Different Microgreen Genotypes Have Unique Growth and Yield Responses to Intensity of Supplemental *PAR* from Light-emitting Diodes during Winter Greenhouse Production in Southern Ontario, Canada

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Abstract. Low natural daily light integrals (DLIs) are a major limiting factor for greenhouse production during darker months (e.g., October to February in Canada). Supplemental lighting (SL) is commonly used to maintain crop productivity and quality during these periods, particularly when the supply chain demands consistent production levels year-round. What remains to be determined are the optimum SL light intensities (LIs) for winter production of a myriad of different commodities. The present study investigated the growth and yield of sunflower (*Helianthus annuus* L., ‘Black oil’), kale (*Brassica napus* L., ‘Red Russian’), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L., ‘Ruby Streaks’), grown as microgreens, in a greenhouse under SL light-emitting diode (LED) photosynthetic photon flux density (*PPFD*) levels ranging from 17.0 to 304 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a 16-hour photoperiod (i.e., supplemental DLIs from 1.0 to 17.5 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$). Crops were sown in a commercial greenhouse near Hamilton, ON, Canada (lat. 43°14' N, long. 80°07' W) on 1 Feb. 2018, and harvested after 8, 11, 12, and 12 days, resulting in average natural DLIs of 6.5, 5.9, 6.2, and 6.2 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ for sunflower, kale, arugula, and mustard, respectively. Corresponding total light integrals (TLIs) ranged from 60 to 188 $\text{mol}\cdot\text{m}^{-2}$ for sunflower, 76 to 258 $\text{mol}\cdot\text{m}^{-2}$ for kale, 86 to 280 $\text{mol}\cdot\text{m}^{-2}$ for arugula, and 86 to 284 $\text{mol}\cdot\text{m}^{-2}$ for mustard. Fresh weight (i.e., marketable yield) increased asymptotically with increasing LI and leaf area increased linearly with increasing LI, in all genotypes. Hypocotyl length of mustard decreased and hypocotyl diameter of sunflower, arugula, and mustard increased with increasing LI. Dry weight, robust index, and relative chlorophyll content increased and specific leaf area decreased in kale, arugula, and mustard with increasing LI. Commercial microgreen greenhouse growers can use the light response models described herein to predict relevant production metrics according to the available (natural and supplemental) light levels to select the most appropriate SL LI to achieve the desired production goals as economically as possible.

Microgreens are an emerging commodity in worldwide markets. They consist of vegetable and herb seedlings that are grown in illuminated environments and harvested at or

before the first true leaf stage (Kyriacou et al., 2016; Xiao et al., 2012). Microgreen production is attractive to greenhouse growers due to the increasing demand and high value, with wholesale prices ranging from \$30 to \$50 US per pound (Treadwell et al., 2016). Microgreens have a limited postharvest shelf life (Berba and Uchanski, 2012; Chandra et al., 2012; Kou et al., 2013); therefore, local production, particularly for fresh-cut products, represents the most promising production strategy (Kyriacou et al., 2016). However, low natural light is a major limiting factor in greenhouse production during darker months in higher-latitude regions (Dorais and Gosselin, 2002). For example, the natural outdoor daily light integrals (DLIs) in southern Canada and northern United States in October through February typically range from 5 to 15 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (Faust and Logan, 2018). With further 25%

to 60% reductions of the outside DLIs due to transmission losses through greenhouse glazing and structural materials (Giacomelli et al., 1988; Lau and Stanley, 1989; Llewellyn et al., 2013; Ting and Giacomelli, 1987), crop-level natural DLIs may be insufficient to grow many commodities. Nonetheless, contemporary consumers expect high-quality greenhouse-grown produce year-round. Because low DLIs are generally associated with reductions in yield and quality, high-quality winter-grown produce can command a substantial price premium. Therefore, supplemental lighting (SL) may be economical for growing high-value commodities during the winter months (Dorais and Gosselin, 2002; Dorais et al., 2017).

High-pressure sodium (HPS) fixtures have been commonly used in greenhouses for SL (Hemming, 2011; Ouzounis et al., 2015). Recently, light-emitting diodes (LEDs) have been increasingly used as an alternative to HPS due to their comparatively high energy efficiency and durability (Mitchell et al., 2015; Morrow, 2008; Nelson and Bugbee, 2014; Pattison et al., 2016). In addition, the potential for spectrum and intensity control with LEDs give growers more power to manipulate their crops with light and save energy when natural lighting is sufficient (Chang et al., 2014; Llewellyn and Zheng, 2018a, 2018b; Morrow, 2008). Considerable research has been devoted to investigating different SL spectrum treatments in greenhouse environments (Bantis et al., 2018) where comparable supplemental light intensities (LIs) have yielded similar crop productivity metrics for LED vs. HPS in many commodities (Currey and Lopez, 2013; Hernández and Kubota, 2015; Llewellyn et al., 2019; Martineau et al., 2012; Poel and Runkle, 2017; Randall and Lopez, 2014).

Increasing LI has generally been associated with increases in fresh weight (FW) and percentage of dry weight (DW), decreases in height, and decreases in specific leaf area (SLA) of juvenile growth stages (including microgreens) of commodities grown in sole-source (SS) (Gerovac et al., 2016; Kitaya et al., 1998; Meng and Runkle, 2019; Park and Runkle, 2018; Samuolienė et al., 2013; Viršilė and Sirtautas, 2013; Yao et al., 2017) and greenhouse (Boivin et al., 1987; Dorais and Gosselin, 2002; Gaudreau et al., 1994; Oh et al., 2010; Rezai et al., 2018; Torres and Lopez, 2011) production environments. These relationships between LI and plant growth have borne out in seedlings of the majority of herbaceous and woody species that have been investigated worldwide (Poorter et al., 2019).

