

Increases in Shoot Tissue Pigments, Glucosinolates, and Mineral Elements in Sprouting Broccoli after Exposure to Short-duration Blue Light from Light Emitting Diodes

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ABSTRACT. Microgreens are specialty leafy crops harvested just above the roots after the first true leaves have emerged and are consumed fresh. Broccoli (*Brassica oleracea* var. *italica*) microgreens can accumulate significant concentrations of cancer-fighting glucosinolates as well as being a rich source of other antioxidant phytochemicals. Light-emitting diodes (LEDs) now provide the ability to measure impacts of narrow-band wavelengths of light on seedling physiology. The carotenoid zeaxanthin has been hypothesized to be a blue light receptor in plant physiology. The objective of this study was to measure the impact of short-duration blue light on phytochemical compounds, which impart the nutritional quality of sprouting broccoli microgreens. Broccoli microgreens were grown in a controlled environment under LEDs using growing pads. Seeds were cultured on the pads submerged in deionized water and grown under a 24-hour photoperiod using red (627 nm)/blue (470 nm) LEDs ($350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at an air temperature of 23 °C. On emergence of the first true leaf, a complete nutrient solution with 42 mg·L⁻¹ of nitrogen (N) was used to submerge the growing pads. At 13 days after sowing, broccoli plantlets were grown under either: 1) red and blue LED light ($350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); or 2) blue LED light ($41 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) treatments for 5 days before harvest. The experiment was repeated three times. Frozen shoot tissues were freeze-dried and measured for carotenoids, chlorophylls, glucosinolates, and mineral elements. Comparing the two LED light treatments revealed the short-duration blue LED treatment before harvest significantly increased shoot tissue β -carotene ($P \leq 0.05$), violaxanthin ($P \leq 0.01$), total xanthophyll cycle pigments ($P \leq 0.05$), glucoraphanin ($P \leq 0.05$), epiprogoitrin ($P \leq 0.05$), aliphatic glucosinolates ($P \leq 0.05$), essential micronutrients of copper (Cu) ($P = 0.02$), iron (Fe) ($P \leq 0.01$), boron (B), manganese (Mn), molybdenum (Mo), sodium (Na), zinc (Zn) ($P \leq 0.001$), and the essential macronutrients of calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) ($P \leq 0.001$). Results demonstrate management of LED lighting technology through preharvest, short-duration blue light acted to increase important phytochemical compounds influencing the nutritional value of broccoli microgreens.

The production of secondary metabolites in plant tissues is determined by an interaction of environmental growth factors with biochemical, physiological, and genetic characteristics (Goldman et al., 1999; Kopsell et al., 2004; Kurilich et al., 1999). The light environment is a significant influential factor on plant secondary metabolite production (Kopsell et al., 2012; Lefsrud et al., 2006), and physiological changes will occur in plants when exposed to varying wavelengths of light (Johkan et al., 2010; Li and Kubota, 2009; Loreto et al., 2009; Samuolienė et al., 2012; Stutte et al., 2009). Irradiance levels can also influence secondary metabolite production in plants such as carotenoid pigment and glucosinolate concentrations (Charron and Sams, 2004; Lefsrud et al., 2006).

Near monochromatic LEDs are one of the most energy-efficient and rapidly developing lighting technologies. One developing application of LED technology is for horticultural plant production in controlled environments (Martineau et al., 2012). Capacities such as spectral composition control, high

light output, and little radiant heat emissions make LEDs the most significant advancement in horticultural lighting since the development of high-intensity discharge lamps (Morrow, 2008). LEDs now provide the ability to measure impacts of narrow-band wavelengths of light on seedling morphology and physiology (Lefsrud et al., 2008).

Carotenoids are produced in the plastids and protect photosynthetic structures by quenching excited triplet Chl (³Chl) to dissipate excess energy and bind singlet oxygen (¹O₂) to inhibit potential oxidative damage (Demmig-Adams et al., 1996; Frank and Cogdell, 1996; Tracewell et al., 2001). Although there is a good understanding of the biosynthetic pathways for carotenoid production in plants (Cunningham, 2002; Kopsell and Kopsell, 2006), the regulatory means by which different plants can alter levels of individual carotenoids largely remains uncertain (Farnham and Kopsell, 2009). Chlorophyll and carotenoid pigments function in light harvesting and photoprotection in plants. Maximum absorption of chlorophyll *a* (Chl *a*) and *b* (Chl *b*) pigments are in the red (663 and 642 nm, respectively) and blue regions (430 and 453 nm, respectively) of the visible light spectrum. In contrast, absorption of the carotenoid pigments of lutein (LUT) and β -carotene (BC) are highest in the blue region at 448 and 454 nm, respectively (Lefsrud et al., 2008; Lichtenthaler, 1987). Light signals are perceived by plants through photoreceptor proteins called phototropins (Briggs and

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Huala, 1999). Two such phototropins, called *phot-1* and *phot-2*, have been identified in *Arabidopsis thaliana* and work together to respond to blue light stimuli (Baum et al., 1999; Briggs and Christie, 2002; Fuchs et al., 2003). Blue light exposure during plant growth is qualitatively required for normal photosynthesis and facilitates quantitative leaf responses similar to those normally associated with higher light intensities (Hogewoning et al., 2010). Xanthophyll carotenoid pigments, specifically zeaxanthin (ZEA), can modulate blue light-dependent responses in plants (Tlalka et al., 1999). Moreover, ZEA is believed to be an important photoreceptor for blue light-activated stomatal operations (Briggs and Huala, 1999). As a result of their light absorption capabilities, specialized pigments have evolved to be vital to light perception and responses in plant species.

