and salvia was measured from each treatment on the two largest leaves of each plant on the harvest dates. Three leaflets of each plant were placed in separate plastic bags with deionized water to prevent desiccation until they were sectioned. The leaves were layered, rolled, and inserted into a handheld microtome (MT.5503, Euromex Microscopes Holland, Arnhem, the Netherlands). Nine to eleven cross sections per sampled plant of each species and treatment were sliced and placed with deionized water on a single-frosted pre-cleaned microscope slide (75 x 25 mm, Corning Glass Works, Corning, NY) with a 28 g cover

slip (VWR Scientific Inc., San Francisco, CA). Wet-mounted fresh sections were examined under 64× magnification on an Olympus Stereo microscope (SZH-ILLD, Olympus American Inc., Center Valley, PA). The thickness of the leaf, away from a vein or a midrib, was measured using the ocular micrometer in the viewfinder for each sample, while maintaining the same magnification. A conversion factor was determined between the viewfinder reticule in the microscope and a stage micrometer.

Statistical analysis. Data were analyzed with SAS (SAS Institute, Cary, NC) means procedure (PROC MEANS), mixed model procedure (PROC MIXED), general linear mixed model procedure (PROC GLIMMIX; Poisson distribution for count data), with the pdmix800 program (Arnold M. Saxton, University of Tennessee) that provided pairwise comparisons between treatments using Tukey's honestly significant test at $P \leq 0.05$.

Results

Leaf number and relative leaf area. In all species, the mean leaf number was similar among treatments and was 10.9, 9.8, 5.6, and 11.6 for impatiens, salvia, tomato, and petunia, respectively (Figure 4.2). Leaf area was greatest in impatiens and petunia under the fluorescent lamps (27.2 and 29.0 cm^2, respectively), under the $R_{80}+R_{80}$ treatment in salvia (34.1 cm^2) and under the $B_{20}+R_{70}+HR_{70}$ in tomato (39.9 cm^2). Leaf area under these treatments was set to 100% and leaf area for the other treatments was calculated relative to these values. Leaf area of impatiens and salvia under treatment $R_{80}+HR_{80}$ was approximately twice that of plants grown with ≥80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light. Petunia leaf area under treatment $R_{80}+HR_{80}$ was 80 to 116% greater than plants under treatments $B_{10}+R_{75}+HR_{75}$ or $B_{80}+R_{40}+HR_{40}$, respectively. Leaf area of all plant species under treatment $R_{80}+HR_{80}$ was similar to that of plants under fluorescent

lamps. In tomato, there was no significant effect of light quality on leaf area and there was a significant ($P \leq 0.001$) interaction between light quality and replication (data not shown).

Seedling height. Impatiens, salvia, and tomato were tallest under the $R_{80}+HR_{80}$ treatment (44, 59, and 101 mm, respectively). Height under the other treatments was calculated relative to these values. Impatiens, salvia, and tomato with ≥10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light were 37 to 48%, 29 to 50%, or 23 to 49% shorter than plants under the $R_{80}+HR_{80}$ treatment, respectively. Stem height of impatiens was similar for all other treatments. Salvia grown under treatment $B_{80}+R_{40}+HR_{40}$ were 22 to 36% shorter than plants grown under treatment $B_{10}+R_{75}+HR_{75}$ or under fluorescent lamps. Similarly, tomato under treatment $B_{80}+R_{40}+HR_{40}$ were 24 to 26% shorter than plants irradiated with 10 or 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light or those grown under fluorescent lamps. Salvia and tomato were of similar height under treatment $R_{80}+HR_{80}$ and fluorescent lamps.

Fresh shoot weight. Fresh shoot weight was greatest for impatiens, salvia, and petunia under the $R_{80}+HR_{80}$ treatment (1.58, 1.26, and 0.98 g, respectively), while it was greatest under treatment $B_{20}+R_{70}+HR_{70}$ for tomato (1.90 g) (Figure 4.3). Fresh shoot weight under the other treatments was calculated relative to these values. Impatiens fresh shoot weight was 53 to 78% greater for plants grown under treatment $R_{80}+HR_{80}$ than for plants grown under ≥80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light or under treatment $B_{10}+R_{75}+HR_{75}$. Fresh shoot weight of salvia under $R_{80}+HR_{80}$ was 65 to 98% greater for plants grown under ≥40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light. Petunia under treatment $R_{80}+HR_{80}$ had 84% greater fresh weight than plants under treatment $B_{80}+R_{40}+HR_{40}$. The fresh shoot weight of tomato was similar among plants under all treatments and there was a significant ($P \leq 0.001$) interaction between light quality and replication (data not shown).