It is often not possible to infer the integrated amount of photosynthetically active radiation (*PAR*) a crop received [i.e., total light integral (TLI); $\text{mol}\cdot\text{m}^{-2}$] in the literature, particularly in greenhouse environments, due to incomplete descriptions of the canopy-level *PPFD* from natural and/or supplemental sources. Although TLI [also known as integrated photosynthetic photon flux (IPPF), Chun et al., 2001] is not yet a commonly-used metric in the scientific community, it can normalize

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quantifications of light “doses” from different production scenarios (e.g., SS vs. greenhouse) and lighting environments (e.g., LI, photoperiod, and lengths of production cycles), thus enabling greater relevance between different studies. When considering yield metrics on an area basis, the relationships between yield and TLI are a direct measure of light use efficiency (LUE) (e.g., $g(\text{marketable biomass}) \cdot \text{mol}^{-1}(\text{PAR})$). A caveat of making comparisons between studies on a TLI-basis is that even if plants receive the same TLI, they may respond differently depending on the dynamics of how light was provided (e.g., $200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for 8 h vs. $100 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for 16 h) and other differences in the growing environment.

Some studies have investigated sufficiently high LI to show distinct maxima in various morphological and physiological parameters, similar to the saturation phenomena of photosynthesis responses to high LI (van Iersel, 2017). He et al. (2019) grew cherry tomato seedlings for 25 d under SS LED LI ranging from 50 to $550 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a 12-h photoperiod (TLI ranged from 54 to $594 \text{ mol} \cdot \text{m}^{-2}$). They showed that FW, DW, and health index [i.e., stem diameter / stem height $\times$ DW, Fan et al. (2013)] all plateaued at $300 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (i.e., TLI of $324 \text{ mol} \cdot \text{m}^{-2}$) and that photoinhibition occurred at higher LI. Rezaei et al. (2018) and Soustani et al. (2014) investigated the effects of different levels of neutral-density shade on growth and morphology. Leaf number, FW, DW, and oil yield of 5-month-old sage (*Salvia officinalis* L.) were all maximized when grown outdoors under 30% shade vs. deeper shade treatments or full sunlight (Rezaei et al., 2018). Soustani et al. (2014) showed that shade level had greater morphological influences than seed size in *Quercus castaneifolia* seedlings, with seedlings grown at moderate levels of shade having the greatest biomass. However, insufficient information was provided, in both of these studies, to ascribe absolute LI levels to their treatments or calculate TLI. Viršilė and Sirtautas (2013) grew borage (*Borago officinalis* L.) as microgreens (no seeding density was provided) for either 10 or 12 d (production times are conflicting) under SS LED LI ranging from 110 to $545 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a 16-h photoperiod (TLI ranged from 63 to $314 \text{ mol} \cdot \text{m}^{-2}$, assuming 10-d production). They found that borage HL decreased and FW increased as PPFD increased from 110 to $440 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, followed by the reverse trends at $545 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Our previous study (Jones-Baumgardt et al., 2019) investigated the SS production of four Brassicaceae microgreens grown under LED LI ranging from 100 to $600 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a 16-h photoperiod (TLI ranged from 58 to $380 \text{ mol} \cdot \text{m}^{-2}$) and found linear reductions in HL with increasing LI, but saturating increases in FW and DW with increasing LI. Gerovac et al. (2016) and Samuolienė et al. (2013) also both investigated the influences of SS LED LI on several Brassicaceae microgreens. Gerovac et al. (2016) reported that there were LI treatment effects in FW in only one of three genotypes and that the maximum LI was insufficient to saturate bio-

mass production in that genotype. They also reported biomass accumulation on a per plant basis, which is not truly reflective of how microgreens are grown and sold commercially (i.e., in very dense canopies). Samuolienė et al. (2013) may have used sufficiently high LI ($545 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) to saturate biomass accumulation but they did not provide biomass accumulation data in their results. Fu et al. (2012a, 2012b) investigated the effects of SS LI, which ranged from 100 to $800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of combined HPS and sunlight dysprosium [i.e., metal halide (MH)] light (with a 14-h photoperiod), on 10-d-old lettuce seedlings that were then harvested 27 d after sowing (TLI ranged from 86 to $685 \text{ mol} \cdot \text{m}^{-2}$). They showed that FW plateaued at LI between 400 and $600 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (i.e., TLI between 343 and $514 \text{ mol} \cdot \text{m}^{-2}$). Notably, they also attempted to relate results from indoor production (i.e., plant factories) to greenhouses, but they did not account for the substantial contributions of natural lighting in the overall incident PAR in most greenhouse environments. Erwin and Gesick (2017) related leaf-level photosynthesis of chard, kale, and spinach to the anticipated optimum LI for production of these commodities based on their light saturation points (LSP). However, they also provided a detailed discussion of the myriad limitations of relating leaf-level measurements to growth and yield in production scenarios.

The goal of SL in a greenhouse production scenario is to balance the increases in yield with the additional costs of inputs (i.e., lighting infrastructure and electricity). Since the efficiency of the conversion of electricity into biomass is extremely sensitive to LI at moderate DLIs (Bugbee and Monje, 1992), it is likely that there is an economic optimum for the SL LI to use in any greenhouse commodity production scenario. This optimum may depend on many other factors, including supply chain demands, geographic location, time of year (i.e., availability of natural light), and many integral aspects of the growing environment (e.g., temperature, VPD, CO_2 concentration, nutrient availability, length of production cycle, etc.). The objective of this study was to quantify the effects of supplemental LED LI during the winter months in a higher-latitude region (Ontario, Canada) on the growth and yield of four economically important greenhouse-grown microgreens: sunflower, kale, arugula, and mustard. By reporting the results in terms of both DLI and TLI, they are hopefully more broadly applicable to disparate production environments. TLI may be a particularly useful metric for comparing the results of different LI trials because it accounts for differences in photoperiod, natural DLI (if natural light is present), supplemental LI, and production time. This is particularly important for greenhouse production systems, where natural DLIs can range by an order of magnitude, depending on weather, season, and geographic region (Faust and Logan, 2018; Poorter et al., 2019).