Cruciferous vegetables are relatively abundant sources of antioxidants with potential anticarcinogenic activity (Kurilich et al., 1999). The bioactive compounds in Brassicas include lutein and β -carotene carotenoids, glucoraphanin and glucobrassicin glucosinolates, quercetin and kaempferol flavonoids, and C and E vitamins (Jeffery and Araya, 2009). Plant carotenoids are the most important source of provitamin A in the human diet. There is increasing evidence that carotenoids can also protect humans against certain specific chronic ailments including cancer, cardiovascular disease, and age-related macular degeneration (Giovannucci, 1999; Mayne, 1996). The most studied bioactive components in the Brassicas are the glucosinolates and their hydrolyzed isothiocyanates (Stoewsand, 1995). Isothiocyanates possess anticarcinogenic activity and may be useful as chemopreventative agents in the human diet. The major aliphatic glucosinolate found in broccoli is glucoraphanin. The isothiocyanate derived from glucoraphanin is sulforaphane, which induces upregulation of phase II detoxification enzymes and is the central cancer-preventative agent in broccoli (Fahey et al., 1997). The vegetable Brassicas are also consumed for their nutritional values of Ca, Mg, P, K, and Fe (Farnham et al., 2000), and many members of the genus are identified as good dietary sources of these nutrients. Calcium and K are two important elements that play critical roles in bone development and cellular metabolism in human nutrition. Brassicas are high in Ca content and low in oxalate compounds that can bind to Ca and reduce absorption (Weaver and Heaney, 1999). Microgreens are specialty leafy crops harvested just above the roots after the first true leaves have emerged and usually consumed fresh as a salad green (Kopsell et al., 2012). Broccoli microgreens are valued for significant concentrations of cancer-fighting glucosinolates (GSs) as well as being a rich source of carotenoid phytochemicals and essential mineral elements. Because of the impacts blue light wavelengths can have on plant physiology and secondary metabolite production, the objective of this study was to measure the impact of short-duration blue light from LEDs on the nutritionally important phytochemical compounds in sprouting broccoli microgreens.

Materials and Methods

SPROUTING BROCCOLI CULTURE AND HARVEST. Hydroponic culture using growing pads (Sure to Grow, Beachwood, OH) of polyethylene terephthalate fibers ($25.4 \times 24.7 \times 0.89$ cm) were used to germinate and grow sprouting broccoli (Mountain Rose Herbs, Eugene, OR) microgreen plants (Kopsell et al., 2012). A 7-g sample of sprouting broccoli seeds was sown evenly onto the growing pad, and two pads were set in a perforated tray

($26 \times 52 \times 6$ cm). Perforated trays were set into solid-bottom trays ($26 \times 52 \times 6$ cm) and filled with deionized water to create a hydroponic tray system for microgreen culture. Four tray systems were placed randomly into each of two controlled environment chambers (Model E15; Conviron, Winnipeg, Manitoba, Canada) to germinate seeds at 23 ± 1 °C in darkness. Deionized water was added at a rate of $200 \text{ mL} \cdot \text{d}^{-1}$ to the bottom tray. Tray systems were equally divided to act as two experimental replications, and chambers acted as experimental treatments. Three complete experimental runs were conducted on 8 Apr. 2011, 28 Apr. 2011, and 23 May 2011. After 3 d, the photoperiod was set at 24 h using LED panels with 470- and 627-nm diodes (Orbital Technologies, Madison, WI). Photosynthetically active radiation for LED lighting was measured with a spectroradiometer (Model SPEC-ultraviolet/PAR; Apogee Instruments, Logan, UT) at the center of each panel, and panel heights from the growing pads were adjusted to maintain an average light intensity of $350 \pm 10 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for the chambers composed of 12% blue and 88% red light. On emergence of the first true leaf 5 d after sowing seeds, a nutrient solution of $42 \text{ mg} \cdot \text{L}^{-1} \text{ N}$ [20% Hoagland's #2 solution (Hoagland and Arnon, 1950)] was used to submerge the growing pads. Each tray received 500 mL of the nutrient solution each day throughout the remainder of the study. At 13 d after sowing seeds, light treatments were initiated. Two chambers served as experimental light treatment units. One chamber was kept at the starting light intensity of $350 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ from LED lights of 470 and 627 nm and represented the red and blue LED preharvest treatment, and one chamber was adjusted to have only 470 nm and represented the blue LED treatment ($41 \pm 2 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) (Fig. 1). Microgreen plants were harvested from both chambers 19 d after sowing seeds. Microgreen stems were cut at the surface of the growing pads and frozen at -80 °C before sample preparation. Temperature and light treatment conditions were measured at the beginning and confirmed at the end of each experimental run.

SHOOT TISSUE PIGMENT EXTRACTION. Microgreen tissues were freeze-dried (Model 6 L FreeZone; LabConCo, Kansas City, MO) at a constant temperature of -25 °C before extraction. Pigments were extracted from freeze-dried tissues and analyzed according to Kopsell et al. (2012). A 0.1-g tissue subsample was rehydrated with 0.8 mL of ultrapure H_2O for 20 min. After incubation, 0.8 mL of the internal standard ethyl- β -8'-apocarotenoate (Sigma-Aldrich, St. Louis, MO) was added to determine extraction efficiency. The addition of 2.5 mL of tetrahydrofuran (THF) was performed after sample hydration. The sample was then homogenized in a tissue grinding tube (Potter-Elvehjem; Kimble Chase-Kontes, Vineland, NJ) using ≈ 25 insertions with a pestle attached to a drill press set at 540 rpm. During homogenization, the tube was immersed in ice to dissipate heat. The tube was then placed into a clinical centrifuge for 3 min at $500 g_n$. The supernatant was removed and the sample pellet was resuspended in 2 mL THF and homogenized again with the same extraction technique. The procedure was repeated for a total of four extractions to obtain a colorless supernatant. The combined supernatants were reduced to 0.5 mL under a stream of N gas (N-EVAP 111; Organomation, Berlin, MA) and brought up to a final volume of 5 mL with acetone. A 2-mL aliquot was filtered through a $0.2\text{-}\mu\text{m}$ polytetrafluoroethylene (PTFE) filter (Econofilter PTFE 25/20; Agilent Technologies, Santa Clara, CA) using a 5-mL syringe (Becton, Dickinson and Co., Franklin Lakes, NJ) before high-performance liquid chromatography (HPLC) analysis.