Dry shoot weight. Dry weight was greatest for salvia and petunia under the R_{80}+HR_{80} treatment (221 and 133 mg respectively), for impatiens under treatment B_{10}+R_{75}+HR_{75} (167 mg), and for tomato under treatment B_{20}+R_{70}+HR_{70} (304 mg). Dry weight under the other treatments was calculated relative to these values. Dry weight of impatiens was essentially the same under treatments that delivered 0 to 40 μmol·m^{-2}·s^{-1} of B light, and all of those were more than twice that of plants grown under fluorescent lamps. Salvia showed a similar but stronger trend for dry weight as for fresh shoot weight. Plants grown under treatment R_{80}+HR_{80} had 70 to 133% greater dry weight than plants grown with ≥40 μmol·m^{-2}·s^{-1} of B light or plants grown under fluorescent lamps. Tomato grown under B_{20}+R_{70}+HR_{70} had 112% greater dry weight than plants grown under fluorescent lamps but was similar among the other treatments. Dry weight of petunia under treatment R_{80}+HR_{80} was 33 to 91% greater than plants under ≥20 μmol·m^{-2}·s^{-1} of B light.

Leaf:stem fresh weight. Salvia and tomato had a decreasing leaf:stem fresh weight ratio with increasing percentage of R light. Plants of both species had a relatively high leaf:stem fresh weight ratio under treatments with ≥80 μmol·m^{-2}·s^{-1} of B light, while a relatively low ratio in the treatment R_{80}+HR_{80}. The leaf:stem fresh weight of impatiens was similar among treatments except for plants grown under fluorescent lighting, which had a 58 to 85% greater leaf:stem weight ratio.

Chlorophyll concentration. Salvia, tomato, and petunia had the greatest chlorophyll concentration under the fluorescent lamps (183, 142, and 138 mg Chl·g^{-1} fresh tissue, respectively), while it was greatest under treatment R_{80}+HR_{80} for impatiens (87.9 mg Chl·g^{-1} fresh tissue) (Figure 4.4). Chlorophyll concentration under the other treatments was calculated relative to these values. Impatiens, salvia, tomato, and petunia had 33 to 44%, 28 to 46%, 51 to

131%, 47 to 145% greater concentration of chlorophyll under fluorescent lamps, respectively, than plants under all other treatments except impatiens under treatment R_{80}+HR_{80}.

Leaf thickness. Salvia leaves were thickest under the B_{160} treatment (0.33 mm) while tomato leaf thickness was the greatest under treatment B_{40}+R_{60}+HR_{60} for tomato (0.31 mm). Leaf thickness under the other treatments was calculated relative to these values. Leaves of salvia were 37 to 43% thinner in plants grown without B light or plants grown under fluorescent lamps compared to plants grown under 100% B light. Tomato leaves were 41% thinner under fluorescent lamps than under the treatment with 25% B light.

Chlorosis score, edema, and flower bud number. Tomato developed chlorosis, edema, or both in some lighting treatments, whereas all other plants developed without any physiological disorders. The subjective chlorosis score generally decreased (i.e., chlorosis became more severe) as the percentage of B light decreased (Figure 4.5). Tomato grown under only B light or fluorescent lamps did not develop edema but it became more common with lower percentages of B light. Impatiens was the only plant that had visible flower buds at the end of the treatments. Impatiens generally developed more flower buds under progressively more B light. For example, under only B light there were 59, 177, or 535% more flower buds than under treatments with 20, 10, or 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light, respectively. Impatiens grown under fluorescent lamps developed a similar number of flower buds as plants grown with 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light.

Discussion

Our objective was to quantify how plant morphology changes in response to light environments with different B:R ratios. Plants acclimate to being grown under only R light, in

the absence of B and FR light, by increasing leaf expansion and developing characters analogous with the shade-avoidance response including increased chlorophyll content and stem length and decreased leaf thickness (Blackman and Wilson, 1951; Eskins, 1992; Franklin and Whitelam, 2005; Grime and Jeffery, 1965; Jarvis, 1964). In our study, leaf area of impatiens, salvia, and petunia grown without B light was much greater than that of plants grown with B light. Similarly, leaf area was 47 to 130% greater in tomato, impatiens, petunia, and salvia grown under only R light compared to the same *PPF* that included ≥25% B light (Chapter 3). Interestingly, in this study, leaf area of all species under only R light was similar to that of plants under fluorescent lamps even though the fluorescent lamps emitted 33 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light, which was more than the $B_{20}+R_{70}+HR_{70}$ treatment. Similarly, lettuce grown at a *PPF* of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ had a 44% greater leaf area under R fluorescent light than under B fluorescent light, while it was similar under R, R+B, or white fluorescent light (Ohashi-Kaneko et al., 2007). In contrast, leaf area of cotton (*Gossypium hirsutum*) was similar between plants grown under 100% B or R LEDs (peaks of 460 or 660 nm), while both were greater than that of plants under B+R (1:3) at the same *PPF* of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Li et al., 2010). These contrasting results with cotton could at least partially be attributed to the low *PPF*, which was less than half of that in our study.