Materials and Methods

An SL gradient experiment was performed at a commercial greenhouse near Hamilton, ON, Canada (lat. $43^\circ 14' \text{N}$, long. $80^\circ 07' \text{W}$). This involved intentionally creating heterogeneity in canopy-level SL PPFD (through careful fixture placement) within a common growing environment, rigorously assessing the spatial variability in canopy-level SL PPFD at high resolution (performed at night), and assigning selected trays of microgreens to very specific fixed coordinates (i.e., PPFD treatments). The application of gradient-type experimental designs for LI investigations is described further in the Discussion section. The greenhouse was a modern facility comprising 28 gutter-connected (i.e., Venlo-style) bays, each measuring $6.4 \text{ m} \times 70.7 \text{ m}$, with the gutters running in the east–west direction. The whole greenhouse was outfitted with mobile sub-irrigation benches, each measuring $5.46 \text{ m} \times 1.61 \text{ m}$, that could be moved throughout the facility on rails. The roof of the entire production area was recently (Spring 2017) refitted with UVA-transmissive acrylic cladding (16mm Acrylite® Alltop ultraviolet transmitting acrylic double-skin sheet; Evonik Cyro Canada Inc., Toronto, ON, Canada). Typical spectral photon flux distribution (300 to 800 nm) at bench level from natural and SL sources are provided in Fig. 1. The experimental growing environment consisted of four adjacent benches that were fixed in place on the rails, with the near side of the experimental plot positioned $\approx 3 \text{ m}$ from the west end of a centrally located greenhouse bay.

Lighting setup. Ten LED fixtures (LX502G; Heliospectra AB, Gothenburg, Sweden) were hung in pairs 1.4 m above the benches near each of the four corners and in the geometric center of the four-bench growing area. The Euclidian coordinates (in meters; from the northeast corner of the experimental area) of the center of each fixture are provided in Table 1. These fixtures had three individually controllable spectrum channels for blue (B), white (W), and red (R) LEDs. The dominant wavelength and full width at half maximum were 445 nm and 20 nm for B and 660 nm and 20 nm for R channels, respectively. The broad-spectrum photon flux from the W channel was described as

Fig. 1. Relative spectral photon flux distribution, at crop level, of natural light (midday) and supplemental light-emitting diode (LED) light.

Table 1. The Euclidian coordinates (in meters) of the center of each fixture relative to the upper left corner of the growing area.

Fixture no.	Distance (m) for each coordinate		
	X (over from left)	Y (down from top)	Z (hang ht)
1	0.25	1.09	1.40
2	1.05	1.09	1.40
3	4.41	1.09	1.40
4	5.21	1.09	1.40
5	2.33	3.59	1.40
6	3.13	3.59	1.40
7	0.25	6.08	1.40
8	1.05	6.08	1.40
9	4.41	6.08	1.40
10	5.21	6.08	1.40

Bench 1	Arugula	283	296	279	248	207	157	110	65	31		27	55	98	146	203	246	278	296	289	Sunflower	
					282	230	169	115	76	41		38	65	103	155	225	278					
		275	298	291	261	214	159	115	75	36		35	62	100	147	206	250	281	292	280		
Bench 2	Arugula	129	140	133	119	113	116	105	85	79		80	78	88	100	110	122	134	142	139	Sunflower	
		25	28	36	61	102	136	170	202	231		226	199	162	122	91	57	36	30	26		
		17	21	35	68	106	157	226	277				280	234	172	116	70	32	21	17		
Bench 3	Kale	17	21	35	70	115	171	236	280			287	233	163	112	68	34	22	18	Mustard		
		22	25	32	55	89	122	164	201	227		229	208	174	139	103	61	34	28		27	
		134	145	134	119	102	92	81	74	75		75	76	85	92	101	118	137	139		125	
Bench 4	Kale	283	299	281	248	208	156	111	70	37		37	69	112	159	207	250	285	303	287	Mustard	
					278	229	165	113	74	41		41	70	108	159	225	277					
		276	304	281	247	207	154	110	65	30		31	59	100	146	201	244	273	292	279		

Fig. 2. Canopy-level supplemental photosynthetic photon flux density (PPFD; μmol·m⁻²·s⁻¹) at the geometric center of each tray location (50 trays per genotype). The four genotypes (sunflower, kale, arugula, and mustard) were randomly assigned to one of four quadrants; which have different background fills and are labeled accordingly. All growth and harvest metrics were taken from plants harvested from a circle with a 5-cm radius (76.4 cm²) located in the center of each tray. The dark grey areas represent border trays and the trays located directly below the lighting fixtures, which were not assessed for growth.

Fig. 3. Gutter-level natural sunlight daily light integrals (DLIs; mol·m⁻²·d⁻¹) from 1 to 13 Feb. 2018. The mean (± SD; n = 8 for sunflower, n = 11 for kale, and n = 12 for arugula and mustard) natural DLIs were 6.5 ± 1.7, 5.9 ± 1.7, 6.2 ± 1.7, and 6.2 ± 1.7 mol·m⁻²·d⁻¹ for sunflower, kale, arugula, and mustard, respectively.

having a 5700 K correlated color temperature (CCT). All channels were operated on full power, providing an SL photosynthetic photon flux ratio (average ± SD, n = 200) of B (400–500 nm), green (G, 500–600 nm), and R (600–700 nm) of B (15.5 ± 1.2); G (11.6 ± 0.6); R (72.9 ± 1.7) (Fig. 1).