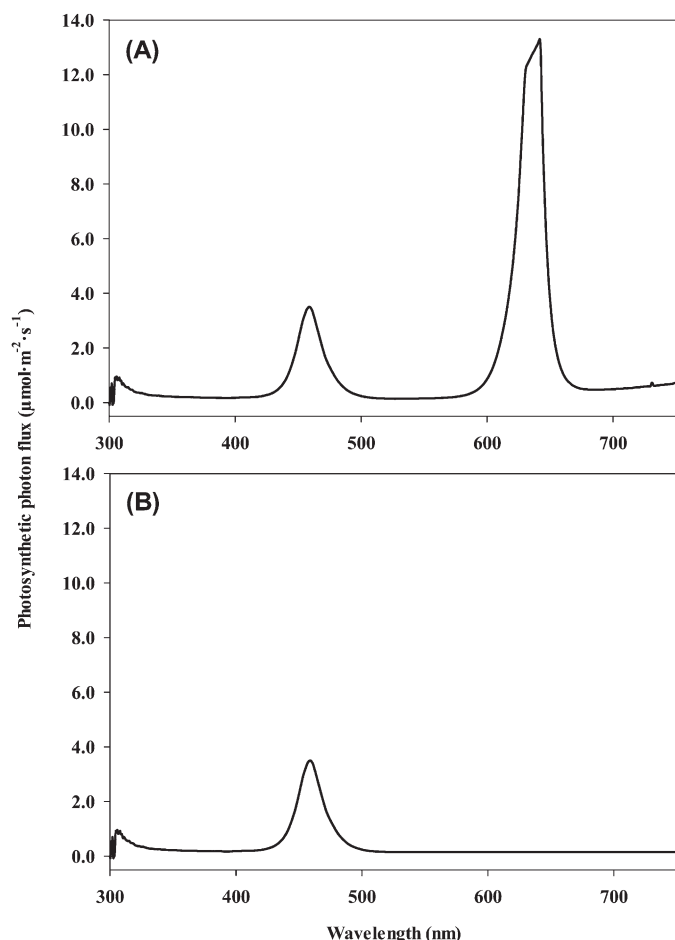


Fig. 1. Spectroradiometer photon flux distribution for the light treatments of: (A) red and blue light-emitting diode (LED) treatment of 627 nm and 470 nm, respectively (light intensity of $350 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); and (B) blue LED treatment of 470 nm only (light intensity of $41 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

SHOOT TISSUE PIGMENT HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS. An Agilent 1200 series HPLC unit with a photodiode array detector (Agilent Technologies) was used for pigment separation. Chromatographic separations were achieved using an analytical scale (4.6 i.d. \times 250 mm) 5- μm , 200- \AA polymeric RP-C₃₀ column (ProntoSIL; MACMOD Analytical, Chadds Ford, PA), which allowed for effective separation of chemically similar pigment compounds. The column was equipped with a 5- μm guard cartridge (4.0 i.d. \times 10 mm) and holder (ProntoSIL) and was maintained at 30 °C using a thermostatted column compartment. All separations were achieved isocratically using a binary mobile phase of 11% methyl *tert*-butyl ether (MTBE), 88.99% MeOH, and 0.01% triethylamine (TEA) (v/v/v). The flow rate was 1.0 mL $\cdot\text{min}^{-1}$ with a run time of 58 min. Eluted compounds from a 10- μL injection were detected at 453 nm (carotenoids and internal standard), 652 nm Chl *a*, and 665 nm (Chl *b*). Data were collected, recorded, and integrated using ChemStation software (Agilent Technologies). Peak assignment for individual pigments was performed by comparing retention times and line spectra obtained from photodiode array detection using external standards [antheraxanthin (ANT), BC, Chl *a*, Chl *b*, LUT, neoxanthin (NEO), violaxanthin (VIO), ZEA from ChromaDex, Irvine, CA].

SHOOT TISSUE GLUCOSINOLATE EXTRACTION. For GS analysis, 0.2 g of freeze-dried tissue sample was combined with 1 mL benzyl GS solution (1 mM) as an internal standard, 2.0 mL MeOH, and 0.1 mL barium-lead acetate (0.6 M) in a 16 \times 100-mm culture tube and shaken at 60 rpm for 1 h. Each tube was then centrifuged at 2000 g_n for 10 min. A 0.5-mL aliquot of supernatant was then added to a 1-mL column containing 0.3 mL DEAE Sephadex A-25 (Sigma-Aldrich). The sample was desulfated by the procedure of Raney and McGregor (1990).