Plants grown in environments with B light can have less biomass accumulation and thicker stems than those under only R light, but responses have varied among species studied (Chapter 3; Johkan et al., 2010; Schuerger et al., 1997). In our study, fresh shoot weight of impatiens, petunia, and salvia was 53 to 98% greater under treatments without B LED light than with ≥50% B light. Biomass allocation between leaves and stems was similar among all treatments except those of plants grown under fluorescent lighting for the shade-tolerant

impatiens, while leaf biomass of the shade-intolerant salvia and tomato was proportionately greater under light with lower B:R ratios. Although fresh shoot weight was similar among treatments grown under only R light or fluorescent lamps, plants grown under only R light had 33 to 133% greater dry weight than plants grown under fluorescent lamps. Thus, plants grown under only R light fixed more carbon than under fluorescent light, which apparently had a higher water content. Contrasting results have been reported in lettuce and komatsuna: lettuce had 28% greater shoot dry weight under white than red fluorescent light, whereas komatsuna had 43% greater dry weight under R light than white light (Ohashi-Kaneko et al., 2006). In strawberry, fresh shoot weight was 42% greater under only R (peak = 660 nm) than only B (peak = 450 nm) LED light at a *PPF* of 45 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Nhut et al., 2003).

In protected climates, the shade-avoidance response can be prevented by low-density spacing of plants to avoid mutual shading and by delivering B light or light with a high R:FR. Phytochrome and cryptochrome photoreceptors perceive R and FR light or B light and UV-A (320–390 nm) radiation, respectively, and mediate extension growth (Liu et al., 2011a; Smith, 2000; Stapleton, 1992). Because very few FR photons were present in the six LED lighting treatments, the stem elongation inhibition we observed from as little as 6.3% B light could be attributed to the B-light-stimulated cryptochrome receptors, which are most stimulated by wavelengths between 390 and 480 nm (Liu et al., 2011a; Ahmad et al., 2002). In *Arabidopsis*, *CRY*1 genes regulate extension growth of seedlings by altering downstream expression of other genes, such as *COP1* and *HY5* (Jiao et al., 2007; Liu et al., 2011a; Yang et al., 2005). *CRY*1 consequently regulates phytohormone distribution of GA (Lui et al., 2011b). The smallest quantity of B light delivered in our treatments was 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$, which was apparently adequate to stimulate cryptochrome receptors. Only 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light was sufficient to

stimulate cryptochrome responses in *Arabidopsis* and barley (*Hordeum vulgare*) (Christopher and Mullet, 1994; Hogewoning et al., 2010; Mochizuki et al., 2004). All plants in our study with ≥25% B light were of similar height, which suggests that cryptochrome became saturated at around 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light. In a previous study, impatiens, tomato, salvia, and petunia grown under ≥25% B light were 41 to 57% shorter than those under only R light (Chapter 3). In contrast, marigold and salvia grown under B LEDs (peak = 440 nm) at a *PPF* of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were approximately twice as tall as plants grown under only R LEDs (peak = 650 nm) at the same intensity (Heo et al., 2002). We cannot explain this discrepancy.

Thinner leaves, also a characteristic of the shade-avoidance response, typically develop under a low R:FR ratio or light deficient in B (Fukuda et al., 2008; Schuerger et al., 1997). In our study, leaves of salvia were 37 to 43% thinner in plants grown without B light or under fluorescent lamps than plants grown under 100% B light, however tomato leaf thickness was similar under the different B:R ratios. The increase in salvia leaf thickness with increasing percentage of B light is consistent with that reported for cucumber and geranium (Fukuda et al., 2008; Schuerger et al., 1997). Plants grown under white fluorescent lamps had relatively thin leaves, contributing to the relatively low dry weight and high water content, compared with those grown under combinations of R and B LEDs. Leaves could be thinner under fluorescent light because of the high proportion of G light, which isn't absorbed by leaves as much as blue or red light. Light quality can also influence leaf orientation; Fukuda et al., (2008) showed that irradiating the adaxial surface of geranium (*Pelargonium zonale*) leaves with light from B+R LEDs were 20% more upright than leaves irradiated with only R light (Fukuda et al., 2008). Plants in our study grown under fluorescent lamps had visually more upright (horizontal) leaves than those grown under our LED treatments, although data was not recorded.

