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Effects of Seed Density and Other Factors on the Yield of Microgreens Grown Hydroponically on Burlap

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Abstract

Microgreens are gaining popularity as a new, nutritious salad crop. Growing microgreens in stacked hydroponic channels may improve efficiency and food safety for microgreens. However, differences between soil and hydroponic production methods for microgreens are not well known, especially when it comes to specific factors, like seed density, light exposure and yield for all the crops used as microgreens. This study explored the yield of six types of microgreens grown on burlap during three years of commercial production in a small educational greenhouse. The varieties, or species, tested in this study included basil, arugula, carrot, and blends of brassicas, radish and mustard. Seeds were sown directly on a single layer of burlap in a hydroponic nutrient film technique (NFT) system. Fresh weights (FW) of the microgreens were recorded after harvest to track the influence of seed density, light levels, growth time and season.

The mean seed density for arugula was 42.9 $g{\cdot}m^{-2}$, and 41.0 $g{\cdot}m^{-2}$ for basil, 57.8 $g{\cdot}m^{-2}$ for carrot, 55.7 $g{\cdot}m^{-2}$ for the mild blend, 51.5 $g{\cdot}m^{-2}$ for the mustard blend and 103.1 $g{\cdot}m^{-2}$ for the radish blend. Basil yields increased when temperatures were high in the spring and summer. In contrast, the mustard blend and arugula microgreens produced lower yields when grown in the spring and summer months compared to winter. Basil grew significantly better in full sun, and radish grew better on average when grown in the shade. The seed densities did not correlate with yield as expected. Light exposure and season appeared to be more influential to microgreen yields than seed density. When compared to other similar studies the seed densities, yields and growing conditions were diverse. This publication aimed to address a gap in knowledge on microgreen production methods.

Keywords: hydroponic, microgreen, burlap

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Introduction

Background and Setting

Microgreens are an agricultural product grown and sold as a salad green, similar to sprouts and baby greens. Microgreens, as the word suggests, are harvested while very small, after 7 to 28 days of growth from seed. Stems and cotyledons (young leaves) are harvested and the root is left behind in the growth medium. The first true leaves may or may not be present, depending on growth rate and preference (Fig. 1). Unlike sprouts, the roots of microgreens are not consumed, making them a safer product in general (Buck, et al., 2003, Reed, et al., 2018; Xiao, et al., 2014). Consumers appreciate microgreens for their surprisingly strong flavors and delicate texture. Chefs typically use them as garnish, toppings and in salads (Fig. 2).

Figure 1. Basil microgreens, harvested at 20 days, with cotyledons and emerging true leaves.

Figure 2. Chefs at Mason Dining using microgreens grown on campus as garnish for an event in 2015. Photo by Evan Cantwell, George Mason University.

Microgreens are gaining popularity as a super food, since they are high in vitamins and nutrients. Microgreens contain more nutrients per pound than mature crops of the same type (Pinto, et al., 2015; Warner, 2012; Weber, 2016; Xiao, et al., 2012). More than 60% of the world's population is malnourished, including developed countries (White & Broadley, 2009). In densely settled areas, the length of shelf life governs what food is available at affordable prices, often to the detriment of nutrition and health. Microgreens can be an accessible source of fresh, nutritious food that can be easily grown in small indoor spaces. Indoor microgreen farming may also be more sustainable than traditional crop production (Weber, 2017).

Conventional agricultural practices overall contribute to global pollution levels (Alley, et al., 2007). Many environmental impacts from farming have been confirmed by scientists, including pollution caused by soil erosion, deforestation, runoff from fertilizers, chemicals, livestock waste, and air pollution (Alley, et al., 2007; Harvey, 2016). The production of microgreens has a smaller environmental impact when compared to the production of mature vegetables, because they require much less time, fertilizer, space, and inputs (Weber, 2016). Environmentalists, farmers and health-conscious individuals are becoming more aware of the value of this up and coming crop.

