ENVIRONMENTAL AND CULTURAL PRACTICES TO OPTIMIZE THE GROWTH AND DEVELOPMENT OF THREE MICROGREEN SPECIES

A Thesis

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Jonathan Asher Allred

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ABSTRACT

The recent popularity of edible microgreens (young seedlings of vegetable and herbs harvested shortly after emergence of the first true leaf) has resulted in increased interest of greenhouse growers to cultivate them for local markets. Their cultivation in hoop-houses, greenhouses and indoor production vertical farms (plant factories), permit growers control over growth parameters. This in turn allows growers to influence crop yield, morphology, days to harvest (DTH) and secondary metabolites, which hold perceived health benefits with consumers. Recommendations for their germination and growth parameters vary widely by seed supplier leaving growers to determine their own ideal cultural practices best suited for their operation. The use of horticultural lighting systems to hasten growth and promote the development of aromatic compounds in microgreens has also received little attention in the published literature. Therefore, it was the objective of this study to quantify the effects of four common cultural practices, four daily light integrals (DLI) and four carbon dioxide (CO_2) concentrations on the growth, morphology, DTH and secondary metabolite production of three microgreen species: arugula (*Eruca sativa* L.), mizuna (*Brassica rapa* L. var. *japonica*) and mustard [*Brassica. juncea* (L.) Czern. 'Garnet Giant'].

We began by evaluating four seed densities (1.1, 1.65, 2.2, 2.75 and 3.3 seeds•cm^{-2}); five fertilizer concentrations (0, 50, 100, 150 or 200 mg N•L^{-1}); four substrate depths (1.8, 3.3, 4.3 and 5.8 cm); and four air temperatures (14, 16, 20 and 22 °C) on fresh weight (FW), fresh weight per plant (FWPP), dry weight (DW), plant height and DTH. FW and FWPP were influenced in equal but opposite quadratic fashions as seed density increased from 1.1 to 3.3 seeds•cm^{-2} where total FW increased while FWPP decreased. FW increased in a quadratic fashion as both fertilizer

concentration and substrate depth increased from 0 to 200 mg $N \cdot L^{-1}$ and 1.8 to 5.8 cm. DTH decreased linearly as air temperatures increased from 14 to 22 °C.

After parameters were established for optimal cultural practices, we sought to quantify the effects of four DLI and four CO_2 levels on the growth, morphology and secondary metabolite content of microgreens. Four levels of DLI (3, 6, 9 and 12 $mol \cdot m^{-2} \cdot d^{-1}$) by four levels of CO_2 (400, 600, 800 and 1000 ppm) were evaluated under a full factorial design. FW increased linearly for mizuna and mustard as DLI and CO_2 increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ and 400 to 1000 ppm. Arugula FW increased in a quadratic fashion as DLI increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ and linearly as CO_2 increased from 400 to 1000 ppm. Dry weight increased linearly for all species as DLI and CO_2 increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ and 400 to 1000 ppm. For mizuna and mustard, DTH decreased in a quadratic fashion while arugula DTH decreased linearly as DLI increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ with no observed influence from CO_2. Total phenolics and total flavonoids increased linearly as DLI increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ where the effect of DLI on phenolic content was dependent on the CO_2 level.

The results of these studies can help growers determine optimal cultural practices to maximize yields, minimize production time and achieve a target crop size based on individual market demand. In addition, results can help growers conclude what combination of DLI and CO_2 can achieve maximum yields at the lowest lighting energy input. Growers can then determine the importance of achieving maximum phenolic and flavonoid compounds and adjust light and CO_2 as needed.

BIOGRAPHICAL SKETCH

Jonathan A. Allred was born in Torrance, California and raised in the small coastal city of Manhattan Beach, California. Since a young child, Jonathan has always enjoyed the outdoors with a particular fondness for the high sierras of California's central mount ranges. From a young age, Jonathan accompanied by his parents; Ernest V. Allred (father) and Karen B. Allred (mother), would take vacations to the areas surrounding Mammoth Mountain and Lake Tahoe, California. These early years and continued trips throughout his young life cemented a passion for the environment and plant sciences. This passion later emerged as an interest in the horticultural sciences where Jonathan studied plant science at California State Polytechnic University of Pomona where he received his B.S. in 2014.

It was at the university's prospective student visitation day where Jonathan met Lisa M. Kapuskar who was visiting the schools animal health science program. The two soon began dating; a defining moment viewed by many as a significant turning point in Jonathan's academic career. As a naturally gifted scholar, Lisa's devotion to her schoolwork positively influenced Jonathan's coursework and strengthened his interests in furthering his education. During Jonathan's time at Cal Poly Pomona, he served as a farm laborer at the university' farm. It was there that Jonathan's interests in greenhouse vegetable production took hold after three years of field-grown vegetable production.

His communication with Dr. Neil S. Mattson of Cornell University's controlled environment agriculture group ultimately led to his acceptance into a master's degree program in the field of horticulture at Cornell. To this day, Jonathan remains eager to learn and build upon the scientific knowledge surrounding advanced greenhouse vegetable production systems.

ACKNOWLEDGEMENTS

I would like to begin by thanking Dr. Neil S. Mattson (Committee Chair; Horticulture Section School of Integrative Plant Science) for dedicating extensive time towards the improvement of my research and academic skillset. His decision to mentor me through shared knowledge, compassion and patience is highly respected by not only me but the academic community as a whole. His active role in extension and community outreach, are traits that I strive to emulate. I am proud to continue my academic and research career with him in the years to come.

Secondly, I would like to thank Dr. Michael B. Timmons (Committee member; Biological and Environmental Engineering) for his enthusiasm towards life and hard work. His ability to convey engineering principals in a manner that is understandable by all has been a valuable tool during my time at Cornell University. I look forward to future collaborations encompassing the principles of both horticulture and engineering.

Thirdly, I would like to thank Dr. Miguel I. Gomez (Committee member; Dyson School of Applied and Agricultural Economics) for his aiding in my comprehension of the economic principals surrounding agricultural operations. His extensive knowledge and calm demeanor make him a joy to be around and I look forward to future collaborations involving the principles of both horticulture and economics.

Fourthly, I would like to thank Francoise Vermeylen (Consultant; Cornell Statistical Consulting Unit) for her tireless efforts in assisting me with the statistical analysis of my data. Her input was highly valued and I am eager to seek her input on future experiments.

Lastly, I would like to wholeheartedly thank Lisa Marie Kapuskar, my beloved fiancée and partner of eight years (March 22, 2009) for her endless support both with conducting experiments and emotionally when the stresses of graduate school were at their peak. May our life's journey continue on a path of endless love and happiness for many more years to come.

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LIST OF ABBREVIATIONS

CE	Catechin equivalent
CO_2	Carbon dioxide
DDI	Double deionized
DLI	Daily light integral
DTH	Days to harvest
DW	Dry weight
EC	Electrical conductivity
FW	Fresh weight
FWPP	Fresh weight per plant
GA	Gibberellin
GAE	Gallic acid equivalent
HPS	High pressure sodium
LAR	Leaf area ratio
LED	Light emitting diode
LI	Light intensity
LN	Liquid nitrogen
LQ	Light quality
PAR	Photosynthetically active radiation
PPF	Photosynthetic Photon Flux
RH	Relative humidity
W	Watt

CHAPTER 1

Effects of Cultural Practices on Yield and Production Time of Three Microgreen Species

Abstract

The recent popularity of edible microgreens (young seedlings of vegetable and herbs harvested shortly after emergence of the first true leaf) has resulted in increased interest of greenhouse growers to cultivate them for local markets. Recommendations for their germination and growth parameters vary widely by seed supplier leaving growers to determine their own ideal cultural practices best suited for their operation. The objective of this study was to quantify the effects of four common cultural practices on the growth, morphology and days to harvest (DTH) of three microgreen species. Arugula (*Eruca sativa* L.), mizuna (*Brassica rapa* L. var. *japonica*) and mustard [*Brassica. juncea* (L.) Czern. 'Garnet Giant'] were evaluated under four seed densities (1.1, 1.65, 2.2, 2.75 and 3.3 seeds•cm^{-2}); five fertilizer concentrations (0, 50, 100, 150 or 200 mg N•L^{-1}); four substrate depths (1.8, 3.3, 4.3 and 5.8 cm); and four air temperatures (14, 16, 20 and 22 °C). Fresh Weight (FW) and fresh weight per plant (FWPP) were influenced in equal but opposite quadratic fashions as seed density increased from 1.1 to 3.3 seeds•cm^{-2} where total FW increased while FWPP decreased. FW increased in a quadratic fashion as both fertilizer concentration and substrate depth increased from 0 to 200 mg N•L^{-1} and 1.8 to 5.8 cm. DTH decreased linearly as air temperatures increased from 14 to 22 °C. The results of this study can help growers determine optimal cultural practices to maximize yields, minimize production time and achieve a target crop size based on individual market demand.

Introduction

Microgreens are a broadly classified edible specialty crop produced for wholesale and retail markets alike. This relatively new category of crops consists of numerous vegetables and herbs harvested shortly after emergence of the first true leaf or leaves but prior to true leaf development and senescence of the cotyledons (Gerovac et al., 2016; Gioia et al., 2016). Growth intervals range between ten and twenty days from seeding to harvest (Lee et al., 2004) with variations attributable to species, cultural and environmental practices. Their presence in local and national markets has been fueled by demand, ease of production and adaptability to both small and large-scale production facilities. With a current wholesale value of US $66 to $110 per kg of clamshell-packaged product (Treadwell et al., 2010), their high value has incentivized commercial greenhouse growers to begin producing them (Gerovac et al., 2016). As with many new market introductions, few formal guidelines exist for production methods or harvest specifications (Murphy and Pill, 2010). Presently, work by Xiao et al. (2012); Xiao et al. (2014) and Xiao et al. (2016) have concentrated on quantifying the post-harvest mineral, sensory and chemical compositions of edible microgreens. The effects of varying light intensity and spectra on the morphology and nutritional composition has been researched by Bazaityte et al. (2014); Gerovac et al. (2016); Kopsell et al. (2012) and Samuoliene et al. (2013). However, minimal published data exists on the effects of other cultural practices and environmental stimuli on the growth and development rate of microgreens.

In practice, production of microgreens can be found in many forms ranging from soilless media in trays to capillary mats in troughs (Treadwell et al., 2010). Gioia et al. (2016) investigated the use of three different fiber mats against a traditional peat-based media concluding that two of the three mats evaluated, provided equivalent FW yields to peat. Further,

two of the three mats had the desirable benefit that plants had roughly half the shoot tissue nitrate concentration (1959 vs. 940 mg•kg^{-1} FW for peat vs. fiber mats). Currently however, we know of no published literature on the effects of substrate height/volume on microgreen yield. Substrate height/volume affects the water and air holding capacity as well as the available water and nutrients and can have pronounced effects on the growth of plants (van Iersel, 1997). This is important because most microgreen growers in the central New York region produce the crop on either a non-organic or organic certified soilless substrate mix (including various combinations of coconut coir, compost, peat, perlite, or vermiculite) in flats (N.S. Mattson, personal communication). A common practice is to fill 10" x 20" trays with substrate, however the substrate height/container volume varies widely by grower with cost savings being the principle factor in substrate height selection. With a commercial peat-lite mix such as LM-111 (Lambert Peat Moss, Rivière-Ouelle, Canada), cost of substrate per square meter of production area at a substrate height of 5.8 cm (container height) based on a 107 L compressed bale cost of $14.68, would be $2.44•m^{-2}. As a result, sizable savings in material costs could be achieved if yield responses to variations in substrate depth are unaffected. Because the crop has such a quick turnover rate, growers are reluctant to use large volume of substrate.

Shoot FW is often the preferred yield determinant for microgreens (Murphy et al., 2010); therefore, it is important that growers be able to produce the highest quality crop at the greatest FW in the shortest period of time. Work by Lee et al. (2004) applied traditional seed treatment methods such as matric seed priming in fine vermiculite or hydrogen peroxide baths to greenhouse grown beet and chard (*Beta vulgaris* L.) microgreens resulting in as high as 2.8-fold greater shoot FW for chard at 11 days after planting as compared to untreated seed. Murphy et al. (2010) reported yield increases in microgreen beets of 98% by 7 days and 144% by 15 days after

planting through pre-germination in fine vermiculite and cultivation in hydroponic nutrient film technique as compared to no seed treatments in a peat-lite mix. The influence of seeding/population density on yield has been well established in traditional agronomic/horticultural crops but little information exists on its effect on microgreens. Murphy and Pill, (2010) investigated the effects of three seeding rates at 0.81, 1.62 and 2.37 seeds•cm^{-2} against a commercially suggested seeding rate of 3.18 seeds•cm^{-2}. The total FW and FWPP were inversely correlated where seeding at rates below the commercially suggested rate resulted in a lower yield per unit area.

Nitrogen-based complete water soluble fertilizers are necessary additions in most traditional greenhouse vegetable crops. Compared with 100 mg N•L^{-1}, a solution fertilization rate of 400 mg N•L^{-1} increased shoot dry weight (DW) of celery (*Apium graveolens* L.), lettuce (*Lactuca sativa* L.), broccoli (*Brassica oleracea* L.), and tomato (*Solanun lycopersicum* L.) transplants by 37%, 38%, 61%, and 38%, respectively (Masson et al., 1991). In microgreen production, it is common for growers to rely on stored reserves in the seeds or pre-plant media fertilization (i.e. starter charge) and an additional water-soluble fertilizer is not always used or only used periodically. Murphy and Pill, (2010) investigated various rates of both pre and post planting fertilization and a combination of the two in microgreen arugula (*Eruca sativa* L.). Optimum FW production was achieved at either 150 mg N•L^{-1} of daily liquid fertilization with 21 N – 2.2 P – 16.6 K or a combination of 75 mg N•L^{-1} of liquid feed plus a pre-plant media incorporation of 1,000 mg N•L^{-1} from calcium nitrate.

Many microgreen growers cultivate the crop as a supplemental income to a larger more diversified greenhouse/farm operation. As a result, germination and growing temperatures vary greatly from the recommended germination temperature 24 °C and growing temperature of 16 °C

stated by the seed suppliers. To our knowledge, no literature exists on the effects of air temperature on the germination and growth of microgreens.

The objective of this work is to build upon the limited published data for microgreen production optimization by investigating parameters for substrate depth, seeding density, growing temperatures, and fertilization rates.

Materials and Methods

Three microgreen species of the Brassicaceae Family; arugula, mizuna (*Brassica rapa* L. var. *japonica*) and mustard [*Brassica. juncea* (L.) Czern. 'Garnet Giant'] (Figure 1) were purchased from Johnny's Selected Seeds company (Winslow, ME USA); lot numbers were 46962, 51583, and 46623, respectively. Species were chosen for their presence in local markets, similarity in seed diameter (± 0.2 mm) and similar number of days from seeding to harvest (± 1 day). Diversity in sensory attributes for both vision and taste were important in species selection as representative subsamples of a larger microgreen population. Arugula was chosen for its strong aroma associated with a peppery or wasabi-like flavor. Mizuna was chosen for its mild flavor and unique morphology of fringed true leaves. Lastly, mustard 'Garnet Giant' was chosen for its deep red pigmentation and moderate spiciness. All treatments were conducted on

Figure 1. Images of microgreen species utilized in this experiment where A, B and C represent arugula, mizuna and mustard.

galvanized steel benches 85 cm from the floor in single layer glass greenhouses located in Ithaca, NY. For all experiments, polystyrene 2401 inserts (53.3 cm x 26.7 cm x 5.7 cm; Dillen-ITML Greenhouse) containing 24 cells (8 cm x 6 cm) per insert, were placed in flats containing drainage holes (54.5 cm x 27.8 cm x 6.2 cm; TO Plastics). A commercial peat-lite mix (LM-111, Lambert Peat Moss, Rivière-Ouelle, Canada) was used for all experiments. An elemental analysis (in $mg \bullet L^{-1}$) of 0.89 nitrogen (N), 12.48 phosphorus (P), 105.62 potassium (K), 33.91 calcium (Ca), 21.42 magnesium (Mg), 84.91 sulfur (S), 0.05 boron (B), 1.36 iron (Fe), 0.13 manganese (Mn), 0.04 copper (Cu), 0.67 zinc (Zn), 0.01 molybdenum (Mo), 0.47 aluminum (Al), 35.82 sodium (Na), 23.46 chloride (Cl), pH: 6.45 and electrical conductivity (EC) ($ms \bullet cm^{-1}$): 0.61 was provided by J. R. Peter's Testing Services (J. R. Peter's Inc., Allentown, PA.). After seeding and watering, trays containing seeds exposed on the media surface were covered with a propagation dome (Curtis Wagner Plastics Corp.) followed by a standard flat placed over the top of the propagation dome to induce darkness. Germination times varied by treatment but in all cases, blackout domes were removed when initial radical and shoot formation had occurred with an average shoot height of 0.5 cm. Propagation domes were later removed when seedling height averaged 1 to 1.5 cm; a germination time period of 48 to 96 hours depending on the treatment. Species were harvested when the subjective average plant height across treatments for the first true leaf measured 1 cm in length. Plants were measured for average plant height (from cell height to the tallest part of a representative plant) and cut at pot height and weighed for FW on a per cell basis. Samples were placed in an oven and dried at 70 °C for 72 hours to obtain sample DW. Cells that exhibited greater than 5% disease pressure (most likely from pathogens causing seedling death) were excluded from statistical analysis.