Plants acclimate to a high B:R ratio by increasing chlorophyll synthesis, as mediated by cryptochrome (Folta and Childers, 2008). Plants in our study had 28 to 145% greater chlorophyll concentration under fluorescent lamps, which emitted 54% G light, than under all treatments except for impatiens grown without B light. In addition, petunia had relatively low chlorophyll content when plants were grown under 25% B light. Similarly, in our previous study, the concentration of chlorophyll in salvia and tomato was relatively high under fluorescent light and relatively low under all other treatments, but it was similar among all treatments for impatiens and petunia (Chapter 3). This suggests that plants grown under fluorescent lamps could have acclimated to the high percentage of G light, which is absorbed by chlorophyll less than R or B light, by increasing chlorophyll biosynthesis, decreasing chlorophyll degradation, or both, to maximize photosynthetic capacity. Other studies have reported similar chlorophyll concentrations between LED light treatments in cherry tomato and lettuce (Liu et al., 2011b). For example, chlorophyll content per leaf area of cherry tomato (*Lycopersicon esculentum* var. *cerasiforme*) was similar among LED treatments delivering B, G, R, R+B (1:1) or R+B+G (3:3:1; peaks = 650, 450, and 520 nm, respectively) at the same *PPF* (Liu et al., 2011b). In contrast to our results, chlorophyll concentration of lettuce were similar among combinations of cool-white fluorescent and R (peak=658 nm) or B (peak=476 nm) LED light at the same *PPF* (Li and Kubota, 2009).

Edema has been correlated with environments deficient in B or FR light or UV-B (280 to 315 nm; Jenkins, 2009) radiation, particularly on plants in the Solanaceae (Lang and Tibbitts, 1983; Massa et al., 2008; Morrow and Tibbitts, 1988; Nilsen, 1971). Tomato grown under only B light or fluorescent lamps did not develop edema and it became more prevalent as the B:R ratio decreased. Similarly, wild tomato (*L. hirsutum*) developed edema on 63% of the leaf area surface

when under R fluorescent lamps, whereas it was absent under B fluorescent lamps at a *PPF* of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Morrow and Tibbitts, 1988). Plants under B light often have greater stomatal conductance than those grown under only R light (Ohashi-Kaneko et al., 2006; Kim et al., 2004). Edema develops when plants take up water more quickly than they can transpire it, so plants in our study under only B light may have had the greatest transpiration rates, which in turn resulted in no edema under that light treatment.

Early flowering can be induced in some species by B light. Impatiens under only B light developed significantly more flower buds under treatments with $\geq$40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ B light than with 0 to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light. *CRY2* cryptochrome receptors can stimulate flowering by promoting downstream flowering genes including *CO* and *FT* (El-Assal et al., 2003; Chaves et al., 2011). This suggests that increasing stimulation of *CRY2* by increasing B light caused impatiens to flower earlier than those with little or no B light. Increased flower number in light that contains B, compared to without B, has been reported in other flowering annual plants. For example, marigold and salvia produced 43 or 100% more flower buds, respectively, under B+R (peak=440, 650 nm) LED light compared to when grown under fluorescent light at the same intensity (Heo et al., 2006). In contrast, marigold and salvia grown under B or R LEDs (peaks=440 and 650 nm, respectively) at a *PPF* of 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ developed a similar number of flower buds, while plants under either treatment had 77 to 86% fewer flower buds than those under fluorescent lamps (Heo et al., 2002). Similarly, impatiens grown under 100% B light had 71 times more flower buds than those grown under only R light (Chapter 3). We terminated experiments before salvia, tomato, or petunia had visible flower buds so don't know whether B light would have had similar effects on flowering as that in impatiens.

We conclude that plants acclimate to only R light by increasing leaf expansion and stem elongation, while plant responses to B light include inhibited extension growth and in some cases, greater leaf thickness and chlorophyll concentration. Subsequently, plants under only R light accumulated more biomass than those of other treatments in part due to the increased surface area for light capture. Approximately 6-13% B light was apparently sufficient to stimulate cryptochrome photoreceptors that inhibited extension growth, thereby reducing leaf size and biomass accumulation. Therefore, including as little as 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of B light in an R-dominant background can elicit desirable growth responses for the production of propagules, herbs, microgreens, and other situations in which compact growth is desired.

APPENDIX

Table 4.1. Actual air and canopy temperatures (°C) as measured by thermocouples and infrared sensors for the six LED-lighting treatments (B: blue, R: red, HR: hyper red) and one fluorescent lighting treatment. The value after each LED type represents the intensity (in $\mu mol \cdot m^{-2} \cdot s^{-1}$) of each waveband. All temperatures had a standard error ±0.1 °C.