The canopy-level SL PPFD and spectral quality were measured (at night) at the geometric center of 200 subplot locations (Fig. 2) using a radiometrically calibrated spectrometer (XR-Flame-S; Ocean Optics, Dunedin, Fla., USA) coupled to a 400-nm × 1.9-m patch cord with a CC3 cosine corrector. Using the Spectrasuite software package, spectral power distribution was measured and converted to photon flux using the PARspec subroutine. The SL PPFD ranged from 17 to 304 μmol·m⁻²·s⁻¹ at crop level over the entire experimental area. These mea-

surements closely matched the IES file-based lighting simulations provided, on a 5.2-cm square grid, by Heliospectra for the whole experimental area (data not shown). The SL PPFD and spectral photon flux distribution were re-measured at each location at the end of the experiment to verify steady-state SL conditions at each sample location. The LEDs were operated on a 16-h photoperiod (0600 to 2200 HR), providing SL DLIs ranging from 1.0 to 17.5 mol·m⁻²·d⁻¹. A sunlight-calibrated quantum sensor (SQ-110; Apogee Instruments, Logan, UT, USA) placed just above the light fixtures in the center of the growing area and tethered to a datalogger (U-12; Onset Computer Corporation, Bourne, MA) collected natural light PPFD data on 120-s intervals throughout the trial. These data were used to calculate the natural DLI within the experimental greenhouse area for each day of the trial (Fig. 3). Although the full four-bench experimental area was covered with trays of microgreens, only the plants harvested from selected locations (for which the SL LI was known) were assessed for growth and yield.

Growing media and seeding. Seeds of sunflower (*Helianthus annuus* L., 'Black oil', 5.99 seeds/g), kale (*Brassica napus* L., 'Red Russian', 367 seeds/g), arugula (*Eruca sativa* L., 641 seeds/g), and mustard (*Brassica juncea* L., 'Ruby Streaks', 612 seeds/g) were grown in fiber trays (23.5 × 48.5 × 3.5 cm) for 8 to 12 d after sowing (DAS). The growing substrate comprised (by volume) 30% peat, 30% compost, 30% coir, and 10% perlite. Soil macronutrient and micronutrient concentrations were comparable to those reported by Jones-Baumgardt et al. (2019). Trays were machine-filled (3.5 cm deep) with substrate and seeded on the day the experiment was started. Seeding rates were 94, 8, 4, and 4 g/tray, resulting in seeding densities of 0.50, 2.58, 2.25, and 2.15 seed/cm² for sunflower, kale, arugula, and mustard, respectively. The experimental area was divided into four quadrants with each quadrant randomly assigned to one of the four genotypes (Fig. 2). Seeded trays of the respective genotypes were placed in the geometric center of each of the 54 subplot locations within each quadrant (Fig. 2). Trays of border plants were placed along the outer columns of each subplot (shaded in grey in Fig. 2). Trays of microgreens were top-irrigated until minimal drainage was observed, starting on 1 Feb. (2018) at 1400 HR and as needed thereafter (following the grower's protocol).

Growth and morphology measurements. Following commercial practices, microgreens were harvested at the visual appearance of true leaves, resulting in harvest dates of 8 DAS for sunflower, 11 DAS for kale, and 12 DAS for arugula and mustard. For each genotype, 50 trays were sampled. The trays located directly under the pairs of LED fixtures were not used in the trial because they potentially had greater amounts of natural LI blocked by the fixtures. For each microgreen tray, one core sample (i.e., full

plants and substrate) was collected in the center (i.e., where SL *PPFD* were initially characterized) of each tray using a cylindrical core sampler (76.4 cm²). Random plants were selected from inside each core to measure each of the following parameters: hypocotyl length (HL), hypocotyl diameter (HD), cotyledon dry weight (CDW), cotyledon leaf area (LA), relative chlorophyll content (RCC), and aboveground FW and DW. Different plants were used for each parameter, except for CDW and LA. HL was measured from the base of the hypocotyl to the shoot apical meristem on five plants. For hypocotyl diameter measurements, five plants were scanned (CanoScan LiDE 25; Canon Inc., Tokyo, Japan) in JPEG format at 297 pixels per inch (PPI) and then ImageJ 1.42 software (<https://imagej.nih.gov/ij/download.html>) was used to process the images to determine HD. LA was assessed on 10 plants with a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln, NE). True leaves were of insignificant size at the time of harvest and therefore only cotyledons were used for LA measurements. Thereafter, the leaves of the 10 plants were pooled, weighed, and dried to a constant weight at 70 °C for 3 d and the CDW was recorded. The specific leaf area (SLA) was determined by using the following equation:

$$\text{Specific leaf area (SLA)} \\ = \text{Cotyledon leaf area (LA)} / \text{Cotyledon dry weight (CDW)}$$

Single cotyledons on each of five plants were used to measure RCC with a SPAD meter (SPAD 502; Spectrum Technologies, Inc., Aurora, IL). All remaining plants from each core were cut just above the substrate level and combined to determine FW and DW. These values were pooled with the respective CFW and CDW data recorded for the subsamples that had been used for SLA analysis. The robust index (RI) of each plant was determined using the following equation:

$$\text{Robust index (RI)} \\ = \text{Hypocotyl diameter (HD)} / \text{Hypocotyl length (HL)} \times \text{Dry weight (DW)}$$

Growing environment. Due to the different lengths of production cycles and daily variations in natural lighting (Fig. 3), each crop was exposed to slightly different average natural DLIs. The average natural DLIs (mean $\pm$ sd) and TLIs were 6.50 ± 1.70 mol·m⁻²·d⁻¹ and 52.0 mol·m⁻² for sunflower, 5.94 ± 1.70 mol·m⁻²·d⁻¹ and 65.3 mol·m⁻² for kale, and 6.20 ± 1.70 mol·m⁻²·d⁻¹ and 74.4 mol·m⁻² for both arugula and mustard. The natural TLIs were calculated by summing the DLI of each full day of production plus half of the DLI for both the start and harvest days, over each respective genotype's production period. The air temperature and relative humidity (RH) targets were set at constant levels of 21 °C and 40%, respectively. Air temperature and RH were recorded (every 120 s) using a greenhouse environmental

control system (Damatex Inc, Montréal, QC, Canada). The average air temperature and RH were 21.0 ± 1.1 °C and $42.0 \pm 4.1\%$ (mean $\pm$ sd), respectively. No supplemental CO₂ was used in the experiment.