Seeding Density Experiment

Cells were filled to the top with LM-111 at a moisture content ratio of 1:1 (peat-lite mix: fertilizer solution) by weight with tap water containing a nutrient solution of 150 mg $N \bullet L^{-1}$ (21 N – 2.2 P – 16.6 K Jack's All-Purpose Liquid Feed, J. R. Peter's Inc., Allentown, PA). Six cells per treatment combination per species were sowed at five rates of 1.1, 1.65, 2.2, 2.75 and 3.3 seeds$\bullet cm^{-2}$ and broadcasted over the media surface. Rates correspond to seed weights per square meter of 18.57, 27.86, 37.14, 46.43 and 55.72 $g \bullet m^{-2}$ for arugula; 21.66, 32.49, 43.32, 54.16, 64.99 $g \bullet m^{-2}$ for mizuna; and 21.08, 31.62, 42.16, 52.7, 63.24 $g \bullet m^{-2}$ mustard. Flats containing seeds were watered to container capacity with tap water containing a nutrient solution of 150 mg $N \bullet L^{-1}$ using a fine mist nozzle. Flats were placed in the greenhouse with an average daily air temperature of 20 °C ± 1 °C. Blackout domes were removed after 36 hours of germination followed by removal of the propagation dome after a total germination time of 48 hours. Subsequent watering was performed as needed using an overhead water-breaking nozzle with tap water containing a nutrient solution of 150 mg $N \bullet L^{-1}$ approximately every three days. Treatments within each species were harvested at the same time period and analyzed for plant height, FW, and FWPP. FWPP was obtained as a calculated value by dividing the FW of each cell by the number of seeds of a given species per cell. Values were then corrected for seed germination percentage provided on the seed package.

Fertilizer Concentration Experiment

Five concentrations of fertilizer solution were tested consisting of 0, 50, 100, 150 or 200 mg $N \bullet L^{-1}$ from the complete water soluble fertilizer as noted above (21 N – 2.2 P – 16.6 K). They were prepared as 1:100 concentration stocks solutions. Eight cells per treatment

combination per species were filled to the top with LM-111 at a moisture content ratio of 1:1 (peat-lite mix: fertilizer solution) by weight with either 0, 50, 100, 150 or 200 mg $N \bullet L^{-1}$. Cells were sowed at a rate of 2.75 seeds$\bullet cm^{-2}$ and broadcast over the media surface. Flats containing seeds were moistened with tap water using a fine mist nozzle. Flats were placed in the greenhouse with an average daily air temperature of 20 °C ± 1 °C. Blackout domes were removed after 36 hours of germination followed by removal of the propagation dome after a total germination time of 48 hours. After seeding, plants were watered about every three days with their corresponding fertilizer treatment, which was applied via subirrigation by placing the tray in a container holding about 3 cm of nutrient solution for about three minutes. Treatments within each species were harvested at the same time period and analyzed for height, FW and DW.

Substrate Depth Experiment

Cell and tray height were modified using scissors to accommodate four substrate depths of 1.8, 3.3, 4.3 and 5.8 cm$\bullet cell^{-1}$ with corresponding volumes of 86.4, 158.4, 206.4 and 278.4 $cm^3 \bullet cell^{-1}$. Decreases in substrate heights were correlated with 25, 50 and 75 % reductions in volume by weight ($V \bullet W^{-1}$) of container capacity saturated media below the manufacturer tray height of 5.8 cm. Media was prepared using LM-111 at a moisture content ratio of 1:3 (peat-lite mix: fertilizer solution) by weight with tap water containing a nutrient solution of 150 mg $N \bullet L^{-1}$. Cells were filled by weight with the 1.8 cm treatment receiving 31 g, 3.3 cm at 63 g, 4.3 cm at 94 g and 5.8 cm at 125 g of media. Eight cells per treatment combination per species were sowed at a rate of 2.75 seeds$\bullet cm^{-2}$ and broadcast over the media surface. Flats containing seeds were watered to container capacity with tap water containing a nutrient solution of 150 mg $N \bullet L^{-1}$ using a fine mist nozzle. Flats were placed in the greenhouse with an average daily air temperature of

20 °C ± 1 °C. Blackout domes were removed after 36 hours of germination followed by removal of the propagation dome after a total germination time of 48 hours. After seeding, plants were watered overhead to container capacity using a water-breaking nozzle with tap water containing a nutrient solution of 150 mg $N \cdot L^{-1}$ about every three days for the 4.3 and 5.8 cm cells to as often as once or twice daily for the 1.8 to 3.3 cm cells. Treatments within each species were harvested at the same time period and analyzed for plant height, FW and DW.

Air Temperature Experiment

Cells were filled to the top with LM-111 at a moisture content ratio of 1:1 (peat-lite mix: fertilizer solution) by weight with tap water containing a nutrient solution of 150 mg $N \cdot L^{-1}$. Cells were sowed at a rate of 2.75 seeds$\cdot cm^{-2}$ and broadcast over the media surface. Flats containing seeds were watered to container capacity with tap water containing a nutrient solution of 150 mg $N \cdot L^{-1}$ using a fine mist nozzle. Ten replicate cells per species were placed in one of four greenhouses connected at gable end in the same greenhouse range, with average daily air temperatures of 14, 16, 20 and 22 °C (± 1 °C). Blackout and germination dome removal times varied by treatment due to variation in germination time by temperature treatment. Blackout domes were removed at 72, 48, 36 and 36 hours, followed by removal of the germination domes at 96, 72, 48 and 48 hours for the 14, 16, 20 and 22 °C treatments, respectively. Treatments within each species were harvested when the subjective average size of the first true leaf measured 1 cm in length across all plants. Treatments were analyzed for DTH.

Statistical Methods

For all experiments, a total of three experimental cycles were conducted over time from April 2015 to April 2017 with six, eight or twelve replications per treatment combination per species per crop cycle. For each experiment, ANOVA at a $P \leq 0.05$ was conducted to determine significant effects of treatments and species on plant FW, DW, plant height and DTH. Regression analyses were conducted as either linear (L) or quadratic (Q) using JMP statistical software (JMP Pro 11; SAS Institute, Cary, NC).

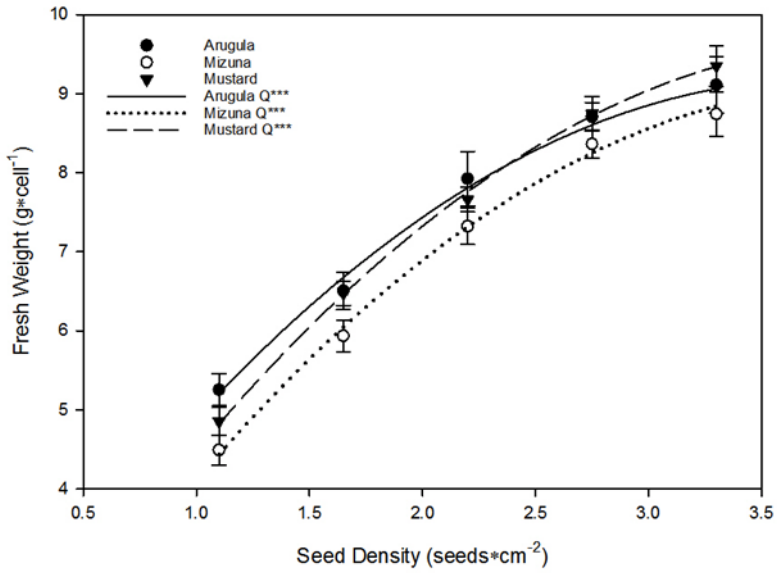

Figure 2.1. Fresh weight per cell of arugula, mizuna and mustard microgreens under five seed density levels of 1.1, 1.65, 2.2, 2.75 and 3.3 seeds•cm^{-2}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

Results

Seeding Density

Fresh Weight. FW for arugula, mizuna and mustard increased in a quadratic fashion as seed density increased from 1.1 to 3.3 seeds•cm^{-2} (Figure 2.1). The slope of the quadratic function was independent of species with reduced gains in FW above 2.75 seeds•cm^{-2}. For example, as seed density increased from 2.75 to 3.3 seeds•cm^{-2}, FW increased by 5% for arugula, 5% for mizuna and 7% for mustard. Greatest FW yield increases occurred between 1.1 and 1.65 seeds•cm^{-2} at 24% for arugula, 32% for mizuna and 33% for mustard. The linear component of the model exhibited an interaction between species and seed density where the effect of density on FW

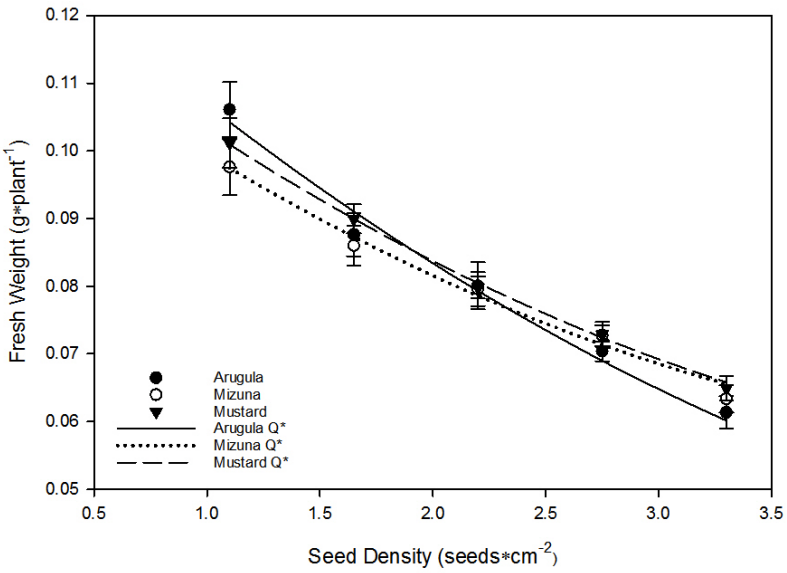

Figure 2.2. Fresh weight per plant per cell of arugula, mizuna and mustard microgreens under five seed density levels of 1.1, 1.65, 2.2, 2.75 and 3.3 seeds•cm^{-2}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

varied by species (Table 1.1). The slope of mizuna was not significantly different from arugula or mustard but mustard (p=0.0207) was significantly different from arugula. Mustard exhibited the largest positive response in FW per unit increase in seed density.

Fresh Weight per Plant. FWPP for all three species decreased in a quadratic fashion as seed density increased from 1.1 to 3.3 seeds•cm^{-2} (Figure 2.2). No significant differences in FWPP were observed at the species level however, species did have a significant impact on the effect on density. For example, Mizuna (p=0.0003) and mustard (p=0.0083) were significantly different from arugula but were not from each other. Arugula was the most responsive in reductions in FWPP as seed density increased from 1.1 to 3.3 seeds•cm^{-2} (Table1.2). For example, FWPP for arugula decreased by 42% as seed density increased from 1.1 to 3.3 seeds•cm^{-2}. In contrast, FWPP for mizuna decreased by 35% while mustard decreased by 36% as seed density increased from 1.1 to 3.3 seeds•cm^{-2}.

Plant Height. Plant height for all three species increased in a quadratic fashion as seed density increased from 1.1 to 3.3 seeds•cm^{-2} (Figure 2.3). The effect of seed density on plant height was independent of species meaning that all three species exhibited the same response in plant height to increasing seed density (Table 1.2). Average plant height increased 25% as seed

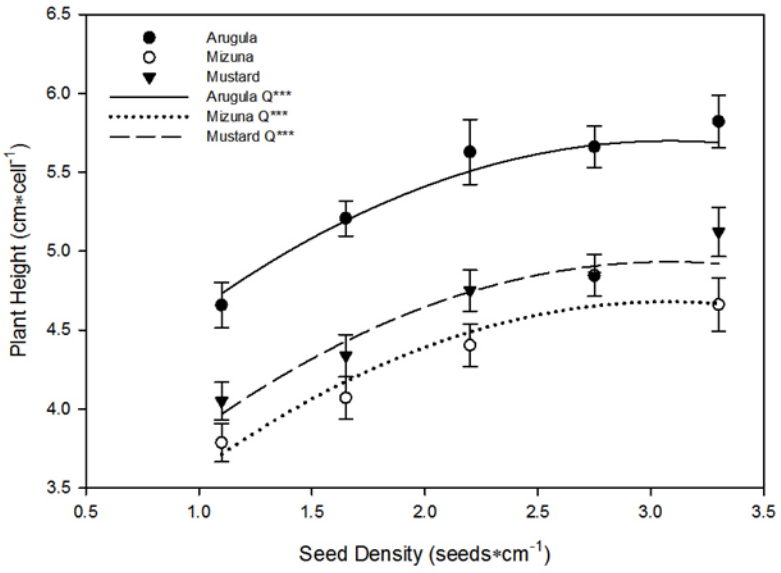

Figure 2.3. Plant height per cell of arugula, mizuna and mustard microgreens under five seed density levels of 1.1, 1.65, 2.2, 2.75 and 3.3 seeds•cm^{-2}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

density increased from 1.1 to 3.3 seeds•cm^{-2} with reduced gains in plant height above 2.2 seeds•cm^{-2}. A species effect on plant height was present where the plant height of mizuna (p=.0009) and mustard (p=.004) were significantly different from arugula but not from each other, where arugula was the tallest of all three species.

Table 1.1. Analysis of variance for the effects of species, density, species•density, density•density and model-accounted variability (R^2).

Seed Density Experiment			
Main Effects & Interactions	*Plant Height*	*Fresh Weight*	*Fresh Weight per Plant*
Species	**	NS	NS
Density	***	***	***
Species•Density	NS	*	***
Density^2	***	***	*
R^2	0.77	0.89	0.84

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 1.2. Regression values for the response of plant height, fresh weight and fresh weight per plant for arugula, mizuna and mustard to per unit changes in seed density (seeds•cm^{-2}) including squared terms for a quadratic model.

Seed Density Experiment			
Plant Height			
	Arugula		
Slope	Esimate[B]	Std. Err.[B]	*P*-value
Intercept	3.361706	0.339974	***[A]
Density	1.5166825	0.201572	***[A]
Density^2	-0.24599	0.046112	***[A]
	Mizuna		
Intercept	2.34181	0.339974	***[A]
Density	1.5166825	0.201572	***[A]
Density^2	-0.24599	0.046112	***[A]
	Mustard		
Intercept	2.596685	0.339974	***[A]
Density	1.5166825	0.201572	***[A]
Density^2	-0.24599	0.046112	***[A]
Fresh Weight			
	Arugula		
Intercept	1.2518914	0.829171	**[A]
Density	4.2097471	0.36036	***[A]
Density^2	-0.558352	0.080624	***[A]
	Mizuna		
Intercept	0.206161	0.829171	*[A]
Density	4.4606696	0.36036	***[A]
Density^2	-0.558352	0.080624	***[A]
	Mustard		
Intercept	0.5364492	0.829171	*[A]
Density	4.5121616	0.36036	***[A]
Density^2	-0.558352	0.080624	***[A]
Fresh Weight per Plant			
	Arugula		
Intercept	0.1350287	0.009495	***[A]
Density	-0.030617	0.00424	***[A]
Density^2	0.0024028	0.000949	*[A]
	Mizuna		
Intercept	0.1221506	0.009495	***[A]
Density	-0.025082	0.00424	***[A]
Density^2	0.0024028	0.000949	*[A]
	Mustard		
Intercept	0.127248	0.009495	***[A]
Density	-0.026553	0.00424	***[A]
Density^2	0.0024028	0.000949	*[A]

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

[A] Significance when treatment effects are at their average value

[B] Estimated response when all other treatment effects are equal to zero

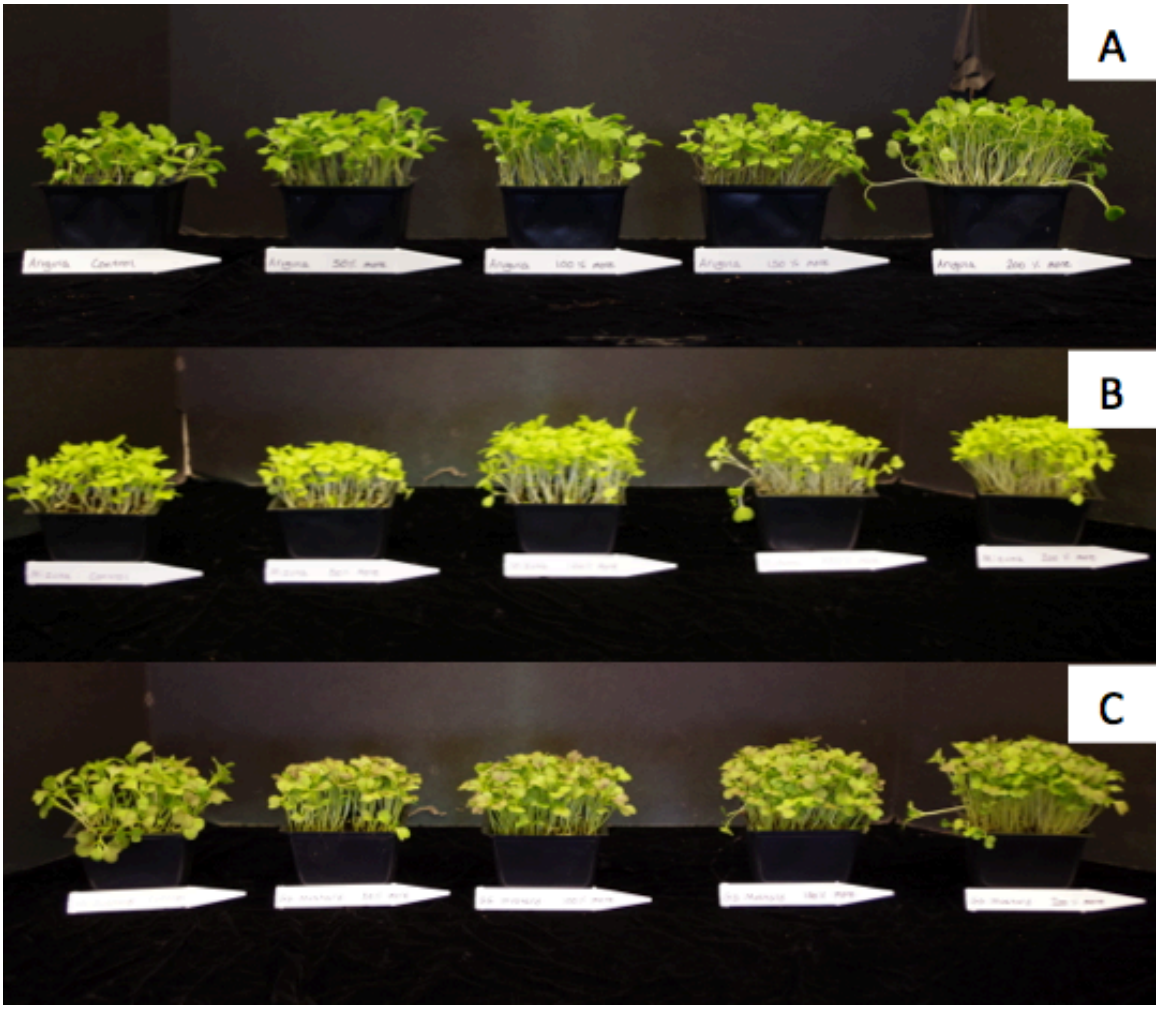

Figure 2.4. Images of arugula (A), mizuna (B) and mustard (C) under five seed densities (seeds•cm^{-2}) 1.1, 1.65, 2.2, 2.75 and 3.3 depicted from left to right.