SHOOT TISSUE GLUCOSINOLATE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS. Extracted desulfoglucosinolates were separated using an HPLC unit with a photodiode array detector (1100 series; Agilent Technologies) using a reverse-phase 4.6 i.d. \times 250 mm, 5- μm Luna C₁₈ column (Phenomenex, Torrance, CA) at a wavelength of 230 nm. The column temperature was set at 40 °C with a flow rate of 1 mL $\cdot\text{min}^{-1}$. The gradient elution parameters were 100% water for 1 min followed by a 15-min linear gradient to 75% water:25% acetonitrile. Solvent levels were then held constant for 5 min and returned to 100% water for the final 5 min. Desulfoglucosinolates were identified by comparison with retention times of authentic standards or previously reported results (Hansen et al., 1995; Kushad et al., 1999). Desulfated forms of sinigrin (2-propenyl GS), epiprogoitrin (2-hydroxy-3-butenyl GS), glucoiberin (3-methylsulfinylpropyl GS), glucoraphenin (4-methylsulphinyl-3-butenyl GS), glucobrassicin (3-indolylmethyl GS), 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl GS), neoglucobrassicin (1-methoxy-3-indolylmethyl GS), and gluconasturtiin (2-phenylethyl) were provided by S. Palmieri (Istituto Sperimentale Industriali, Bologna, Italy). Gluconasturtiin (2-phenylethyl GS) was purchased from LKT Laboratories (St. Paul, MN). Response factors used were from the International Organization for Standardization Method 9167-1.

SHOOT TISSUE MINERAL ELEMENT ANALYSIS. A 0.5-g subsample of ground freeze-dried tissue was combined with 10 mL HNO₃ (70%) and sealed in a closed vessel microwave digestion system (ETHOS series; Milestone, Shelton, CT). Digestion procedures followed those for organically based matrices (U.S. Environmental Protection Agency, 1996). Digestions were diluted with 2% HNO₃/0.5% HCl (v/v), and elemental measurements were made using an inductively coupled plasma-mass spectroscopy (ICP-MS) system (7500ce; Agilent Technologies). The ICP-MS system was equipped with an octapole collision/reaction cell, ChemStation software (7500 ICP-MS; Agilent Technologies), a micromist nebulizer, a water-cooled quartz spray chamber, and an autosampler (ASX-510; CETAC, Omaha, NE). The instrument was optimized daily in terms of sensitivity (Li, Y, Tl), level of oxide (Ce), and doubly charged ion (Ce) using a tuning solution containing 10 $\mu\text{g}\cdot\text{L}^{-1}$ of Li, Y, Tl, Ce, and Co in a 2% HNO₃/0.5% HCl (v/v) matrix.

STATISTICAL ANALYSIS. Data sets were analyzed using statistical software (Version 9.2; SAS Institute, Cary, NC) with differences between LED lighting treatments determined by *t* test ($\alpha = 0.05$). Because of the cultivation method on the grow pads, broccoli microgreen biomass data are presented on the basis of grams of fresh weight (FM) per gram seed weight. Microgreen tissue pigment data are presented on a FM basis, whereas tissue glucosinolate and tissue mineral element data are presented on a dry mass basis.

Results

The LED lighting treatments did not impact broccoli microgreen FM shoot biomass. Broccoli microgreen shoots grown

under the red and blue LED treatment averaged 11.3 g FM per gram seed weight, whereas broccoli microgreens grown under the blue LED treatment averaged 10.6 g FM per gram seed weight. Broccoli microgreens differed in morphology between LED light treatments. Microgreen grown under the red and blue LED treatment was shorter and had a waxy blush to the leaf cuticle, whereas microgreens grown under the blue LED treatment for 5 d preharvest caused plants to elongate slightly and exhibit a lighter green color.

Concentrations of Chl *a* and total chlorophyll in the broccoli microgreen tissues did not differ between the red and blue LED and blue LED treatments (Table 1). However, the blue LED treatment caused a 17.6% decrease in Chl *b* concentrations. Decreases in broccoli microgreen tissue Chl *b* resulted in a significant increase in the chlorophyll *a* to *b* ratio under the blue LED treatment (Table 1).

Broccoli microgreen tissue BC concentrations increased 20.7% ($P \leq 0.05$) after exposure to the blue LED treatment. Tissue concentrations of VIO increased 41.4% ($P \leq 0.01$) after exposure to the blue LED treatment. The blue LED treatment did not impact concentrations of LUT, ZEA, or NEO in the broccoli microgreen tissues. Concentrations of total xanthophyll

Table 1. Mean values for chlorophyll shoot tissue pigments in sprouting broccoli microgreens grown under light-emitting diode (LED) treatment conditions of 627 nm and 470 nm (red and blue LED treatment) at 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or a 5-d preharvest light treatment of only 470 nm (blue LED treatment) at an average of 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.^z

Shoot tissue pigment	Red and blue LED treatment	Blue LED treatment	$P > t ^y$
	[mean \pm SE (mg/100 g fresh weight)]		
Chlorophyll a	40.80 \pm 2.90	36.75 \pm 2.90	NS
Chlorophyll b	16.40 \pm 0.95	13.50 \pm 0.95	0.04
Total chlorophyll	57.20 \pm 3.81	50.25 \pm 3.81	NS
	[mean \pm SE (pigment ratio)]		
Chlorophyll <i>a</i> to <i>b</i>	2.47 \pm 0.06	2.71 \pm 0.06	≤ 0.01

^zValues represent three complete experimental runs, eight replications of composite samples of sprouting plants for each light treatment per run.

^ySignificance based on paired *t* tests.

NS = nonsignificant.

cycle pigments (ZEA + ANT + VIO) increased 28.3% ($P \leq 0.05$) following the blue LED treatment, which was the result of the large increases in VIO in the broccoli microgreen tissues (Table 2). The pigment ratio of ZEA + ANT/ZEA + ANT + VIO decreased 30% ($P \leq 0.01$) in the shoot tissues after exposure to the blue LED treatment (Table 2).

Concentrations of tissue GSs in the broccoli microgreen tissues were impacted by LED lighting treatment. Total aliphatic GS concentrations increased 35.1% ($P \leq 0.05$) in the shoot tissues after exposure to the blue LED treatment. Individual aliphatic GS concentrations positively impacted by the blue LED treatment were glucoraphenin ($P \leq 0.05$) and epiprogoitrin ($P \leq 0.05$), increasing 37.4% and 55.6%, respectively. Concentrations of sinigrin and glucoiberin were not impacted by the LED lighting treatment. Total or individual concentrations of shoot tissue indole GSs in the broccoli microgreens were also unaffected by the LED lighting treatment. Concentrations of gluconasturtiin increased by 400% ($P \leq 0.05$) after the microgreen tissues were exposed to the blue LED treatment (Table 3).