	Replication 1		Replication 2		Replication 3	
Light quality treatment	Air	Canopy	Air	Canopy	Air	Canopy
B_{160}	21.2	20.6	21.2	20.4	21.4	21.1
$B_{80}+R_{40}+HR_{40}$	20.8	21.1	20.8	20.8	21.9	20.8
$B_{40}+R_{60}+HR_{60}$	20.6	20.4	20.9	20.3	21.4	21.3
$B_{20}+R_{70}+HR_{70}$	21.4	20.6	20.4	20.1	21.5	21.4
$B_{10}+R_{75}+HR_{75}$	21.0	20.5	20.6	20.7	21.2	21.0
$R_{80}+HR_{80}$	20.7	20.4	20.4	20.3	21.4	21.5
Fluorescent	21.7	21.9	21.9	21.5	21.5	21.7

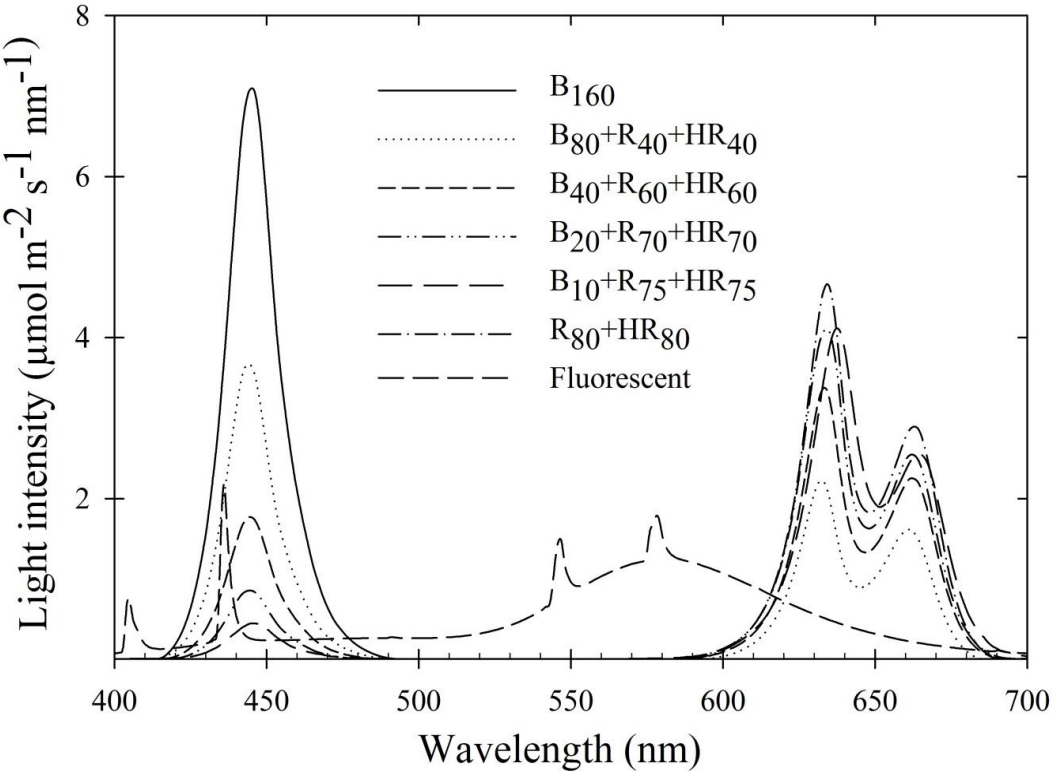

Figure 4.1. The spectral distribution of six light quality treatments delivered by blue (B), red (R), and hyper red (HR) LEDs and one treatment delivered by cool-white fluorescent lamps, each delivering a *PPF* of 160 μmol·m^{-2}·s^{-1}. The value after each LED type represents its intensity (in μmol·m^{-2}·s^{-1}).