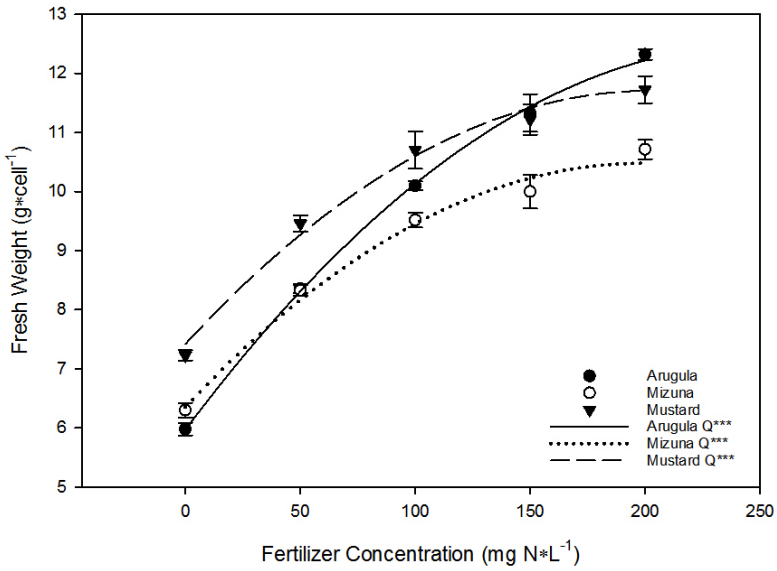

Figure 3.1. Fresh weight per cell of arugula, mizuna and mustard microgreens under five fertilizer concentrations of 0, 50, 100, 150 or 200 mg N•L^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

Fertilizer Concentration

Fresh Weight. FW of arugula, mizuna and mustard increased in a quadratic fashion as fertilizer concentration increased from 0 to 200 mg N•L^{-1} (Figure 3.1). For example, the mean FW for mizuna increased 70% as fertilizer concentration increased from 0 to 200 mg N•L^{-1} with greatest gains in FW occurring between 0 and 100 mg N•L^{-1}: a 50% increase. The quadratic function was independent of species however; the effect of fertilizer on the linear component of the model did vary by species (Table 2.1). There were no significant differences between mizuna and mustard but both mizuna and mustard (p<0.0001) were significantly different from arugula at an average fertilizer rate. Arugula exhibited the largest response in increasing FW yield of 106% as fertilizer increased from 0 to 200 mg N•L^{-1}. This response was 51% greater than the equivalent treatment response for mizuna and 70% greater than the response of mustard. For all three species, greatest FW was observed at 200 mg N•L^{-1}.

Dry Weight. DW of arugula, mizuna and mustard increased linearly as fertilizer concentration increased from 0 to 200 mg N L^{-1} (Figure 3.2). Arugula exhibited the largest increase in DW of 51% as fertilizer concentration increased from 0 to 200 mg N•L^{-1}. The effects of fertilizer concentration on DW varied by species where mizuna (p<0.0001) and mustard

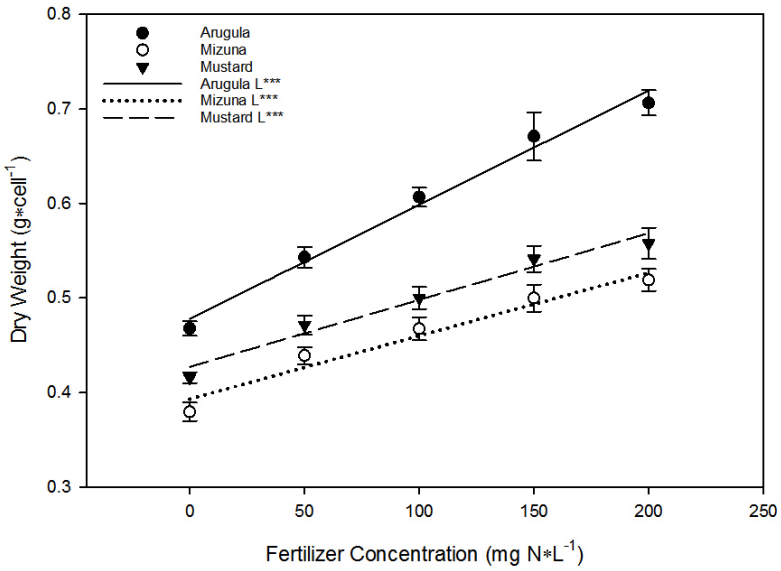

Figure 3.2. Dry weight per cell of arugula, mizuna and mustard microgreens under five fertilizer concentrations of 0, 50, 100, 150 or 200 mg N•L^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at *P* ≤ 0.05, 0.01, or 0.001, respectively.**

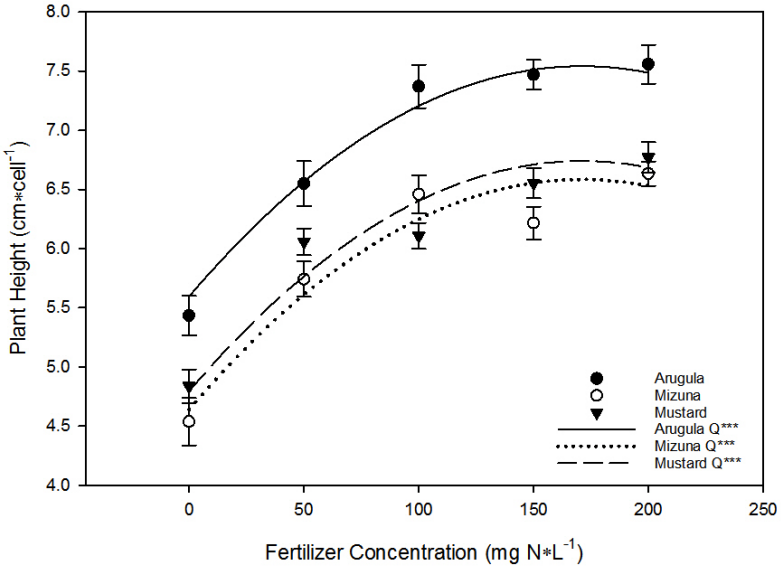

Figure 3.3. Plant height per cell of arugula, mizuna and mustard microgreens under five fertilizer concentrations of 0, 50, 100, 150 or 200 mg N•L^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at *P* ≤ 0.05, 0.01, or 0.001, respectively**

(p<0.0001) were significantly less influenced than arugula to increases in fertility (Table 2.2). DW increased by 37% for mizuna and 34% for mustard as fertilizer concentration increased from 0 to 200 mg N•L^{-1}.

Plant Height. Plant height for arugula, mizuna and mustard increased in a quadratic function as fertilizer concentration increased from 0 to 200 mg N•L^{-1} (Figure 3.3). For all three species, little to no gain in plant height was observed above 150 mg N•L^{-1}. For example, average plant height for arugula increased by 39% as fertilizer concentration increased from 0 to 200 mg N•L^{-1}. An increase of only 3% in plant height occurred as fertilizer concentration increased from 100 to 200 mg N•L^{-1} for arugula. The effect of fertilizer concentration on plant height

remained constant across species with the only difference in plant height between species attributable to species variation (Table 2.1). The plant heights for mizuna (p=0.0186) and mustard (p=0.0366) were significantly different from arugula but not from each other where arugula was always taller than mizuna and mustard.

Table 2.1. Analysis of variance for the effects of species, fertilizer, species•fertilizer, fertilizer^2 and model-accounted variability (R^2).

Fertilizer Concentration Experiment			
Main Effects & Interactions	*Plant Height*	*Fresh Weight*	*Dry Weight*
Species	*	*	**
Fertilizer	***	***	***
Species•Fertilizer	NS	***	***
Fertilizer^2	***	***	NS
R^2	0.71	0.92	0.88

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 2.2. Regression values for the response of plant height, fresh weight and dry weight for arugula, mizuna and mustard to per unit changes in fertilizer concentration (mg N•L^{-1}) including squared terms for a quadratic model.

Fertilizer Concentration Experiment			
Plant Height			
		Arugula	
Slope	Esimate[B]	Std. Err.[B]	*P*-value
Intercept	5.5955667	0.218652	***[A]
Fertilizer	0.0227551	0.001512	***[A]
Fertilizer^2	-0.00006649	0.000007249	***[A]
		Mizuna	
Intercept	4.6393642	0.218659	***[A]
Fertilizer	0.0227551	0.001512	***[A]
Fertilizer^2	-0.00006649	0.000007249	***[A]
		Mustard	
Intercept	4.7948397	0.218662	***[A]
Fertilizer	0.0227551	0.001512	***[A]
Fertilizer^2	-0.00006649	0.000007249	***[A]
Fresh Weight			
		Arugula	
Intercept	5.9674717	0.989867	*[A]
Fertilizer	0.0520661	0.001992	***[A]
Fertilizer^2	-0.000104	0.000008857	***[A]
		Mizuna	
Intercept	6.3517051	0.989868	*[A]
Fertilizer	0.0414543	0.001989	***[A]
Fertilizer^2	-0.000104	0.000008857	***[A]
		Mustard	
Intercept	7.4201752	0.989868	*[A]
Fertilizer	0.0422526	0.001989	***[A]
Fertilizer^2	-0.000104	0.000008857	***[A]
Dry Weight			
		Arugula	
Intercept	0.4778417	0.048445	**
Fertilizer	0.0012081	0.000053	***
		Mizuna	
Intercept	0.3929333	0.048445	*
Fertilizer	0.0006693	0.00005289	***
		Mustard	
Intercept	0.4270988	0.048446	**
Fertilizer	0.0007083	0.00005289	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

[A] Significance when treatment effects are at their average value

[B] Estimated response when all other treatment effects are equal to zero

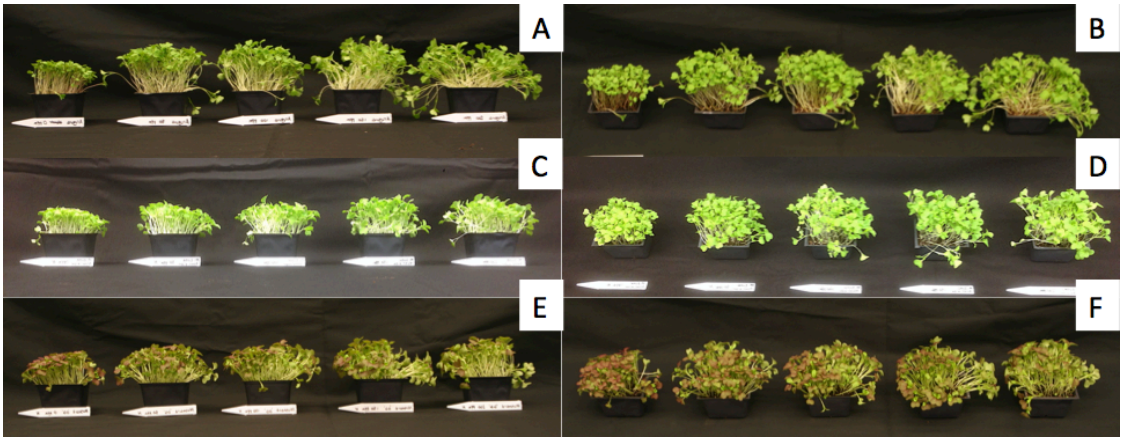

Figure 3.4. Images of arugula, mizuna and mustard under five fertilizer concentrations of 0, 50, 100, 150 and 200 mg N•L^{-1} . A-B, C-D and E-F represent arugula, mizuna and mustard, where A, C and E are in the horizontal plane and B, D and F are in the vertical plane. Within A-F, fertilizer concentrations of 0 to 200 mg N•L^{-1} are left to right.

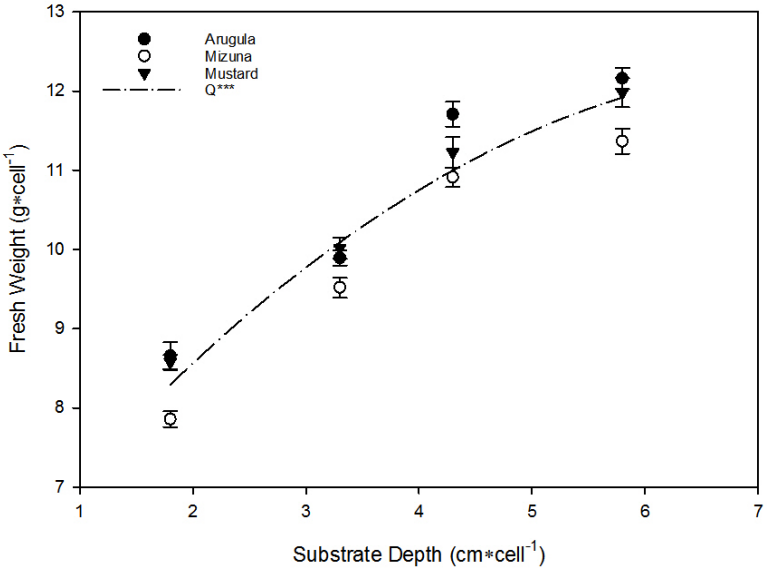

Figure 4.1. Fresh weight per cell of arugula, mizuna and mustard microgreens under four substrate depths of 1.8, 3.3, 4.3 and 5.8 cm•cell^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at *P* ≤ 0.05, 0.01, or 0.001, respectively.**

Substrate Depth

Fresh Weight. FW for arugula, mizuna and mustard increased in a quadratic fashion as substrate depth increased from 1.8 to 5.8 cm (Figure 4.1). For example, FW for arugula, mizuna and mustard increased by 41, 45 and 40% as substrate depth increased from 1.8 to 5.8 cm with no significant difference between species.

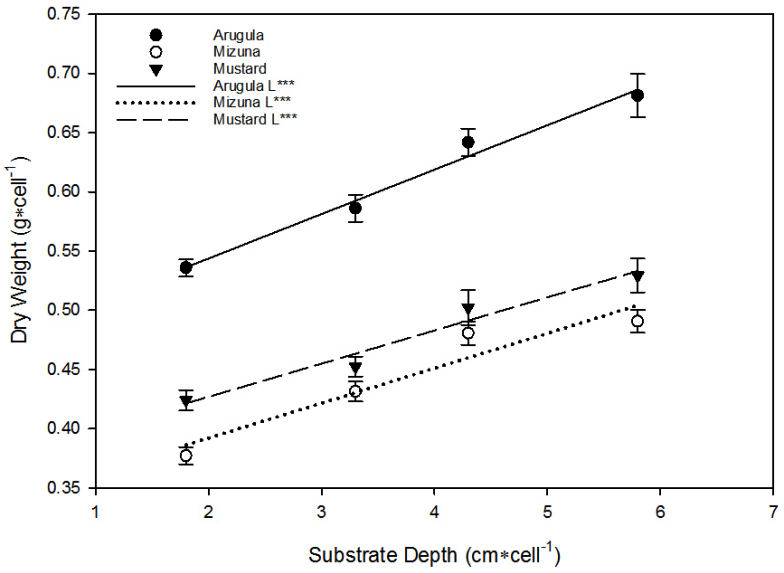

Figure 4.2. Dry weight per cell of arugula, mizuna and mustard microgreens under four substrate depths of 1.8, 3.3, 4.3 and 5.8 cm•cell^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

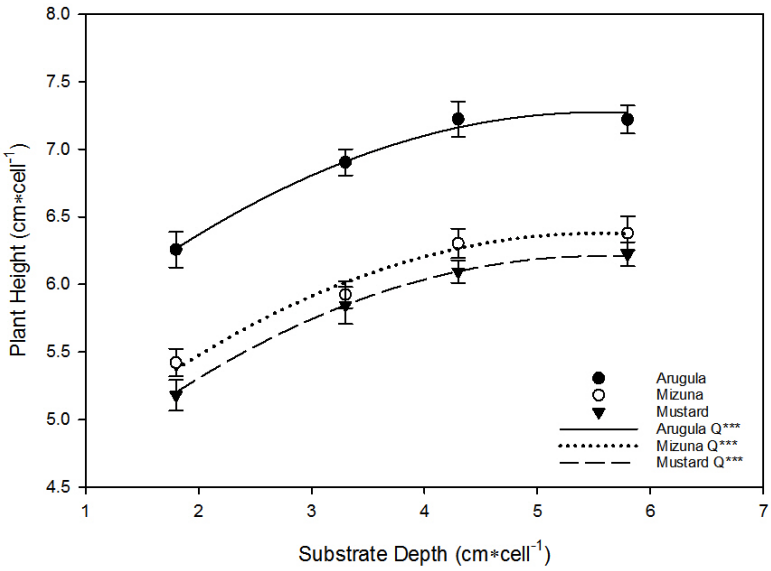

Figure 4.3. Plant height per cell of arugula, mizuna and mustard microgreens under four substrate depths of 1.8, 3.3, 4.3 and 5.8 cm•cell^{-1}. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

Dry Weight. DW for arugula, mizuna and mustard increased linearly as substrate depth increased from 1.8 to 5.8 cm (Figure 4.2). The effect of species on DW was not significant; however there was a significant interaction between substrate depth and species (Table 3.1). The slope of the interaction term for mizuna (p=0.0489) and mustard (p=0.0195) at an average substrate depth, were significantly different from arugula but not from each other. For example, the estimated increase in DW per mm increase in substrate depth was 30% greater in arugula than the average per unit increase for mizuna and mustard.

