The Use of Light-Emitting Diodes for Microgreen Production in Controlled Environments

by

Chase S. Jones-Baumgardt

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ABSTRACT

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Chase Jones-Baumgardt
University of Guelph, 2019

Advisors:
Dr. Youbin Zheng

Light-emitting diodes (LEDs) are currently used as sole-source (SS) and supplemental (SL) lighting for microgreen production in indoor and greenhouse environments, respectively. The objectives of this study were to: 1) investigate the effect of SS LED light intensity (LI) on growth, yield, quality, and phytochemical and nitrate content; 2) quantify the effects of SL LED LI during the winter months on the growth and yield of microgreens. In the indoor environment, as SS LI increased fresh weight (FW), dry weight, total and reduced ascorbate and total anthocyanins increased in all genotypes, whereas total phenolic content was elevated in kale and arugula. Higher SS LI proportionally decreased hypocotyl length (HL) in all genotypes. In the greenhouse, leaf area and FW increased with increasing daily light integrals (DLI) in all genotypes. With the exception of sunflower, the dry weight, robust index, and relative chlorophyll content increased with increasing DLI, whereas specific leaf area decreased.
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LIST OF ABBREVIATION, SYMBOLS AND NOMENCLATURE

B – Blue
DAS – Days after sowing
DLI – Daily light integral (mol·m⁻²·d⁻¹)
FR – Far red
g – gram
G – Green
HA – Hue angle
HD – Hypocotyl diameter
HL – Hypocotyl length
HPS – High pressure sodium
LA – Leaf area
LED – Light-emitting diode
mg – milligram
PAR – Photosynthetically active radiation
PFR – Photon flux ratio
PPFD – Photosynthetic photon flux density (μmol·m⁻²·s⁻¹)
R – Red
RH – Relative humidity
RI – Robust index
SD – Standard deviation
SE – Standard error
SL – Supplemental
SLA – Specific leaf area
SS – Sole-source
TLI – Total light integral (mol·m²)
TPFD – Total photon flux density (μmol·m⁻²·s⁻¹)
UV-A – Ultraviolet A
UV-B – Ultraviolet B
W – White
CHAPTER ONE
INTRODUCTION

1.1 MICROGREENS

The term “microgreens” refers to seedlings of vegetables or herbs, that are harvested just above the soil or soilless growing substrate after the cotyledons have fully developed, either before or after their true leaves have emerged (Treadwell et al., 2016). They have a relatively short production cycle, growing for approximately 7 to 21 d after seeding; they are harvested later than sprouts and earlier than baby greens (Treadwell et al., 2016). Depending on the genotype, the height of microgreens range between 2.5 to 7.6 cm (Xiao et al., 2012). There are over 100 genotypes of microgreens that are grown for commercial purposes (Treadwell et al., 2016); however, the most commonly grown genotypes belong to Brassicaceae (e.g., broccoli, kale and cabbage), because of their ease of germination, high phytochemical content, vibrant colors, and unique flavours and textures. In addition, microgreens are derived from the Asteraceae, Lamiaceae, Apiaceae, and Amaranthceae families (Kyraciou et al., 2016).

1.2 HISTORY OF MICROGREENS

In the late 1900s microgreens were initially used to garnish, add flavour, texture and color to a wide variety of dishes including salads, soups and sandwiches in upscale restaurants. In 1999 the first published example of microgreens was in the New York Times (Burros, 1999) and by the early 2000’s microgreens were used in upscale restaurants across the United States (Himmel, 2001). Originally, microgreens were not available in the marketplace because of their short shelf-life and high-price (Hansen,
By 2000, microgreens were available in the California marketplace for about $60 a pound (Hansen, 2000). At this time, microgreens were also being sold uncut to enhance shelf-life in order for long-distance transport to consumers. As supply has risen to meet demand, market prices have further dropped, with microgreens valued at $30 to $50 a pound in Florida (Treadwell et al., 2016). There are presently no available industry statistics for Canadian microgreens; however, local greenhouse producer Greenbelt Microgreens has reported prices that are similar to those in the United States. The appeal of eating microgreens for home consumption has further increased because of reduced market prices and their high phytochemical content.

1.3 MICROGREEN PHYTOCHEMICAL CONTENT
Microgreens are well known for their high phytochemical content and possible bioactive value. It is known that ascorbate levels decrease in baby spinach when the harvest window is extended from 17 to 30 d after germination (Bergquist et al., 2006). Similarly, Lester et al., (2010) found that ascorbate, carotenoids, folate, α-tocopherol and phyloquinone of younger baby spinach leaves are greater than mature spinach leaves. Brassicaceae microgreen genotypes contain high concentrations of polyphenols (Sun et al., 2013). Furthermore, two week-old lettuce microgreens contain higher mineral content and lower nitrate content than lettuce grown for ten weeks (Pinto et al., 2015). Xiao et al. (2012) demonstrated that 25 commercially grown microgreen genotypes contain higher amounts of ascorbate, carotenoids, phyloquinone, and tocopherols than their mature counterparts (US Department of Agriculture, 2011). Most recently, it was determined that 30 genotypes of microgreens have high levels of macro- (e.g., Ca and Mg) and micro-elements (e.g., Fe and Zn) (Xiao et al., 2016).
1.4 CURRENT PRODUCTION PRACTICES OF MICROGREENS

Microgreens are grown in a variety of growth environments (i.e., outdoor, indoor, and greenhouse), substrates (i.e., soil, soilless, and hydroponic), with different fertilizers (i.e., organic and inorganic), and at different scales of productions (i.e., small and large) (Kyriacou et al., 2016). They can be grown indoors where all of the photosynthetic lighting is generated artificially, in a greenhouse with or without supplemental (SL) lighting or outdoors with ambient lighting.

1.5 GENERAL RESPONSE OF PLANTS TO LIGHT INTENSITY

Understanding the relationships between light intensity (LI) and growth of various plant commodities is important, since LI directly affects photosynthetic rates. This is relevant in SL and sole-source (SS) production systems, where infrastructure and electricity required for crop lighting (and associated heat management) are major input costs. In higher plants, the relationship between LI and photosynthesis is linear at lower LI (van Iersel, 2017); and is thus considered to be ‘light limited’. At higher LI, the photosynthetic response to increasing LI levels is saturated, indicating that other factors (e.g., CO₂ concentration, temperature, plant water status, and stomatal conductance) have become limiting (van Iersel, 2017). Although, the hyperbolic shape of this ‘light response curve’ (i.e., LI vs. photosynthesis) is common to all photosynthetic organisms and lighting environments, plants can acclimate to their lighting environments by varying morphology, and photosynthetic efficiency (Givnish, 1988; Bailey et al., 2001). For example, the specific leaf area of seedlings (i.e., g(dw)·cm⁻²) and LI tend to be negatively correlated (Givnish et al., 2004; Matos et al., 2009; Sims and Pearcy, 1994), and thus it may be
possible to use higher microgreen seeding densities to generate greater yields in a given growing area. Furthermore, some plants (e.g., lettuce and ginger) increase the production of photo-protective compounds (e.g., anthocyanins, phenolics, ascorbate, and carotenoids) under higher LI, which may also be beneficial to human health (Craver et al., 2017; Ghasemzadeh et al., 2010; Oh et al., 2009; Samuolienė et al., 2013). Therefore, increasing LI can potentially elevate the nutritional value of microgreens in concert with increasing yields.

1.6 IMPORTANCE OF LIGHTING FOR MICROGREEN GROWTH AND PHYTOCHEMICAL AND NITRATE CONTENTS

Light is one of the most influential environmental factors for plants, especially in controlled growth environments. It is well known that LI, light quality, and photoperiod can regulate plant growth and phytochemical content, and therefore are key components of the lighting environment (Carvalho and Folta, 2014; Ouzounis et al., 2015). Light requirements of plants are specific to the genotype, developmental stages, environmental conditions and target of yield, quality and phytochemical content. To date, studies conducted worldwide have determined specific light regimes (i.e., LI, quality and photoperiod) for plant production, but there is still a lack of information regarding the effects of LI, light quality and photoperiod for growth and phytochemical content of microgreens of different genotypes. Therefore, detailed studies on light regimens are needed to obtain more specific production goals to allow growers to take better advantage of using light.
1.7 EFFECT OF LIGHT INTENSITY ON MICROGREEN GROWTH

There is limited and variable information available regarding the effects of different SL and SS LI on the growth of microgreens. Recent research has investigated the relationship between SS LI and growth in the following *Brassica* microgreens: kohlrabi, mizuna, mustard, red pak choi and tatsoi (Gerovac et al., 2016; Samuolienè et al., 2013). On the whole, the measured growth parameters were similar across seedlings of various *Brassica* genotypes (Gerovac et al., 2016; Samuolienè et al., 2013), although the magnitude of the effects may be influenced by genotype. Furthermore, Gerovac et al. (2016) reported fresh weight (FW) on a per-plant basis, which may not be an accurate representation of the effect of LI on yield, since microgreens are grown as dense canopies in a commercial production scenario. Moreover, since dry weight (DW) data were not presented, the relationships between LI and biomass production cannot be easily established. Samuolienè et al. (2013) used unique wavelengths of red (R) light-emitting diodes (LEDs) (peaking at 638 and 665 nm) and far red (FR) (peaking at 735 nm). They described the photon flux ratio (PFR, representing light spectrum) of blue (B), R, and FR for their lighting treatments, but the reported values were taken using an instrument (RF-100, Sonopan, Poland) that is known to have varying wavelength sensitivity (with about 35% greater sensitivity at 740 nm vs. 450 nm), meaning that the PFR was poorly defined and the absolute total photon flux density (TPFD) levels were likely inaccurate. The authors also did not describe the following: plot uniformity distribution, whether true replication occurred and both FW and DW were not reported, preventing the ability to determine the effect of LI on yield.

From the aforementioned studies it appears that further clarification regarding the
effect of LI on microgreens’ growth using economically important parameters is needed. Furthermore, it seems that microgreens’ growth responses to LI could be genotype-specific. In the literature, there are no microgreen studies investigating the effect of SL LI on growth parameters. Determination of the effect of LI on the growth of different microgreen genotypes in a SL and SS environment is required.

1.8 EFFECT OF LIGHT INTENSITY ON MICROGREEN PHYTOCHEMICAL AND NITRATE CONTENTS

Recent research has investigated the relationship between SS LI and phytochemical content, including nitrate, in the Brassica microgreens kohlrabi, mizuna, mustard, red pak choi and tatsoi (Brazaityte et al. 2015; Craver et al., 2017; Samuolienė et al., 2013). Total carotenoid content of mustard (Brassica juncea ‘Garnet Giant’) was reduced as LI increased from 105 to 330 μmol·m⁻²·s⁻¹, regardless of PFR, whereas total chlorophyll content decreased as LI increased with most PFRs (Craver et al., 2017). By contrast, increases in chlorophyll content index (Samuolienė et al., 2013) and total carotenoids (Brazaityte et al., 2015) of mustard (Brassica juncea L. ‘Red Lion’) were evident with an increase in LI. While not microgreen research, in general nitrate accumulation in some leafy vegetables is associated with lower LI compared to higher LI (Chadjaa et al., 1999; Fallovo et al., 2011; Gaudreau et al., 1995; Proietti et al., 2004; Trejo-Téllez et al., 2019), although other research has found nitrate accumulation increased as LI increases (Fallovo et al., 2009; Fallovo et al., 2011) or was unaffected (Cantliffe, 1972; Parks et al., 2008).

Recent research has demonstrated a connection between phytochemical and nitrate contents and SS LI. However, the available research regarding the effects of LI on
phytochemical and nitrate contents in microgreens is variable and may be genotype-specific. Since optimal levels of phytochemicals vary with genotype, more research is needed to determine the phytochemical responses to SS LI of other economically important genotypes.

1.9 THESIS RESEARCH GOAL AND OBJECTIVES

Considering the research available regarding the effects of LI on microgreen production is limited, variable, and may be genotype-specific, the goal of this thesis was to determine the effect of LI on growth, yield, quality, and phytochemical and nitrate contents of economically important microgreens in controlled environments.

The overall hypothesis of the study was:

The growth, yield, quality and metabolite responses of microgreens to LED LI (i.e., SL and SS) could be genotype-specific.

The objectives of the study were to:

1. Investigate the influence of SS LED LI, ranging from 100 to 600 μmol·m⁻²·s⁻¹ (with a 16 h photoperiod) on growth, yield, and quality of commercially-relevant Brassicaceae microgreens.

2. Investigate the influence of SS LED (B15:R85) LI, ranging from 100 to 600 μmol·m⁻²·s⁻¹ on the levels of ascorbate metabolites, total carotenoids, total chlorophyll and its individual components, total phenolics, anthocyanins, and nitrate of four economically-important and organically grown Brassicaceae microgreens.
3. Quantify the effects of SL LED LI during the winter months in higher latitude regions (e.g., southern Ontario) on the growth and yield of four economically-important greenhouse-grown microgreens: sunflower, kale, arugula and mustard.

This study was intended to provide microgreen producers information to plan and use their lighting technology as efficiently as possible in controlled environments (i.e., indoor and greenhouse). It is clear that light regimes are specific to the crop and environmental condition, and thus this research is needed by the scientific community and will add to the body of knowledge of microgreens.

**Note:** All experimental chapters in this thesis (2, 3 and 4) follow the Journal of the American Society for Horticultural Science (HortScience) style guidelines and are intended to be submitted to scientific journals for publication. Chapter 2 has been reviewed and accepted by the journal HortScience.
1.10 LITERATURE CITED


CHAPTER TWO
INTENSITY OF SOLE-SOURCE LIGHT-EMITTING DIODES AFFECTS THE GROWTH, YIELD, AND QUALITY OF BRASSICACEAE MICROGREENS

Additional index words. PPFD, TLI, controlled environment, kale, cabbage, arugula, mustard

ABSTRACT
Indoor farming is an increasingly popular approach for growing leafy vegetables, and under this production system artificial light provides the SS of radiation for photosynthesis and light signalling. The present study examined the effects of SS LI on growth, yield, and quality of kale (Brassica napus L. ‘Red Russian’), cabbage (Brassica oleracea L.), arugula (Eruca sativa L.), and mustard (Brassica juncea L. ‘Ruby Streaks’) microgreens grown in a walk-in growth chamber. SS LEDs were used to provide six target photosynthetic photon flux density (PPFD) treatments: 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ with a PFR of B15: R85 and 16-h photoperiod. As LI increased from 100 to 600 μmol·m⁻²·s⁻¹, FW increased by 36%, 56%, 76%, and 82% for kale, cabbage, arugula, and mustard, respectively. Similarly, DW increased by 65%, 69%, 122%, and 145% for kale, cabbage, arugula, and mustard, respectively as LI increased from 100 to 600 μmol·m⁻²·s⁻¹. Increasing LI decreased hypocotyl length and color indicated by hue angle linearly in all genotypes. Growers can use the results of this study to optimize SS LI for their production systems, genotypes, and production goals.
2.1 INTRODUCTION

Microgreens are an emerging culinary trend due to their unique appearances and textures, intense flavours, and high phytonutrient densities (Xiao et al., 2012). Collectively, these crops are seedlings of vegetables and herbs, which are germinated and grown in lit environments and then harvested and consumed at an immature growth stage. Microgreens are densely seeded and harvested shortly after the cotyledons have fully developed; either with or without the emergence of the first true leaves depending on species. Harvesting is usually done 7 to 20 d after seeding, when they are 2.5 to 7.5 cm in height (Treadwell et al., 2016). Greenhouse and indoor growers have become interested in microgreen production due to increasing demand, short production cycles, and high market value; with wholesale prices ranging from $30 to $50 USD per pound (Treadwell et al., 2016). The Brassicaceae family are especially popular to grow as microgreens due to their ease of germination, short growth cycles, varying colors, distinct flavours, and high phytochemical concentrations (Xiao et al., 2012)

Microgreens can be grown in a myriad of production scenarios including: outdoor, indoor, and greenhouse environments in soil or soilless growing systems (Kyriacou et al., 2016). Indoor farming is an increasingly popular approach for growing leafy vegetables as it allows the producers the greatest potential for manipulating the growing environment to modify taste and morphology, based on market preferences (Despommier, 2013) and producing highly uniform crops year-round. Light is one of the most influential environmental factors on plant growth and morphology, especially in indoor farming where artificial lighting provides SS of radiation for photosynthesis and light signalling. Historically, the most commonly-used artificial light sources for controlled
environment crop production have been fluorescent tubes in SS environments (Kozai, 2013) and high intensity discharge lamps such as high pressure sodium (HPS) in greenhouses (Ouzounis et al., 2015; Singh et al., 2015). Horticultural light-emitting diodes (LEDs) have become viable replacements for these older technologies due to their potential for high energy efficiency and durability, long lifetime, and low radiant heat emissions directed towards the crop (Mitchell and Stutte, 2017). It is also possible to adjust the intensity and spectrum with some horticultural LED systems (Llewellyn and Zheng, 2018), providing growers with additional tools with which they may be able to use light to manipulate crop growth, morphology, and phytochemical production (e.g., photoprotective pigments).