The blue LED treatment caused some of the most significant impacts on accumulations of macronutrient and micronutrient elements in the shoot tissues of the broccoli microgreens. Exposure to the blue LED treatment caused significant ($P \leq 0.001$) increases in shoot tissue P (53.9%), K (65.1%), Mg (53.0%), Ca (37.6%), and S (53.5%) when compared with the red and blue LED treatment (Table 4). Similarly, exposure to the blue LED treatment caused significant ($P \leq 0.001$) increases in shoot tissue B (29.3%), Mn (46.8%), Mo (55.6%), Na (60.6%), and Zn (35.3%). Increases also occurred for shoot tissue Cu (49.8%; $P = 0.02$) and Fe (63.8%; $P \leq 0.01$), although at lower significance levels (Table 4).

Discussion

Light stimuli are the most important signals controlling plant growth and developmental processes. Blue light signals control central processes such as phototropisms, suppression of stem elongation, chloroplast movements, stomatal operations, and genetic expression (Baum et al., 1999). Improvements in LED lighting technologies have increased literary contributions to the scientific database on the impacts of narrow-band wavelength on plant physiology. Many studies now demonstrate the

Table 2. Mean values for carotenoid shoot tissue pigments in sprouting broccoli microgreens grown under light-emitting diode (LED) treatment conditions of 627 nm and 470 nm (red and blue LED treatment) at 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or a 5-d preharvest light treatment of only 470 nm (blue LED treatment) at an average of 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.^z

Shoot tissue pigment	Red and blue LED treatment	Blue LED treatment	$P > t ^y$
	[mean \pm SE (mg/100 g fresh weight)]		
β -carotene	2.17 \pm 0.19	2.62 \pm 0.11	≤ 0.05
Lutein	4.31 \pm 0.18	3.92 \pm 0.33	NS
Zeaxanthin (ZEA)	0.04 \pm 0.002	0.03 \pm 0.002	NS
Antheraxanthin (ANT)	0.35 \pm 0.02	0.34 \pm 0.03	NS
Violaxanthin (VIO)	0.99 \pm 0.08	1.40 \pm 0.12	≤ 0.01
ZEA + ANT + VIO	1.38 \pm 0.09	1.77 \pm 0.15	≤ 0.05
Neoxanthin	1.22 \pm 0.06	1.02 \pm 0.09	NS
	[mean \pm SE (pigment ratio)]		
ZEA+ANT/ZEA+ANT+VIO	0.30 \pm 0.02	0.21 \pm 0.01	≤ 0.01

^zValues represent three complete experimental runs, eight replications of composite samples of sprouting plants for each light treatment per run.

^ySignificance based on paired *t* tests.

NS = nonsignificant.

Table 3. Mean values for shoot tissue glucosinolates in sprouting broccoli microgreens grown under light-emitting diode (LED) treatment conditions of 627 nm and 470 nm (red and blue LED treatment) at 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or a 5-d preharvest light treatment of only 470 nm (blue LED treatment) at an average of 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.^z

Glucosinolate	Red and blue LED treatment	Blue LED treatment	$P > t $ ^y
	[mean \pm SE ($\mu\text{g}\cdot\text{g}^{-1}$ dry mass)]		
Aliphatic glucosinolates	5.95 \pm 0.76	8.04 \pm 0.64	\leq 0.05
Sinigrin	0.22 \pm 0.04	0.24 \pm 0.03	NS
Epiprogoin	0.09 \pm 0.02	0.14 \pm 0.01	\leq 0.05
Glucoiberin	1.58 \pm 0.20	2.07 \pm 0.18	NS
Glucoraphenin	4.06 \pm 0.52	5.58 \pm 0.46	\leq 0.05
Indole glucosinolates	9.35 \pm 0.80	9.20 \pm 0.53	NS
Glucobrassicin	1.80 \pm 0.31	1.87 \pm 0.22	NS
4-Hydroxyglucobrassicin	6.34 \pm 0.58	5.93 \pm 0.35	NS
Neoglucobrassicin	0.43 \pm 0.08	0.63 \pm 0.08	NS
Aromatic glucosinolates	0.004 \pm 0.004	0.02 \pm 0.007	\leq 0.05
Gluconasturtiin	0.004 \pm 0.004	0.02 \pm 0.007	\leq 0.05
Total glucosinolates	15.31 \pm 1.24	17.26 \pm 1.07	NS

^zValues represent three complete experimental runs, eight replications of composite samples of sprouting plants for each light treatment per run.

^ySignificance based on paired t tests.

NS = nonsignificant.

impact of blue wavelengths on secondary metabolic pathways among specialty crops.

The FM biomass of the broccoli microgreens was unaffected by the blue LED treatment in the current study. Similarly, Yorio et al. (2001) showed that lettuce (*Lactuca sativa*) shoot biomass did not differ among cool-white fluorescent, red (600 to 700 nm) + blue (400 to 500 nm), or red (600 to 700 nm) light from LEDs when plants were cultured in nutrient solutions under a total photosynthetic photon flux of 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. However, the same authors did show significant decreases in spinach (*Spinacea oleracea* cv. Nordic IV) and radish (*Raphanus sativus* cv. Cherriette) shoot biomass under red + blue and red light when compared with the cool-white fluorescent light.