Plant Height. Plant height increased for arugula, mizuna and mustard in a quadratic fashion as substrate depth increased from 1.8 to 5.8 cm (Figure 4.3). The effect of substrate depth on plant height did not vary by species with average plant height across all species increasing by 18% as substrate depth increased from 1.8 to 5.8 cm. Plant height did vary by species where mizuna (p=0.0297) and mustard (p=0.0149) were significantly shorter than arugula by an average of 1 cm across all substrate depths but were not significantly different from each other.

Table 3.1. Analysis of variance for the effects of species, substrate, species•substrate, substrate^2 and model-accounted variability (R^2).

Substrate Depth Experiment			
Main Effects & Interactions	*Plant Height*	*Fresh Weight*	*Dry Weight*
Species	*	NS	NS
Substrate	***	***	***
Species•Substrate	NS	NS	*
Substrate^2	***	***	NS
R^2	0.79	0.83	0.89

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 3.2. Regression values for the response of plant height, fresh weight and dry weight for arugula, mizuna and mustard to per unit changes in substrate depth (cm•cell^{-1}).

Substrate Depth Experiment			
Plant Height			
	Arugula		
Slope	Esimate[B]	Std. Err.[B]	*P*-value
Intercept	5.0636649	0.267708	***[A]
Substrate	0.7959202	0.086262	***[A]
Substrate^2	-0.071601	0.0112	***[A]
	Mizuna		
Intercept	4.1688732	0.267708	***[A]
Substrate	0.7959202	0.086262	***[A]
Substrate^2	-0.071601	0.0112	***[A]
	Mustard		
Intercept	3.9994483	0.267788	***[A]
Substrate	0.7959202	0.086262	***[A]
Substrate^2	-0.071601	0.0112	***[A]
Fresh Weight			
	Arugula; Mizuna; Mustard		
Intercept	5.435257	0.703136	**[A]
Substrate	1.7988614	0.18596	***[A]
Substrate^2	-0.117396	0.024144	***[A]
Dry Weight			
	Arugula		
Intercept	0.4688717	0.053196	***
Substrate	0.0374926	0.002839	***
	Mizuna		
Intercept	0.3331415	0.053244	**
Substrate	0.0294694	0.002896	***
	Mustard		
Intercept	0.3708944	0.053207	**
Substrate	0.0280133	0.002868	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

[A] Significance when treatment effects are at their average value

[B] Estimated response when all other treatment effects are equal to zero

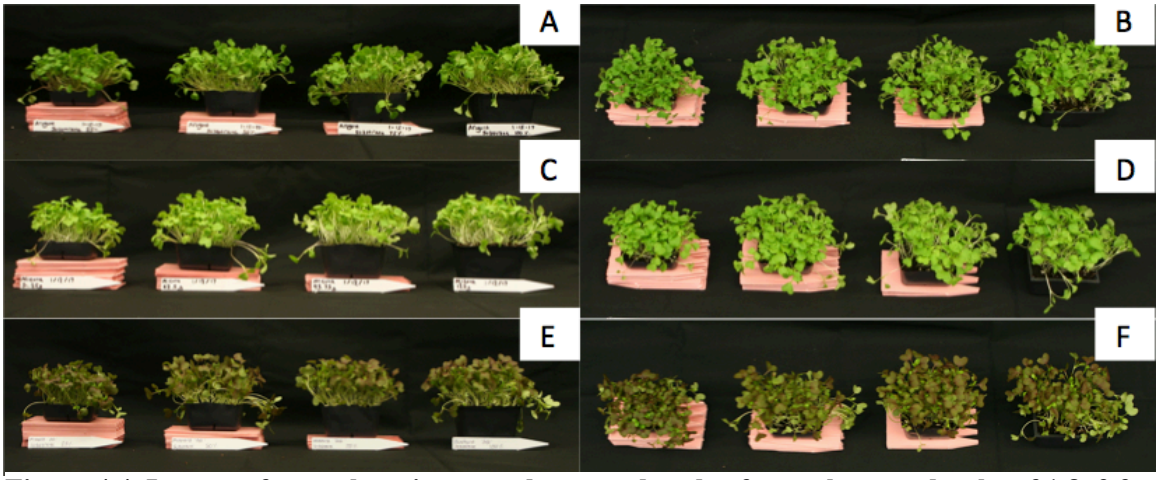

Figure 4.4. Images of arugula, mizuna and mustard under four substrate depths of 1.8, 3.3, 4.3 and 5.8 cm•cell^{-1}. A-B, C-D and E-F represent arugula, mizuna and mustard, where A, C and E are in the horizontal plane and B, D and F are in the vertical plane. Within A-F, substrate depths of 1.8 to 5.8 cm•cell^{-1} are left to right.

Air Temperature

Days to Harvest. DTH for arugula, mizuna and mustard decreased linearly as air temperature increased from 14 to 22°C (Figure 5.1). The effect of temperature on DTH remained constant across all three species (Table 4.2). DTH varied by species where Mizuna (p=0.0141) and mustard (p=0.0499) were ready for harvest significantly earlier than arugula but DTH did not differ between them. For example, DTH for arugula decreased by an average of 7 days from 19 to

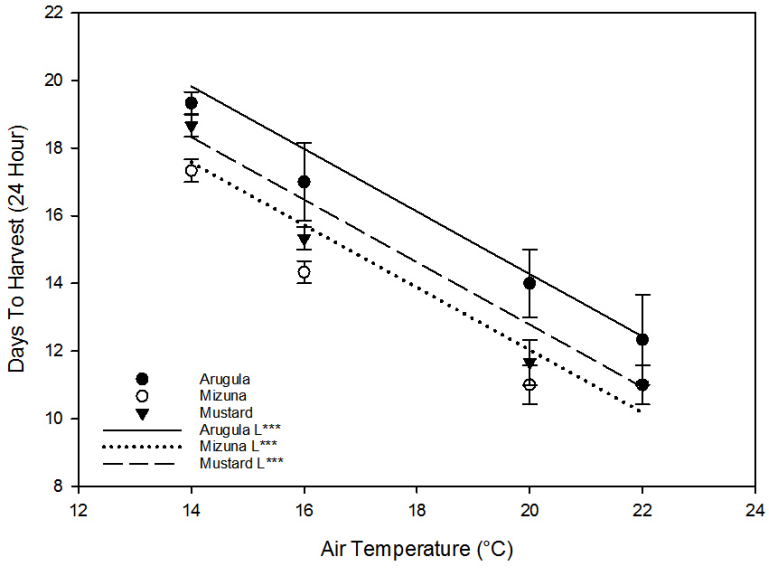

Figure 5.1. Days to harvest of arugula, mizuna and mustard microgreens under four air temperatures of 14, 16, 20 and 22°C. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

12 days as temperatures increased from 14 to 22°C. Mizuna DTH decreased by an average of 6 days while mustard decreased by an average of 8 days from a starting point of 17 and 19 days, respectively, as temperatures increased from 14 to 22°C.

Table 4.1. Analysis of variance for the effects of species, temperature and model-accounted variability (R^2).

Air Temperature Experiment	
Main Effects & Interactions	*Days To Harvest*
Species	*
Temperature	***
R^2	0.92

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 4.2. Regression values for the response of DTH for arugula, mizuna and mustard to per unit changes in air temperature (°C).

Air Temperature Experiment			
	Days To Harvest		
		Arugula	
Slope	Esimate[B]	Std. Err.[B]	*P*-value
Intercept	32.786508	1.188546	***
Temperature	-0.925397	0.055246	***
		Mizuna	
Intercept	30.536508	1.188546	***
Temperature	-0.925397	0.055246	***
		Mustard	
Intercept	31.286508	1.188546	***
Temperature	-0.925397	0.055246	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.
[A] Significance when treatment effects are at their average value
[B] Estimated response when all other treatment effects are equal to zero

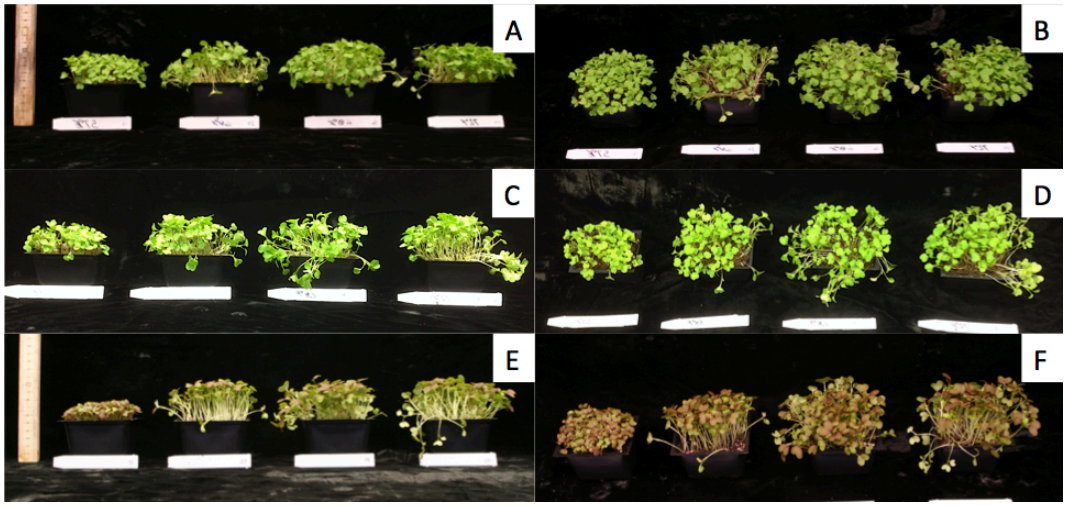

Figure 5.2. Images of arugula, mizuna and mustard under four air temperatures of 14, 16, 20 and 22°C taken when the first temperature treatment was ready to harvest (22°C) showing variation in development rate. A-B, C-D and E-F represent arugula, mizuna and mustard, where A, C and E are in the horizontal plane and B, D and F are in the vertical plane. Within A-F, air temperatures of 14 to 22°C are left to right.

Discussion

Seeding Density

Recommendations for specific seeding rates vary widely by seed supply company and typically are not specified according to plant species/cultivar. For example, Johnny's Seeds recommends 2.14 g per 10" x 20" tray at 1.1 seeds•cm^{-2}, while Mountain Valley Seed Company (Salt Lake City, UT USA) (not evaluated) suggests a seeding rate of 28.34 g per 10" x 20" tray at 12.3, 10.5 and 10.8 seeds•cm^{-2} for arugula, mizuna and mustard, respectively. Our seed density experiment which deviated from the recommended seeding rates found on Johnny's Seed packages illustrated that incremental increases in seed density result in a quadratic increase in FW per unit area over recommended rates.

Also observed was a quadratic decrease in FWPP as seed density increased from 1.1 to 3.3 seeds•cm^{-2}. This increase in population FW and simultaneous reduction in FWPP as observed in this experiment has been previously documented. Murphy and Pill (2010) reported similar

findings with population FW increasing linearly and FWPP decreasing linearly as seed density increased from 0.81 to 3.18 seeds•cm^{-2}. The higher seed density of 3.3 seeds•cm^{-2} explored in this experiment could explain the observed quadratic response not found in other literature on microgreen seed density. The significant decrease in FWPP observed in arugula can also be seen in the lowest population FW increase of the three species, as represented by the smallest per unit increase in FW as a response to increasing seed density. Observations throughout the experiment noted the particular sensitivity of arugula to changes in environmental stimuli. Observations (not shown) found that arugula exhibited a smaller leaf area and more tender tissue as compared to mizuna and mustard with increasing seed density. Arugula also appeared to have greater disease susceptibility particularly at higher seed densities, which is one of the reasons why the recommended rates are lower than optimal yield densities.

Response of plant height to increasing seed density, although not significantly different by species, points to hypocotyl elongation as a mechanism to outcompete neighboring seedlings for light and growing space. In crowded plants, branching is reduced and plant height is increased relative to biomass, stem diameter, and leaf area (Schmitt and Wulff, 1993). This phenomenon is clearly evidenced in the relationship between increased population FW, decreased FWPP, increased plant height and observed traits associated with reduced leaf area and more tender tissue.

Fertilizer Concentration

Many small to medium sized greenhouse growers in central New York are highly diversified in respects to the crops they grow. Based on our observations, many growers utilize the same growing media and fertility treatments across crop species (such as vegetable

transplants and bedding plants). As a result, microgreens produced in these operations are subject to potentially suboptimal fertility regimes. For all three species, a quadratic response in FW to fertilizer concentration was found with optimal concentration between 150 to 200 mg $N \bullet L^{-1}$. These results are well supported by Murphy and Pill, (2010) who found that a constant liquid feed of 150 mg $N \bullet L^{-1}$ resulted in the optimal FW yields for microgreen arugula. Although FW for all species is highest at 200 mg $N \bullet L^{-1}$, the quadratic model illustrates a decreasing yield return with increasing fertilizer rates above 150 mg $N \bullet L^{-1}$.

This phenomenon was not reciprocated in DW yields which exhibited a linear response for all three species as nitrogen increased from 0 to 200 mg $N \bullet L^{-1}$. To our knowledge, no literature exists on the effects of complete water-soluble fertilizer concentration on microgreen DW yields. One explanation for the lack of collinearity between FW and DW could be reduced water uptake at the highest fertilizer concentration due to osmotic stress. Albornoz and Lieth (2015) demonstrated a relationship between increasing fertilizer water EC and decreased FW and DW in lettuce where reductions in FW were observed beyond an EC of 1.2 $ds \bullet m^{-1}$ while DW did not begin decreasing until an EC of 4.8 $ds \bullet m^{-1}$. However, we did not observe any obvious symptoms of water stress in any of the treatments. Further work would need to be conducted in this area to determine the relationship.

To our knowledge, the response of microgreen plant height to fertilizer concentration has not been investigated in the literature. Our results showed a quadratic increase in plant height for all three species as fertilizer increased from 0 to 200 mg $N \bullet L^{-1}$, with the effects being independent of species. The effects of fertilizer concentration on the plant height of greenhouse grown lettuce shows a very similar trend. Work by Soundy et al. (2005) illustrated a quadratic response of plant height of lettuce as nitrogen increased from 0 to 120 mg $N \bullet L^{-1}$, with no gains in

plant height above 90 mg $N \bullet L^{-1}$. Results for the three species of microgreens in this experiment all appear to have little to no gain in plant height above 100 mg $N \bullet L^{-1}$.

Substrate Depth

Substrate depth (i.e. root volume) treatments impact both physical properties (moisture holding capacity and air porosity) as well as total nutrients. All of these parameters influenced the growth responses we observed. Work by Cardoso et al. (2015) found that fresh mass of lettuce transplants decreased by 27.6% for plants grown under severe root confinement as compared to no confinement. Van Iersel (1997) found that growth of salvia (*Salvia splendens*) in 7.3 ml cells was reduced because of a low net assimilation rate compared to plants in 55, 160 and 510 ml pots. Plants in the 510 ml containers grew faster, exhibited greater lateral branching, larger leaf area and earlier flowering due to a larger leaf area ratio and lesser degree of root restriction. These results correlate well with our findings. With FW for all three species increasing in a quadratic fashion as substrate depth increased from 1.8 to 5.8 cm (86.4 to 278.4 cm^3), the level of root confinement can be assumed to be changing at the same slope. It was observed that the 5.8 cm treatment did not contain any roots in the bottom of the cell which may explain the reduced gain in FW observed above 4.3 cm due to the roots already having an adequate level of water and nutrients at the 4.3 cm depth. For all replications, an excess or deficiency in moisture content by treatment was observed. Cells at a substrate height of 1.8 cm dried out more frequently and needed more frequent irrigation than the 5.8 cm treatment which exhibited excess moisture and the occurrence of disease pressure in select replicates of arugula.

DW did not share the same correlation with FW as represented by a linear increase in DW as substrate depth increased from 1.8 to 5.8 cm. Van Iersel (1997) also reported a linear

response of shoot dry mass to increasing substrate volume but did not investigate shoot FW or plant height. The response of plant height to increasing substrate depth also fits the assumption well. The species independent quadratic response of plant height shows reduced gains in plant height above a substrate height of 4.3 cm. Based on the results and supporting literature, it is reasonable to conclude that a smaller root mass as a result of lower substrate heights had a large effect on shoot FW, DW and plant height.