Although many studies have been conducted worldwide to investigate the use of LED technologies for growing a myriad of horticultural commodities in SS systems, there are still few robust studies related to the use of LEDs for the production of many important microgreens. The focus of the present study is on SS LED LI, and the effects on yield and morphology of Brassicaceae microgreens. This study relates relevant crop production metrics to both instantaneous intensity of PAR, defined as PPFD (μmol·m⁻²·s⁻¹), and accumulated light over the complete production cycle, defined as total light integral (TLI, mol·m⁻²). An advantage of using TLI to quantify a crop’s exposure to photosynthetic light is its potential to normalize yield results from different production scenarios (e.g., SS vs. greenhouse) and lighting environments (e.g., LI, photoperiod, and lengths of production cycles). Although, TLI does not seem to be a commonly-used metric in the scientific community, some greenhouse growers utilize light sums (typically
from logged outdoor global radiation data) to assist with their production decisions, particularly regarding the use of SL lighting.

Several research groups have explored the relationships between SS LI and various growth and yield metrics of Brassicaceae microgreens. Samuoliene et al., (2013) investigated the effects of five LED total photon flux density (TPFD, 400 to 800 nm) levels ranging from 110 to 545 μmol·m⁻²·s⁻¹ (16 h photoperiod) on growth and phytochemical content of red pak choi, kohlrabi, tatsoi, and mustard. Their trial used modules comprised of LEDs with peak wavelengths at 455, 638, 660, and 735 nm, respectively. They described the PFR for their lighting treatments but the PFR was in conflict with their referenced paper (Tamulaitis et al., 2005). Their PFR and TPFD levels were reportedly measured using a quantum sensor (RF-100, Sonopan, Poland) known to have varying wavelength sensitivity; meaning that PFR and absolute PPFD were likely inaccurate. Furthermore, the authors did not describe plot-level uniformity distribution, it is unclear whether true statistical replication occurred, and neither FW nor DW data were reported, meaning readers cannot evaluate how LI affected crop yield (microgreen commodities are normally sold on a FW basis) or biomass (DW) metrics. Gerovac et al. (2016) reported on a factorial experiment that investigated three PFR (B13:G0:R87:FR0, B8:G18:R74:FR0, and B9:G0:R84:FR7) and three TPFD levels (105, 210, and 315 μmol·m⁻²·s⁻¹), all with 16 h photoperiod, on the production of kohlrabi, mizuna, and mustard microgreens. Their results showed FW increased up to 34% as LI increased from 105 to 315 μmol·m⁻²·s⁻¹, depending on genotype and PFR. However, mustard only had LI treatment effects under two of the PFR and kohlrabi showed no LI treatment effects, regardless of PFR. It should be noted that FW was reported on a per-plant basis, which
may not be a true reflection of LI treatment effects on yield. Since microgreens are typically grown as dense canopies (i.e., leaf area index \( \geq 1 \)), yield data would be more appropriately represented on a per unit area basis (e.g., g\( \cdot \)m\(^{-2} \)). Gerovac et al. (2016) also reported about a 25% increase in percent DW for kohlrabi and mustard as LI increased from 105 to 315 \( \mu \text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \), regardless of PFR. However, percent DW relates to plant water content at harvest, and is not definitively indicative of crop yield. Moreover, since actual DW data were not presented, the relationships between LI and biomass production cannot be readily ascertained from this study. Both Samuolenë et al., (2013) and Gerovac et al. (2016) found that hypocotyl length (HL) generally decreased with increasing LI, although results were inconsistent across all LI x genotype combinations. Gerovac et al. (2016) speculated that the presence of FR (700 to 800 nm) in one of their PFR treatments could have induced shade avoidance responses characteristic of growing environments with low PFR of red (R, 600 to 700 nm) to FR (R:FR) in some of the genotype x LI treatment combinations. However, shade avoidance responses are normally observed when plants are exposed to R:FR that are substantially lower than parity (Mah et al., 2018; Blom et al., 1995; Fletcher et al., 2005). Therefore, a shade avoidance response was highly unlikely in this growing environment, particularly since the shoot apical meristem of individual plants would not have been subject to substantial amounts of vegetated shade. While not a microgreens study, Potter et al. (1999) reported responses of canola (\textit{Brassica napus} L. ‘Westar’) seedlings to SS PPFD ranging from 25 to 500 \( \mu \text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \). Although the seedlings were grown on a per-cell basis in a germination tray (versus a dense microgreen canopy), the responses of height and biomass metrics are revelatory to how Brassicaceae microgreens respond to increasing LI. They found approximately a 2-times reduction in height and increase in aboveground biomass,
respectively, as LI increased from 150 to 500 $\mu$mol·m$^{-2}$·s$^{-1}$, when harvested 16 to 17 d after sowing. They attributed the decreases in HL at higher LI to decade-level decreases in concentrations of endogenous gibberellins.

In SS production environments, the use of higher LI to increase crop yields and quality must be balanced against the higher input costs of lighting infrastructure and energy, in order to maximize profit. From the aforementioned studies, there clearly exists a need for further clarity on how Brassicaceae microgreens respond to LED LI in SS production environments; particularly regarding economically-relevant production metrics such as HL, and fresh and dry yield per unit area. The objectives of this study were to investigate the influence of SS LED LI, ranging from 100 to 600 $\mu$mol·m$^{-2}$·s$^{-1}$ (with a 16 h photoperiod and PFR of B15:R85) on growth, yield, and quality of commercially-relevant Brassicaceae microgreens and develop mathematical models in order to describe these relationships.

2.2 METHODOLOGY

2.2.1. Growing Media and Seeding

Seedlings of kale (*Brassica napus* L. ‘Red Russian’), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby Streaks’) were grown in fibre trays (23.5 x 48.5 x 3.5 cm) for 10 to 11 d after sowing. The growing substrate was comprised of 30% peat, 30% compost, 30% coir, and 10% perlite by volume. The substrate analysis indicated that the macronutrient concentrations (mg·kg$^{-1}$(DW)) were: 1410 K, 1390 Ca, 329 P, 295 Mg, 220 S, and 42 N-NO$_3$; and the micronutrient concentrations (mg·kg$^{-1}$(DW)) were: 218 Na, 47 Fe, 10 Mn, 9.4 Zn, 0.7 B, and 0.6 Cu.
Trays were machine-filled with substrate and pre-seeded at Greenbelt Microgreens (Lynden, Ont., Canada) on the day each replicate was started. Seeding rates were the same as used in production at Greenbelt Microgreens. Per-tray seeding rates varied according to seed size and were: 8 g for kale (2.78 mg•seed⁻¹), 10 g for cabbage (2.84 mg•seed⁻¹), and 4 g for arugula (1.56 mg•seed⁻¹), and mustard (1.63 mg•seed⁻¹), resulting in seeding densities ranging from 2.15 to 3.09 seeds•cm⁻². Trays of microgreens were top-irrigated with well water as needed.

2.2.2. Growth Chamber Environment
The experiment was conducted at the University of Guelph (Ont., Canada), with sequential replications starting on 13 Oct., 14 Nov., and 12 Dec. (2017), respectively. A 29 m² walk-in growth chamber was divided into six 1.5 m² treatment plots, surrounded by opaque white vertical blinds to prevent light contamination between treatments, while still allowing sufficient airflow to maintain uniform temperature and humidity throughout the growth chamber. Each plot was divided into four subplots that were the same dimensions as a single tray. The four species were each randomly assigned to one of the subplots within each plot. Tray positions rotated clockwise daily, while maintaining a constant aspect, to enhance uniformity of crops’ exposure to light. The growing area for each light treatment was 0.46 m² and was centered directly below the lights.

2.2.3. Lighting Setup
LED arrays (LX601C, Heliospectra AB, Gothenburg, Sweden) were used to provide six PPFD treatments: 100, 200, 300, 400, 500, and 600 μmol•m⁻²•s⁻¹ (Table 2.1), measured at the level of the top of the substrate. These fixtures have four separately-controllable
spectrum channels: B (peak 445 nm), R (peak 660 nm), white (W, 5700K broad spectrum), and FR (peak 735 nm). Only the B and R channels were used in the present study, with the fixtures configured to provide a PFR of B15:R85. For each treatment, two LED arrays, spaced 36 cm apart (on-center, with the long sides of the fixtures parallel), were mounted 56.5 cm above the top of the substrate (measured from the bottom of the LED array). For the 600 μmol·m⁻²·s⁻¹ treatment, a third fixture was centered between the other two at the same elevation and aspect, which resulted in 1 cm gaps between the near sides of adjacent fixtures. LI and spectral quality were measured using a radiometrically-calibrated UV-VIS Flame spectrometer (Ocean Optics, Dunedin, Fla., USA) coupled to a 400 nm x 1.9 m patch cord with a CC3 cosine corrector and tethered to a laptop running the Spectrasuite software package. Spectral power distribution was converted to photon flux using the PARspec subroutine. Prior to commencing each replicate, LI and B:R were measured within 25 locations in each plot, on a 16.7 x 16.7 cm square grid, centered under the LED arrays (Table 1). Intensity and uniformity measurements were repeated after harvest to confirm that the average PPFD of B:R did not change more than ± 3%, within each plot, over the course of each respective replicate.

The LED arrays and growth chamber environmental control were set to the same daily 16-h light (L) / 8-h dark (D) photoperiod with L/D temperature setpoints of 21 °C / 17 °C and a constant 80% relative humidity (RH) setpoint. Three data loggers (HOBO U12-013; Onset Computer Corporation, Bourne, Mass., USA) were used to record air temperature and RH every 120 s. For each replicate, the loggers were positioned on the bench adjacent to the microgreen trays in three randomly-selected plots. Halfway through each trial, the logger locations were switched to the other three plots to provide plot-level
environmental data for each plot for half of the duration of each replication. This resulted in 18 unique data-collection events which, when the means for each event were averaged, resulted in L/D temperatures and RH (mean ± SD), of 21 ± 0.42 °C / 17 ± 0.31 °C and RH of 86 ± 1.9%. Supplemental CO₂ was not used in the experiment and the mean chamber CO₂ concentration (mean ± SD) during the daylight period was 470 ± 22 ppm.

2.2.4. Growth and Morphology Measurements
Growth and morphology measurements were taken 10 d after sowing (DAS) for kale and cabbage and 11 DAS for arugula and mustard. Five representative plants from each sub-plot were selected to determine HL, which was measured from the base of the hypocotyl to the shoot apical meristem using a ruler. Three sub-samples, each comprised of five representative plants from the remaining plants in each sub-plot, were collected to measure leaf area (LA) using a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln, Nebr., USA). If visible, true leaves were of insignificant size and therefore only cotyledons were used for LA measurements. To measure fresh and dry yield from each subplot, three additional representative samples (i.e., full plants and substrate) were collected using a cylindrical core sampler (76.4 cm²). All plants within each core were cut just above substrate level and combined to determine FW. Each sample was dried in an oven at 70 °C for 3 d to constant weight and DW were recorded.

2.2.5. Digital Image Analysis of Cotyledons
Five representative cotyledons (1 per plant from 5 plants) per sub-plot were scanned (CanoScan LiDE 25; Canon Inc., Tokyo, Japan) in JPEG format at 297 pixels per inch (PPI). ImageJ 1.42 software (https://imagej.nih.gov/ij/download.html) was used to
determine red, green, and blue (RGB) values for each image, which were then converted into hue and saturation values using the formulas outlined by Karcher and Richardson (2003). Hue angle (HA) refers to a position on a continuous circular scale (0° to 360°) while, saturation refers to the purity of a color with 0% and 100% representing gray and full saturation, respectively (Karcher and Richardson, 2003). A calibration curve was obtained by fitting a linear regression equation to hue angle values measured by scanning 12 color chips from the Munsell Color Charts for Plant Tissues (GretagMacbeth LLC, New Windsor, N.Y., USA) with varying hue angles, ranging from green to red (5Y 6/6, 2.5GY 6/6, 5GY 6/6, 7.5GY 6/6, 2.5G 6/6, 5G 6/6, 5Y 6/6, 2.5GY 6/6, 5GY 6/6, 7.5GY 6/6, 2.5G 6/6, and 5G 6/6). The present study’s hue angle values were corrected based on the resulting linear regression equation: \( y = 0.993x + 10.7 \) (\( r^2 = 0.996 \)) where \( y \) = actual hue angle of Munsell Color Chart for Plant Tissues color chips and \( x \) = hue angle quantified by digital image analysis.

2.2.6. Statistical Analysis

The experiment was a randomized complete block design with six (6) LI treatments, four (4) microgreen genotypes, and three (3) consecutive replications. Data were analyzed using R statistical software (RStudio 1.1.453; Auckland, New Zealand). HL, leaf area, HA, and seedling color saturation were individually analyzed using linear regressions whereas, FW and DW were individually analyzed using asymptotic light response curves (described below), with both the independent (i.e., PPFD and TLI) 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 \( y = a + be^{cx} \) (Delgado et al., 1993), where \( y, x, a, \) and \( e \) represent yield, LI (i.e., PPFD or TLI),
estimated maximum yield, and Euler’s number, respectively. The parameters for a, b, and c were derived through non-linear regressions. All regression analyses were evaluated at $P \leq 0.05$ level of statistical significance. The best-fit model regressions and equations are only presented for production and harvest indices with significant regressions. Normality of residuals (normally distributed residuals) and homoscedasticity of variances (variance around the regression is uniform for all values of the predictor variable, which is $x$) were confirmed by using the Shapiro-Wilk and Levene test, respectively.
Table 2. Mean PPFD from R and B light (and combined) delivered from SS LEDs to achieve a target light ratio (%) of R85:B15 with target PPFD levels of 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹. Mean values reported are the average of 25 spectral scans before each respective replicate.