The blue LED treatment resulted in significantly higher BC and VIO concentrations in the shoot tissue of the broccoli microgreens (Table 2). Li and Kubota (2009) supplemented cool-white fluorescent light with different LED wavelengths to determine impacts of supplementation on phytochemical contents in 'Red Cross' baby leaf lettuce. The authors showed that white light supplemented with blue light (476 nm) resulted in significantly higher tissue anthocyanins, xanthophylls, and BC when compared with the white light control or white light supplemented with green light (526 nm). The total carotenoid pigment content in photosynthetic bacteria (*Rhodospseudomonas palustris*) was highest when cultured under blue light (470 nm) as compared with white light from incandescent, halogen, and fluorescent lamps and the individual narrow-band wavelengths of yellow (595 nm), red (630 nm), or green (520 nm) (Kuo et al., 2012). Both of these studies support the finding in the current project in which blue light exposure increased carotenoid concentrations in broccoli microgreen tissues.

Ma et al. (2012) evaluated carotenoid contents and gene expression within the carotenoid metabolic pathway in satsuma mandarin (*Citrus unshiu*) following postharvest irradiation with blue (470 nm) or red (660 nm) LED light and compared values

Table 4. Mean values for shoot tissue macronutrient elements and micronutrient elements in sprouting broccoli microgreens grown under light-emitting diode (LED) treatment conditions of 627 nm and 470 nm (red and blue LED treatment) at 350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or a 5-d preharvest light treatment of only 470 nm (blue LED treatment) at an average of 41 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.^z

Mineral element	Red and blue LED treatment	Blue LED treatment	$P > t $ ^y
	[mean \pm SE (mg·g ⁻¹ dry mass)]		
Macronutrients			
Phosphorus	3.80 \pm 0.09	5.85 \pm 0.20	\leq 0.001
Potassium	12.31 \pm 0.33	20.33 \pm 0.80	\leq 0.001
Magnesium	3.47 \pm 0.06	5.31 \pm 0.16	\leq 0.001
Calcium	10.25 \pm 0.20	14.10 \pm 0.33	\leq 0.001
Sulfur	5.05 \pm 0.11	7.75 \pm 0.20	\leq 0.001
Micronutrients			
Boron	51.08 \pm 1.44	66.03 \pm 2.27	\leq 0.001
Copper	5.70 \pm 0.63	8.54 \pm 0.95	0.02
Iron	26.31 \pm 1.40	43.10 \pm 6.03	\leq 0.01
Manganese	33.14 \pm 1.41	48.66 \pm 2.40	\leq 0.001
Molybdenum	0.63 \pm 0.02	0.98 \pm 0.04	\leq 0.001
Sodium	126.00 \pm 3.81	202.30 \pm 7.40	\leq 0.001
Zinc	27.25 \pm 1.20	36.86 \pm 1.42	\leq 0.001

^zValues represent three complete experimental runs, eight replications of composite samples of sprouting plants for each light treatment per run.

^ySignificance based on paired t tests.

with fruits stored under dark conditions. Exposure of the fruit to continuous blue light (50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 6 d resulted in significantly higher concentrations of flavedo BC, α -carotene, all-*trans* VIO, and LUT. Expression of genes within the carotenoid metabolic pathway was highest at 3 d of irradiance exposure when compared with 6 d of exposure. Postharvest blue light exposure resulted in significantly higher expression of mRNA levels within the citrus flavedo for two isoforms of phytoene synthase (PSY), ξ -carotene desaturase (ZDS), and one of two isoforms of lycopene β -cyclase (LCYb). Expression of zeaxanthin epoxidase (ZEP) was very high in fruits irradiated with both red and blue light. The first step in the carotenoid metabolic pathway is the condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene through PSY. Two structurally similar enzymes, phytoene desaturase and ZDS, make the conversions of phytoene to lycopene. The carotenoid pathway branches at the cyclization reactions of lycopene, mediated by LCYb and lycopene ϵ -cyclase, to produce carotenoids with either two β -rings (e.g., BC, ZEA, ANT, VIO, and NEO) or carotenoids with one β -ring and one ϵ -ring (e.g., α -carotene and LUT), respectively. Epoxidation reactions converting ZEA to VIO are controlled by ZEP (Cunningham, 2002; Kopsell and Kopsell, 2006). Data from Ma et al. (2012) could indicate that increases in broccoli microgreen tissue BC and VIO in the current study may be the result of increases in gene expression of key enzymes within the carotenoid metabolic pathway caused by blue light exposure.

Total aliphatic GS in the broccoli microgreen tissues was significantly increased after exposure to the blue LED treatment, predominantly caused by increases in glucoraphenin (Table 3). Glucoraphenin is hydrolyzed into the anticarcinogenic isothiocyanate sulforaphane by the cruciferous enzyme myrosinase. Recently, Clarke et al. (2011) demonstrated higher bioavailability of sulforaphane and its metabolites in human subjects consuming

broccoli sprouts as compared with prepared broccoli supplements. Their work further illustrates the nutritional impacts of whole foods vs. monomolecular supplements. Previous work in our group demonstrated the influence of light intensity on glucosinolate concentrations within Brassica species (Charron and Sams, 2004; Lefsrud et al., 2006). Reflective mulches have also been used to direct different narrow-band wavelength of light into crop canopies. Antonious et al. (1996) established that blue-, green-, and white-colored mulches reflected 25%, 7%, and 41% of incoming solar radiation, respectively, in the blue (450 ± 5 nm) wavelengths. The authors found that total glucosinolate concentrations in turnip (*B. rapa*) were significantly higher for plants cultured on the blue-colored mulches. Gluconastutiin concentrations in the broccoli microgreen tissues were also significantly increased after exposure to the blue LED treatment (Table 3). There is one example present in the literature in which gluconastutiin concentrations in watercress (*Nasturtium officinale*) were significantly higher after exposure to red light as compared with white light (metal halide lamps at 400 μmol·m⁻²·s⁻¹) (Engelen-Eigles et al., 2006). These data provide more evidence of the ability of narrow-band wavelengths, especially blue light, to increase GS production in specialty crops. Total GSs in the broccoli microgreens were elevated slightly under the blue LED treatments but did not differ significantly from the red and blue LED treatment (Table 3). Increases were found under the blue LED treatment for many aliphatic GS and the aromatic GS gluconasturtiin. However, none of the indole GSs were impacted by the LED treatments (Table 3). It may be possible that the blue LED treatment impacted aliphatic and aromatic side-chain additions and not the heteroaromatic groupings. Furthermore, the blue LED treatment may have had no effect on GS biosynthesis from the amino acid tryptophan (indole GS), whereas GS biosynthesis from other amino acids (aliphatic and aromatic GS) was positively impacted. In either case, the exact mechanisms remain unknown.