Air Temperature

Similar to seed density, seed company recommendations for germination and growing temperatures are not species specific. For example, Johnny's Seeds recommends 24 °C for germination and 16 °C from germination until harvest. Although it is known that the optimal temperature for net photosynthesis decreases as the crop develops (Seginer et al., 1991); this model is more appropriately applicable to crops after development on the first true leaves and greater net photosynthetic potential is achieved. The range of temperature treatments did not exceed the peak net photosynthetic temperature of approximately 24 °C as defined by Seginer et al. (1991). The ability of the highest temperature treatments to maintain a maximum photosynthetic rate and leaf area ratio (LAR) development rate is well represented by the linear decrease in DTH for all species. DTH variations by species that were independent of temperature could signify genetic variation in maturation time. This is further supported by the significant difference between mizuna and arugula and mustard and arugula but not mizuna and mustard. The lower temperature treatments had significantly longer DTH, which was due to a slower development rate. Results on FW, DW, and plant height were not presented due to the high

variability associated with the data as a result of the significantly differently growing time that treatments were given to reach the defined harvest size.

Conclusion

Variations in the cultural practices associated with the cultivation of microgreens can have a significant effect on FW and DW yields, plant height and DTH. By altering the growing environment for seed density, fertilizer concentration, substrate depth and air temperature, growers can maximize yield, minimize production time and achieve a target crop size based on individual market demand. With exception of the FW response to seed density and treatments where species had no effect, mizuna and mustard exhibited no statistical differences from one another in their response to treatments. This may suggest that these particular crops of the same genus can be assumed to be equal within the parameters of the experiments conducted in this research. If further research finds this more broadly applicable, the potential to designate microgreen production protocols based on genus rather than species could offer growers a simple solution to maximizing yields. In addition, when new varieties are released, growers would have a basis for starting cultivation, making it possible to bring new varieties to market sooner and at a higher quality than may have been previously possible. Although a full factorial experiment was not conducted in our study and should be a subject for future research, our results may have value for growers seeking to incorporate production protocols by examining the feasibility of modifying their cultural practices to emulate the parameters explored in this as well as supporting research. For example, based on our findings, initial parameters might be to sow seed at a rate of 2.75 seeds•cm^{-2} over a peat-lite media at a height of 4.3 cm, fertilized with a complete liquid feed fertilizer at a rate of 150 mg N•L^{-1} and grown in a greenhouse with an average daily

temperature of 22 °C. If a substrate height/volume lower than 3.3/158.4 (seeds•cm^{-2}/cm^2) is used, growers should consider using an automated watering system to avoid the potential of rapid root zone moisture loss beyond the crops permanent wilting point. Depending on individual market designation of crop morphological state, shortest DTH may be the most significant factor in a grower's decision-making process. Because of this, factors determined to yield greatest FW or shortest DTH, within the scope of this research may not be applicable to all microgreen producers. The discrepancy between the quadratic and linear models of FW and DW warrants further investigation. However, since FW is often of greatest concern to growers and consumers, management decisions should be made based on profitable yields which are most greatly associated with the FW regression models.

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CHAPTER 2

Effects of Daily Light Integral and Carbon Dioxide Concentration on the Growth, Development and Secondary Metabolites of Three Microgreen Species

Abstract

Microgreen cultivation in hoop-houses, greenhouses and indoor production vertical farms (plant factories) has been increasing in recent years due to their rising popularity and perceived health benefits with consumers. Daily light integral (DLI) targets for microgreen production vary widely between publications with no formal recommendations by seed supply companies. Additionally, to our knowledge, no published literature exists on the effects of carbon dioxide (CO_2) supplementation on the growth and development of these crops. The objective of this study was to quantify the effects of four DLI and four CO_2 treatments on the growth, morphology and secondary metabolite content of three microgreen species: arugula (*Eruca sativa* L.), mizuna (*Brassica rapa* L. var. *japonica*) and mustard [*Brassica. juncea* (L.) Czern. 'Garnet Giant']. All species were grown in a commercial peat-lite mix in walk-in growth chambers with plants inside mini-chambers for CO_2 control. Four levels of DLI (3, 6, 9 and 12 $mol•m^{-2}•d^{-1}$) by four levels of CO_2 (400, 600, 800 and 1000 ppm) were evaluated under a full factorial design. Fresh weight (FW) increased linearly for mizuna and mustard as DLI and CO_2 increased from 3 to 12 $mol•m^{-2}•d^{-1}$ and 400 to 1000 ppm. Arugula FW increased in a quadratic fashion as DLI increased from 3 to 12 $mol•m^{-2}•d^{-1}$ and linearly as CO_2 increased from 400 to 1000 ppm. Dry weight (DW) increased linearly for all species as DLI and CO_2 increased from 3 to 12 $mol•m^{-2}•d^{-1}$ and 400 to 1000 ppm. For mizuna and mustard, days to harvest (DTH) decreased in a quadratic fashion while arugula DTH decreased linearly as DLI increased from 3

to 12 mol•m^{-2}•d^{-1} with no observed influence from CO_2. Total phenolics and total flavonoids increased linearly as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} where the effect of DLI on phenolic content was dependent on the CO_2 level. The results of this study can help growers determine combinations of DLI and CO_2 to achieve maximum yields at the lowest lighting energy input. Growers can then determine the importance of achieving maximum phenolic and flavonoid compounds and adjust light and CO_2 as needed.

Introduction

Microgreens are a broadly classified specialty crop produced for wholesale and retail markets alike. This relatively new category of crops consists of numerous vegetables and herbs harvested shortly after emergence of the first true leaf or leaves but prior to true leaf development and senescence of the cotyledons (Gerovac et al., 2016; Gioia et al., 2016). Growth intervals range between ten and twenty days from seeding to harvest (Lee and Pill, 2004) with variations attributable to species, cultural and environmental practices. Their presence in local and national markets has been fueled by consumer demand, ease of production and adaptability to many types (high tunnel, greenhouse, indoor vertical farms) and scales of production facilities. With a current wholesale value of US $66 to $110 per kg of clamshell-packaged product (Treadwell et al., 2010), their high value have incentivized commercial greenhouse growers to begin producing them (Gerovac et al., 2016). Production of microgreens can be found in many forms ranging from soilless media in trays to capillary mats in troughs (Treadwell et al., 2010) with some growers incorporating supplemental or sole-source lighting into their production systems.

In Northern latitudes of North America and Europe, light quantity is often a limiting

factor for the growth of plants in greenhouses during the winter months (Duke et al., 1975). The use of supplemental lighting providing photosynthetically active radiation (PAR, radiation from 400 to 700 nm wavelength) in greenhouse production systems and its effects on plant yield has been well established. Work by Albright et al. (2000) found a linear relationship between shoot DW and total accumulated light as DLI increased from 8 to 22 mol•m^{-2}•d^{-1} in greenhouse-grown butterhead lettuce (*Lactuca sativa* L.). Kitaya et al. (1998) also reported a linear increase in percent DW of lettuce plug transplants with increasing DLI in two categories from 5.8 to 17.3 and 8.6 to 25.9 mol•m^{-2}•d^{-1}. Light quality or spectral distribution as referring to the wavelengths of light ranging from ultraviolet A (UVA, 315 to 400 nm) to far red (FR, 705 to 740 nm) have a significant effect on plant morphology (Pocock, 2015). In general, target DLIs vary by crop ranging from 12 to 17 mol•m^{-2}•d^{-1} for hydroponically grown lettuce, 15 to 30 mol•m^{-2}•d^{-1} for greenhouse peppers (*Capsicum annuum* L.) and tomatoes (*Solanum lycopersicum* L.) and 15 to 30 mol•m^{-2}•d^{-1} for greenhouse cucumbers (*Cucumis sativus* L.) (Mattson, 2017).

DLI targets for microgreens have only recently been investigated but it is well documented that spectral light quality (LQ) and DLI can have significant effects on the growth, morphology and nutritional quality of microgreens. Gerovac et al. (2016) found that increasing DLI from 6 to 18 mol•m^{-2}•d^{-1} decreased hypocotyl length by 32% for Kohlrabi (*Brassica oleracea* L.), while simultaneously increasing FW and percent DW by 34 and 19% in Mustard. Brazaityte et al. (2015) found that in general, *Brassicaceae* microgreens accumulated more total carotenoids at higher DLI's of 19 to 25 mol•m^{-2}•d^{-1} as compared to 12.7 mol•m^{-2}•d^{-1}. However, the use of supplemental or sole-source lighting for plant production can amount to significant monthly energy consumption. Harbick and Albright, (2016) found energy use for supplemental greenhouse lighting of butterhead lettuce varied by location from 2,943 GJ•ha^{-1}•y^{-1} in Phoenix,

AZ to 9,402 GJ•ha^{-1}•y^{-1} in Helena, MT at a target DLI of 17 mol•m^{-2}•d^{-1} and a light efficacy of 1.70 µmol/J. Today's most efficacious electric light emitting diode (LED) lights have an efficacy of 2.46 µmol/J but their initial cost remains high as compared to high pressure sodium (HPS) fixtures (Nelson and Bugbee, 2014). In plant factories electricity for lighting constitutes one of the greatest costs associated with indoor vertical production systems (Goto, 2012). Harbick and Albright, (2016) quantified these costs at 36,500 GJ•ha^{-1}•y^{-1} for head lettuce at a DLI of 17 mol•m^{-2}•d^{-1}; making plant factories significantly more energy intensive than traditional greenhouses for the range of climates in the continental United States.

The benefits of CO_2 enrichment on plant growth is well known. Kitaya et al. (1998) concluded that increasing levels of CO_2 from 400 to 800 ppm significantly increased percent DW of lettuce plug transplants while Park et al. (2012) found that increasing CO_2 from 350 to 1000 ppm resulted in increased plant size and FW of lettuce. Additionally, it has been shown that different combinations of DLI and CO_2 enrichment can result in the same fresh and dry mass of lettuce by either increasing DLI and reducing CO_2, or decreasing DLI and increasing CO_2 (Both et al., 1997). Subsequent work has modeled this relationship using computer algorithms resulting in a 15% savings in electricity cost•m^{-2}•y^{-1} for supplemental lighting over a constant PAR integral at ambient CO_2 (Ferentinos et al., 2000). The intent of this work is to investigate the use of supplemental CO_2 as a means to reduce DLI thresholds in microgreen production systems.

To our knowledge, no published data exists on the effects of both DLI and CO_2 concentration on the growth and secondary metabolite content of microgreens. Results could aid greenhouse growers in establishing DLI and CO_2 values based on a target FW or DW. Additionally, results could have particular implications for application in plant factories where a DLI could be set at a minimum threshold and CO_2 could be utilized to make up the difference in

growth, thereby reducing electricity costs for electrical lighting.

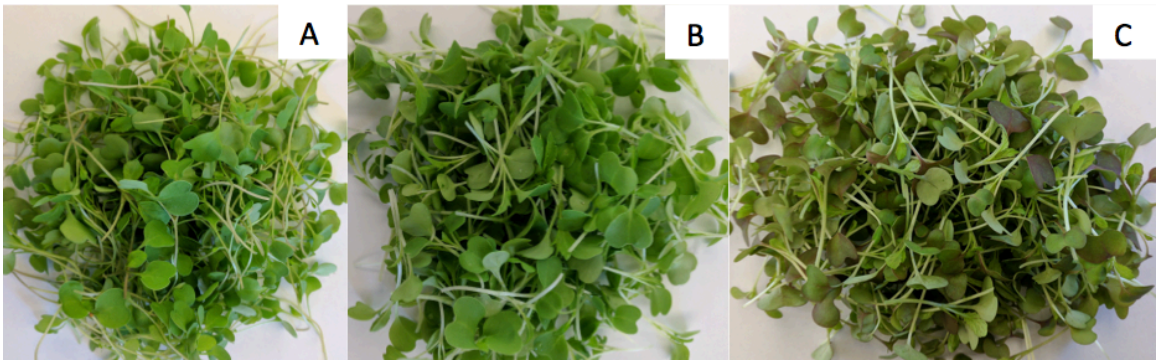

Figure 3. Images of microgreen species utilized in this experiment where A, B and C represent arugula, mizuna and mustard.

Materials and Methods

Three microgreen species of the Brassicaceae Family; arugula (*Eruca sativa* L.), mizuna (*Brassica rapa* L. var. *japonica*) and mustard [*B. juncea* (L.) Czern. 'Garnet Giant'] (Figure 1) were purchased from Johnny's Selected Seeds company (Winslow, ME USA); lot numbers were 46962, 51583, and 46623, respectively. Species were chosen for their presence in local markets, similarity in seed size (± 0.2 mm) and similar number of days from seeding to harvest (± 1 day). Diversity in sensory attributes for both vision and taste were important in species selection as representative subsamples of a larger microgreen population. Arugula was chosen for its strong aroma associated with a peppery or wasabi-like flavor. Mizuna was chosen for its mild flavor and unique morphology of fringed true leaves. Lastly, mustard 'Garnet Giant' was chosen for its deep red pigmentation and moderate spiciness.

Plant Material and Culture

Preliminary experiments were conducted to determine cultural parameters such as seeding density, fertilizer and substrates that resulted in good plant performance. A media-based

growing system was created using polystyrene 2401 inserts (53.3 cm x 26.7 cm x 5.7 cm; Dillen-ITML Greenhouse) placed in flats containing drainage holes (54.5 cm x 27.8 cm x 6.2 cm; TO Plastics). 24 cells (8 cm x 6 cm) per insert were filled to the top resulting in a volume per cell of 278.4 cm^3 with a commercial peat-lite mix (LM-111, Lambert Peat Moss, Rivière-Ouelle, Canada) at a moisture content ratio of 1:1 (peat-lite mix: fertilizer solution) by weight using a 150 mg $N•L^{-1}$ liquid fertilizer (21 N – 2.2 P – 16.6 K Jack's All-Purpose Liquid Feed, J. R. Peter's Inc., Allentown, PA). Cells were seeded at a rate of 2.75 seeds$•cm^{-2}$ resulting in 125 seeds per cell. Seeds were counted by weight after determining the average weight of three sets of 100 seeds per species resulting in weights per cell of 0.223 g for arugula, 0.26 g for mizuna and 0.253 g for mustard. Seeding times were staggered by species to ensure identical development stage when they were transferred from germination chambers to treatment chambers. Mustard was the first to be seeded followed by mizuna two hours after and arugula six hours after mustard. Flats containing seeds were irrigated with tap water containing a nutrient solution of 150 mg $N•L^{-1}$ using a fine mist nozzle. Seeds were left exposed on the media surface and covered with a propagation dome (Curtis Wagner Plastics Corp.) followed by a light restrictive standard flat (TO Plastics) placed on top of the propagation dome. After seeding, plants were watered about every three days with tap water containing nutrient solution of 150 mg $N•L^{-1}$, which was applied via subirrigation by placing the tray in a container holding about 3 cm of nutrient solution for about three minutes.

Germination Environment

Trays were placed at random on plastic benches in a walk-in controlled environment chamber (M1 Walk-in; Environmental Growth Chambers, Chagrin Falls, OH) 75 cm from the

floor. Trays were oriented with lengthwise edges touching. Dummy flats were placed at each end of the row to reduce temperature edge effects. Seeds were germinated between 15 Jan. 2016 and 20 Oct. 2016 for several different crop cycles as described below. Germination took place in darkness under an average daily temperature, relative humidity (RH) and CO_2 concentration of 23 ± 0.5 °C, 75 ± 10%, 400, 600, 800 & 1000 ± 50 ppm, respectively. Seedlings were transferred to their respective treatments once approximately 95% of seedlings were 1 cm in height yielding germination times of 42 hours for arugula, 46 hours for mizuna and 48 hours for mustard. Crop height percentage was based on average representative cells. After the designated germination period, trays were divided into two sections of 12 cells per species. Cells were transferred to half flats (27.8 cm x 27.8 cm x 6.2 cm; TO Plastics) and assigned to one of four CO_2 treatments within a DLI treatment at random.

Growth and Treatment Environment

Treatments were conducted within two walk-in chambers, each equipped with two mini-chambers constructed of a rigid transparent acrylic (100 cm x 68 cm x 46 cm) with plate glass lids. Mini-chambers were oriented to assure equal light integrals between mini-chambers. A 3,840 W scalable overhead luminaire array of T5 fluorescent tubes (Pentron High Output ECO; Sylvania, Wilmington, MA USA) provided irradiance levels at plant height of 130, 235 and 330 $\mu mol \bullet m^{-2} \bullet s^{-1}$ at 1, 2 and 3 activated light banks, respectively. Mini-chambers were placed over a wood framed PVC covered platform with the growing surface 98 cm below the array. The orientation of the mini-chambers was transverse to the orientation of the walk-in chambers with each mini-chamber containing an instrument cluster at one end to monitor CO_2, temperature and RH. PAR was recorded by one quantum sensor (SQ-110; Apogee Instruments Inc., Logan, UT

USA) within each mini-chamber. A 15 W computer fan in each mini-chamber was used to distribute air to maintain uniform CO_2, temperature and RH across the chamber. Sensors were wired to a custom control program (LabVIEW; National Instruments, Houston, TX USA) for monitoring and control of mini-chamber conditions. One quantum sensor (LI-190R; LI-COR Inc., Lincoln, NE USA) in each walk-in chamber was wired to an environmental control system (Argus Control Systems Ltd. Surrey, British Columbia, Canada) for PAR control within each walk-in chamber. SQ-110 sensors were calibrated against LI-190R sensors to standardize output values and used solely for observational purposes. Four DLI treatments of 3, 6, 9 and 12 $mol \bullet m^{-2} \bullet d^{-1}$ were administered over a 14 hour ± 0.5 hour photoperiod. Shade cloth (50%) (Griffin Greenhouse Supply, Tewksbury, MA) was used in the lowest light treatment to maintain the target photoperiod. During each crop run, walk-in chambers were maintained at the same DLI and within each mini-chamber, four CO_2 treatments of 400, 600, 800 and 1000 ppm were provided.