<table>
<thead>
<tr>
<th>Target</th>
<th>Blue (400–500 nm)</th>
<th>Red (600–700 nm)</th>
<th>Total</th>
<th>DLI²</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>15.1 ± 0.8</td>
<td>83.5 ± 4.1</td>
<td>98.6 ± 4.9</td>
<td>5.8</td>
</tr>
<tr>
<td>200</td>
<td>30.4 ± 2.0</td>
<td>169 ± 10.6</td>
<td>199 ± 12.6</td>
<td>11.5</td>
</tr>
<tr>
<td>300</td>
<td>45.2 ± 2.5</td>
<td>255 ± 13.0</td>
<td>300 ± 15.5</td>
<td>17.3</td>
</tr>
<tr>
<td>400</td>
<td>60.5 ± 3.6</td>
<td>340 ± 18.8</td>
<td>401 ± 22.4</td>
<td>23</td>
</tr>
<tr>
<td>500</td>
<td>74.3 ± 5.2</td>
<td>427 ± 27.3</td>
<td>501 ± 24.2</td>
<td>28.9</td>
</tr>
<tr>
<td>600</td>
<td>90.5 ± 4.1</td>
<td>508 ± 22.7</td>
<td>599 ± 26.8</td>
<td>34.6</td>
</tr>
</tbody>
</table>

²PPFD = photosynthetic photon flux density (μmol·m⁻²·s⁻¹)

³The three rows within each target PPFD level represent PPFD means ± SD for the respective treatments (n=25) for replicate 1, 2, and, 3, respectively.

⁴DLI = daily light integral (mol·m⁻²·d⁻¹)
2.3 RESULTS

Results are discussed primarily on the basis of PPFD effects, in order to be most relatable to similar studies. However, since PPFD and TLI are proportional in this study (because LI and photoperiod are fixed levels), the models are also presented in terms of TLI (Fig. 2.1) and the respective models share a common r². It is worth mentioning that the growing period for arugula and mustard was 1-d (~9%) longer than cabbage and kale. Therefore, while the PPFD levels (on the x-axes) are the same for all genotypes, the TLI for cabbage and kale are smaller than for arugula and mustard. Therefore, with respect to PPFD, the time-weighted effects of LI on harvest metrics may be more pronounced between some genotypes than the harvest data indicated.

As LI increased from 100 to 600 μmol·m⁻²·s⁻¹, HL decreased linearly by 24%, 23%, 37%, and 62% for kale, cabbage, arugula, and mustard, respectively (Fig. 1A–D). There were no treatment effects on per-plant leaf area (Figs. 1E–H). Both FW and DW increased asymptotically as LI increased. As LI increased from 100 to 600 μmol·m⁻²·s⁻¹, FW increased by 36%, 56%, 76%, and 82% for kale, cabbage, arugula, and mustard, respectively (Fig. 1I–L). As LI increased from 100 to 600 μmol·m⁻²·s⁻¹, DW increased by 65%, 69%, 122%, and 145% for kale, cabbage, arugula, and mustard, respectively (Fig. 1M–P). As LI increased from 100 to 600 μmol·m⁻²·s⁻¹, hue angle decreased by 35, 19, 19, and 25° for kale, cabbage, arugula, and mustard, respectively (Fig. 1Q–T). Saturation of kale and arugula was not influenced by LI (Fig. 1U and 1W), however saturation of cabbage and mustard increased by 18% and 36%, respectively, as LI increased from 100 to 600 μmol·m⁻²·s⁻¹ (Fig. 1V and 1X).
Figure 2.1. Hypocotyl length (A–D), leaf area (E–H), fresh weight (I–L), dry weight (M–P), hue angle (Q–T), and saturation (U–X) of kale (Brassica napus L. ‘Red Russian’), cabbage (Brassica oleracea L.), arugula (Eruca sativa L.), and mustard (Brassica juncea L. ‘Ruby Streaks’) microgreens grown under PPFD levels of 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ delivered from SS LEDs with a B to R PFR of B15:R85. Data and
models are also presented in terms of TLI (mol·m⁻²), which is the integral of PPFD for the entire production period. All regression analyses were evaluated at \( P \leq 0.05 \) level of statistical significance. The best-fit model regressions and equations are only presented for production and harvest indices with significant regressions. Error bars indicate \( \pm \) SE of three separate experiments.

2.4 DISCUSSION

From a production standpoint, microgreens represent a unique commodity in that the input biomass (i.e., the seeds) and the harvested crop (i.e., aboveground biomass of young plant at or before the first true leaf stage) may not be that different. Unlike more mature plant organisms, embryos of germinating seeds rely solely on stored energy resources prior to the development and activation of the photosynthetic machinery. The transition from heterotrophic to autotrophic growth is a dynamic process that is influenced by a myriad of factors such as seed size, planting depth, temperature, and the availability of PAR. Although, there were no direct measurements made on photosynthesis or the balance of autotrophic vs. heterotrophic growth in this study, the modeled relationships between LI and harvest metrics (Fig. 1) clearly showed the autotrophic proclivity of the crops by the time of harvest.

Although an increased LI tends to be commonly associated with more compact growth (i.e., shorter internodes) in mature vegetative plant tissues (Burkholder, 1936; Butler, 1963; Yeh and Hsu, 2004; Zervoudakis et al., 2012), the influence of LI on HL of young seedlings has not been as well documented. Some studies on seedlings of floriculture and vegetable crops have presented mixed results on the effects of LI on HL
(Craver et al., 2018; Craver et al., 2019; Hernández and Kubota, 2014; Poel and Runkle, 2017; Pramuk and Runkle, 2005; Randall and Lopez, 2015), usually with either reductions or no change in HL with increasing LI. Seedlings of *Brassica* genotypes all tend to grow shorter under higher LI (Gerovac et al., 2016; Potter et al., 1999; Samuolienė et al., 2013), although the magnitude of the effects may be influenced by genotype, seed size, seeding density, and the time between sowing and harvest. When harvested between 10 and 15 days after sowing (DAS), Gerovac et al. (2016) reported reductions in HL of up to 30% in kohlrabi, mizuna, and mustard microgreens (seeding rate between 12 and 20 mg·cm⁻²) as LI increased from 105 to 315 μmol·m⁻²·s⁻¹, under most genotype-by-PFR combinations. Samuolienė et al. (2013) reported reductions in HL ranging between 9% and 28% in 10-d old mustard, red pak choi, tatsoi, and kohlrabi microgreens (sown at between 5 and 10 mg·cm⁻²) when grown at 545 vs. 110 μmol·m⁻²·s⁻¹. By contrast, Potter et al. (1999) showed approximately 3-fold shorter HL, both 7 and 14 DAS, in canola (*Brassica napus* L. ‘Westar’) seedlings grown (one seedling per cell) at 500 vs. 150 μmol·m⁻²·s⁻¹. Given these varying responses of similar genotypes to LI under different planting densities, plant responses to vegetated shade may be antagonistic to the full phenotypic expression of LI-induced reductions in HL; such as in the production of endogenous gibberellins (Potter et al., 1999). The differences between genotypes in their LI effects on HL may have also been influenced by the availability of stored energy resources (i.e., size of seed). To illustrate this, kale and cabbage, the two tallest genotypes (for a given LI) in the present study, had approximately twice the seed size and also had higher seeding densities than arugula and mustard. High LI-mediated reductions in HL could have important ramifications for microgreen production as shorter HL may increase overall crop robustness and lengthen postharvest shelf life; however
shorter HL can also increase difficulty of harvesting, particularly if harvesting is done by machine. Depending on the importance of HL at harvest, relative to other production metrics (e.g., yield), it may be possible to counteract the LI-induced reductions in HL by using targeted light spectrum treatments such as a reduced PFR of R to FR light (R:FR) (Mah et al., 2018; Blom et al., 1995; Hisamtsu et al., 2008; Fletcher et al., 2005) or monochromatic B light treatments (Hata et al., 2013; Hernández and Kubota, 2016; Kim et al., 2014; Kong et al., 2018) to stretch the plants.

It can be argued that the most important harvest metric for commercial microgreen production is fresh yield (i.e., kg(FW)·m⁻²), as microgreens are typically sold on a ‘per-FW’ basis. In this study, all genotypes in the present study showed asymptotic trends of increasing FW with increasing LI. Kale and cabbage had higher yield at a given LI than arugula and mustard, which may have been partly due to their higher seed sizes and seeding densities. The FW data (on a per plant basis) reported by Gerovac et al. (2016) only showed treatment effects on some genotype x PFR combinations. There were no LI treatment effects in kohlrabi or mizuna regardless of PFR and 19% and 34% increase in FW in mustard for the PFRs with 7% FR and 18% green (G, 500 to 600 nm), respectively, as LI increased from 105 to 315 μmol·m⁻²·s⁻¹. Their FW results for mizuna do not agree with their text (15% increase in FW regardless of PFR) and graphical results (no difference in FW), as LI increased from 105 to 315 μmol·m⁻²·s⁻¹. Based on the asymptotic models of FW response to LI in the present study, the crops in Gerovac et al. (2016) should have been well below the saturation point on their respective light response curves, and thus readily responded to increasing LI with increasing biomass production. However, it is still not apparent how increasing LI by a factor of 3 (i.e., increasing DLI
from 6 to 18 mol·m⁻²·d⁻¹) did not have substantial LI treatment effects on fresh yield for many genotype x PFR combinations in the Gerovac et al. (2016) study. This may indicate that other (unknown) factors related to growing environment or crop husbandry may have been limiting plant growth and yield in their experiments. Notably, kohlrabi, which was the genotype that showed the lowest FW response to increasing LI, also had the largest seeds (i.e., about 2-times higher mass per seed than mizuna and mustard) based on current information from their seed supplier (Johnny’s Selected Seeds, Winslow, ME). Unfortunately, the Samuolienė et al. (2013) paper did not present any FW data for their crops. Both Gerovac et al. (2016) and Samuolienė et al. (2013) presented ‘percent DW’ data, but this metric was essentially a measure of water content at the time of harvest and did not relate directly to biomass production. The trend of increasing percent DW (i.e., decreasing water content) with increasing LI in the present study (data not directly shown) were similar to the trends reported by both Gerovac et al. (2016) and Samuolienė et al. (2013). These observations may be indicative of inherently reduced water content in plants grown under higher LI or may be an artifact of inadequate irrigation strategies in some studies, perhaps related to disparate water demand of plants grown under large LI ranges.

DW yield data, though less directly applicable to commercial production goals than FW, is the most widely used metric in academia for assessing treatment effects on biomass accumulation, particularly in foliar tissues. In the present study, DW followed similar trends as FW. Overall, a 6-times increase in LI resulted in a 1.6 to 2.5-times increase in (aboveground) DW production. When an asymptotic model was applied to the aboveground DW data presented in Potter et al. (1999), there was approximately a 3-
times increase in canola yield over the same range of LI (model extrapolated to 600 μmol·m⁻²·s⁻¹). However, since their plants were harvested 17 DAS, the TLI was >35% higher than the TLI in the present study (for a given PPFD); therefore, over a comparable TLI their increase in aboveground DW would be closer to 2-times. This comparison exemplifies why extreme caution is required when relating results of different LI trials on the basis of PPFD or (to a lesser-extent) DLI. Furthermore, although it has been said that 1% more light should result in a concomitant 0.5% to 1% increase in crop yields (Marcelis et al., 2006), this is unlikely to be achieved for microgreens production as growing cycles are short and seed storage likely provides a disproportionate contribution to the total harvest biomass. The net gains in DW were 47.0 g·m⁻², 45.1 g·m⁻², 63.7 g·m⁻², and 64.7 g·m⁻² for kale, cabbage, arugula, and mustard, respectively, as LI increased from 100 to 600 μmol·m⁻²·s⁻¹. When considering these gains in DW along with the initial seed (input) mass of the different genotypes, it would appear that seedlings of smaller-seeded genotypes, such as arugula and mustard, may have considerably higher phenotypic plasticity for biomass accumulation responses to increasing LI, even when accounting for their higher DAS (i.e., higher TLI). This may be an important consideration for growers to balance when considering the various input costs associated with the two main drivers of harvestable biomass accumulation in microgreens production: initial seed resources and photosynthetic light inputs.

There were no LI treatment effects on leaf area for any of the genotypes. Although the relative biomass allocation to hypocotyl and leaf tissues were not assessed in this study, it is likely that higher LI treatments produced plants with greater leaf thickness, since this trend has been observed in other species and production scenarios.
Further, since tissue water potential and morphological factors that can influence the rate of postharvest water loss may also have a substantial influence on shelf life, more research is needed to elucidate how varying LI affects leaf morphology (particularly leaf thickness and stomatal densities) and relate these metrics to shelf life.

The general decreases in hue angle observed in the cotyledons of all genotypes were indicative of increases in the proportions of yellow and red pigments in the cotyledons of kale, cabbage, and arugula (all green colored plants), and purple (likely associated with anthocyanins) in the cotyledons of mustard (reddish-purple colored). Only mustard cotyledons had LI treatment effects on saturation (i.e., purity of color relative to grey), with increasing saturation at higher LI. These observations may be indicative of increased concentrations of carotenoids and anthocyanins at higher LI. Carotenoids in the chloroplast play an essential photoprotective role in reducing photooxidative damage to the photosynthetic apparatus caused by excess LI (Krinsky, 1979). Chloroplast-specific carotenoids are synthesized in response to light to protect against photoinhibition (Bou-Torrent et al., 2015; Llorente et al., 2017; Toledo-Ortiz et al., 2010). Anthocyanins have also been shown to screen photosynthetic tissues from high LIs (Smillie and Hetherington, 1999) and increased anthocyanin synthesis has been shown to occur under high LI (Krol et al., 1995; Mancinelli, 1983). It is also likely that higher LI reduced the quality of the visual appearance of Brassicaceae microgreens; human perception of plant color was beyond the scope of this study. Since plant color is the most important visual indicator of plant quality and health (Barrett et al., 2010), it is
possible that deleterious effects of higher LI on the visual appearance of Brassicaceae microgreens may somewhat offset any increases in yield.

Overall, the magnitude of the morphological and yield responses expressed by the four genotypes indicated that arugula and mustard exhibited greater levels of phenotypic plasticity to LI than kale and cabbage. This may have important production implications as growers may wish to assign different levels of lighting infrastructure and energy budget to growing commodities that exhibit greater yield responses to increasing LI. Photoperiod and spectrum were kept at fixed levels, with varying PPFD levels in the present study. Lighting at lower intensities for longer photoperiods (up to and including continuous lighting) and gradually increasing the LI as the crop matures (either using dimmers or by moving the plants between zones with different LI) are other common methods for reducing costs associated with crop lighting. In some cases, crops are even kept in the dark until germination is complete and the cotyledons have unfolded. These additional strategies also deserve attention in the scientific community. As more data becomes available, expression of lighting levels in terms of TLI will permit more relevant comparisons between studies of similar crops grown in different production scenarios.
2.4.1. Conclusion

Overall, the results of this study demonstrated how SS LI can affect the interplay between the most relevant factors of growth, yield and quality for several economically-important genotypes of Brassicaceae microgreens. Indoor growers of Brassicaceae microgreens can use these results, especially the light response curves for yield, to help determine the economic optimum LI to target for their production systems, genotypes and production goals.
2.5 LITERATURE CITED


enhances responsiveness to gibberellins and promotes stem extension in chrysanthemum. J. Biotechnol. 83:695–700.


Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience. 50:705–713.


CHAPTER THREE
INTENSITY OF SOLE-SOURCE LIGHT-EMITTING DIODES AFFECTS PHYTOCHEMICAL CONTENT OF BRASSICACEAE MICROGREENS

Additional index words. PPFD, microgreens, light intensity, controlled environment, kale, cabbage, arugula, mustard

ABSTRACT
It is desirable for growers to have the ability to influence phytochemical and nitrate contents of microgreen genotypes by manipulating LI from LEDs. However, research regarding the effects of SS LED LI on the desirable phytochemical and undesirable nitrate content in Brassicaceae microgreens is limited, variable and may be genotypic-specific. This study examined the effects of varying SS LED LI on the phytochemical and nitrate contents of organically grown kale (Brassica napus L. ‘Red Russian’), cabbage (Brassica oleracea L.), arugula (Eruca sativa L.), and mustard (Brassica juncea L. ‘Ruby Streaks’) microgreens in a walk-in growth chamber. SS LEDs were used to provide six PPFD treatments: 100, 200, 300, 400, 500, and 600 μmol·m²·s⁻¹ with a PFR of B15:R85 and 16-h photoperiod. Phytochemical measurements included ascorbate metabolites, total carotenoids, total chlorophyll and its individual components, and total phenolics and anthocyanins. The total and reduced ascorbate and total anthocyanins of all genotypes increased as LI increased from 100 to 600 μmol·m²·s⁻¹. Total phenolic content of kale and arugula increased as LI increased. Conversely, the levels of total chlorophyll and its individual components in cabbage and arugula, and nitrate content of mustard generally decreased as LI increased. Growers can adapt the SS light regimens,
including LI, from this study to influence the phytochemical and nitrate contents of all microgreen genotypes and mustard microgreens, respectively, based on desired preferences; producing Brassicaceae microgreens with potential added value compared to natural grown Brassicaceae microgreens.

3.1 INTRODUCTION

Microgreens are a novel specialty crop. The term microgreens is a broad description for the immature aerial tissues of vegetables belonging to several families of plants, including those of the Amaranthaceae, Apiaceae, Asteraceae and Brassicaceae (Kyraciou et al., 2016). Typically, microgreens are harvested once the cotyledons have fully expanded and after the appearance of the first true leaves. They have become a culinary trend due to their vibrant colors, unique textures and intense flavors. Microgreens are considered “functional foods” (foods containing health promoting or disease preventing additives) as several genotypes have high phytochemical content (Xiao et al., 2012); there is also evidence that these levels can be higher than their mature counterparts (Pinto et al., 2015). The consumption of phytochemicals from fruits and vegetables is associated with the reduced risk of a number of chronic diseases including cardiovascular diseases and cancer (van Horn et al., 2008; Virgili and Marino, 2008). Epidemiological evidence suggests that these health benefits are due to their antioxidant activity (Podségdek, 2007). Brassicaceae crops possess a complex mixture of phytochemicals, including those with high antioxidant activity, such as vitamin C (i.e., ascorbate), carotenoids and phenolic compounds (e.g., anthocyanins) (Martinez-Sanchez et al., 2008). In addition to their antioxidant effects, these phytochemicals have other benefits for human health. For example, vitamin C is essential to maintain overall health, as it is needed for many
biological functions (e.g., collagen and carnitine biosynthesis) (Davey et al., 2000; Naidu 2003). Carotenoids are vitamin A precursors in the human diet, which is required for vision, differentiation of epithelial cells, maintenance of cell function for growth, protection of immune system and reproduction (Mayne, 1996; Rao and Rao, 2007).

Phenolic compounds possess anti-inflammatory and antimicrobial effects in humans (Cartea et al., 2011; Ozcan et al., 2014). Moreover, anthocyanins are phenolics that function as pigments in fruits and flowers, thus serving as attractants for pollinators and seed dispersers. Anthocyanin accumulate in leaves of mature Arabidopsis thaliana L. plants (a member of the Brassicaceae) subjected to soil nutrient deficiency and high light abiotic stress (Lillo et al., 2008). In humans, anthocyanins improve visual and brain function (Tsuda, 2011).

Leafy vegetables, including members of the Brassicaceae, are an important component of the human diet, but are also known to accumulate nitrate (Santamaria, 2006). The average nitrate content of leafy vegetables ranges from 0.14 to 4.7 mg·g⁻¹ (Alexander et al., 2008). Nitrate itself is generally non-toxic (Mensinga et al., 2003), but is converted to nitrite in the human body which can pose serious health risks (Pannala et al., 2003; Spiegelhalder et al., 1976). In the USA, the maximum recommended amount of nitrate consumption is approximately 7.0 mg·kg⁻¹ bodymass per day (Mensinga et al., 2003), whereas in Europe this is set at 3.7 mg·kg⁻¹ bodymass per day (Joint F. A. O. WHO Expert Committee on Food Additives, 2002; Scientific Committee on Food, 1997). Therefore, a reduction in nitrate levels within Brassicaceae vegetables that are already rich in phytochemicals is highly desirable for consumers.
Plant growth, morphology, and phytochemical content can be influenced by the quality of the light under which they are grown, both within and beyond the photosynthetic active radiation (PAR) spectrum, (Goto, 2012). Plant photoreceptors have been identified that respond to wavelengths of ultraviolet (UV, <300 nm) and FR (700 to 800 nm) radiation, both of which have been shown to induce photomorphogenic and biochemical responses in some genotypes (Casal and Smith, 1989; Chen and Chory, 2011; Demotes-Mainard et al., 2016; Rizzini et al., 2011; Christie et al., 2012). Within the PAR spectrum, R (600 to 700 nm) and B (400 to 500 nm) light have higher quantum yield than G (500 to 600 nm) in 22 plant genotypes (McCree, 1972). Unlike monochromatic R or B light, combinations of R and B light increase productivity in cucumber (Hogewoning et al., 2010), lettuce (Wang et al., 2016; Yorio et al., 2001), radish and spinach (Yorio et al., 2001), pepper (Brown et al., 1995), and rice (Matsuda et al., 2004; Ohashi-kaneko et al., 2006). Thus, the possibility remains that R and B combinations may be necessary in SS environments for normal growth and development of many commodities. It is for these reasons that many horticultural LED fixtures are largely comprised of R and B wavelengths, including the systems commonly used for research on the SS production of microgreens (Craver et al., 2017; Gerovac et al., 2016; Kopsell et al., 2014; Samuolienë et al., 2013). Typically, horticultural LED studies include a higher proportion of R than B light. A major challenge with interpreting the results of SL and SS LED lighting research is the diversity in the PFRs of B and R (B:R) used, both among LED treatments and between different trials (Ouzounis et al., 2015). B:R commonly range from B25:R75 to B5:R95 in relevant LED lighting literature; therefore B15:R85 was chosen for the present study to ensure relevant comparisons to most other studies.
LI influences the accumulation of phytochemicals and nitrate in plants. It has been demonstrated that LI can affect the phytochemical content of lettuce (Oh et al., 2009; Zhou et al., 2011), mustard (Makus and Lester, 2002), kale (de Azevedo and Rodriguez-Amaya, 2005; Lefsrud et al., 2006) and spinach (Lefsrud et al., 2006; Li et al., 2009). Typically, carotenoid levels were reduced in various genotypes with an increase in LI, although the magnitude of the effect appears to be influenced by genotype. In general, exposure to a lower LI compared to a higher LI is associated with nitrate accumulation in some leafy vegetables (Chadjaa et al., 1999; Fallovo et al., 2011; Gaudreau et al., 1995; Proietti et al., 2004; Trejo-Téllez et al., 2019), although, some studies have reported that nitrate accumulation increases as LI increases (Fallovo et al., 2009; Fallovo et al., 2011) or is unchanged (Cantliffe, 1972; Parks et al., 2008). Recent research has investigated the relationship between LI and phytochemical content, including nitrate, in the Brassica microgreens kohlrabi, mizuna, mustard, red pak choi and tatsoi (Brazaityte et al. 2015; Craver et al., 2017; Samuolienė et al., 2013). For example, Craver et al. (2017) found total carotenoid content of mustard (Brassica juncea ‘Garnet Giant’) was reduced as LI increased from 105 to 330 μmol·m⁻²·s⁻¹, regardless of PFR, whereas total chlorophyll content decreased as LI increased with most PFRs. By contrast, increases in chlorophyll content index (Samuolienė et al., 2013) and total carotenoid content (Brazaityte et al., 2015) of mustard (Brassica juncea L. ‘Red Lion’) were evident with an increase in LI. Furthermore, total chlorophyll content of kohlrabi (Brassica oleracea var. gongylodes) decreased with LI up to 220 μmol·m⁻²·s⁻¹ under B8:G18:R74:FR0, whereas the levels of total carotenoids and total phenolics were unaffected, regardless of PFR, and total anthocyanin levels increased with augmented LI under all PFR (Craver et al., 2017). Conversely, Samuolienė et al. (2013) demonstrated LI had no effect on the chlorophyll
content index, total phenolics and anthocyanins in kohlrabi (*Brassica oleracea* var. gongylodes). From the aforementioned studies, research available regarding the effects of LI on the desirable phytochemical and undesirable nitrate content in leafy vegetables and Brassicaceae microgreens is variable and may be genotypic-specific. To date, there is not much known on the phytochemical and nitrate accumulation in kale, cabbage, arugula and mustard microgreen genotypes in response to LI and grown using organic substrate and fertilizer.

In this study, we tested the hypothesis that SS LED LI affects the phytochemical and nitrate contents of Brassicaceae microgreens in a genotypic manner. The objectives of this study were to investigate the influence of SS LED (B15:R85) LI, ranging from 100 to 600 μmol·m⁻²·s⁻¹ on the levels of ascorbate metabolites, total carotenoids, total chlorophyll and its individual components, total phenolics, total anthocyanins, and nitrate content of four economically-important and organically grown Brassicaceae microgreens.

### 3.2 METHODOLOGY

#### 3.2.1. Growing Condition and Lighting Setup

Frozen (-80 °C) microgreen tissues from the previous LI experiment (see Chapter 2) were used to perform phytochemical and nitrate analysis in this study. The growing media and seeding, growth chamber environment, and lighting setup were described in section 2.2.1., 2.2.2., and 2.2.3., respectively. Briefly, seeds of kale (*Brassica napus* L. ‘Red Russian’), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby Streaks’) were sown on organic substrate under an LED PFR of B15:R85 at a fixed LI. Microgreens were cultivated in a walk-in growth chamber set to 21 °C day (16 h)
and 17 °C night temperature (8 h), and a constant RH of 80%. The growth chamber was sub-divided into 6 sub-plots of varying PPFD treatments of 100, 200, 300, 400, 500 and 600 μmol·m⁻²·s⁻¹, with each sub-plot containing one tray of each microgreen genotype. The aforementioned experiment was performed in triplicate.

3.2. Sample Preparation and Storage

For each microgreen genotype and each LI treatment, aerial tissues (~5 g fresh weight) were collected 10 d after sowing for kale and cabbage and 11 d after sowing for arugula and mustard from each of three sub-samples, flash frozen in liquid N₂ and ground into a fine powder using an ice-cold mortar and pestle. The flash frozen microgreen powders were stored at -80 °C for up to 8 months prior to phytochemical and nitrate extraction (as described under sections 3.2.3 to 3.2.8).

3.2.3. Ascorbate Metabolite Assays

For all four microgreen genotypes, subsamples of each light treatment replicate were assessed for the levels of ascorbate (total and reduced) and dehydroascorbate according to an established method by Flaherty et al. (2018) with minor modifications. For each LI treatment replicate subsample, 200 mg of cryogenic microgreen powder was mixed with 500 µL of 6% (w/v) meta-phosphoric acid and homogenized with a pre-chilled mortar and pestle and 100 mg of silica sand to facilitate grinding. The homogenate was transferred to a pre-chilled 1.5 mL microfuge tube, and centrifuged at 13,000 g for 10 min at 4 °C. The clarified supernatant was passed through a 0.45 μm polytetrafluoroethylene (PTFE) filter (PTFE Syringe filters 0.45 μm/13 mm; Mandel Scientific Inc, Guelph, Ontario, Canada) prior to analysis with HPLC (1200 series;
Agilent Technologies, Palo Alto, CA, USA). For dehydroascorbate analysis, 50 µL of the filtered extract was combined with 25 µL of 2 M Tris base containing 400 mM dithiothreitol (DTT) in a 1.5 mL microfuge tube and incubated for 15 min at room temperature. The chemical reduction was terminated by the addition of 25 µL 8.5% (v/v) ortho-phosphoric acid.

Ascorbate extracts treated with and without DTT (1 µL each) were separately analyzed by injection on a Restek Ultra Aqueous C18 column (150 x 4.6 mm, 5 µm particle; Chromatographics Specialties Inc, ON, Canada) attached to an HLPC and maintained at a constant temperature of 20 °C. For both extracts, ascorbate was eluted isocratically using 20 mM ortho-phosphoric acid at a flow rate of 1.0 mL·min⁻¹ over 10 min and detected with a diode array detector at 254 nm. The total ascorbate in the DTT-treated extract was compared to an authentic range of ascorbate standard, whereas reduced ascorbate levels were assessed in the non-DTT treated extract. Dehydroascorbate was calculated by subtracting the reduced ascorbate in the non-DTT treated extract from that in the DTT-treated extract. For each genotype and LI treatment, ascorbate metabolites were corrected using a spike-and-recovery assessment (a known amount of ascorbate is added to a representative duplicate subsample).

3.2.4. Chlorophyll and Carotenoid Determination

For all four microgreen genotypes and their LI treatment replicate subsamples, the levels of chlorophyll and carotenoid pigments, and total phenolic content were assessed from frozen (-80 °C) tissues following two separate methanolic extractions. Briefly, for each
LI treatment replicate subsample, a 200 mg portion of the frozen microgreen powder was resuspended with 1 mL of ice-cold methanol and vortexed for 1 min. The homogenate was returned to ice for 5 min, vortexed for 1 min, centrifuged at 4 °C for 5 min at 21,000 g, and the supernatant was collected in a 2 mL microfuge tube. For each methanolic extract, chlorophyll \( a \), chlorophyll \( b \) and carotenoid pigments, absorbance was measured spectrophotometrically (SpectraMax 384 Plus Microplate Reader; Molecular Devices, Sunnyvale, CA, USA) at 665, 652 and 476 nm and final tissue concentrations were calculated using equations as described by Lichtenthaler and Buschmann (2001).

3.2.5. Total Phenolic Content Determination

For each LI treatment replicate subsample, a 20 mg portion of the frozen tissue powder was resuspended with 1 mL of ice-cold methanol and total phenolics were extracted using the conditions outlined under section 3.2.4. Duplicate aliquots (25 μL) of the supernatant were each transferred to a separate well within a 96-well microplate, and then mixed with 125 μL of 10% (v/v) Folin-Ciocalteau (FC) reagent. This was followed by the addition of 125 μL of 7.5% (w/v) \( \text{Na}_2\text{CO}_3 \) and incubation at room temperature for 10 min. Absorbance was measured spectrophotometrically at 765 nm and compared to a known range of gallic acid and expressed as gallic acid equivalents.