Blue light acts as a powerful signal controlling stomatal operation. In intact leaves, blue light is up to 20 times more effective than red light in opening stomata (Sharkey and Raschke, 1981; Shimazaki et al., 2007). Blue light exposure can also cause significant changes in guard cell membrane transport activity through variations in Ca²⁺, K⁺, and H⁺ fluxes and corresponding impacts on pH conditions (Babourina et al., 2002). Exposure to the blue LED treatment in the current study resulted in significant increases in all of the essential elements measured in the broccoli microgreen tissues (Table 4). The impacts of blue light on stomatal opening and membrane transport activity may be the underlying cause for such increases in macronutrient and micronutrient accumulations in the broccoli microgreen tissues.

The results described in this article clearly show a relationship between blue light exposure and responses within primary and secondary metabolic pathways in broccoli microgreen tissues. Simple applications of preharvest blue light resulted in significant increases in nutritionally important carotenoids, GSs, and mineral elements in the broccoli microgreens, most notably increases in BC, glucraphanin, K, Mg, and Fe. Because of the potential impacts on human health, increasing concentrations of primary and secondary metabolites in specialty microgreen crops would be of value to consumers.

Literature Cited

Antonious, G.F., M.J. Kasperbauer, and M.E. Byers. 1996. Light reflected from colored mulches to growing turnip leaves affects

glucosinolate and sugar contents of edible roots. *Photochem. Photobiol.* 64:605–610.

Babourina, O., I. Newman, and S. Shabala. 2002. Blue light-induced kinetics of H⁺ and Ca²⁺ fluxes in etiolated wild-type and phototropin-mutant *Arabidopsis* seedlings. *Proc. Natl. Acad. Sci. USA* 99:2433–2438.

Baum, G., J.C. Long, G.I. Jenkins, and A.J. Trewavas. 1999. Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca²⁺. *Proc. Natl. Acad. Sci. USA* 96:13554–13559.

Briggs, W.R. and J.M. Christie. 2002. Phototropins 1 and 2: Versatile plant blue-light receptors. *Trends Plant Sci.* 7:204–210.

Briggs, W.R. and E. Huala. 1999. Blue-light photoreceptors in higher plants. *Annu. Rev. Cell Biol.* 15:33–62.

Charron, C.S. and C.E. Sams. 2004. Glucosinolate content and myrosinase activity in rapid-cycling *Brassica oleracea* grown in a controlled environment. *J. Amer. Soc. Hort. Sci.* 129:321–330.

Clarke, J.D., A. Hsu, K. Riedl, D. Bella, S.J. Schwartz, J.F. Stevens, and E. Ho. 2011. Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. *Pharmacol. Res.* 64:456–463.

Cunningham, F.X., Jr. 2002. Regulation of carotenoid synthesis and accumulation in plants. *Pure Appl. Chem.* 74:1409–1417.

Demmig-Adams, B., A.M. Gilmore, and W.W. Adams, III. 1996. In vivo functions of carotenoids in higher plants. *FASEB J.* 10:403–412.

Engelen-Eigles, G., G. Holden, J.D. Cohen, and G. Gardner. 2006. The effect of temperature, photoperiod, and light quality on gluconastutiin concentrations in watercress (*Nasturtium officinale* R. Br.). *J. Agr. Food Chem.* 54:328–334.

Farnham, M.W., M.A. Grusak, and M. Wang. 2000. Calcium and magnesium concentration of inbred and hybrid broccoli heads. *J. Amer. Soc. Hort. Sci.* 125:344–349.

Farnham, M.W. and D.A. Kopsell. 2009. Importance of genotype on carotenoid and chlorophyll levels in broccoli heads. *HortScience* 44:1248–1253.

Fahey, J.W., Y. Zhang, and P. Talalay. 1997. Broccoli sprouts: An excellent rich source of inducers of enzymes that protect against chemical carcinogens. *Proc. Natl. Acad. Sci. USA* 94:10367–10372.

Frank, H.A. and R.J. Cogdell. 1996. Carotenoids in photosynthesis. *Photochemistry* 63:257–264.

Fuchs, I., K. Philippar, K. Ljung, G. Sandberg, and R. Hedrich. 2003. Blue light regulates an auxin-induced K⁺-channel gene in the maize coleoptile. *Proc. Natl. Acad. Sci. USA* 100:11795–11800.

Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and prostate cancer: Review of the epidemiologic literature. *J. Natl. Cancer Inst.* 91:317–331.

Goldman, I.L., A.A. Kader, and C. Heintz. 1999. Influence of production, handling, and storage on phytonutrient content of foods. *Nutr. Rev.* 57:S46–S52.

Hansen, M., P. Møller, H. Sørensen, and M. Cantwell de Trejo. 1995. Glucosinolates in broccoli stored under controlled atmosphere. *J. Amer. Soc. Hort. Sci.* 120:1069–1074.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agr. Expt. Sta. Circ.* No. 347.