Despite the excitement around microgreens, there is still a major gap in published research on how to grow different varieties of microgreens. The term variety as it relates to microgreens means the different crops used for microgreens and cultivars of those species, such as red cabbage or yellow frill mustard. Most of the scientific research focuses on analyzing the nutrition concentrations or microbial safety of microgreens. The articles that focus on microgreen yield are often simple, only discuss one variety, are anecdotal and/or not peer-reviewed. Most academic publications on microgreens rely on loose medium or soil to grow them. One scientist compared burlap production of microgreens with other growth mediums, but it was only for broccoli microgreens (Di Gioia, et al., 2016). More information and extended research are needed to learn about the different varieties and the efficacy of burlap production methods for microgreens.

Microgreens have the ability to influence the world by providing fresh, local, and nutritious produce to malnourished populations, while also providing an educational tool to teach about sustainable agriculture and healthy diet choices. A shift in consumer demand and food culture would be required before this new food crop becomes widely accepted. This future of attitudes around food is developing in Universities. For example, a recent article lists the top 75 Schools for Food, showing what matters most to young consumers (The Daily Meal, 2018). A lot of the dining programs featured in the article have fresh, local ingredients and sustainable features, like composting and campus farms.

On that list of schools, coming in at number 21, was George Mason University (GMU), a large public university in northern Virginia, U.S.A., right outside of Washington, D.C.. Students at GMU were demanding more fresh and local food options on campus. Proof came as student clubs forming around the topic and in student applications for grant projects with the Office of Sustainability to create more gardens and aquaponics systems. The dining services corporation at GMU, Sodexo, received feedback repeatedly from students asking for fresher ingredients and healthier options.

To meet the students' call for fresher food on campus the staff at GMU decided to start a project to create a hydroponic greenhouse program that could grow food on campus to serve in the dining halls (Fig. 3). I had just graduated in 2014 when they hired me to start the project. I had previous horticulture experience from maintaining the student-led garden for 3 years, and I was a regular volunteer in the greenhouse alongside its former staff in the College of Science.

I did all the budgeting, planning, and design to retrofit the existing vacant greenhouse on campus to support a hydroponic food production system. In 2014 and 2016, GMU funded over $35,000 total in grants to build the system and purchase supplies. The operation was deemed safe for crop production by Sodexo staff. Since it relies on hydroponics in a protected indoor environment, it was safer than sourcing food grown under field conditions with compost. A food safety protocol, requiring worker health and hygiene, was created to adhere to Sodexo's food safety guidelines.

GMU already had a liability contract with Sodexo so the administrative staff signed an addendum and agreed to cover the cost of taxes to make the project happen. Sodexo paid an annual sum in return for the produce grown by sustainability staff and student participants in the greenhouse. GMU's facilities department agreed to pay the staff salary to run the greenhouse program year-round, through the Office of Sustainability. Since then, the project has turned it into the Greenhouse and Gardens Program, whose mission is to grow food on campus and educate students

Figure 3. Exterior of the Presidents Park Greenhouse at George Mason University's campus in Fairfax, VA. Photo by Evan Cantwell.

about sustainable agriculture. The managers at Mason Dining are able to source about 10% of all their ingredients from local farms within a 250-mile radius, and the campus greenhouse provides a portion of this local food from a quarter mile away.

Since 2015, I have been managing the greenhouse and the hydroponic food production systems in it, with huge success. We grow hydroponic lettuce, basil, microgreens, tomatoes and assorted herbs. We planned to grow microgreens from the beginning, since they are profitable and attractive to chefs. The microgreens are served in the dining halls on campus as a salad green (Fig. 4).

Figure 4. Microgreens mixed with the lettuce in the salad bar on campus.