Statistical analysis. Data were analyzed using R statistical software (RStudio 1.1.453; Auckland, New Zealand). HL, HD, LA, SLA, RCC, and RI were analyzed using linear regressions and FW and DW were analyzed using asymptotic light response curves, with both the independent (i.e., LI) and dependent (i.e., production and harvest indices) values as continuous variables. The relationships between LI and yield (FW or DW) were determined using the asymptotic model $y = a + be^{(x/a)}$ (Delgado et al., 1993), where y , x , a , and e represent yield, LI (i.e., DLI or TLI), estimated maximum yield, and Euler's number, respectively. The parameters for a , b , and c were derived from nonlinear regressions. All regression analyses were evaluated at a significance level of $P \leq 0.05$. The best fit model equations are only presented for parameters with significant regressions, whereas overall means (i.e., pooled data from total LI ranges) are presented when there were no treatment effects. Normality of residuals and homoscedasticity of variances were confirmed using the Shapiro-Wilk and Levene tests, respectively.

Results

To make the results of this study most directly relatable to those of similar studies, the results are discussed primarily in terms of average DLI. However, both DLI and TLI models are presented when applicable. Because the growing periods were not consistent across all genotypes, growth and yield measurements modeled by TLI may provide more relevant comparisons between genotypes within the present study and also to results from other studies that have different production times and lighting conditions.

Sunflower. There were no effects of LI on HL, DW, SLA, RI, and RCC in sunflower (Table 2). As average DLI increased from 7.5 to 23.5 mol·m⁻²·d⁻¹, HD and LA increased linearly by 7.8% and 11%, respectively. FW increased asymptotically by 15% as average DLI increased from 7.48 to 23.5 mol·m⁻²·d⁻¹.

Kale. There were no effects of LI on HL and HD in kale (Table 2). LA, RI, and RCC increased linearly by 17%, 60%, and 18%, respectively, and SLA decreased linearly by 27% as average DLI increased from 6.9 to 23.5 mol·m⁻²·d⁻¹. FW and DW increased asymptotically by 43% and 64%, respectively, as average DLI increased from 6.9 to 23.5 mol·m⁻²·d⁻¹.

Arugula. There was no effect of LI on HL in arugula (Table 2). HD, LA, RI, and RCC increased linearly by 16%, 40%, 123%, and 21%, respectively, and SLA decreased linearly by 30% as average DLI increased from 7.2 to 23.4 mol·m⁻²·d⁻¹. FW and DW increased asymptotically by 67% and 85%, respectively, as average DLI increased from 7.2 to 23.4 mol·m⁻²·d⁻¹.

Mustard. As average DLI increased from 7.2 to 23.7 mol·m⁻²·d⁻¹, HD, LA, RI, and RCC increased by 12%, 20%, 102%, and 17%, respectively (Table 2). HL and SLA decreased linearly by 8.5% and 40%, respectively, as average DLI increased from 7.2 to 23.7 mol·m⁻²·d⁻¹. FW and DW increased asymptotically by 61% and 78%, respectively, as average DLI increased from 7.2 to 23.7 mol·m⁻²·d⁻¹.

Discussion

Gradient designs. Greenhouse lighting trials often involve plots of different, reasonably homogeneous intra-plot LI levels (from natural and/or supplemental sources) within a common growing environment (Dansereau et al., 1998; Lopez and Runkle, 2008; Oh et al., 2010; Torres and Lopez, 2011). Treatment plots are typically separated in space to minimize light contamination from adjacent plots, which often limits the quantity of experimental plots and also can create variations in other environmental conditions (e.g., temperature, humidity, and air flow). Physical barriers, such as the opaque blinds commonly used in SS lighting research, have limited use in the greenhouse environment because they can also strongly influence the natural lighting levels at crop level. Furthermore, the requirement for proper replication of treatments inherently limits the number of LI treatment levels that can be investigated. Sometimes consecutive replication methods are used to increase the number of possible treatment levels and/or replications within the available plots (Gaudreau et al., 1994; Gent, 2014; Hoshi et al., 2011; Sublett et al., 2018; Tani et al., 2014; Zhang et al., 2019). However, because there can be considerable temporal variations in natural lighting (Hoshi et al., 2011; Lopez and Runkle, 2008; Poel and Runkle, 2017; Poorter et al., 2019), concurrent experimental designs are highly preferable for LI research trials in greenhouse environments. Furthermore, a regression analysis of data from replicated designs invariably has aggregations of data points at relatively few discreet levels of the independent variable (i.e., LI) with the need for interpolation between these levels. In contrast, gradient-type ecological approaches can outperform replicated designs when evaluating biological responses along a continuous variable (Kreyling et al., 2018), such as LI. Although technically more difficult to arrange than plots of replicated treatments, gradient designs, such as what was used in the present study, have been successfully used to establish supplemental LI effects on year-round greenhouse production (e.g., cut roses) (Bredmose, 1993, 1994).

Yield. Microgreens are a unique commodity group in that seeding densities are very high (Murphy and Pill, 2010; Kyriacou et al., 2016), production times are extremely short (Treadwell et al., 2016), and input biomass (i.e., seed weight) and marketable yield (i.e., FW of aboveground biomass) may be of similar magnitudes (Jones-Baumgardt et al., 2019). Before activation of the photosynthetic machinery (i.e., transition to autotrophic

Table 2. Effect of daily light integrals (DLIs) and total light integrals (TLIs) ranging from 6.9 to 23.7 mol·m⁻²·d⁻¹ and 59.9 to 284 mol·m⁻², respectively, on growth and yield measurements of sunflower (*Helianthus annuus* L., ‘Black oil’), kale (*Brassica napus* L., ‘Red Russian’), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L., ‘Ruby Streaks’) microgreens.