3.2.6. Total Anthocyanin Determination

Total anthocyanins were extracted using acidified methanol as described by Roepke and Bozzo (2015). For each of the four microgreen genotypes, a 100 mg portion of frozen powder from each LI treatment replicate subsample was resuspended in 500 μL of 9:1:10 methanol:acetic acid:MilliQ water in a 1.5 mL microfuge tube, vortexed for 1 min, and
then agitated on an orbital shaker (Adams™ Nutator; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 20 min prior to centrifugation at 16,000 g for 10 min at room temperature. The pellet was re-extracted twice as described above, and the supernatants were pooled and partitioned against 1.5 mL of chloroform. For the partitioning step, the mixture was vortexed for 1 min and then the phases were separated in a clinical centrifuge at 2,500 g for 2 min. The acidified methanol phase was collected into a 1.5 mL microfuge tube and dried under a vacuum (Savant SC100 Speed Vac Concentrator with RH40-12 Rotor; Savant Instruments Inc., Farmingdale, NY, USA). The dried anthocyanin residue was resuspended in 200 μL of methanol containing 0.1% (v/v) HCl. Absorbance was monitored at 530 nm with a spectrophotometer and expressed as cyanidin 3-glucoside equivalents using a molar absorptivity ($\varepsilon$) of 34,300 L·mol$^{-1}$·cm$^{-1}$ (Siegelman and Hendricks, 1958).

3.2.7. Nitrate Analysis

Nitrate was extracted from cryogenic frozen powders of all four microgreens using a procedure by Hachiya and Okamoto (2017). For each LI treatment replicate subsample, a 50 mg portion of the frozen powder was resuspended with 500 μL of MilliQ water, incubated at 100 °C for 20 min and then centrifuged at 20,400 g for 10 min at room temperature. Afterward, 10 μL of the supernatant was mixed with 40 uL of 0.05% (w/v) salicylic acid in sulfuric acid in a 2 mL microfuge tube and incubated at room temperature for 20 min. To determine non-specific background, the incubation was performed in the absence of salicylic acid. In either case, 1 mL of 8% (w/v) NaOH was added at the end of the incubation period, and absorbance was measured at 410 nm with a spectrophotometer and compared to an authentic range of nitrate standard.
3.2.8. Statistical Analysis

The experiment was a randomized complete block design with 6 LI treatments, 4 genotypes, and 3 consecutive replications. Data were analyzed using R statistical software (RStudio 1.1.453; Auckland, New Zealand) with treatment effects determined by one-way analysis of variance (ANOVA). Means separations were performed using Tukey’s Honestly Significant Difference (HSD) test at $P \leq 0.05$. Normality of residuals (normally distributed residuals) and homoscedasticity of variances (variance around the regression is uniform for all values of the predictor variable, which is $x$) were confirmed by using the Shapiro-Wilk and Levene test, respectively. Outliers were detected and removed in nitrate content data using Lund’s Test of Studentized Residuals.
3.3 RESULTS

3.3.1. Effect of LI on Ascorbate Metabolites in Microgreens

Total ascorbate increased with increasing LI (Figs. 3.1A–D). Total ascorbate levels in kale and mustard were respectively 94% and 71% higher at a LI of 500 than at 100 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \), but no further increase in these levels were apparent at the highest LI (Figs. 3.1A and D). Total ascorbate levels in cabbage were 75% greater at 600 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \) than at the lowest LI used in this study (Fig. 3.1B). Total ascorbate in arugula was 65% greater at 400 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \) than at 100 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \), with no additional increases at the two highest LIs (Fig. 3.1C).

Overall, the levels of the reduced form of ascorbate increased with an increase in LI (Figs. 3.1E–H). The content of reduced ascorbate in kale and cabbage were 105% and 54% higher, respectively, at LI of 500 than at 100 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \), and no further increase in these metabolite levels were apparent at 600 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \) (Figs. 3.1E and F). Reduced ascorbate levels of arugula and mustard were 64% and 58% higher, respectively, as LI increased from 100 to 400 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \), with no additional increases at the two highest LIs (Figs. 3.1G and H).

There was no effect of LI on dehydroascorbate content in kale, cabbage and arugula (ranging between 0.017 to 0.106 mg·g FW\(^{-1}\)) (Figs. 3.1I–L). Dehydroascorbate of kale, cabbage and arugula represented 4.77%, 11.7%, and 5.04%, respectively, of the total ascorbate measured. Dehydroascorbate content of mustard grown under 500 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \) was 138% higher than the levels detected in plants cultivated under 200 \( \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \) (Fig. 3.1L).
Figure 3. 1. Total ascorbate (A–D), reduced ascorbate (E–H) and dehydroascorbate content (I–L) of kale (Brassica napus L. ‘Red Russian’), cabbage (Brassica oleracea L.), arugula (Eruca sativa L.), and mustard (Brassica juncea L. ‘Ruby Streaks’) microgreens grown under varying PPFD levels. Each microgreen genotype was cultivated under PPFDs 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ delivered from SS LEDs with a PFR of B15:R85. Data represent the mean ± SE of three separate experiments. For each microgreen genotype, means sharing the same letter are not statistically different by Tukey’s Honestly Significant Difference test at $P \leq 0.05$. 
3.3.2. Effect of LI on Carotenoid and Chlorophyll Contents in Microgreens

There was no effect of LI on chlorophyll \( a \), chlorophyll \( b \) and total chlorophyll in kale and mustard. Chlorophyll \( a \), chlorophyll \( b \) and total chlorophyll of cabbage decreased 24\%, 30\% and 25\%, respectively, when LI increased from 100 to 400 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), with no further changes in the levels of these pigments at LI of 500 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) or higher (Figs. 3.2B, F and J). Chlorophyll \( a \) and total chlorophyll of arugula decreased 22\% and 21\%, respectively, when LI increased from 100 to 400 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), with no further decreases at higher LIs (Figs. 3.2C and K). Chlorophyll \( b \) of arugula decreased 30\% as LI increased from 100 to 500 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \), with no further decreases at the highest LI (Fig. 3.2G). Overall, there was no effect of LI on carotenoid content in all microgreen genotypes (ranging between 0.077 to 0.126 \( \text{mg} \cdot \text{g FW}^{-1} \)) (Figs. 3.2M–P).
Figure 3.2 Chlorophyll a (A–D), chlorophyll b (E–H), total chlorophyll (I–L) and total carotenoid content (M–P) of kale (*Brassica napus* L. ‘Red Russian’), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby Streaks’) microgreens grown under varying PPFD levels. Each microgreen genotype was cultivated under PPFDs 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ delivered from SS LEDs with a PFR of B15:R85. Data represent the mean ± SE of three separate experiments. For each microgreen genotype, means sharing the same letter are not statistically different by Tukey’s Honestly Significant Difference test at \( P \leq 0.05 \).
3.3.3. Effect of LI on Total Phenolic and Anthocyanin Contents in Microgreens

There was no effect of LI on the total phenolic content in cabbage and mustard. The total phenolic content of kale was 62% higher at a LI of 500 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$ versus 100 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$, with no further increases at the highest LI (Fig. 3.3A). The total phenolic content of arugula increased 62% as LI increased from 100 to 600 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$ (Fig. 3.3C).

Overall, total anthocyanin levels increased as LI increased (Figs. 3.3E–H), but the responses were genotype-specific. The total anthocyanin levels of kale, arugula and mustard increased 788%, 361% and 162%, respectively, as LI increased from 100 to 600 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$ (Figs. 3.3E, G, and H). In cabbage, total anthocyanin levels were 147% higher at a LI of 500 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$ versus 100 $\mu$mol$\cdot$m$^{-2}\cdot$s$^{-1}$, with no further increases at the highest LI (Fig. 3.3F).
Figure 3. 3. Total phenolic (A–D) and total anthocyanin (E–H) content of kale (*Brassica napus* L. ‘Red Russian’), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby Streaks’) microgreens grown under varying PPFD levels. Each microgreen was cultivated under PPFDs 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ delivered from SS LEDs with a PFR of B15:R85. Total phenolic content is expressed as gallic acid equivalent, whereas total anthocyanins are expressed as cyanidin 3-glucoside equivalents. Data represent the mean ± SE of three separate experiments. For each microgreen genotype, means sharing the same letter are not statistically different by Tukey’s Honestly Significant Difference test at $P \leq 0.05$. 

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3.3.4. Nitrate Content

There was no effect of LI on nitrate content in kale, cabbage and arugula genotypes; nitrate levels ranged between 0.022 and 0.308 mg·g FW⁻¹ (Table 3.1). Nitrate content of mustard decreased 51% when LI increased from 100 to 300 μmol·m⁻²·s⁻¹, with no further decreases at higher LIs (Table 3.1).

Table 3.1. Nitrate content of kale (*Brassica napus* L. ‘Red Russian’), cabbage (*Brassica oleracea* L.), arugula (*Eruca sativa* L.), and mustard (*Brassica juncea* L. ‘Ruby Steaks’) microgreens grown under varying PPFD levels. Each microgreen was cultivated under PPFDs 100, 200, 300, 400, 500, and 600 μmol·m⁻²·s⁻¹ delivered from SS LEDs with a PFR of B15:R85.

<table>
<thead>
<tr>
<th>PPFD (μmol·m⁻²·s⁻¹)</th>
<th>Kale (mg·g⁻¹ FW⁻¹)</th>
<th>Cabbage (mg·g⁻¹ FW⁻¹)</th>
<th>Arugula (mg·g⁻¹ FW⁻¹)</th>
<th>Mustard (mg·g⁻¹ FW⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.051 ± 0.023 a</td>
<td>0.235 ± 0.101 a</td>
<td>0.308 ± 0.126 a</td>
<td>0.101 ± 0.007 a</td>
</tr>
<tr>
<td>200</td>
<td>0.048 ± 0.024 a</td>
<td>0.264 ± 0.113 a</td>
<td>0.177 ± 0.117 a</td>
<td>0.094 ± 0.006 a</td>
</tr>
<tr>
<td>300</td>
<td>0.039 ± 0.015 a</td>
<td>0.190 ± 0.046 a</td>
<td>0.085 ± 0.060 a</td>
<td>0.050 ± 0.008 b</td>
</tr>
<tr>
<td>400</td>
<td>0.043 ± 0.003 a</td>
<td>0.143 ± 0.036 a</td>
<td>0.103 ± 0.065 a</td>
<td>0.022 ± 0.002 b</td>
</tr>
<tr>
<td>500</td>
<td>0.038 ± 0.009 a</td>
<td>0.087 ± 0.032 a</td>
<td>0.080 ± 0.036 a</td>
<td>0.033 ± 0.006 b</td>
</tr>
<tr>
<td>600</td>
<td>0.021 ± 0.007 a</td>
<td>0.135 ± 0.090 a</td>
<td>0.041 ± 0.017 a</td>
<td>0.032 ± 0.005 b</td>
</tr>
</tbody>
</table>

²PPFD = photosynthetic photon flux density (μmol·m⁻²·s⁻¹)

³Data represent the mean ± SE of three separate experiments

⁴Treatment means sharing the same letter are not statistically different by Tukey’s Honestly Significant Difference test at $P \leq 0.05$

⁵FW= fresh weight
3.4 DISCUSSION

Increased ascorbate content in leaves is a hallmark response to increasing LI. Ascorbate content in microgreens is important for human health, as we lack the capacity to synthesize ascorbate and are fully reliant on edible plants for dietary sources (Davey et al., 2000; Naidu 2003). In this study, total and reduced ascorbate levels were increased by increasing LI, although the LI corresponding to the maximum ascorbate levels varied with genotype. Similarly, Makus and Lester (2002) reported ‘Tendergreen’ and ‘Florida Broadleaf’ mustard greens grown under full sunlight have 28% more ascorbate than plants grown under 50% sunlight. Although the light regimens in Samuolienė et al. (2013) were similar to those of this study, the ascorbate contents in red pak choi and tasoI were maximal in plants grown under 100 μmol·m⁻²·s⁻¹, whereas ascorbate content of kohlrabi decreased with increasing LI, and mustard ascorbate was unaffected by LI. The phytochemical composition in Brassicaceae microgreens varies with genotype (Xiao et al., 2019). Genetic differences in Brassicaceae microgreens could explain the variability in ascorbate content as a function of LI treatments between this study and previous research by Samuolienė et al. (2013). Ascorbate plays an important photoprotectant role in scavenging reactive oxygen species produced during photosynthesis and for the dissipation of excess light energy (Asada, 1999; Muller-Moule et al., 2002). Ascorbate synthesis can increase at higher LI in leaves; however, the light regulatory mechanisms of ascorbate synthesis remain unclear (Yabuta et al., 2007; Zhang et al., 2009). Thus, the increased accumulation of ascorbate in kale, cabbage, arugula and mustard microgreens at higher LI could represent a photoprotective mechanism against high LI.
Carotenoids are yellow, orange and red pigments present in certain fruit and vegetables (Rao and Rao, 2007), and in the chloroplasts they play an essential photoprotective role in reducing photooxidative damage to the photosynthetic apparatus caused by excess light (Krinsky, 1979). In this study, there were no LI treatment effects on total carotenoid content across all microgreen genotypes. Similarly, Craver et al. (2017) revealed there is no effect of LI on total carotenoid content in kohlrabi and mizuna, regardless of PFR. Conversely, scientific evidence showed that total carotenoid content increased as LI increased in some genotypes. Total carotenoid content of mustard, red pak choi and tasoï was maximal at a LI of 330 to 440 µmol·m⁻²·s⁻¹, but decreased at the highest LI (Samuolienė et al., 2013). Chloroplast-specific carotenoids are synthesized in response to light in order to minimize photoinhibition (Bou-Torrent et al., 2015; Llorente et al., 2017; Toledo-Ortiz et al., 2010). Previous research has also found that total carotenoid content decreased as LI increased in some microgreen genotypes. For example, Craver et al. (2017) found that total carotenoid content of mizuna grown under B9:G0:R84:FR7 was 22% less at 220 µmol·m⁻²·s⁻¹ than at 105 µmol·m⁻²·s⁻¹. Makus and Lester (2002) reported total carotenoid content of ‘Tendergreen’ and ‘Florida Broadleaf’ mustard greens grown under full sunlight was 31% less than plants subjected to 50% sunlight. Kopsell et al. (2012) found that total carotenoid content of ‘Florida Broadleaf’ mustard microgreens was 13% less at 463 µmol·m⁻²·s⁻¹ than at 275 µmol·m⁻²·s⁻¹. Under high LI, photodegradation of pigment molecules and dilution of total carotenoids can be linked to increases in plant mass (Lefsrud et al., 2006). It is tempting to speculate that a simultaneous action of carotenoid synthesis and a dilution and photodegradation of total carotenoids with an increase in plant mass (which was found in section 2.3.1) at higher
LIs may have resulted in no change in total carotenoid content in all four microgreen genotypes investigated in this study.