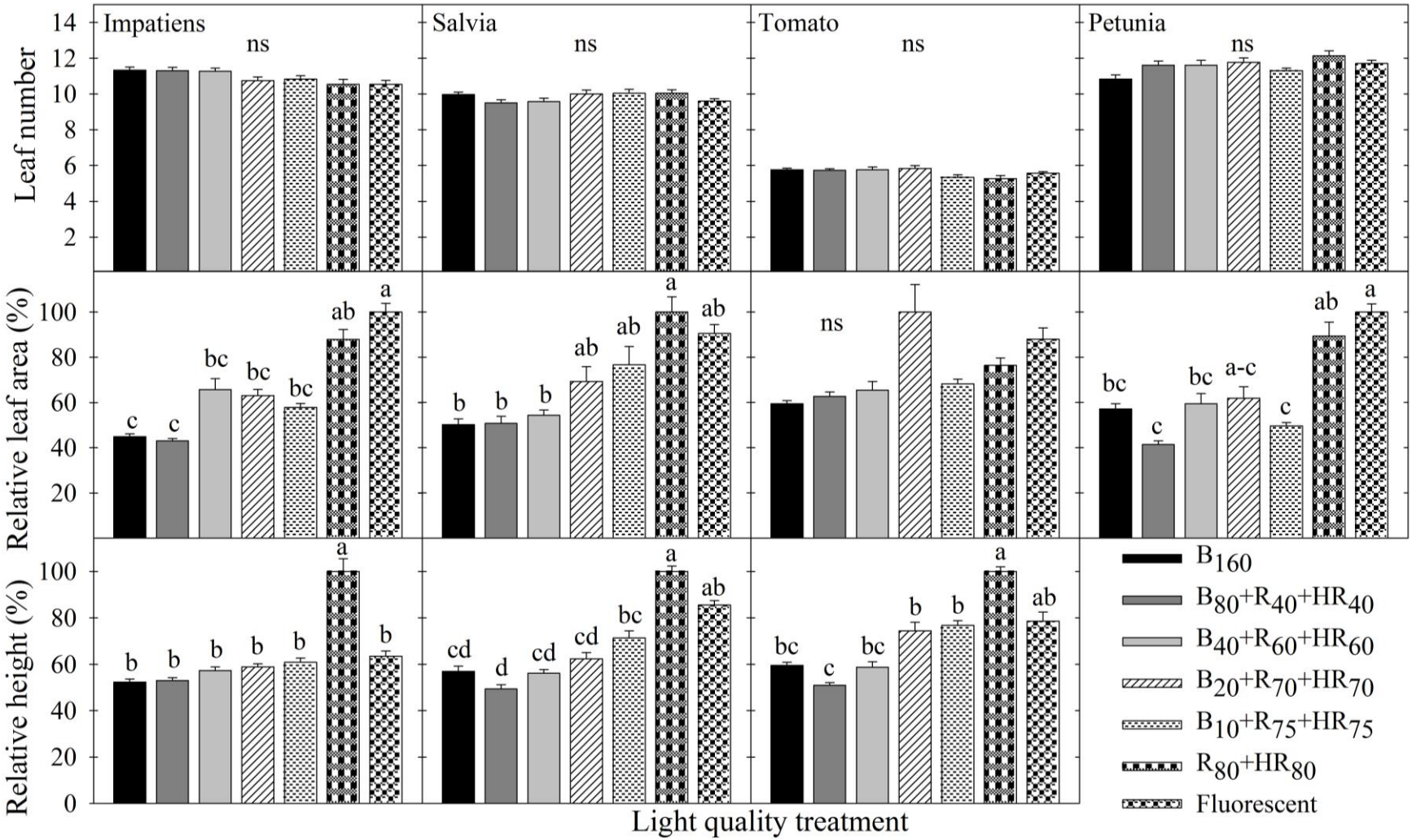

Figure 4.2. Pooled mean leaf area, leaf number, and height of four seedling crops grown under six light quality treatments delivered by LEDs (B: blue, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The value after each LED type represents its intensity (in $\mu mol \cdot m^{-2} \cdot s^{-1}$). Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