Growth Measurements

Species were harvested when the representative average first true leaf measured 1 cm in length. Plants were measured for average plant height (from cell height to the tallest part of representative plants) and cut at cell height and weighed for FW on a per cell basis. For each treatment, 1 g FW from each replicate was collected as a representative treatment sample and frozen in liquid nitrogen (LN) for analysis of total flavonoids and phenolics. Remaining tissue was then weighed to account for loss and dried at 70 °C for 72 hours to obtain sample DW. DW values were adjusted to account for tissue removed for total flavonoids and phenolics.

Total Phenolic Analysis

Frozen samples ground in LN were analyzed for total phenolics using the Folin-Ciocalteu Phenol colorimetric method described by Singleton et al. (1999) with some alterations. In summary, 200 µl of sample extract (0.25 g•ml^{-1} 80% acetone) was brought up to 2.8 ml with double deionized (DDI) water. A total of 200 µl of Folin-Ciocalteu reagent was added, vortexed and allowed to sit in darkness at 23 °C for six minutes. 2 ml of 7% (w•v^{-1}) Na_2CO_3 was added and allowed to sit in darkness at 23 °C for 90 minutes before reading the absorbance at 750 nm. Samples were read against a blank containing all reagents with DDI water substituted for the extract. Standards were prepared by substituting the extract for 0, 10, 50, 100, 250, 500, 750 and 1000 mg•L^{-1} of a gallic acid standard. Total phenolic content was expressed in mg of gallic acid equivalents (GAE)•100 g^{-1} FW.

Total Flavonoid Analysis

Frozen samples ground in LN were analyzed for total flavonoids using the aluminum chloride colorimetric method described by Marinova et al. (2005) with some alterations. In brief, 1 ml of sample extract (0.25 g•ml^{-1} 80% acetone) was brought up to 5 ml with DDI water and vortexed. A total of 300 µl of 5% $NaNO_2$ was added to each sample, vortexed and allowed to stand in darkness at 23°C for five minutes. Then, 300 µl of 10% $AlCl_3$ was added, vortexed and placed in darkness at 23°C for six minutes. Lastly, 2 ml of 2 M NaOH was added and the final volume was brought up to 10 ml with DDI water. Samples were vortexed and placed in darkness at 23°C for 30 minutes before reading the absorbance at 510 nm. Samples were read against a blank containing all reagents with DDI water substituted for the extract. Standards were prepared by substituting the extract for 0, 10, 50, 100, 250, 500, 750 and 1000 mg•L^{-1} of a catechin

standard. Total flavonoid content was expressed in mg of catechin equivalents (CE)•100 g^{-1} fresh weight.

Statistical Methods

A total of three experimental cycles consisting of twelve runs were conducted. At each run, two walk-in chambers were set to one of four DLI treatments. Within each DLI, four CO_2 treatments were set in one of four mini-chambers each containing the three species. The experiment was designed as a multi-level mixed effects model containing five unique levels of nested parameters (trial, run, walk-in chamber, mini-chamber and species). The effects of DLI, CO_2 and species on FW, DW and plant height were analyzed using a one-way mixed effects ANOVA at $P \leq 0.05$. Analysis of treatment effects on DTH, total phenolics, and total flavonoids were analyzed as mean values averaged by species across replications within a treatment in a reduced one-way mixed effects ANOVA at $P \leq 0.05$. The software for all statistical analysis was JMP (JMP Pro 11; SAS Institute, Cary, NC).

Results

Fresh Weight

FW for mizuna and mustard increased linearly as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} (Figure 2.1). An interaction existed between DLI and species where the effects of DLI varied by species (Table1.1). For example, as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at a CO_2 concentration of 400 ppm, mean FW increased by 27% for mizuna and 35% for mustard. For arugula, DLI exhibited a quadratic response with increasing gains in FW as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} respectively. However, reduced gains in yield were most evidenced between

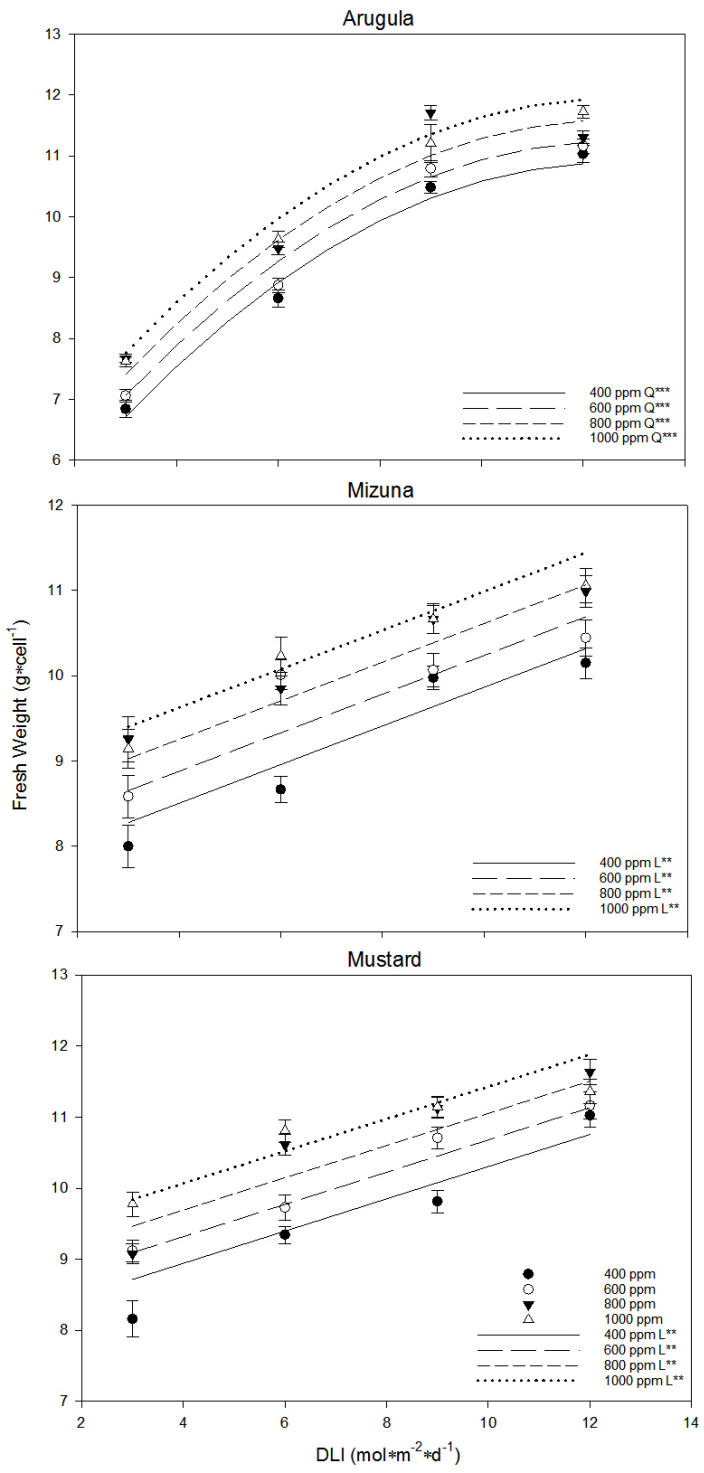

Figure 4.1. Fresh weight per cell of arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} at four CO_2 concentrations of 400, 600, 800 and 1000 ppm. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

9 and 12 mol•m^{-2}•d^{-1} with a 5% increase in FW as compared to a 27% increase from 3 and 6 mol•m^{-2}•d^{-1} and 21% from 6 and 9 mol•m^{-2}•d^{-1}. For all three species, the effects of CO_2 on FW were linear between 400 and 1000 ppm. The impact of CO_2 on FW for all three species was smaller than the impact of DLI with the degree of the difference varying by species and DLI (Table 1.2). However, CO_2 levels above 400 ppm could be used at lower DLIs to achieve a FW equivalent to 400 ppm CO_2 at a higher DLI. For example, the average FW for mizuna and mustard at 1000 ppm CO_2 and a DLI of 3, 6 and 9 mol•m^{-2}•d^{-1} was greater than the average FW at 400 ppm CO_2 and a DLI of 6, 9 and 12 mol•m^{-2}•d^{-1}, respectively.

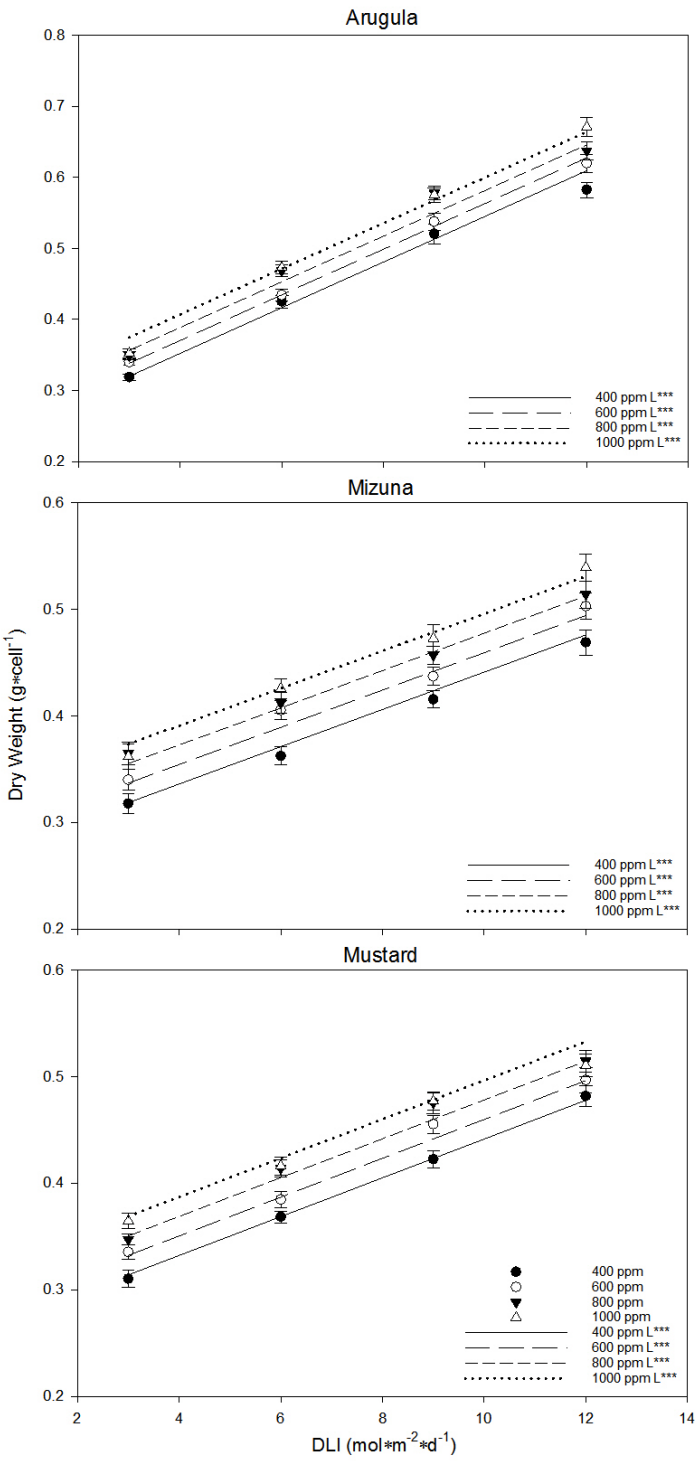

Figure 2.2. Dry weight per cell of arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} at four CO_2 concentrations of 400, 600, 800 and 1000 ppm. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

Dry weight

DW for arugula, mizuna and mustard increased linearly as DLI and CO_2 increased from 3 to 12 mol•m^{-2}•d^{-1} and 400 to 1000 ppm (Figure 2.2). The effect of DLI on DW varied by species however, not all species were significantly different from each other. At an average DLI, the slope of mizuna was not significantly different from mustard but both species were significantly different from arugula (P<0.0001). For example, as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at a CO_2 concentration of 400 ppm, mean DW increased by 48% for mizuna and 55% for mustard as compared to 83% for arugula. Elevated levels of CO_2 were effective in maintaining DW at lower DLI levels. For example, the average DW for mizuna and mustard at 1000 ppm CO_2 and a DLI of 3, 6 and 9 mol•m^{-2}•d^{-1} was equal to the average

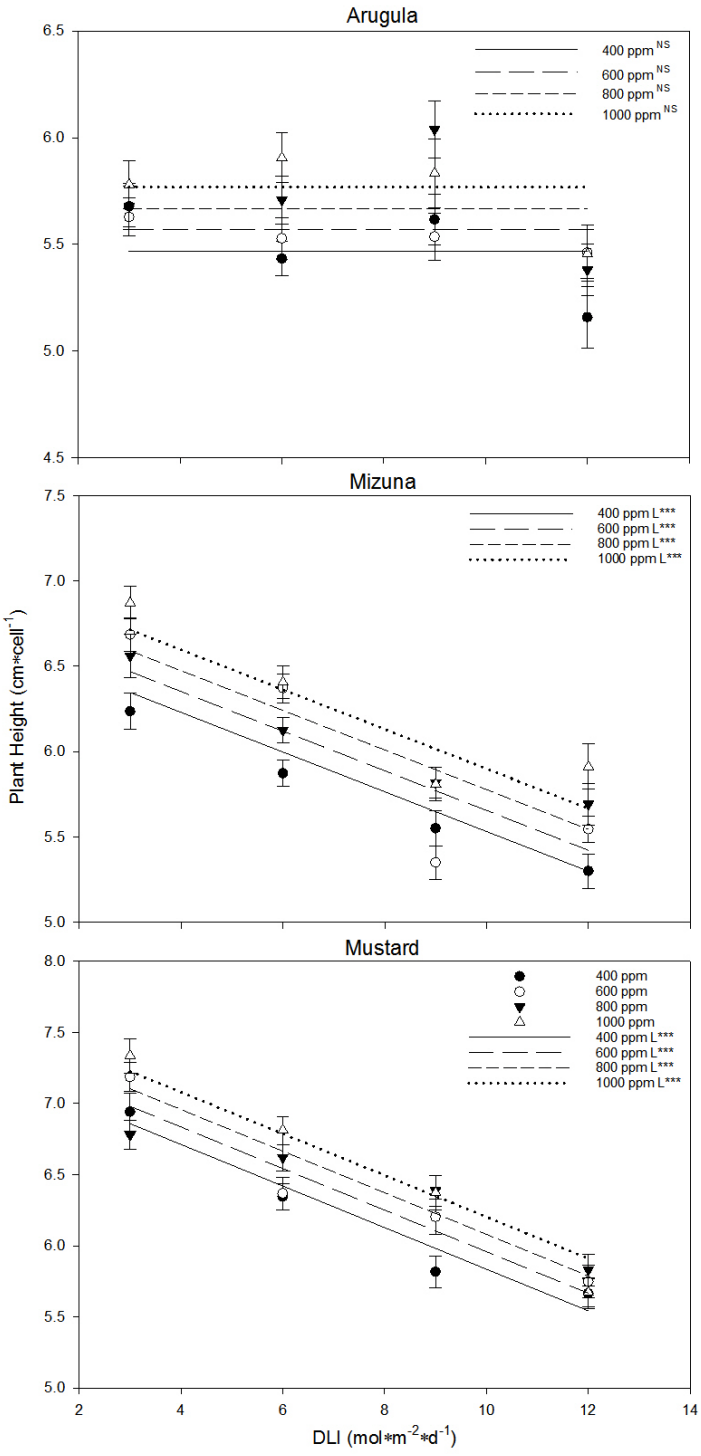

Figure 2.3. Plant height per cell of arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} at four CO_2 concentrations of 400, 600, 800 and 1000 ppm. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

DW at 400 ppm CO_2 and a DLI of 6, 9 and 12 mol•m^{-2}•d^{-1}, respectively.

Plant Height

Average plant height for mizuna and mustard decreased linearly as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} (Figure 2.3). Decreases amounted to reductions in plant height of 0.9 cm for mizuna and 1.3 cm for mustard as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at 400 ppm CO_2. An interaction between DLI and species was present where the slopes of mizuna and mustard were significantly different from arugula but not from each other. CO_2 also had a significant effect on plant height exhibiting a linear increase in plant height as concentrations increased from 400 to 1000 ppm. This resulted in an average increase in in plant height across all species and DLI levels of 0.38 cm as

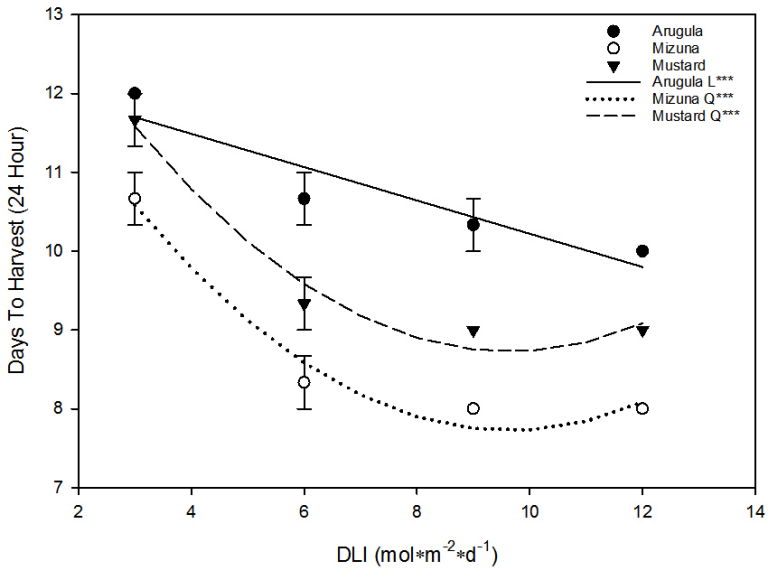

Figure 2.4. Days to harvest per for arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} for all CO_2 levels. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at *P* ≤ 0.05, 0.01, or 0.001, respectively.**

CO_2 increased from 400 to 1000 ppm.