Chlorophyll content is closely associated with human perception of the green pigmentation of leaves (Barrett et al., 2010). Chlorophyll accumulation is also an important indicator of how plants respond to increasing LI. There was no impact of LI on the levels of chlorophyll \(a\), chlorophyll \(b\) and total chlorophyll content in kale and mustard, whereas all chlorophyll levels decreased as LI increased in cabbage and arugula. In the scientific literature, there has been much debate on the impact of LI on chlorophyll content. For example, relative chlorophyll content was 12% greater cultivated under 315 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\) relative to lower LIs (105 and 210 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\)) using a PFR combination of B8:G18:R74:FR0 (Gerovac et al., 2016). Similarly, the chlorophyll content index of mustard and red pak choi was higher with an increased LI, but LI had no effect on the same pigment index of tatsoi and kohlrabi (Samuolienë et al., 2013). Conversely, Kopsell et al. (2012) found that the respective content of total chlorophyll, chlorophyll \(a\), and chlorophyll \(b\) in mustard shoot tissues was 38%, 18%, and 28% lower at a LI of 463 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\) than at 275 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\). Likewise, Makus and Lester (2002) reported a 38% decrease in leaf total chlorophyll levels in two greenhouse grown cultivars of mustard under ambient light versus 50% shade. Similarly, Craver et al. (2017) revealed that LI affects total chlorophyll content but in a manner that is consistent with genotype x PFR combinations. For example, a 20% decrease in total chlorophyll levels was evident in kohlrabi grown under B8:G18:R74:FR0 when LI was increased from 105 to 220 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\); whereas a 22% to 34% decrease in total chlorophyll levels are evident in mizuna and mustard grown under varying PFRs as LI increased from 105 to 315 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\).
Genetic variation influences phytochemical synthesis and degradation in 
Brassicaceae microgreens (Xiao et al., 2019). Similarly, genetic variability may explain the different optimal LI for chlorophyll accumulation in the microgreens evaluated in our study. In this study, previously discussed photodegradation and dilution effect (Lefsrud et al., 2006) of total carotenoid content as LI increases may have also resulted in decreased chlorophyll content.

Phenolic compounds are plant secondary metabolites that include phenolic acids, flavonoids, stilbenes and tannins (Ozcan et al., 2014). In our study, total phenolic content of kale and mustard was respectively highest at 500 and 600 μmol·m⁻²·s⁻¹ relative to all other LIs, whereas there was no impact of LI on the total phenolic content in cabbage and arugula. The genotype related changes in total phenolic content as a function of LI are in agreement with previous research. Total phenolic content of ginger leaves was 20% to 23% greater at 790 μmol·m⁻²·s⁻¹ relative to plants cultivated at 310 μmol·m⁻²·s⁻¹ (Ghasemzadeh et al., 2010). Similarly, the total phenolic content of kohlrabi, tatsoi and red pak choi was greatest at a LI of 440 μmol·m⁻²·s⁻¹, whereas the total phenolic content in mustard spiked at 220 μmol·m⁻²·s⁻¹ relative to microgreens cultivated under 330 and 100 μmol·m⁻²·s⁻¹ (Samuolienė et al., 2013). Conversely, Craver et al. (2017) reported no impact of LI on total phenolic content in kohlrabi, regardless of PFR. The total phenolic compound composition can vary considerably between Brassica genotypes (Podsedek, 2007), possibly causing differences in total phenolic content of microgreens.

Anthocyanins are flavonoid pigments that are responsible for the red, blue and purple color of plant tissues (e.g., leaves, stems and fruit) (Cartea et al., 2011). In this
study, total anthocyanin levels increased proportionally with an increase in LI supplied during growth, regardless of microgreen genotypes, although the finite levels were generally greater in mustard as compared to cabbage and kale, and the smallest levels were apparent in arugula. The differences in total anthocyanin content of microgreens grown under a range of LIs could be a consequence of genetic variability, as the levels of other phytochemicals are impacted by genotype (Xiao et al., 2019). The effect of LI on total anthocyanin accumulation in Brassicaceae microgreens is inconsistent across the scientific literature. For example, Samuoliené et al. (2013) found that total anthocyanin levels of kohlrabi, tatsoi, and red pak choi were 27% to 55% greater at 330 or 440 μmol·m⁻²·s⁻¹ as compared to 220 μmol·m⁻²·s⁻¹. Additionally, total anthocyanin content of mustard was 29% higher under 110, 330, 440 and 545 μmol·m⁻²·s⁻¹ as compared to 220 μmol·m⁻²·s⁻¹. The total anthocyanin levels in kohlrabi microgreens grown under B13:G0:R87:FR0 and B8:G18:R74:FR0 were respectively, augmented 18% and 31% at 315 μmol·m⁻²·s⁻¹ relative to plants grown at 105 μmol·m⁻²·s⁻¹ (Craver et al., 2017).

Moreover, total anthocyanin levels of kohlrabi grown under B9:G0:R84:FR7 increased 14% as LI increased from 105 to 220 μmol·m⁻²·s⁻¹ (Craver et al., 2017). Anthocyanins can act to screen photosynthetic tissues from high LI in seed pods of the deciduous tree Bauhinia variegata L. (Smillie and Hetherington, 1999) and increased anthocyanin synthesis occurs under high LI in jack pine seedlings (Krol et al., 1995; Mancinelli, 1983). The accumulation of anthocyanins under high LI is associated with the upregulation of anthocyanin biosynthesis gene transcripts in Arabidopsis thaliana L. (Lillo et al., 2008). The possibility remains that increased total anthocyanin levels in kale, cabbage, arugula and mustard microgreens is under transcriptional control of anthocyanin biosynthesis.
genes and the accumulation of these phenolic metabolites may be indicative of increased photoprotection against high LI.

Low nitrate levels can add value to fruits and vegetables in the marketplace, specifically microgreens that are already popular for their high phytochemical content. Previous research has demonstrated that nitrate levels in kohlrabi, tatsoi, red pak choi and mustard microgreens cultivated under varying LI ranged between 0.05 and 0.38 mg·g FW\(^{-1}\) (Samuoliene et al., 2013); similar nitrate levels were evident in the microgreens analyzed in this study. Moreover, in this study mustard nitrate content decreased 51% as LI increased from 100 to 600 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\), whereas, there were no effects of LI on nitrate levels of all other microgreen genotypes. Samuoliene et al. (2013) demonstrated that nitrate content of mustard microgreens decreased 15% to minimum levels when LI increased from 220 to 330 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\) with no further decreases at higher LI. In a greenhouse environment, Trejo-Téllez et al. (2019) reported increasing LI from 385 to 516 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\) decreased nitrate content in mature mustard by 36%. In plants, nitrate is reduced to nitrite by nitrate reductase, which is activated by light and inhibited in the absence of light (Lillo, 1994). An increase in nitrate reductase activity in response to increasing LI would provide a rationale for the decrease in nitrate levels observed in mustard microgreens analyzed in this study. However, LI had little impact on nitrate levels in kale, cabbage, and arugula microgreens. This was consistent with the results of Trejo-Téllez et al. (2019) showing that nitrate levels in mature mizuna were little changed in response to increasing LI from 385 to 516 \(\mu\)mol·m\(^{-2}\)·s\(^{-1}\). Moreover, the accumulation of nitrate in Swiss chard shoots was similar under three LI (57%, 46% and 27% of outdoor ambient light) in both winter and spring (Parks et al., 2008). LI above 450 to 900
μmol·m⁻²·s⁻¹ had no effect on spinach leaf nitrate concentrations (Cantliffe, 1972). Together, these findings indicated that additional environmental factors influenced nitrate accumulation. The N supplied during cultivation is known to influence leaf nitrate concentration (Cantliffe 1972; Parks et al., 2008). Given these varying responses between similar genotypes in their LI effects on nitrate content, the possibility remains that nitrate uptake and accumulation in the plant may be dependent on genetic variability (Umar and Iqbal, 2007). More research is required to address the relationship between genotype, nitrogen supply and LI in order to produce microgreens with the lowest nitrate content.
3.4.1. Conclusion.

Herein we demonstrated that SS LI affected the phytochemical content of all microgreen genotypes, but only affected nitrate content in mustard microgreens. As LI increased from 100 to 600 μmol·m⁻²·s⁻¹ total and reduced ascorbate and total anthocyanins of all genotypes increased. Total phenolic content of kale and arugula increased as LI increased. In contrast, total chlorophyll and its individual components of cabbage and arugula, and nitrate content of mustard generally decreased as LI increased. Indoor commercial microgreen growers can use the results of this study to manipulate and control specific phytochemicals, by the LI used, possibly adding market value and utility. These results can also serve other researchers who investigate the effect of LI on phytochemical and nitrate contents of microgreens.
3.5 LITERATURE CITED


CHAPTER FOUR
INTENSITY OF SUPPLEMENTAL LIGHT-EMITTING DIODES AFFECTS MICROGREEN GROWTH AND YIELD DURING WINTER GREENHOUSE PRODUCTION IN HIGHER LATITUDE REGIONS

Additional index words. DLI, PPFD, controlled environment, sunflower, kale, arugula, mustard

ABSTRACT
In higher latitude regions, a major limiting factor for greenhouse production in the winter is low natural DLI. SL light is commonly used to maintain crop productivity and quality during these darker months, particularly when the supply chain demands consistent production levels year-round. What remains to be determined are the optimum SL LIs for winter production of different commodity production scenarios. 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’) microgreens grown in a greenhouse under SL LED PPFD levels ranging from 17 to 304 μmol·m⁻²·s⁻¹ with a 16 h photoperiod (i.e., DLI from 0.98 to 17.5 mol·m⁻²·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 8 DAS for sunflower, 11 DAS for kale, and 12 DAS for arugula and mustard resulting in mean natural DLIs of 6.50, 5.94 and 6.20 mol·m⁻²·d⁻¹, respectively. Leaf area and fresh weight increased with increasing DLI in all genotypes. Relative chlorophyll content, dry weight and robust index of kale, arugula and mustard increased with increasing DLI. Specific
leaf area of kale, arugula and mustard decreased as DLI increased. Hypocotyl diameter of
sunflower, arugula and mustard increased as DLI increased. Hypocotyl length of mustard
decreased with increasing DLI. Commercial microgreen greenhouse growers can use the
light response models described herein to make predictions of relevant production
measurements according to the available light levels during winter in higher latitude
regions; enabling growers to optimize the choice of the most appropriate SL LI to achieve
the desired production goals as economically as possible.

4.1 INTRODUCTION
Microgreens are an emerging commodity in worldwide markets; microgreens consist of
vegetable and herb seedlings that are harvested later than sprouts and earlier than baby
greens. Microgreen production is attractive to greenhouse and indoor growers due to
increasing demand and high value, with wholesale prices ranging from $30 to $50 USD
per pound (Treadwell et al., 2016). However, microgreens have a limited postharvest
shelf-life (Chandra et al., 2012; Kou et al., 2013), and thus local production may
represent a promising strategy for preservation of microgreen quality relative to those
shipped over large distances. However, low natural light level is a major limiting factor in
greenhouse plant production during winter months in higher latitude regions. During the
winter months (i.e., October through February), natural outdoor DLIs in southern Canada
and northern United States typically range between 5 and 15 mol·m⁻²·d⁻¹ (Both and Faust,
2017; Faust and Logan, 2018). However, only 30% to 60% of natural light in the outside
atmosphere reaches the crop within the greenhouse due to transmission losses through
greenhouse glazing materials and blockage from structural materials; during the winter,
this culminates in natural DLIs as low as 1.5 to 4.5 mol·m⁻²·d⁻¹ at the level of the crop
Nonetheless, contemporary consumers expect high quality fruit and vegetables year-round. Since low DLIs during winter months is generally associated with reductions in yield, high quality winter-grown produce can command a substantial price premium. Therefore, SL lighting has been commonly used for growing high-value commodities during the winter months (Dorais et al., 2017), such as microgreens.

HPS fixtures have been commonly used in greenhouses for SL lighting (Hemming, 2011; Ouzounis et al., 2015; Singh et al., 2015); however, within recent years LEDs have been increasingly used as an alternative to HPS fixtures due to their potential for comparatively high-energy efficiency and durability, and low radiant heat emissions directed towards the crop (Mitchell and Stutte, 2017; Zheng, 2016). In addition, the adjustable spectrum and intensity of LEDs offer a more attractive alternative for horticultural applications, despite their relatively high input cost (Llewellyn and Zheng, 2018a;b; Mitchell and Stutte, 2017). In greenhouse applications, dimmable LEDs enable the SL LI to be adjusted in response to fluctuating natural light levels. This reduces energy use as compared to static HPS technologies and helps to maintain a more uniform lighting environment throughout the whole photoperiod, minimizing light stress on crops. At comparable LIs, LED combinations comprised of B (400 to 500 nm) and R (600 to 700 nm) yield similar crop productivity as compared to HPS fixtures for lettuce (Martineau et al., 2012), tomato (Dueck et al., 2012), cucumber transplants (Hernández and Kubota, 2015), ornamentals cuttings (Currey and Lopez, 2013), seedlings (Poel and Runkle, 2017; Randall and Lopez, 2014) and cut flowers (Llewellyn et al., 2019). SS lighting differentially affects crop productivity in plants grown under varying light
spectrums (Gerovac et al., 2016; Hernández and Kubota, 2016; Hogewoning et al., 2010; Kim et al., 2004), whereas the SL lighting spectrum has no impact on crop productivity, at least during the natural photoperiod, possibly because the spectral effects of SL light are suppressed by the broad spectrum of the natural light.

DLI is important for the growth and yield of various greenhouse crops, as an increase in LI directly corresponds to increased photosynthetic rates. SL lighting tends to enhance shoot growth (i.e., shoot biomass and stem diameter) when natural DLI levels are low at juvenile stages of the following commodities: celery (Masson et al., 1991), lettuce (Cathey and Campbell, 1977; Masson et al., 1991), broccoli (Masson et al., 1991) tomato (Boivin et al., 1987; Canham, 1972; Masson et al., 1991; McCall, 1991; Newton, 1966), baby’s breath (Islam and Willumsen, 2001), petunia (Cathey and Campbell, 1977; Oh et al., 2010), and yellow trumpetbush (Torres and Lopez, 2011). Furthermore, an increase in shoot biomass and decreased HL are apparent with increasing DLI in Brassica microgreens (Gerovac et al., 2016; Samuolienė et al., 2013) and seedlings (Potter et al., 1999) grown under SS conditions. Previously, our lab investigated the influence of SS LED (B15:R85) DLI, ranging from 5.76 to 34.6 mol·m⁻²·d⁻¹ (over a 16 h photoperiod) on growth, yield, and quality of four economically-important Brassicaceae microgreens: kale, cabbage, arugula, and mustard (Jones-Baumgardt et al., 2019). Therein, an increase in FW corresponded with increasing DLI in kale, cabbage, arugula and mustard, followed by a genotype-specific saturation of this growth parameter at a higher DLI (i.e., typical light response curves) of 400, 400, 200, and 400 μmol·m⁻²·s⁻¹, respectively. Alterations in DW as a function of DLI are similar to those shown for FW, although the magnitude of the DLI treatment effects are more pronounced and are not readily saturated at higher
DLI. Varying magnitudes of growth, yield and quality (i.e., visual appearance) measurements of different genotypes to increasing DLI indicate that some commodities exhibit a greater range of phenotypic plasticity to photosynthetic light levels.

In this study, we tested the hypothesis that the response of growth and yield to SL LED LI of greenhouse-grown microgreens may be genotype-specific. The objectives of this study were to quantify the effects of SL LED LI during the winter months in higher latitude regions (e.g., southern Ontario) on the growth and yield of four economically-important greenhouse-grown microgreens: sunflower, kale, arugula and mustard.