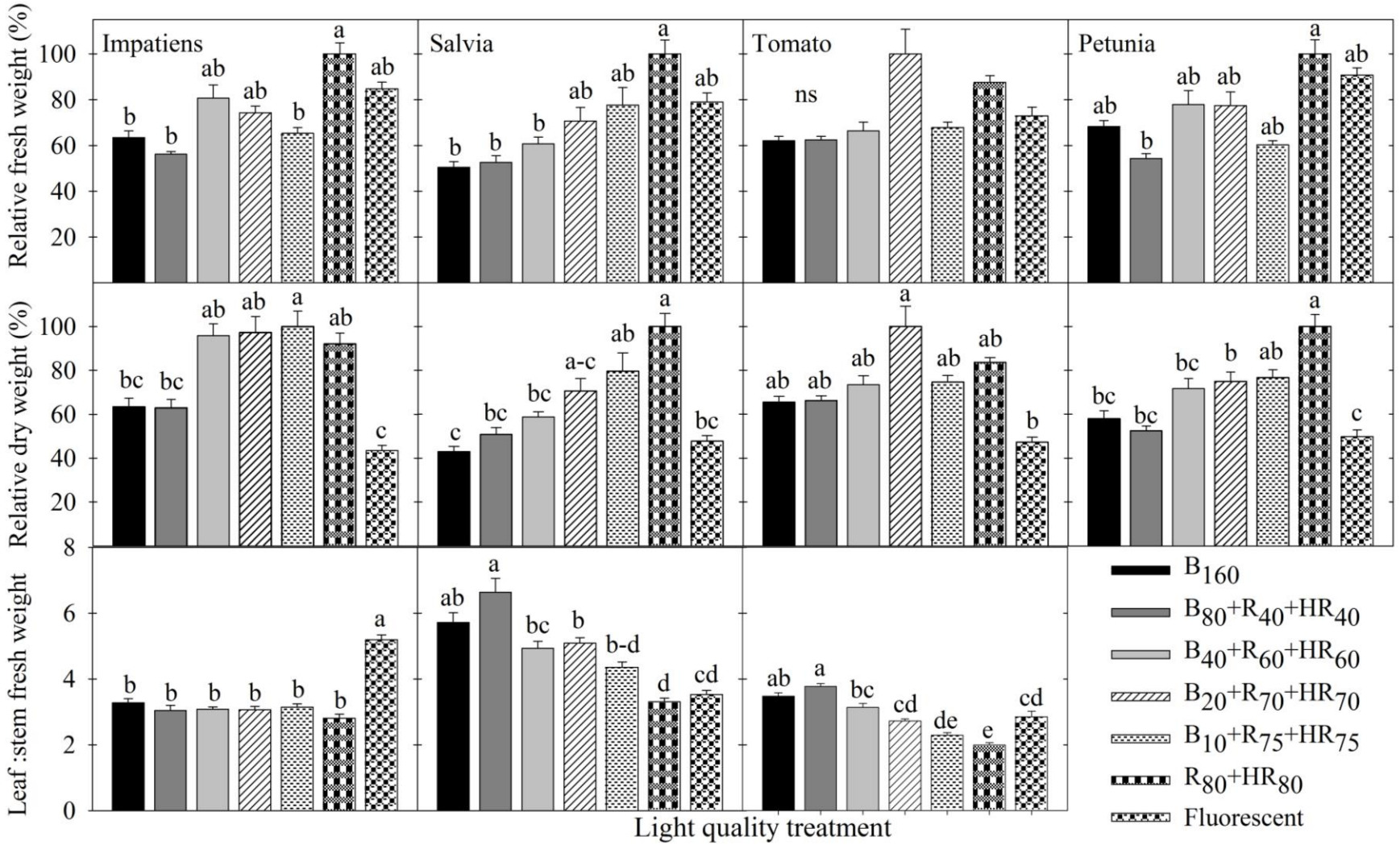

Figure 4.3. Pooled mean fresh and dry shoot weights for four seedling crops and leaf:stem fresh shoot weight ratio for four seedling crops grown under six light quality treatments delivered by LEDs (B: blue, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The value after each LED type represents its intensity (in $\mu mol \cdot m^{-2} \cdot s^{-1}$). Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