Hogewoning, S.W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. *J. Expt. Bot.* 61:3107–3117.

Jeffery, E.H. and M. Araya. 2009. Physiological effect of broccoli consumption. *Phytochem. Rev.* 8:283–298.

Johkan, M., K. Shoji, F. Goto, S. Hashida, and T. Yoshihara. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. *HortScience* 45:1809–1814.

Kopsell, D.A. and D.E. Kopsell. 2006. Accumulation and bioavailability of dietary carotenoids in vegetable crops. *Trends Plant Sci.* 11:499–507.

- Kopsell, D.A., D.E. Kopsell, M.G. Lefsrud, J. Curran-Celentano, and L.E. Dukach. 2004. Variation in lutein, β -carotene, and chlorophyll concentrations among *Brassica oleracea* cultivars and seasons. *HortScience* 39:361–364.
- Kopsell, D.A., N.I. Pantanizopoulos, C.E. Sams, and D.E. Kopsell. 2012. Shoot tissue pigment levels increase in ‘Florida Broadleaf’ mustard (*Brassica juncea* L.) microgreens following high light treatment. *Sci. Hort.* 140:96–99.
- Kuo, F., Y. Chien, and C. Chen. 2012. Effect of light sources on growth and carotenoid content of photosynthetic bacterial *Rhodospseudomonas palustris*. *Bioresour. Technol.* 113:315–318.
- Kurilich, A.C., G.J. Tsau, A. Brown, L. Howard, B.P. Klein, E.H. Jeffery, M. Kushad, M.A. Wallig, and J.A. Juvik. 1999. Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *J. Agr. Food Chem.* 47:1576–1581.
- Kushad, M.M., A.F. Brown, A.C. Kurilich, J.A. Juvik, B.P. Klein, M.A. Wallig, and E.H. Jeffery. 1999. Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *J. Agr. Food Chem.* 47:1541–1548.
- Lefsrud, M.G., D.A. Kopsell, D.E. Kopsell, and J. Curran-Celentano. 2006. Irradiance levels affect growth parameters and carotenoid pigments in kale and spinach grown in a controlled environment. *Physiol. Plant.* 127:624–631.
- Lefsrud, M.G., D.A. Kopsell, and C.E. Sams. 2008. Irradiance from distinct wavelength light-emitting diodes affect secondary metabolites in kale. *HortScience* 43:2243–2244.
- Li, Q. and C. Kubota. 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environ. Exp. Bot.* 67:59–64.
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* 34:350–382.
- Loreto, F., T. Tsonev, and M. Centritto. 2009. The impact of blue light on leaf mesophyll conductance. *J. Expt. Bot.* 60:2283–2290.
- Ma, G., L. Zhang, M. Kato, K. Yamawaki, Y. Kiriwa, M. Yahata, Y. Ikoma, and H. Matsumoto. 2012. Effect of blue and red LED light irradiation on β -cryptoxanthin accumulation in the flavedo of citrus fruits. *J. Agr. Food Chem.* 60:197–201.
- Martineau, V., M.G. Lefsrud, M. Tahera Naznin, and D.A. Kopsell. 2012. Comparison of supplemental greenhouse lighting from light emitting diode and high pressure sodium light treatments for hydroponic growth of Boston lettuce. *HortScience* 47:477–482.
- Mayne, S.T. 1996. β -carotene, carotenoids, and disease prevention in humans. *FASEB J.* 10:690–701.
- Morrow, R.C. 2008. LED lighting in horticulture. *HortScience* 43:1947–1950.
- Raney, J.P. and D.I. McGregor. 1990. Determination of glucosinolate content by gas liquid chromatography of trimethylsilyl derivatives of desulfated glucosinolates. *Proc. Oil Crops Network.* p. 21–23.
- Samuolienė, G., R. Sirtautas, A. Brazaitytė, and P. Duchovskis. 2012. LED lighting and seasonality effects antioxidant properties of baby leaf lettuce. *Food Chem.* 134:1494–1499.
- Sharkey, T.D. and K. Raschke. 1981. Separation and measurement of direct and indirect effects of light on stomata. *Plant Physiol.* 68:33–40.
- Shimazaki, K., M. Doi, S.M. Assmann, and T. Kinoshita. 2007. Light regulation of stomatal movement. *Annu. Rev. Plant Biol.* 58:219–247.
- Stoewsand, G.S. 1995. Bioactive organosulfur phytochemicals in *Brassica oleracea* vegetables—A review. *Food Chem. Toxicol.* 33:537–543.
- Stutte, G.W., S. Edney, and T. Skerritt. 2009. Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. *HortScience* 44:79–82.
- Tlačka, M., M. Runquist, and M. Fricker. 1999. Light perception and the role of the xanthophyll cycle in blue-light-dependent chloroplast movements in *Lemna trisulca* L. *Plant J.* 20:447–459.
- Tracewell, C.A., J.S. Vrettos, J.A. Bautista, H.A. Frank, and G.W. Brudvig. 2001. Carotenoid photooxidation in photosystem II. *Arch. Biochem. Biophys.* 385:61–69.
- U.S. Environmental Protection Agency. 1996. Microwave assisted acid digestion of siliceous and organically based matrices, Method #3052. U.S. Environ. Protection Agency, Washington, DC.
- Weaver, C.M. and R.P. Heaney. 1999. Calcium, p. 141–155. In: Shils, M.E., J.A. Olsen, M. Shike, and A.C. Ross (eds.). *Modern nutrition in health and disease*. 9th Ed. Williams & Wilkins, Baltimore, MD.
- Yorio, N.C., G.D. Goins, H.R. Kagie, R.M. Wheeler, and J.C. Sager. 2001. Improving spinach, radish, and lettuce growth under red light-emitting diodes (LEDs) with blue light supplementation. *HortScience* 36:380–383.