Measurement	Regression equation ^z		Avg ^w	r ²
	DLI ^y	TLI ^x		
Sunflower				
Hypocotyl length (cm)			9.38 ± 0.08	
Hypocotyl diameter (mm)	y = 0.0176x + 3.64	y = 0.00220x + 3.64		0.130
Fresh weight (kg·m ⁻²)	y = 7.00 – 2.79e ^{-0.0188x}	y = 7.00 – 2.79e ^{-0.00235x}		0.119
Dry weight (g·m ⁻²)			397 ± 6	
Leaf area (cm ² /plant)	y = 0.0203x + 3.12	y = 0.00253x + 3.12		0.0865
Specific leaf area (cm ² ·g ⁻¹ (DW))			136 ± 2	
Robust index			16.6 ± 0.4	
Relative chlorophyll content			67.8 ± 0.8	
Kale				
Hypocotyl length (cm)			9.45 ± 0.09	
Hypocotyl diameter (mm)			1.75 ± 0.01	
Fresh weight (kg·m ⁻²)	y = 2.57 – 2.40e ^{-0.156x}	y = 2.57 – 2.40e ^{-0.0141x}		0.389
Dry weight (g·m ⁻²)	y = 130 – 112e ^{-0.0957x}	y = 130 – 112e ^{-0.00870x}		0.597
Leaf area (cm ² /plant)	y = 0.0230x + 2.06	y = 0.00209x + 2.06		0.106
Specific leaf area (cm ² ·g ⁻¹ (DW))	y = -4.65x + 325	y = -0.422x + 325		0.237
Robust index	y = 0.0516x + 1.07	y = 0.00469x + 1.07		0.545
Relative chlorophyll content	y = 0.333x + 28.6	y = 0.0303x + 28.6		0.338
Arugula				
Hypocotyl length (cm)			7.75 ± 0.07	
Hypocotyl diameter (mm)	y = 0.0122x + 1.17	y = 0.00101x + 1.17		0.233
Fresh weight (kg·m ⁻²)	y = 2.73 – 2.15e ^{-0.0654x}	y = 2.73 – 2.15e ^{-0.00545x}		0.716
Dry weight (g·m ⁻²)	y = 206 – 173e ^{-0.0293x}	y = 206 – 173e ^{-0.00244x}		0.819
Leaf area (cm ² /plant)	y = 0.0504x + 1.76	y = 0.00420x + 1.76		0.513
Specific leaf area (cm ² ·g ⁻¹ (DW))	y = -6.72x + 428	y = -0.560x + 428		0.351
Robust index	y = 0.0767x + 0.515	y = 0.00639x + 0.515		0.774
Relative chlorophyll content	y = 0.350x + 26.2	y = 0.0291x + 26.2		0.235
Mustard				
Hypocotyl length (cm)	y = -0.0421x + 8.59	y = -0.00351x + 8.59		0.148
Hypocotyl diameter (mm)	y = 0.0114x + 1.50	y = 0.000949x + 1.50		0.193
Fresh weight (kg·m ⁻²)	y = 2.09 – 2.55e ^{-0.163x}	y = 2.09 – 2.55e ^{-0.0135x}		0.580
Dry weight (g·m ⁻²)	y = 120 – 135e ^{-0.127x}	y = 120 – 135e ^{-0.0106x}		0.661
Leaf area (cm ² /plant)	y = 0.0249x + 1.87	y = 0.00207x + 1.87		0.154
Specific leaf area (cm ² ·g ⁻¹ (DW))	y = -9.64x + 468	y = -0.803x + 468		0.675
Robust index	y = 0.0828x + 0.787	y = 0.00690x + 0.787		0.745
Relative chlorophyll content	y = 0.274x + 25.9	y = 0.0228x + 25.9		0.358

^zGrowth and yield measurements with significant regressions ($P \leq 0.05$) are presented as equations.

^yDaily light integral (DLI; mol·m⁻²·d⁻¹).

^xTotal light integral (TLI; mol·m⁻²).

^wGrowth and yield measurements without treatment effects. The means ± SE (n = 50) are presented.

growth), plant embryos rely solely on stored energy resources (i.e., heterotrophic growth). Therefore, during microgreens' abbreviated lifetimes, both heterotrophic and autotrophic growth may have substantial contributions to the marketable yield. In the present study, arugula and mustard seed were approximately half the size of kale seed and two decades (100×) smaller than sunflower seed. Although the LI-mediated responses of the different genotypes cannot be directly compared due to the differences in seed sizes, seeding densities, and production periods (all of which followed commercial production protocols), it can be generalized that genotypes with larger seeds had shorter production times in conjunction with lower magnitudes of LI-mediated growth and yield responses. This suggests that genotypes with smaller seeds may present greater levels of phenotypic plasticity in response to increasing LI (Jones-Baumgardt et al., 2019), probably due to reduced reliance on stored energy resources in the seed. Therefore, the phenotypic plasticity of yield responses to increasing LI is an important production consideration when balancing the input costs of seed and lighting infrastructure among different commodities.