Days to Harvest

DTH for arugula, mizuna and mustard exhibited a quadratic response with a decreasing number of days from seeding to harvest as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} (Figure 2.4). For example, for all three species, harvest time was reduced by two days (48 hours) irrespective of total crop time as DLI increased from 3 to 12 mol•m^{-2}•d^{-1}. DTH varied by species depending on the DLI where all three species were significantly different from each other. A CO_2 variable was not included in the model as all replications of a DLI treatment for each species were harvested simultaneously across all CO_2 levels. This was because it appeared that CO_2 concentration did not influence developmental stage and so all CO_2 treatments within a DLI were deemed ready to harvest at the same time.

Total Phenolics

Total phenolics increased linearly for arugula, mizuna and mustard as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} (Figure 2.5). The effect of CO_2 on total phenolics was not significant but the slope of DLI for all three species was dependent on CO_2 where the effect of DLI

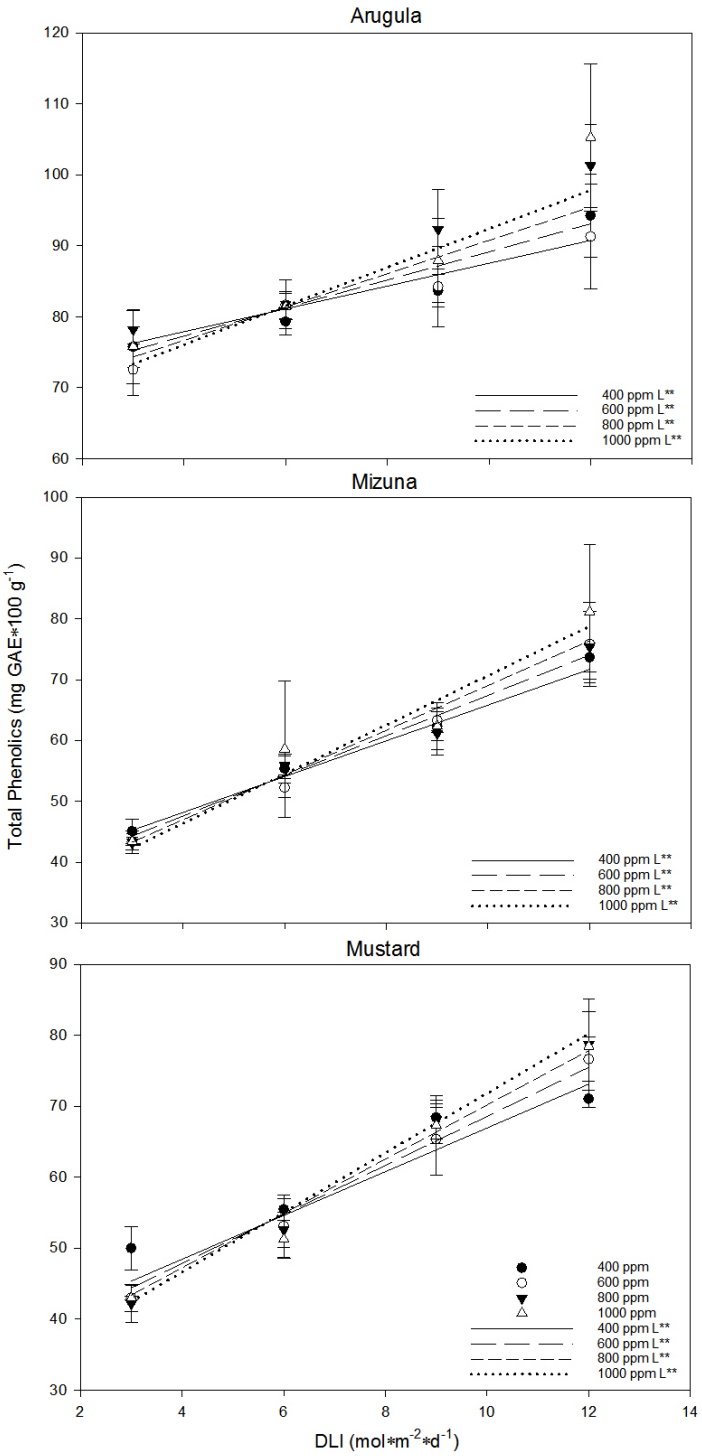

Figure 2.5. Total phenolic content in mg GAE•100 g^{-1} fresh weight tissue of arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} at four CO_2 concentrations of 400, 600, 800 and 1000 ppm. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq 0.05$, 0.01, or 0.001, respectively.**

increased as CO_2 increased from 400 to 1000 ppm (Table 1.1). Arugula exhibited the greatest total phenolic content significantly different from both mizuna and mustard (Table 1.2); increasing from an average of 75.75 to 94.27 and 75.82 to 105.26 mg•100 g^{-1} as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at 400 and 1000 ppm CO_2, respectively. The effect of DLI on total phenolics of mizuna was not significantly different from mustard however; both were significantly different from arugula in their response to the effects of DLI on total phenolics (P<0.0001). For example, total phenolics for mizuna increased from an average of 45.05 to 73.64 and 43.04 to 81.14 mg•100g^{-1} as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at 400 and 1000 ppm CO_2. This resulted in mizuna having a 55% and 28% greater increase in total phenolics over

arugula as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} at 400 and 1000 ppm CO_2, respectively.

Total Flavonoids

Total flavonoids for all three species increased linearly as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} (Figure 2.6). Arugula exhibited the greatest total flavonoid content out of the three species at 3 mol•m^{-2}•d^{-1} but was the least influenced by changes in DLI. For example, total flavonoid content of arugula increased by 36% as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} averaged across all CO_2 levels within a DLI treatment. Mizuna and mustard exhibited the lowest flavonoid contents at 3 mol•m^{-2}•d^{-1} and the highest at 12 mol•m^{-2}•d^{-1} exhibiting an increase of 105% for mizuna and 86% for mustard as DLI increased from 3 to 12 mol•m^{-2}•d^{-1} averaged across all CO_2 levels within a DLI treatment. The effect of DLI on flavonoid content was significantly different by species with mizuna and mustard (P<.0001) significantly different from arugula but not from each other.

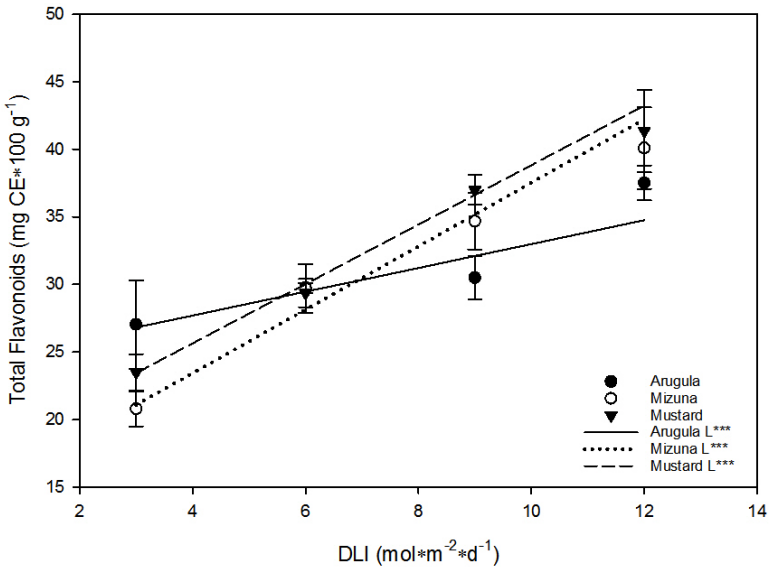

Figure 2.6. Total flavonoid content in mg CE•100 g^{-1} fresh weight tissue for arugula, mizuna and mustard microgreens under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} for all CO_2 levels. Error bars indicate ± SE. Linear (L) or quadratic (Q) represented as NS, *, **, * Nonsignificant at $P \leq$ 0.05, 0.01, or 0.001, respectively.**

Table. 1.1. Analysis of variance for the effects of species, DLI, CO_2, species•DLI, DLI•CO_2, DLI•DLI, species•DLI and model-accounted variability (R^2).

ANOVA Table						
Main Effects & Interactions	*Plant Height*	*Fresh Weight*	*Dry Weight*	Days To Harvest	Total Phenolics	Total Flavonoids
Species	***	NS	***	***	***	***
DLI	***	***	***	***	***	***
CO_2	***	***	***	NS	NS	NS
Species•DLI	***	***	***	NS	***	***
DLI•CO_2	NS	NS	NS	NS	**	NS
DLI^2	NS	NS	NS	**	NS	NS
Species•DLI^2	NS	*	NS	*	NS	NS
R^2	0.73	0.87	0.89	0.98	0.93	0.89

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

Table 1.2. Regression values for the response of plant height, fresh weight and dry weight for arugula, mizuna and mustard to per unit changes in DLI (1 mol•m^{-2}•d^{-1}) and CO_2 (1 ppm).

Regression Table			
Plant Height			
	Arugula[Z]		
Slope	Esimate[B]	Std. Err.[B]	*P*-value
Intercept	5.2688191	0.187947	***
CO_2	0.0004998	0.000134	***
	Mizuna		
Intercept	6.4513461	0.16832	***[A]
DLI	-0.116458	0.017595	***[A]
CO_2	0.0006122	0.000118	***
	Mustard		
Intercept	7.051886	0.168402	***[A]
DLI	-0.146188	0.0176	***[A]
CO_2	0.0006122	0.000118	***
Fresh Weight			
	Arugula		
Intercept	2.9866762	1.059584	***[A]
DLI	1.1453781	0.318291	***[A]
DLI^2	-0.045561	0.020887	*[A]
CO_2	0.0017558	0.000235	***
	Mizuna[Z]		
Intercept	6.8462091	0.457586	***
DLI	0.2269619	0.051651	**
CO_2	0.0018733	0.000233	***
	Mustard[Z]		
Intercept	7.2847852	0.457615	***
DLI	0.2269619	0.051651	**
CO_2	0.0018733	0.000233	***
Dry Weight			
	Arugula		
Intercept	0.1871125	0.018339	***[A]
DLI	0.0321518	0.002068	***[A]
CO_2	0.000091479	0.000009844	***
	Mizuna		
Intercept	0.2293383	0.018338	***[A]
DLI	0.0175093	0.002068	***[A]
CO_2	0.000091479	0.000009844	***
	Mustard		
Intercept	0.2227329	0.01834	***[A]
DLI	0.0182197	0.002068	***[A]
CO_2	0.000091479	0.000009844	***

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respictively.
[A]. Significance when treatment effects are at their average value
[B]. Estimated response when all other treatment effects are equal to zero
[Z]. Reduced model

Table 1.3. (Continued) regression values for the response of days to harvest, total phenolics and total flavonoids for arugula, mizuna and mustard to per unit changes in DLI (1 mol•m^{-2}•d^{-1}) and CO_2 (1 ppm) including interactions and squared terms for a quadratic model.

Regression Table Continued			
Days To Harvest			
		Arugula	
Slope	Esimate[B]	Std. Err[B]	*P*-value
Intercept	12.333333	0.334166	***
DLI	-0.211111	0.040673	***
		Mizuna	
Intercept	13.75	0.690507	***[A]
DLI	-1.25	0.209979	***[A]
DLI^2	0.0648148	0.012037	***[A]
		Mustard	
Intercept	14.75	0.690507	***[A]
DLI	-1.25	0.209979	***[A]
DLI^2	0.0648148	0.01378	***[A]
Total Phenolics			
		Arugula	
Intercept	75.665247	4.97436	***[A]
DLI	0.8652021	0.54688	***[A]
CO_2	-0.010455	0.004619	NS
DLI•CO_2	0.0018547	0.000568	***[A]
		Mizuna	
Intercept	40.579256	4.979539	***[A]
DLI	2.2011953	0.538642	***[A]
CO_2	-0.010455	0.004619	NS
DLI•CO_2	0.0018547	0.000568	***[A]
		Mustard	
Intercept	40.295923	4.979539	***[A]
DLI	2.3415842	0.538642	***[A]
CO_2	-0.010455	0.004619	NS
DLI•CO_2	0.0018547	0.000568	***[A]
Total Flavonoids			
		Arugula	
Intercept	24.165072	1.567264	***[A]
DLI	0.8830256	0.196193	***[A]
		Mizuna	
Intercept	14.012917	1.517047	***[A]
DLI	2.3528333	0.184649	***[A]
		Mustard	
Intercept	16.827083	1.517047	***[A]
DLI	2.2019167	0.184649	***[A]

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001 respectively.

[A]. Significance when treatment effects are at their average value

[B]. Estimated response when all other treatment effects are equal to zero

[Z]. Reduced model

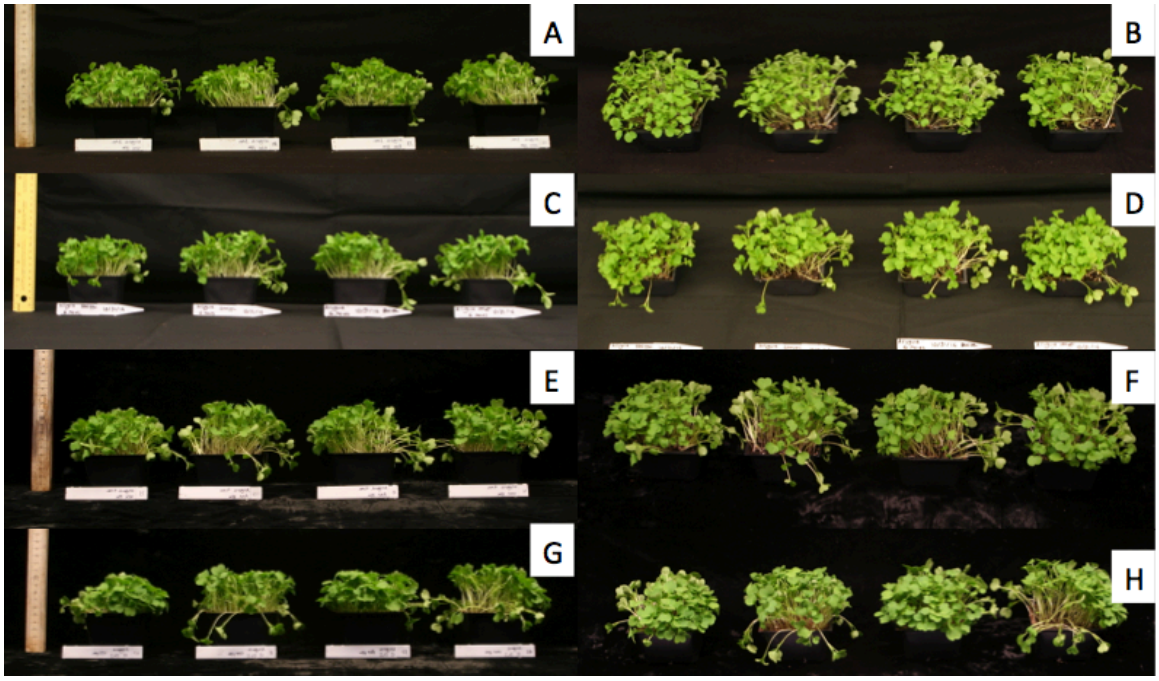

Figure 6.1. Images of arugula under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} and four CO_2 levels of 400, 600, 800 and 1000 ppm. A-B, C-D, E-F and G-H represent a DLI of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} where A, C, E and G are in the horizontal plane and B, D, F and H are in the vertical plane. Within A-H, CO_2 concentrations of 400 to 1000 ppm are left to right.

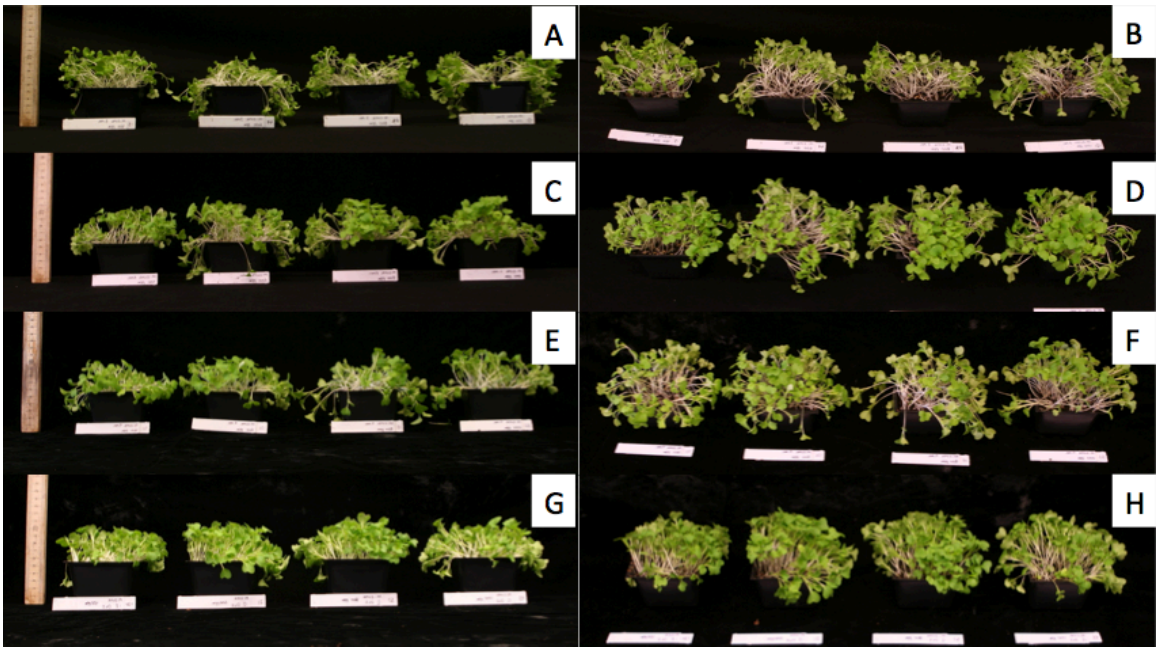

Figure 5.2. Images of mizuna under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} and four CO_2 levels of 400, 600, 800 and 1000 ppm. A-B, C-D, E-F and G-H represent a DLI of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} where A, C, E and G are in the horizontal plane and B, D, F and H are in the vertical plane. Within A-H, CO_2 concentrations of 400 to 1000 ppm are left to right.