4.2. METHODOLOGY

4.2.1. Experimental Setup Rationale

Typically, greenhouse SL research trials involve homogeneous plots of different SL PPFD levels within a common growing environment (Lopez and Runkle, 2008; Oh et al., 2010; Torres and Lopez, 2011). Treatment plots are separated in space to minimize light spillover from adjacent plots, which often limits the quantity of experimental plots. Physical barriers, such as opaque white vertical blinds are commonly used in SS lighting research to separate treatment plots but, have limited use in the greenhouse environment as they can also strongly influence the natural lighting at plot level. Furthermore, the requirements for proper replication inherently limits the number of LI treatment levels that can be investigated. Sometimes consecutive replication methods are utilized to increase the number of treatment levels within the available plots. However, since there can be considerable temporal variations in natural lighting in greenhouse environments, concurrent experimental designs are highly preferable for lighting research in greenhouse
Regression analysis of data from either of these designs have aggregations of data points at a few discreet levels of the independent variable (i.e., LI) with the requirement to interpolate between these levels. In contrast, gradient-style ecological designs can outperform replicated designs when quantifying responses along a continuous variable (Kreyling et al., 2018). A gradient experimental design was used in the current study, which was performed at a commercial greenhouse in Lynden, Ont., Canada. This involved intentionally creating heterogeneity in canopy-level SL PPFD (through selective fixture placement) within a common growing environment, rigorously assessing the spatial variability in SL PPFD at high resolution (done at night) and assigning selected trays of microgreens to specific fixed coordinates (i.e., PPFD treatments) within the main plot. Although the whole cultivation area was populated with crops at equivalent densities using commercial growing practices, only the plants from selected locations (for which the SL LI was known) were assessed at harvest.

4.2.2. Lighting Setup

Ten LED arrays (LX502G, Heliospectra AB, Gothenburg, Sweden) were hung in pairs (1.4 m above the canopy) near each of the four corners and the center of the growing area (Fig. 4.1). The Euclidian coordinates (in m) of the center of each fixture, relative to the upper left corner of the growing area as shown in Fig. 4.2 are provided in Table 4.1. These fixtures contained three separate-controllable spectrum channels for B (peak 445 nm), R (peak 660 nm) and white (W, 5700K broad spectrum) LEDs. All channels were operated on full power, providing a SL PFR of B (400 to 500 nm), G (500 to 600 nm), and R (600 to 700 nm) of B17:G13:R70 and SL PPFD ranging from 17 to 304 μmol·m⁻²·s⁻¹ at crop level over the 216 subplot locations (Fig. 4.2). At night SL PPFD and spectral
quality were measured at canopy-level at the geometric center of all 216 subplot locations using a radiometrically-calibrated spectrometer (XR-Flame, Ocean Optics, Dunedin, Fla., USA) coupled to a 400 nm x 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 resulting measurements matched IES file-based lighting simulations (on a 5.2-cm square grid) provided by Heliospectra for the whole plot (data not shown). The SL PPFD and B:G:R were re-measured at the end of the experiment to verify steady LI at each sample location. The LED arrays had a 16-h photoperiod (0600 to 2200 HR), providing SL PPFD ranging from 17 to 304 μmol·m⁻²·s⁻¹, translating to a SL DLI of 0.98 to 17.5 mol·m⁻²·d⁻¹. A sunlight-calibrated quantum sensor (SQ-110, Apogee Instruments, Logan, Utah, USA) placed just above the light fixtures in the center of the growing area and tethered to a HOBO datalogger (U-12, Onset Computer Corporation, Bourne, Mass.) collected natural light PPFD data on 120 s intervals throughout the trial. These data were used to calculate the natural DLI inside the experimental greenhouse area for each day of the trial (Fig. 4.3).

4.2.3. Growth Media and Seeding

Seeds of sunflower (*Helianthus annuus* L., ‘Black oil’), kale (*Brassica napus* L., ‘Red Russian’), arugula (*Eruca sativa* L.,) and mustard (*Brassica juncea* L., ‘Ruby Streaks’) were grown in fibre trays (23.5 x 48.5 x 3.5 cm) for 8 to 12 DAS, as described below. The growing substrate was comprised of 30% peat, 30% compost, 30% coir and 10% perlite by volume. Soil macronutrient and micronutrient concentrations were comparable to a previous study (see section 2.2.1). Trays were machine-filled with substrate and seeded on the day the experiment was started. Per-tray seeding rates varied according to seed size and were 94 g for sunflower, 8 g for kale, and 4 g for arugula, and mustard.
Trays of microgreens were top-irrigated as needed with water, until minimal drainage was observed.

4.2.4. Greenhouse Environment

The experiment was conducted in a commercial greenhouse in Lynden, Ont., Canada (lat. 43°14′N, long. 80°07′W), with the experiment starting (i.e., seeded trays initially watered-in) on 1 Feb. (2018) at 1400 HR. Due to different harvest dates, each crop was exposed to different natural DLI (Fig. 4.3). Each plot was randomly assigned to one of four genotypes and seeded trays of the respective genotypes were placed in the geometric center of each subplot. Trays of border plants were placed along the outer columns of each subplot (shaded in grey in Fig. 4.2).

The air temperature and relative humidity (RH) were controlled and recorded (every 120 s) using a greenhouse environmental control system (Damatex Inc, Montréal, Quebec, Canada). The average air temperatures and RH were 21.0 ± 1.1 °C and 42.0 ± 4.1% (mean ± SD), respectively. No supplemental CO₂ was used in the experiment.

4.2.5. Growth and Morphology Measurements

Following commercial practices, microgreens were harvested as per true leaf emergence, resulting in harvest 8 DAS for sunflower, 11 DAS for kale, and 12 DAS for arugula and mustard. For each genotype, 50 trays were sampled, and the four trays with the highest SL LI (> 304 μmol·m⁻²·s⁻¹) were not used as these were placed directly under LED arrays, blocking natural LI to the microgreens, possibly causing the lowest natural LI. For each microgreen tray, one ‘core’ sample (i.e., full plants and substrate) was collected in the
centre (i.e., where SL PPFD was characterized) of each tray using a cylindrical core sampler (76.4 cm²). Random plants were selected from inside each core for HL, LA, FW of LA, DW of LA, hypocotyl diameter (HD), relative chlorophyll content (RCC), total sample FW and DW measurements. HL was measured from the base of the hypocotyl to the shoot apical meristem on five plants. LA was assessed on ten plants, with a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln, Nebr., USA). Thereafter, the ten plants were pooled to determine the FW of plants used for LA assessment, and then they were dried to a constant weight at 70 °C for 3 d and the DW was recorded. The specific LA (SLA) per plant was determined by using the equation:

\[
\text{SLA} = \frac{\text{LA}}{\text{Leaf DW}}
\]

For HD measurements, five plants were scanned (CanoScan LiDE 25; Canon Inc., Tokyo, Japan) in JPEG format at 297 pixels per inch (PPI). ImageJ 1.42 software (https://imagej.nih.gov/ij/download.html) was used to determine HD. Five plants were used to measure RCC with a SPAD meter (SPAD 502; Spectrum Technologies, Inc., Aurora, IL, USA). All remaining plants from each core were cut just above the substrate level and combined to determine FW and DW as described above. These values were pooled with the respective FW and DW data recorded for the subsamples used for LA analysis.

The robust index (RI) of each plant was determined using the equation:

\[
\text{RI} = \left( \frac{\text{HD}}{\text{HL}} \right) \times \text{DW}
\]
4.2.6. Statistical Analysis

Data were analyzed using R statistical software (RStudio 1.1.453; Auckland, New Zealand). HL, HD, FW, DW, LA, SLA, RI, and RCC measurements were individually analyzed using linear regressions. All analyses for linear regressions were evaluated at \( P \leq 0.05 \) level of statistical significance. The best-fit model equations are only presented for variables with significant linear regressions, whereas overall means (pooled data from total DLI range) are presented when there were no treatment effects. Normality of residuals (normally distributed residuals) and homoscedasticity of variances (variance around the regression is uniform for all values of the predictor variable, which is x) were confirmed by using the Shapiro-Wilk and Levene test, respectively.
Figure 4. 1. Schematic of the experimental growing area, comprised of 216 subplot locations (blue) and border plants (grey) lit by 10 LX502G LED arrays (numbered in the orange rectangles).
Figure 4. 2. Canopy-level SL PPFD (μmol·m⁻²·s⁻¹) at the center of all 216 subplot locations. The colors yellow and grey represent the 16 tray locations not chosen for sampling and border trays, respectively. The colors green, purple, blue and red represent the sunflower, kale, arugula and mustard microgreen plots, respectively.

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Table 4. 1. The Euclidian coordinates (in m) of the center of each fixture, relative to the upper left corner of the growing area.

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<th>Fixture no. (from Fig. 2)</th>
<th>Distance (m) for each coordinate</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X (over from left)</td>
<td>Y (down from top)</td>
<td>Z (hang height)</td>
</tr>
<tr>
<td>1</td>
<td>0.25</td>
<td>1.09</td>
<td>1.40</td>
</tr>
<tr>
<td>2</td>
<td>1.05</td>
<td>1.09</td>
<td>1.40</td>
</tr>
<tr>
<td>3</td>
<td>4.41</td>
<td>1.09</td>
<td>1.40</td>
</tr>
<tr>
<td>4</td>
<td>5.21</td>
<td>1.09</td>
<td>1.40</td>
</tr>
<tr>
<td>5</td>
<td>2.33</td>
<td>3.59</td>
<td>1.40</td>
</tr>
<tr>
<td>6</td>
<td>3.13</td>
<td>3.59</td>
<td>1.40</td>
</tr>
<tr>
<td>7</td>
<td>0.25</td>
<td>6.08</td>
<td>1.40</td>
</tr>
<tr>
<td>8</td>
<td>1.05</td>
<td>6.08</td>
<td>1.40</td>
</tr>
<tr>
<td>9</td>
<td>4.41</td>
<td>6.08</td>
<td>1.40</td>
</tr>
<tr>
<td>10</td>
<td>5.21</td>
<td>6.08</td>
<td>1.40</td>
</tr>
</tbody>
</table>
Figure 4.3. Gutter-level natural sunlight DLI (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 6.50 ± 1.70, 5.94 ± 1.70 and 6.20 ± 1.70, respectively.
4.3. RESULTS

4.3.1. Sunflower

There was no effect of DLI on HL, DW, SLA, RI and RCC in sunflower (Table 4.2). As DLI increased from 6.9 to 23.7 mol·m⁻²·d⁻¹, HD, FW and LA increased linearly by 7.84%, 15.0% and 10.5%, respectively.

4.3.2. Kale

There was no effect of DLI on HL and HD in kale (Table 4.2). FW, DW, LA, RI and RCC increased linearly by 37.3%, 59.1%, 17.3%, 60.4% and 18.0%, respectively, while, SLA decreased linearly by 26.6% as DLI increased from 6.9 to 23.7 mol·m⁻²·d⁻¹.

4.3.3. Arugula

There was no effect of DLI on HL in arugula (Table 4.2). HD, FW, DW, LA, RI and RCC increased linearly by 16.2%, 61.4%, 80.6%, 40.0%, 123% and 20.5%, respectively, whereas, SLA decreased linearly by 29.5% as DLI increased from 6.9 to 23.7 mol·m⁻²·d⁻¹.

4.4.4. Mustard

As DLI increased from 6.9 to 23.7 mol·m⁻²·d⁻¹, HD, FW, DW, LA, RI and RCC increased by 12.1%, 48.1%, 63.5%, 20.4%, 102%, and 17.1%, respectively (Table 4.2). HL and SLA decreased linearly by 8.51% and 40.2%, respectively, as DLI increased from 6.9 to 23.7 mol·m⁻²·d⁻¹.
Table 4.2. Effect of DLI\textsubscript{s} ranging from 6.9 to 23.7 mol\textperiodcentered m\textsuperscript{2}\textperiodcentered d\textsuperscript{-1} on growth and yield measurements of sunflower (\textit{Helianthus annuus} L., ‘Black oil’), kale (\textit{Brassica napus} L., ‘Red Russian’), arugula (\textit{Eruca sativa} L.) and mustard (\textit{Brassica juncea} L., ‘Ruby Streaks’) microgreens.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Regression equation or (r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunflower</td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>9.38 ± 0.08</td>
</tr>
<tr>
<td>Hypocotyl diameter (mm)</td>
<td></td>
</tr>
<tr>
<td>Fresh weight (kg\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 0.0176x + 3.64) 0.124</td>
</tr>
<tr>
<td>Dry weight (g\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 0.0408x + 4.28) 0.119</td>
</tr>
<tr>
<td>Leaf area (cm\textsuperscript{2}\textperiodcentered plant\textsuperscript{-1})</td>
<td>397 ± 6</td>
</tr>
<tr>
<td>Specific leaf area (cm\textsuperscript{2}\textperiodcentered g\textperiodcentered DW\textsuperscript{-1})</td>
<td>(y = 0.0203x + 3.12) 0.0865</td>
</tr>
<tr>
<td>Robust index</td>
<td>16.6 ± 0.4</td>
</tr>
<tr>
<td>Relative chlorophyll content</td>
<td>67.8 ± 0.8</td>
</tr>
<tr>
<td>Kale</td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>9.45 ± 0.09</td>
</tr>
<tr>
<td>Hypocotyl diameter (mm)</td>
<td>1.75 ± 0.01</td>
</tr>
<tr>
<td>Fresh weight (kg\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 0.0428x + 1.62) 0.345</td>
</tr>
<tr>
<td>Dry weight (g\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 2.73x + 58.7) 0.576</td>
</tr>
<tr>
<td>Leaf area (cm\textsuperscript{2}\textperiodcentered plant\textsuperscript{-1})</td>
<td>(y = 0.0230x + 2.06) 0.106</td>
</tr>
<tr>
<td>Specific leaf area (cm\textsuperscript{2}\textperiodcentered g\textperiodcentered DW\textsuperscript{-1})</td>
<td>(y = -4.65x + 325) 0.237</td>
</tr>
<tr>
<td>Robust index</td>
<td>(y = 0.0516x + 1.07) 0.545</td>
</tr>
<tr>
<td>Relative chlorophyll content</td>
<td>(y = 0.333x + 28.6) 0.338</td>
</tr>
<tr>
<td>Arugula</td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>7.75 ± 0.07</td>
</tr>
<tr>
<td>Hypocotyl diameter (mm)</td>
<td>(y = 0.0122x + 1.17) 0.233</td>
</tr>
<tr>
<td>Fresh weight (kg\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 0.0533x + 1.08) 0.701</td>
</tr>
<tr>
<td>Dry weight (g\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 3.25x + 45.2) 0.816</td>
</tr>
<tr>
<td>Leaf area (cm\textsuperscript{2}\textperiodcentered plant\textsuperscript{-1})</td>
<td>(y = 0.0504x + 1.76) 0.513</td>
</tr>
<tr>
<td>Specific leaf area (cm\textsuperscript{2}\textperiodcentered g\textperiodcentered DW\textsuperscript{-1})</td>
<td>(y = -6.72x + 428) 0.351</td>
</tr>
<tr>
<td>Robust index</td>
<td>(y = 0.0767x + 0.515) 0.774</td>
</tr>
<tr>
<td>Relative chlorophyll content</td>
<td>(y = 0.350x + 26.2) 0.235</td>
</tr>
<tr>
<td>Mustard</td>
<td></td>
</tr>
<tr>
<td>Hypocotyl length (cm)</td>
<td>(y = -0.0421x + 8.59) 0.148</td>
</tr>
<tr>
<td>Hypocotyl diameter (mm)</td>
<td>(y = 0.0114x + 1.50) 0.193</td>
</tr>
<tr>
<td>Fresh weight (kg\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 0.0417x + 1.16) 0.519</td>
</tr>
<tr>
<td>Dry weight (g\textperiodcentered m\textsuperscript{2})</td>
<td>(y = 2.77x + 54.1) 0.617</td>
</tr>
<tr>
<td>Leaf area (cm\textsuperscript{2}\textperiodcentered plant\textsuperscript{-1})</td>
<td>(y = 0.0249x + 1.87) 0.154</td>
</tr>
<tr>
<td>Specific leaf area (cm\textsuperscript{2}\textperiodcentered g\textperiodcentered DW\textsuperscript{-1})</td>
<td>(y = -9.64x + 468) 0.675</td>
</tr>
<tr>
<td>Robust index</td>
<td>(y = 0.0828x + 0.787) 0.745</td>
</tr>
<tr>
<td>Relative chlorophyll content</td>
<td>(y = 0.274x + 25.9) 0.358</td>
</tr>
</tbody>
</table>
Growth measurements with significant (P ≤ 0.05) linear regressions over a range of DLIs (both natural and SL DLI; x) from 6.9 to 23.7 mol·m⁻²·d⁻¹ are presented as equations; for measurements without treatment effects, means ± SE of 50 experimental units.