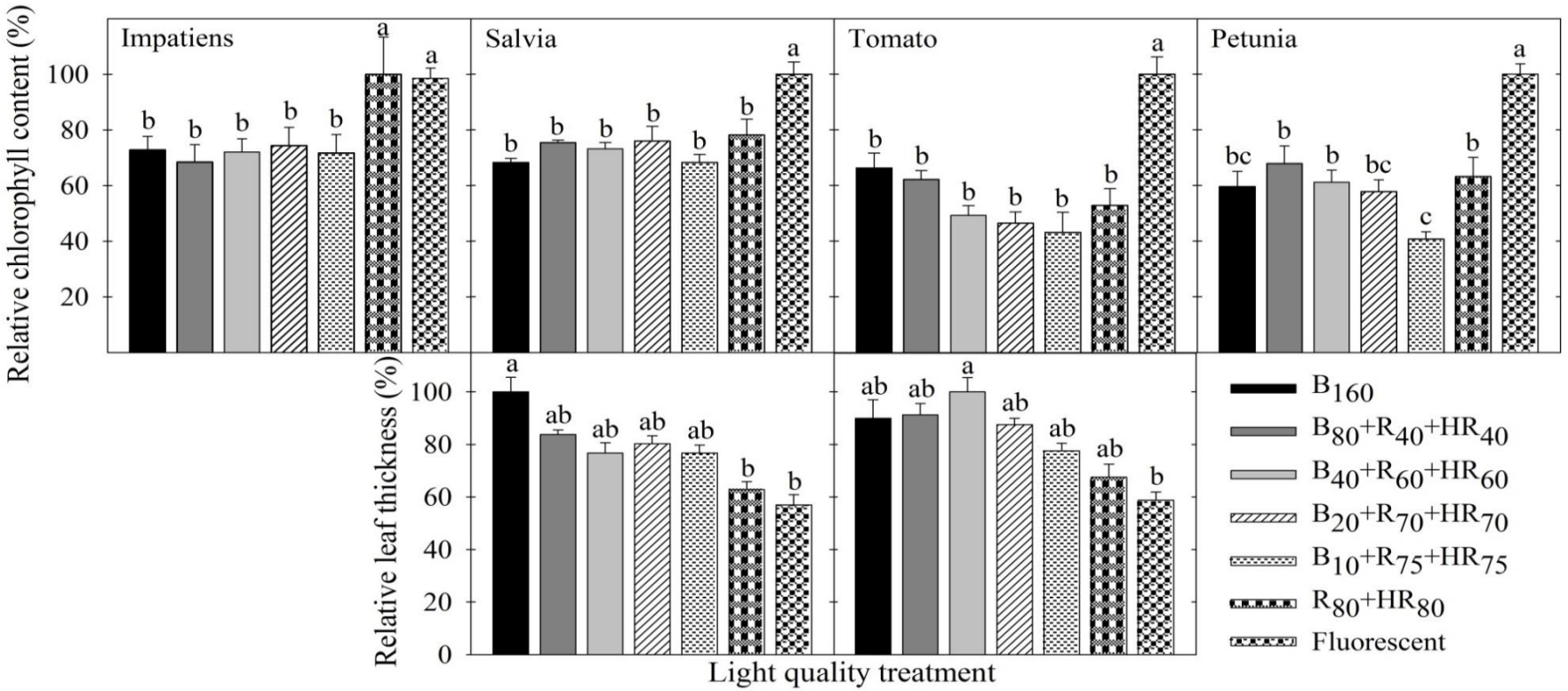

Figure 4.4. Pooled mean chlorophyll concentrations for four seedling crops or pooled leaf thickness for salvia and tomato grown in six light treatments delivered by LEDs (B: blue, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. The value after each LED type represents its intensity (in $\mu mol \cdot m^{-2} \cdot s^{-1}$). Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

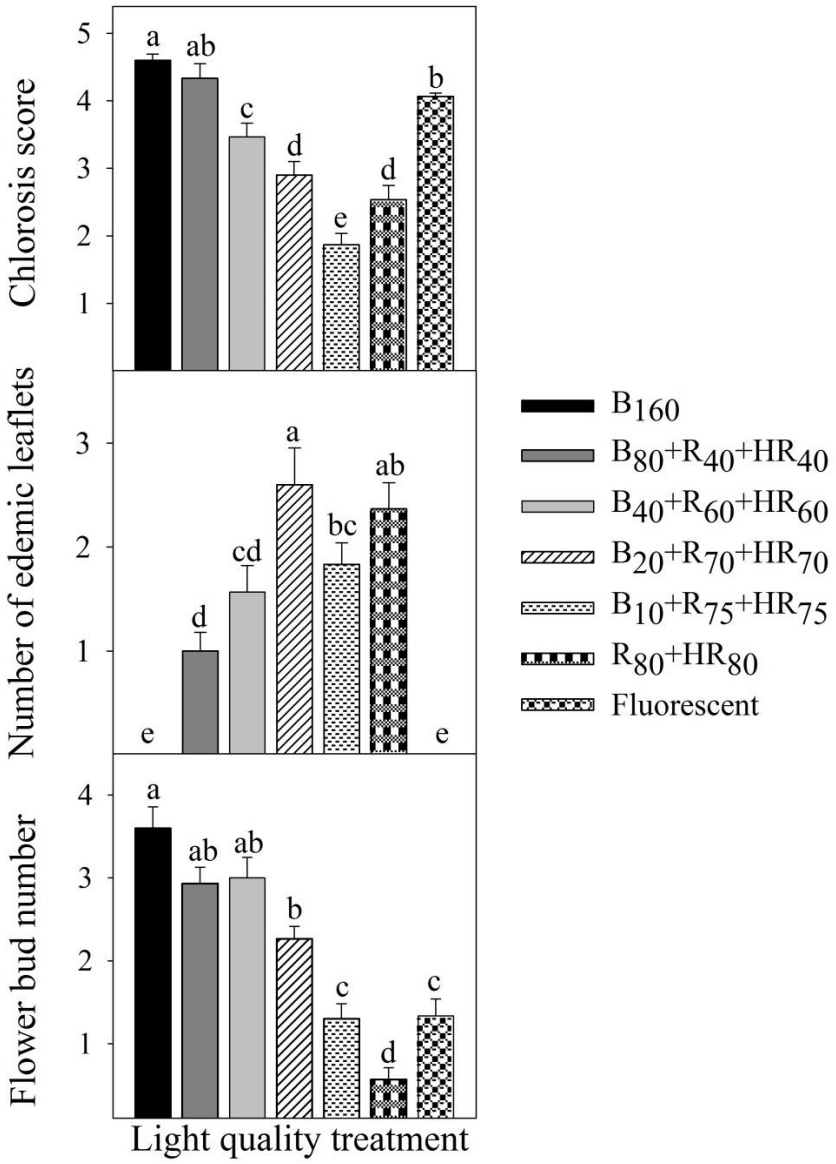

Figure 4.5. Pooled mean chlorosis score and number of leaves exhibiting edema for tomato and flower bud number for impatiens under six light quality treatments delivered by LEDs (B: blue, R: red, HR: hyper red) or one treatment delivered by cool-white fluorescent lamps at the same *PPF*. Chlorosis score (1= most chlorotic, 5= least chlorotic). The value after each LED type represents its intensity (in $\mu mol \cdot m^{-2} \cdot s^{-1}$). Means sharing a letter are not statistically different by Tukey's honestly significant difference at $P \leq 0.05$. Error bars indicate standard error.

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