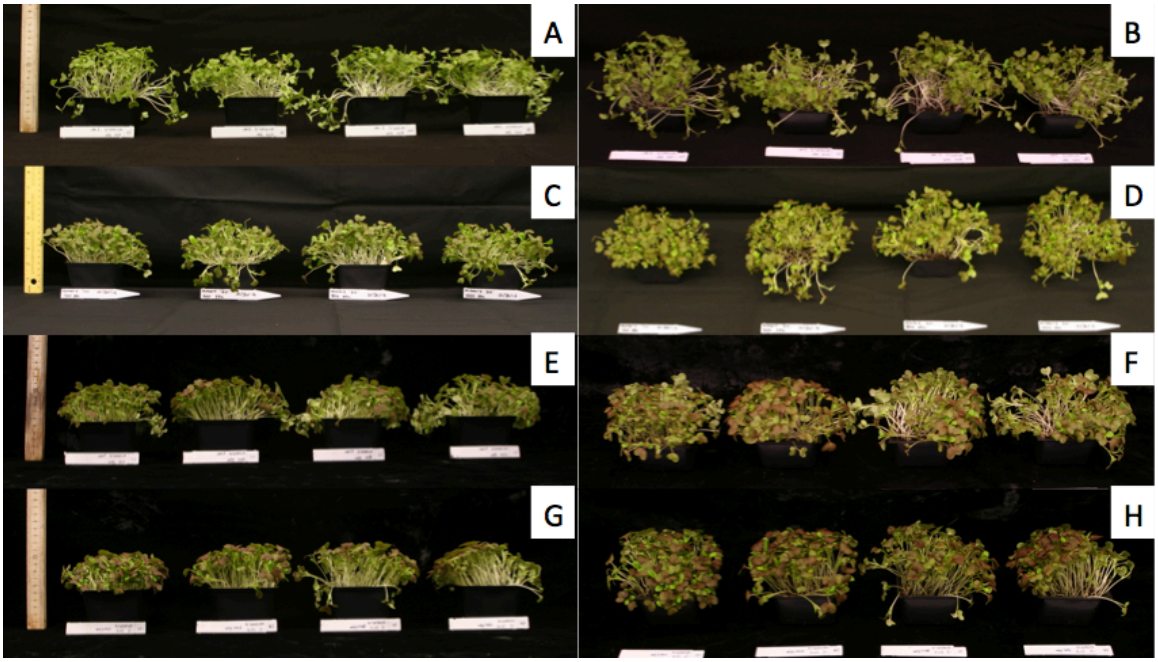

Figure 3.3. Images of mustard under four DLI levels of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} and four CO_2 levels of 400, 600, 800 and 1000 ppm. A-B, C-D, E-F and G-H represent a DLI of 3, 6, 9 and 12 mol•m^{-2}•d^{-1} where A, C, E and G are in the horizontal plane and B, D, F and H are in the vertical plane. Within A-H, CO_2 concentrations of 400 to 1000 ppm are left to right.

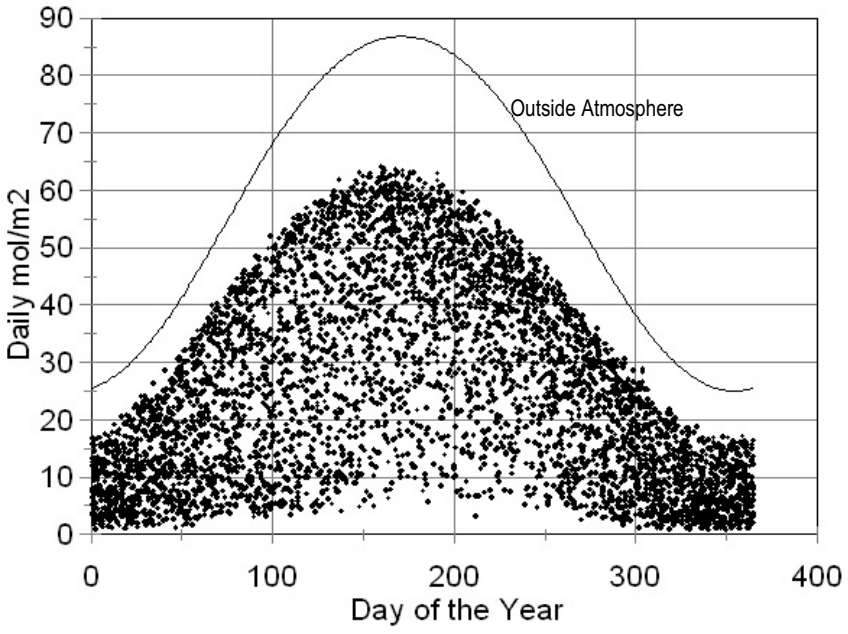

Figure 4. Average daily (Julian day) outdoor solar photosynthetic photon flux (PPF) in mol•m^{-2} for Ithaca, NY from 1983 to 1996 (Albright et al., 2000)

Discussion

Fresh Weight

In the production of microgreens, shoot FW is often the preferred yield determinant (Murphy et al., 2010). In greenhouses, PAR in the form of solar radiation is provided free of cost to the grower. However, the DLI associated with solar PAR may not always be sufficient for plant growth. Using Ithaca, NY as an example, daily outdoor PAR integrals exhibit variation in orders of magnitude between the largest and smallest values ranging from as high as 60+ $mol \bullet m^{-2} \bullet d^{-1}$ to as low as < 1 $mol \bullet m^{-2} \bullet d^{-1}$ (Figure 4; Albright et al., 2000). These variations in DLI over the course of a crops growth cycle can result in irregular growth rates per day, which can have significant effects on crop quality and harvest dates. One of the reasons that growers often object to supplemental lighting for microgreens is due to the assumption that stored reserves present in the seed are sufficient to propel the crop from seeding to harvest irrespective to changes in DLI.

Although microgreens can be produced in the absence of supplemental lighting and under the influence of variable solar DLI, our results showed that increases in DLI from 3 to 12 $mol \bullet m^{-2} \bullet d^{-1}$ have a significantly positive effect on FW yields. Zheng et al. (2011) concluded that even though a maximum net photosynthetic rate of castor (*Ricinus communis* L.) cotyledons was not reached until thirteen days of development, cotyledons were still considered to be powerful photosynthetic organs unmatched by the first true leaves until 18 days after their development. In the case of microgreens harvested in this experiment at a maximum of twelve days after seeding, they still exhibited a significant linear increase in FW for mizuna and mustard and a quadratic increase in FW for arugula as DLI increased from 3 to 12 $mol \bullet m^{-2} \bullet d^{-1}$. Similar results were described by Gerovac et al. (2016) who found that increases in DLI from 6 to 18 $mol \bullet m^{-2} \bullet d^{-1}$

resulted in significant increases in FW for mizuna, mustard and kohlrabi. However, to our knowledge no published data exists involving regression analysis on the effects of DLI on FW production of microgreens.

FW yield response also increased significantly with increasing CO_2 concentration. For all three species, FW increased linearly as CO_2 increased from 400 to 1000 ppm independent of all other parameters. Ruhil et al. (2015) found that CO_2 enrichment of field grown mustard plants significantly increased FW yields by 81 to 103% per season as CO_2 concentration increased from 385 to 585 ppm. Becker and Klaring, (2016) reported a 72% increase in the FW of oak leaf lettuce as CO_2 concentration increased from 200 to 1000 ppm. In our results, increases in FW yields as a function of increasing CO_2 was found to be supplementary to DLI where high CO_2 levels at a lower DLI matched or exceeded the growth of ambient CO_2 at the next highest DLI treatment. This phenomenon was well documented by Both et al. (1997), who concluded that for head lettuce, DLI levels can be reduced if CO_2 concentrations are increased with results of equivalent FW and DW yields. For example they concluded that a DLI/CO_2 ratio of 17/350, 16/420, 15/480, 14/630, 13/820, 12/1270 and 11/1600 ($mol \bullet m^{-2} \bullet d^{-1}$/ppm) all produced the same biomass by 35 days of growth.

Dry Weight

The response of DW to changes in DLI and CO_2 was similar to that of FW with the exception of the absence of a quadratic function for DLI. The linear increase in DW for all three species as DLI and CO_2 increased from 3 to 12 $mol \bullet m^{-2} \bullet d^{-1}$ and 400 to 1000 ppm was highly significant. Gerovac et al. (2016) reported percent DW increases for kohlrabi and mustard from 6.7 to 8.7% and 5 to 6.7% as DLI increased from 6 to 18 $mol \bullet m^{-2} \bullet d^{-1}$. Similar findings were also

described by Samuoliene et al. (2013) where red pak choi and tatsoi (*Brassica rapa* L.) exhibited a significantly higher DW at a DLI of 19, 23.3 and 31.4 mol•m^{-2}•d^{-1}; an increase of 26, 34 and 34% for red pak choi and 19, 17 and 21% for tatsoi over a control of 12.7 mol•m^{-2}•d^{-1}. What is not known however is whether these responses are linear, quadratic or another function associated with a regression analysis due to the categorical analysis of the data. Although DW is often of minimal concern to growers, it is still a necessary parameter in the interpretation of biomass (fixed carbon) response to treatments.

The relationship between FW and DW is well represented in this data by the effects of CO_2 on FW and DW yields where the responses for both were equally significantly. Lopez et al. (2015) found that an increase in CO_2 concentration from 400 to 700 ppm significantly increased DW of green and red lettuce by 42 and 62%. Along with FW, Ruhil et al. (2015) found that CO_2 enrichment increased plant DW by 93, 99, and 112% by season as CO_2 concentration increased from 385 to 585 ppm. Kitaya et al. (1998) also observed a DW increase between 10 and 100% in lettuce plug transplants as CO_2 concentration increased from 400 to 800 ppm with the degree of the response dependent on photoperiod.

Plant Height

It is well documented that increasing light intensity (LI) and DLI have a significant effect on decreasing internode elongation. Gibberellins (GA), which play a role in the activation of hypocotyl elongation, have been shown to decrease in concentration with increasing LI (Potter et al., 1999). Gerovac et al. (2016) published similar findings, where hypocotyl length of mizuna and mustard were significantly reduced by 80% and 37% as DLI increased from 6 to 18 mol•m^{-2}•d^{-1}, respectively. For arugula, the effect of DLI on plant height was not significant

suggesting that GA production in arugula may be less influenced by increases in DLI as were mizuna and mustard. The response of decreasing plant height of 0.12 cm for mizuna and 0.15 cm for mustard for each increase in 1 $mol \bullet m^{-2} \bullet d^{-1}$ of light, shared an inversely correlated response with CO_2; which for all three species significantly increased plant height in a linear fashion by 0.06 cm per 100 ppm CO_2. This response could be utilized to counteract the plant height reducing effect of increasing DLI while simultaneously increasing both FW and DW. Ruhil et al. (2015) found increases of 12, 14 and 17% in the plant height of mustard as CO_2 concentration increased from 385 to 585 ppm. To our knowledge, no published data exists on the consumer-preferred ratio of hypocotyl length to cotyledon size.

Days to Harvest

The importance of crop timing cannot be over-stated. Delivering a product to market on time and at an appropriate and constant size and weight are fundamental to the long-term success of any horticultural business. Varying DLI as explored within the parameters of this experiment, can significantly influence DTH of arugula, mizuna and mustard microgreens. The linear decrease in DTH for arugula and quadratic decrease for mizuna and mustard with increasing DLI further support an argument for controlling DLI in microgreen production. In addition, a faster turnover period per crop cycle would allow for more crop cycles per year, which could increase profits. For this experiment, a designated development stage for crop harvest was defined to maintain consistency between replications. Depending on individual market and grower preference, development stage designation may change, altering the number of days to harvest for each species.

Total Phenolics

Light, amongst various other environmental factors, is one of the most important variables in influencing phytochemical concentrations within the plant (Samuoliene et al., 2012). This is because high irradiance levels creating mild photo-induced stress activate photo-protective mechanisms, which influence the production of primary and secondary metabolites in plants (Samuoliene et al., 2013). The effect of increasing DLI on the total phenolic content of microgreens was significant for all three species with the degree being dependent on species. Xiao et al. (2015) found total phenolic content of mustard 'Dijon' to be 150 mg•100 g^{-1} gallic acid equivalents per DW mass of tissue at an unknown DLI. Samuoliene et al. (2013) found peak production of total phenols occurred between 23.3 and 31.4 mol•m^{-2}•d^{-1} for tatsoi and 19 to 23.3 mol•m^{-2}•d^{-1} for kohlrabi. Total phenolic content increased from approximately 35 to 45 mg•100 g^{-1} for tatsoi and 40 to 60 mg•100 g^{-1} gallic acid equivalents per FW mass of tissue as DLI increased from 6.3 to 31.4 mol•m^{-2}•d^{-1}. It is unclear why the discrepancy in total phenolics varies so widely by publication. One explanation could be the laboratory analysis method used for determination of total phenolics, which also varies with publication. In addition, specific species or crop growth stage at time of analysis could play a role in reported phenolic values.

Total Flavonoids

The pathway for the biosynthesis of flavonoids is reported to be directly linked to the carbohydrate status of plants, which is enhanced by elevated photosynthetic rates (Becker and Klaring, 2016). Additionally, flavonoids have been shown to increase during periods of biotic and abiotic stress (Winkel-Shirley, 2002; Dixon, 1995) that may be imposed by changes in lighting (Mattson and Harwood, 2012). Decreasing flavonoid concentrations with increasing

physiological plant age as observed by Becker et al. (2014) appears to hold true when comparing the total flavonoid content of microgreens to baby leaf arugula. Total flavonoids of baby leaf arugula as described by Mattson and Harwood, (2012) had a concentration of total flavonoids of 27.1 mg CE•100 g^{-1} FW at a DLI of 13 mol•m^{-2}•d^{-1}. In comparison, total flavonoids of microgreen arugula observed under 12 mol•m^{-2}•d^{-1} in this experiment had a concentration of 37.5 mg CE•100 g^{-1} FW. With the exception of the absence of an influence on CO_2 on total flavonoids, the significant linear increase in total flavonoids with increasing DLI followed the same trends as total phenolics.

Conclusion

Changes in DLI and CO_2 can have significant effects on the FW and DW yields, plant height, DTH and secondary metabolite content of microgreens. The lower DLI ranges explored in this work provide a better understanding on the effects of light on microgreen growth as well as establishment of lower-end tolerance levels for DLI in microgreen crops. The ability to supplement low DLI with elevated CO_2 to achieve equivalent growth of high DLIs at a low CO_2 can be of significant benefit to greenhouse growers in areas such as Ithaca, NY where solar DLI, averages less than 10 mol•m^{-2}•d^{-1} during the winter months.

The response of both FW and DW to CO_2 holds particular implications for plant factories and vertical production greenhouses where light is the most expensive and limiting resource to plant growth. However, combinations of low DLI and high CO_2 such as 3/1000 (mol•m^{-2}•d^{-1}/ppm) should be avoided as both low DLI and high CO_2 values promote significant increases in plant height causing crop lodging and intertwinement (Figure 3.1 A-B; 3.2 A-B; 3.3 A-B). If trays are grown containing individual cells with the intention of retail/wholesale sale on

a per cell basis, division of cells containing intertwined shoot can result in plant damage and loss of plant material. If flats are grown for mass harvest where no division of shoots are required, then a 3/1000 (mol•m^{-2}•d^{-1}/ppm) ratio can serve as an adequate target for microgreen production in such environments. In such cases, extensive hypocotyl elongation can be remedied by harvesting higher up the shoot at the expense of FW.

Specific targets for the parameters of DLI and CO_2 for microgreens vary by species, geography, growing structure and grower goals but in general, within the parameters used in our study greatest growth, average plant height, lowest DTH and highest secondary metabolite content can be achieved for all three species at a DLI of 12 mol•m^{-2}•d^{-1} and a CO_2 concentration of 1000 ppm. However, given the ability of higher CO_2 at lower DLIs to achieve equivalent or greater growth than a low CO_2 at a high DLI, FW can be lower by as little as 4.5, 3.8 or 2% for arugula, mizuna or mustard as DLI and CO_2 drop from 12 mol•m^{-2}•d^{-1} at 1000 ppm to 9 mol•m^{-2}•d^{-1} at 1000 ppm. Future work should be conducted on the origin of FW and DW production by tissue under changes to DLI and CO_2. Results could aid growers in determining the source to sink of environmental inputs with the goal to establish more targeted efforts for the most efficient return on investment.

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