4.4 DISCUSSION

Compact growth (i.e., shorter internodes) in mature vegetable tissues is commonly associated with increased DLI (Burkholder, 1936; Butler, 1963; Yeh and Hsu, 2004; Zervoudakis et al., 2012). However, the effects of DLI on HL of young seedlings remains largely unknown. In this study, there was no effect of DLI on the HL for sunflower, kale and arugula, whereas HL of mustard generally decreased as DLI increased. These results are consistent with studies on seedlings of floriculture and vegetable crops, where a reduction or no change in HL was apparent with increasing DLI in SL (Craver et al., 2019; Hernández and Kubota, 2014; Lopez and Runkle, 2008; Poel and Runkle, 2017; Pramuk and Runkle, 2005; Randall and Lopez, 2015) and SS growing environments (Craver et al., 2018). In contrast, HL of Brassica microgreens were typically shorter with increasing DLI (Gerovac et al., 2016; Jones-Baumgardt et al., 2019; Samuolienė et al., 2013). Gerovac et al. (2016) reported, approximately 27% to 40% reductions in mustard HL as DLI increased from 6.05 to 18.1 μmol·m⁻²·s⁻¹, regardless of PFR. Similarly, HL was respectively 16% and 34% shorter in mustard grown under 19.0 and 25.3 mol·m⁻²·d⁻¹ than seedlings cultivated under 6.34 mol·m⁻²·d⁻¹ (Samuolienė et al., 2013). Moreover, Jones-Baumgardt et al. (2019) found a 25% reduction in HL of mustard microgreens grown in walk-in growth chambers under 17.3 versus 5.76 mol·m⁻²·d⁻¹. This study revealed a 6% decrease in mustard HL as DLI increased from 6.05 to 18.1 mol·m⁻²·d⁻¹. It is worth mentioning that in this study the absolute length of the mustard hypocotyls...
grown at approximately 6.05 mol·m⁻²·d⁻¹ in the greenhouse was 3.1 to 5.1 cm taller than the mustard grown under comparable DLI in a growth chamber (Gerovac et al. 2016; Jones-Baumgardt et al., 2019; Samuolienė et al., 2013). Elongated hypocotyls (≥ 5 cm) are an important growth attribute for many commercial microgreen growers as it facilitates their mechanical harvesting. In natural unfiltered sunlight, the R:FR at the beginning and end of the photoperiod can be as low as 0.6 (Demotes-Mainard et al., 2016). A general response to a low R:FR is hypocotyl extension, promoting the shade avoidance response (Blom et al., 1995; Fletcher et al., 2005; Hisamtsu et al., 2008; Mah et al., 2018). The lower magnitude of the reduction in mustard HL could be associated with a low R:FR; if it is assumed that the R:FR in natural unfiltered sunlight is 0.6, this would approximate a R:FR of 1.33 at a SL LI of 17 μmol·m⁻²·s⁻¹ and a PFR of 70% R from LED arrays. This R:FR in our greenhouse experiment was lower than those reported for previous SS environment experiments, or where no FR light was used (Gerovac et al., 2016; Jones-Baumgardt et al., 2019; Samuolienė et al., 2013). Furthermore, increased temperature may be a possible explanation for hypocotyl elongation in this study, where there was a constant average air temperature of 21 °C, which was higher than the average air temperature of 21 °C day/ 17 °C night reported for previous studies (Samuolienė et al., 2013; Gerovac et al., 2016; Jones-Baumgardt et al., 2019) (D/N; 16 h/8 h). Gray et al. (1998) showed that increased temperature promoted auxin synthesis, resulting in increased hypocotyl elongation in Arabidopsis seedlings. Alternatively, G light present from both SL and natural light in combination with R and B light may have also caused an increase in hypocotyl elongation in the microgreens investigated in this study. Similarly, Folta (2004) found that G in combination with B and R reversed the inhibition of hypocotyl elongation in Arabidopsis seedlings.
A fundamental goal for commercial microgreen production is to design growth conditions that maximize FW, since microgreens are sold on a FW basis. On the whole, our study revealed that the FW of sunflower, kale, arugula and mustard increased linearly as DLI increased. Specifically, sunflower and kale had higher yield at a given DLI than arugula and mustard, which may have been partly due to their larger seed sizes. Although there is a paucity of information on the impact of SL LI on microgreen FW, an increased DW is evident at juvenile stages of development in canola, celery, lettuce, broccoli and tomato in response to an increase in the SL LI (Boivin et al., 1987; Canham, 1972; Cathey and Campbell, 1977; Masson et al., 1991; McCall, 1991; Newton, 1966; Potter et al., 1999). Similarly, in our study, the DW of kale, arugula and mustard increase was proportional to the increase in DLI supplied during growth.

Most plants exhibit a proportional increase in photosynthetic rates, with increasing LI; this indicates that photosynthetic and growth rates are light-limited. Furthermore, increases in LI up to the light saturation point (largely determined by the genotype and growth environment) is associated with a saturation in photosynthetic and growth rates, indicating that factors other than LI have become limiting. In this study, kale, arugula and mustard microgreens required high light for maximum growth, since FW and DW did not begin to level off to a maximum at approximately 23.7 mol·m⁻²·d⁻¹. This is consistent with previous research on SS cultivated microgreens, where the FW of kale and mustard is maximal at DLIs in excess of 23.0 mol·m⁻²·d⁻¹ (Jones-Baumgardt et al., 2019). For arugula, this contradicts previous SS research which demonstrated that the maximal FW is evident at a DLI greater than 11.5 mol·m⁻²·d⁻¹ (Jones-Baumgardt et al.,
Different growing conditions such as light spectrum, plant water stress, RH, temperature, CO₂ concentrations, and fluctuating natural LI can have substantial impacts on their light response curves. In this study, FW of kale and mustard did not level off at the highest investigated SL LI, however SL LI above 304 μmol·m⁻²·s⁻¹ was not economically-viable for microgreen greenhouse growers.

Chlorophyll is the green pigment in plants and plays a vital in photosynthesis as it absorbs light energy and converts it into chemical energy required for CO₂ fixation (Francis, 2010). Thus, chlorophyll content can directly influence photosynthesis in the leaves (Croft et al., 2017) and thereby affect plant FW and DW. The biosynthesis of chlorophyll is induced by light (Eckhardt et al., 2004). In this study, RCC of kale, arugula and mustard increased linearly as DLI increased. It is likely that higher DLI increased FW and DW of kale, cabbage and arugula microgreens, by increasing the amount of chlorophyll in the cotyledons.

Unlike thinner leaves, thicker leaves are more structurally resistant against wind, physical damage and herbivores (Onoda et al., 2011). In this study, a negative linear relationship between DLI and SLA was evident for kale, arugula and mustard. This was similar to the decreased SLA that was apparent with an increased DLI in other genotypes and growing environments (e.g., outdoor) (Matos et al., 2009; Sims and Pearcy, 1994; Torres and Lopez, 2011). Short shelf-life (Chandra et al., 2012) is one of the major limiting factors for commercial microgreen production. Microgreens short shelf-life was generally induced from the stress associated with mechanical damage due to cutting and handling during harvest and postharvest (Cantwell and Suslow, 2002). This implied that
limiting mechanical damage during harvest could increase the shelf-life of microgreens. Clarkson et al. (2003) found that salt-stressed baby salad leaves of spinach and lettuce containing lower SLA, scored 50% higher on the organoleptic scale than control treatment (no salt-stress) seven days after harvest. It is tempting to speculate that lower SLA, which is associated with higher DLI, increases shelf-life by decreasing the vulnerability of the plant to physical damage. More research is needed to determine the relationship between leaf thickness and shelf-life.

Overall, the magnitude of the morphological and yield responses expressed by the four genotypes indicated that arugula and mustard exhibited greater levels of phenotypic plasticity to LI than kale and cabbage. The differences between genotypes in their SL LI effects on growth and yield parameters may have been influenced by the availability of stored energy resources (i.e., size of seed). For example, the larger (sunflower and kale) and smaller (arugula and mustard) -seeded genotypes (sunflower and kale) affected on average 57% and 94%, respectively, of the measured growth and yield parameters, as SL LI increased. In addition, Thomson and Miller (1961) reported that dark- and light-grown pea seedlings growth and morphology were comparable over the first 14 to 16 d of growth. Moreover, pea seedlings grown in the dark for 23 d resume growth when transferred to light (Low, 1970). This shows that during juvenile growth light has no effect on pea seedlings, suggesting that food stores in the cotyledons support the growth of pea seedlings in the dark. Sunflower seed size is similar to that of peas, and like peas, their growth and yield were little affected by increasing DLI.
4.4.1. Conclusion

In this study we described how a wide range of SL LI (both low and high) in a greenhouse during winter months in higher latitude regions affected the growth and yield measurements of sunflower, kale, arugula and mustard microgreens. Commercial microgreen greenhouse growers can use the light response models described herein to make predictions of relevant production measurements according to the available light levels during winter in higher latitude regions. This would enable growers to optimize the choice of the most appropriate SL LI to achieve the desired production goals as economically as possible.
4.5 LITERATURE CITED


Randall, W.C. and R.G. Lopez. 2015. Comparison of bedding plant seedlings grown under sole-source light-emitting diodes (LEDs) and greenhouse supplemental lighting from LEDs and high-pressure sodium lamps. HortScience. 50:705–713.


CHAPTER FIVE
GENERAL CONCLUSIONS AND DISCUSSION

The primary objective of this study was to provide growers with the ability to manipulate production metrics and phytochemical and nitrate contents of microgreens by varying LI in controlled environments. Although, microgreens response to light is not only dependent on LI but also light quality, photoperiod and environmental conditions. Previous research on microgreens and leafy vegetables was used to determine setpoints for these parameters in each experiment. Currently, majority of microgreen growers estimate and/or hypothesize the amount of SS or SL LI to use when growing microgreens.

In Chapter 2 and 3, we investigated the effect of SS LED LI on the growth, yield, quality, and phytochemical and nitrate contents of Brassicaceae microgreens (kale, cabbage, arugula and mustard). In general, as SS LED LI increased, FW, DW, total and reduced ascorbate and total anthocyanins of all genotypes increased. Total phenolic content of kale and arugula increased as SS LED LI increased. In contrast, HL and HA of all genotypes, total chlorophyll and its individual components of cabbage and arugula, and nitrate content of mustard generally decreased as SS LED LI increased. The SS LED LI used in the study did not have any effect on LA and carotenoid content of all genotypes, total chlorophyll and its individual components of kale and mustard, phenolic content of cabbage and mustard and nitrate content of kale, cabbage and arugula.

It is well-known that plants grown under different lighting environments, and different genotypes, can have different light responses. Such as to have different leaf morphology, structure, biochemistry and the growth and yield measurements can respond
to light differently. Therefore, we evaluated a range of SL LED LI treatments on the
growth and yield of economically important microgreens (sunflower, kale, arugula and
mustard) during winter greenhouse production in higher latitude regions like southern
Ontario in Chapter 4. LA and FW increased with increasing DLI in all genotypes. DW,
RI and RCC of kale, arugula and mustard increased with increasing DLI. SLA of kale,
arugula and mustard decreased as DLI increased. HD of sunflower, arugula and mustard
increased as DLI increased. HL of mustard decreased as DLI increased.

Overall, the aforementioned results from Chapter 2, 3 and 4 show the response of
growth, yield, quality and phytochemical and nitrate contents to LI may be genotype-
specific. The magnitude of the LI treatment effects on growth, yield, quality and
phytochemical and nitrate contents varied by genotype. Specifically, the magnitude of the
growth, yield and quality responses of all genotypes to LI indicated that arugula and
mustard (smaller-seeded genotypes) exhibited greater phenotypic plasticity to SS and SL
LI than kale, cabbage and sunflower (larger-seeded genotypes).

This study provided baseline data on the effects of SS and SL LED LI on growth,
yield, quality and phytochemical and nitrate contents of microgreens grown in controlled
environments. However, there are still interesting research questions that need to be
answered.

Given the variation in growth, yield, quality and phytochemical and nitrate
contents to LI between microgreen genotypes in the study and aforementioned previous
studies, more research is needed to determine genotype-specific responses to LI of other
economically important microgreens. This will enable growers to group microgreen genotypes based on the LI needed to achieve desired characteristics and assign different levels of lighting infrastructure and energy budgets to microgreens that exhibit greater phenotypic plasticity to increasing LI. Furthermore, because genotypes differ in growth, yield, quality and phytochemical and nitrate contents to LI, identifying optimal LI is dependent on which parameters are measured, desired and genotype selected. The relationship between growth and phytochemical content and taste and appearance may help in determining optimal LI, because taste and appearance are important parameters determining consumer acceptance.

In all Chapters photoperiod and spectrum were at fixed levels, with varying LI levels from LED arrays. Lower LI for longer photoperiods, gradually increasing LI as the crop matures, and crops kept in the dark until germination is complete and cotyledons have unfolded are strategies to reduce costs associated with controlled environment crop lighting and deserve more attention in the scientific community. In Chapter 2 and 3, the SS LED light spectrum was B15:R85; however, other wavelengths and PFRs have been shown to influence photomorphogenesis. More research is needed to determine if altering the B15:R85 or adding G, FR, ultraviolet A, ultraviolet B, or a combination of wavelengths may enhance microgreens growth, yield, quality and phytochemical and nitrate contents. By providing optimal LI, quality and photoperiod desired microgreens will be produced. In the greenhouse trial, SL lighting mostly occurred during the natural photoperiod, causing the SL lighting spectrum to have no impact on crop productivity, possibly because the spectral effects of SL light are supressed by the broad spectrum of the natural light. Instead of competing with the natural spectrum, light regimens (intensity,
spectrum and photoperiod) at the end of day (near the end of the natural photoperiod or during darkness) using LEDs may be an advantageous strategy for manipulating microgreen, growth and yield in greenhouse environments.