Because microgreens are sold on a FW basis, a major goal of commercial microgreen production is to provide growth conditions that maximize FW (Murphy and Pill, 2010) while still maintaining relatively high LUE. Once the LI responses on biomass accumulation start to level off (i.e., approach saturation) at higher LI, LUE decreases accordingly. The maximum LI in some other studies on microgreens and leafy vegetables were insufficiently high to show saturating responses in FW and DW (Gerovac et al., 2016; Lefsrud et al., 2006; Sago, 2016; Samuolienė et al., 2013). Conversely, Fu et al. (2012a, 2012b) showed saturation of FW of romaine lettuce grown in SS at LI ranging from 100 to 800 μmol·m⁻²·s⁻¹. Because saturation occurred between 400 and 600 μmol·m⁻²·s⁻¹, the authors recommended a target SL of 400 μmol·m⁻²·s⁻¹ for winter greenhouse lettuce production, and they further recommended that shading should be provided in summer when ambient PPFD exceeded 600 μmol·m⁻²·s⁻¹. However, they may not have fully accounted for the probable inter-treatment canopy temperature differences in their setup due to varying proximity to SS fixtures (HPS and MH), which may have contributed to photoinhibition and higher tran-

spiration rates under their highest LI treatments (Berry and Bjorkman, 1980; He et al., 2019). Furthermore, their recommendations for greenhouse production environments did not account for the differences in lighting dynamics between an SS research environment and a greenhouse environment. While no clear maxima LI for yield were found in either the present study or in Jones-Baumgardt et al. (2019), the modeled asymptotic relationships between LI and yield do illustrate the trend of diminishing returns at higher LI, particularly for larger-seeded genotypes. These models should help growers pinpoint a lighting strategy that is optimum for their respective growing systems, specific commodities, and production goals.

Light use efficiency will also change quite dynamically over time, as the cotyledons unfold and become increasingly photosynthetically active. Although it is not uncommon to begin illuminating crops at the time of sowing (such as in the present study), using high levels of SL during this period could be wasted energy because SL will assuredly be used more efficiently once the seedlings begin to transition to autotrophic behavior. Therefore, if the time between germination

and the transition to photosynthetic activity were known (probably the first few days after sowing, depending on genotype), then it is conceivable that the TLI levels in the yield equations presented herein could be retroactively adjusted to simulate a protocol whereby SL is provided only between cotyledon unfolding and harvest, which would increase LUE accordingly. Alternatively, because flowering is not important for microgreens and, for a given DLI, plants grown at a low LI for a long photoperiod generally accumulate more biomass than plants grown at a high LI for a short photoperiod (Kitaya et al., 1998; Oda et al., 1989; Ohyama et al., 2005; Sysoeva et al., 2010), continuous lighting may be possible for many genotypes, which could increase yield and/or shorten production time (Lefsrud et al., 2006; Sago, 2016). Supplemental lighting could also be applied only during the night, which may improve overall crop LUE and could dramatically reduce time-of-use energy costs associated with supplemental lighting during daytime hours (Sysoeva et al., 2010). Furthermore, increasing LI in a stepwise fashion, as the crop matures and canopy closes, may also save energy without dramatically affecting yield. This can (easily) be accomplished by using the dimming capabilities of most commercial LEDs, or (if logistically possible) by moving crops through various greenhouse sections that have been outfitted with increasing densities of SL fixtures. More research is needed to evaluate the efficacy of these other lighting strategies for greenhouse production of microgreens.

Morphology. Elongated hypocotyls (≥ 5 cm) are an important growth attribute for many commercial microgreen growers because taller plants facilitate mechanical harvesting processes but may reduce the robustness of the harvested tissues. Although compact growth behaviors in mature plant tissues are commonly associated with increased LI (Poorter et al., 2019; Yeh and Hsu, 2004; Zervoudakis et al., 2012), juvenile plants have shown either reductions or no changes in HL with increasing LI in both greenhouses (Craver et al., 2019; Hernández and Kubota, 2014; Lopez and Runkle, 2008; Poel and Runkle, 2017; Pramuk and Runkle, 2005) and SS growing environments (Craver et al., 2018; Kitaya et al., 1998; Meng and Runkle, 2019; Potter et al., 1999; Viršilė and Sirtautas, 2013). There were only LI treatment effects in HL in mustard in the present study; while Gerovac et al. (2016), Jones-Baumgardt et al. (2019), and Samuolienė et al. (2013) all reported general reductions in HL with increasing SS LI in Brassicaceae microgreens. In mustard, the LI treatment effects on HL were only approximately half the magnitude (over the same TLI range) as was reported in Jones-Baumgardt et al. (2019). Furthermore, the absolute lengths of the hypocotyls were markedly longer in the present study than in any of these SS studies. This indicates that other aspects of the growing environment may have overshadowed the LI treatment effects on HL. Be-

cause all three of the aforementioned SS studies had the same daytime temperature (21 °C) but 4 °C (i.e., 17 °C vs. 21 °C) cooler nighttime temperatures than in the present study, it is possible that the higher nighttime temperature in the greenhouse environment conveyed a thermomorphogenic effect on hypocotyl elongation (Halliday and Davis, 2016; Heggie and Halliday, 2005). In high-density plantings, HL can also be influenced by photoreceptor-mediated neighbor sensing induced by light quality changes within the canopy (i.e., shade avoidance) (Fiorucci and Fankhauser, 2017; Park and Runkle, 2018; Roig-Villanova and Martínez-García, 2016). Shade avoidance responses related to low red to far red ratios (R:FR) should not generally occur in SS environments, unless FR wavelengths comprise a significant portion of the SS spectral photon flux distribution. Conversely, low R:FR are more likely in greenhouse environments because natural sunlight is relatively high in FR [i.e., R:FR ≈ 1.1 (Llewellyn et al., 2013)].

In both the present study and Jones-Baumgardt et al. (2019), the increases in DW in the Brassicaceae genotypes were disproportionate to FW with increasing LI. More specifically, the %DW (i.e., DW/FW) increased with increasing TLI; a common trend in microgreens (Gerovac et al., 2016; Jones-Baumgardt et al., 2019; Samuolienė et al., 2013) and other seedlings (Kitaya et al., 1998; Potter et al., 1999). Concurrent with increasing %DW were decreasing SLA and increasing HD (in arugula and mustard), indicating increasing thickness of above-ground tissues (i.e., stems and cotyledons). The negative linear relationships between LI and SLA in kale, arugula, and mustard were similar to the LI effects on SLA in many other commodities and growing environments (Fan et al., 2013; Gent, 2014; Sims and Percy, 1994; Tani et al., 2014; Torres and Lopez, 2011). Short shelf life is one of the major limiting factors for commercial microgreen production (Chandra et al., 2012). For example, Clarkson et al. (2003) found that salt-stressed baby spinach and lettuce, which has lower SLA (i.e., thicker leaves), scored 50% higher on the organoleptic scale than control treatment (no salt stress) 7 d after harvest. More research is needed to evaluate how these morphological changes may affect palatability of marketable tissues, but it is very likely that they are concomitant with potential reductions in tissue damage during harvest and processing and general increases in post-harvest shelf life (Cantwell and Suslow, 2002; Hodges and Toivonen, 2008).

With the much longer hypocotyls observed in the present study vs. microgreens grown in SS, such as in Gerovac et al. (2016), Jones-Baumgardt et al. (2019), and Samuolienė et al. (2013), a more holistic assessment of plant robustness may be appropriate to appraise how LI affects the postharvest quality of harvested tissues. RI [adapted from Fan et al. (2013)] which relates individual components of hypocotyl volume

(i.e., HL and HD) to DW, may serve as a proxy for plant robustness. To contextualize the term, for a given DW, increases in HL and decreases in HD both confer reductions in robustness (i.e., tall and/or spindly plants). Despite there being no treatment effects on HD in kale and HL in kale and arugula in the present study, increasing LI still increased RI in all three Brassicaceae genotypes. Future research may be able to more directly evaluate how RI relates to aspects of postharvest quality, such as shelf life and palatability.

Chlorophyll. CC is closely associated with human perception of the green pigmentation of leaves (Barrett et al., 2010) and is also an important indicator of how plants respond to increasing LI. Both destructive and nondestructive methods are used to measure CC (Kalaji et al., 2016). Destructive measures usually report CC in terms of per weight (FW or DW) or per LA bases (Richardson et al., 2002), depending on the study's objectives. However, these different bases may result in quite different interpretations of the various studies' results, depending on leaf morphology. To illustrate this, Hernández and Kubota (2014) found that CC of cucumber seedlings increased, on a per LA basis, under SL treatments (vs. no SL) at two natural DLIs. Conversely, Makus and Lester (2002) reported a 60% decrease in CC, on a per DW basis, in two greenhouse-grown cultivars of mustard under ambient light vs. 50% shade. Additionally, Kopsell et al. (2012) reported substantially lower CC, on a per FW basis, in mustard microgreens grown under 463 vs. 275 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of SS fluorescent light. However, because leaf morphology characteristics (e.g., SLA) are unknown, direct comparisons of their CC results are difficult. Furthermore, RCC indices are frequently presented as an alternative to destructive CC measurements due to ease of measurement (Kalaji et al., 2016; Richardson et al., 2002). However, RCC indices also do not account for varying leaf morphology (e.g., SLA) between treatments. In the present study, when considering the magnitude of the RCC treatment effects within the context of decreasing SLA with increasing LI, it appeared that CC on a per DW basis decreased by 12% to 25% in the Brassicaceae genotypes as TLIs increased 86 to 254 $\text{mol}\cdot\text{m}^{-2}$. Tani et al. (2014) reported both increases in SLA (i.e., decreases in specific leaf mass) and decreases in RCC of lettuce grown in a greenhouse under solar panels (i.e., shading) vs. ambient lighting, regardless of time of year. At higher LI, it is conceivable that the intensity of green hues (not measured in the present study) may have increased on a per LA basis, but the total chlorophyll content decreased on a per DW basis, such as was reported by Jones-Baumgardt et al. (2019).

While the present study was performed during early February, when natural light levels were quite low, the TLI models presented herein may also be relevant to other times of the year and production

environments. For example, in Southern Ontario, Canada, the typical “supplemental lighting season” extends from early October through March, when the natural lighting levels can vary by a factor of 4 (i.e., from 5 to 20 mol·m⁻²·d⁻¹) (Faust and Logan, 2018). With an understanding of the ambient lighting dynamics within their production facility, a grower can use the presented TLI models to predict their marketable yield for a given SL intensity and, therefore, determine other aspects of their production protocols (e.g., the growing area, length of production, temperature, CO₂ concentration, etc.) required to achieve their production goals. Furthermore, although saturation was not observed during the trial, it is conceivable that TLIs in excess of ≈300 mol·m⁻² may be excessive for producing microgreens. Locally, this TLI level is achievable (assuming 10-d production cycle and 75% light transmission) with natural light alone during the summer months (i.e., May through August) when the natural DLI may be >40 mol·m⁻²·d⁻¹ (Faust and Logan, 2018). Furthermore, shading (e.g., retractable curtains or whitewash) may be required to reduce canopy-level LI (also greenhouse temperatures) during periods of excessive LI to reduce photoinhibition.

In most light-limiting greenhouse crop production scenarios (e.g., winter production), SL will increase yield and quality. How much SL to provide is largely related to the commodities’ LUE. Although the crops in the present study all showed LI treatment effects on yield, LUE are quite low relative to many other commodities due to relatively high seeding densities, short production times, and the heterotrophic/autotrophic paradox. While this may be a compelling argument against using high intensities of SL for microgreens, their high value and year-round demand may offset the relatively low LUEs, compared with other crops, particularly for smaller-seeded crops.

Conclusion

This study investigated the responses of four microgreen genotypes to various levels of supplemental LED lighting. The relationships between LI (expressed both in terms of average DLIs and TLIs) and key morphological parameters are presented and discussed. Commercial greenhouse growers can use the light response models described herein to make predictions of relevant microgreen production metrics according to the available light levels during winter in high-latitude regions; enabling growers to optimize the choice of the most appropriate SL levels to achieve their desired production goals, as economically as possible.

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