We harvest every week of the year, except during holiday breaks, and chefs serve the produce in the campus dining halls. Students can eat the fresh produce from within the salad bars, sauces, and specialty entrees in the dining halls on campus. In return for the produce, Sodexo pays an annual contribution of $20,000, based on expected yield and current market values. These funds are granted despite any losses in yield, so that we may cover the cost of supplies and utilities to run the operation. This allows us to focus on education and outreach.

Every semester, students receive course credit for volunteering, through internships and class assignments. The School of Integrative Studies at GMU requires experiential learning credits for many degree programs, including the Environmental and Sustainability Studies major (ESS). Students earning ESS degrees have the option to earn their internship credits through our program. I teach the interns and student staff about the standard operating procedures (SOP's) for food safety, and they help me to train and lead the volunteers.

The ESS program is our greatest source of labor and student involvement. Students from other departments also come to volunteer and tour the greenhouse, including nutrition, biology, environmental science, global affairs, business, engineering, and art. We have had hundreds of visitors and tour groups, including guests from China, other universities and local grade schools, military consulting companies, community programs, as well as veterans, who join us to help and learn about sustainable food production.

Figure 5. Students volunteer in the greenhouse to help with the harvest, while earning credit for their class.

Visitors can volunteer and students can earn course credit when they help with weekly operations (Fig. 5). We rely on these free sources of labor to run the program.

Visitors can access many educational opportunities through the greenhouse. Volunteering offers hands on learning, through seed sowing, harvesting and pest management, through tours, free tastings, classroom lectures and other collaborations with professors, too. There are many articles published about the greenhouse program at GMU, and I receive invitations throughout each year to speak at events and give tours. For example, several articles from GMU, and other news outlets, like City Farmer and Horti Daily feature my story. I was invited to speak at a TedX Talk in Tysons Corner. For a full list of my presentations and articles, as well as a link to watch my TedX Talk, please view the appendix at the end of this manuscript.

The popularity of this topic suggests that there is a rising demand for knowledgeable and experienced leaders in the field of sustainable agriculture and urban food production in the DMV area (District of Columbia, Maryland and Virginia, U.S.). I aim to help educate future generations of farmers through my program in Fairfax, Virginia, and through this online publication.

The data I collected over the past three years of microgreens production reflect my processes of trial and error in operating a hydroponic greenhouse and food production system. The trends in the data provide insights on microgreen production that may be helpful for hobbyists, teachers, and business owners.

Objectives of Research

- To investigate three years of production data from several microgreen varieties to examine the efficacy of the hydroponic burlap system as compared to current methods.
- To study the influence of season, low light levels and seed density on the yield of microgreens grown on burlap in a hydroponic rack system.
- To educate growers about hydroponic microgreen production and to provide information on expected outcomes, and ways to increase yield and system efficiency.
- To address the literature gap in topics related to microgreens such as the efficacy of burlap and the effects of seed density for different varieties.
- To increase knowledge on the dietary and nutritional benefits of microgreens.
- To provide possible correlations in the data to maximize fresh weight and quality of microgreens year-round.

Definition of Terms

Burlap: A biodegradable cloth mat made with loosely woven strands of jute, and/or other fibrous plant material, like kenat.

DTH: Days to Harvest, meaning the number of days between the time of seed sowing and harvest.

FW: Fresh Weight, meaning the weight of the microgreens right after harvest.

Hydroponics: A method of growing plants in soil substitutes like burlap, coconut coir and peat moss using recirculating water that contains aqueous fertilizer and adjusted pH levels.

Microgreens: An edible green harvested at the seedling stage, containing the hypocotyl (upper stem), cotyledon and young true leaf (Fig. 6).

NFT: Nutrient Film Technique; a type of hydroponic growing method which uses a channel and a shallow stream of water containing nutrients that runs along the bottom of the channel surface. The water makes contact with the roots and soilless medium from underneath to water and feed the plant.

Variety: The species of vegetable used for microgreens, may include the cultivar or not. Examples; garnet red mustard, and arugula.

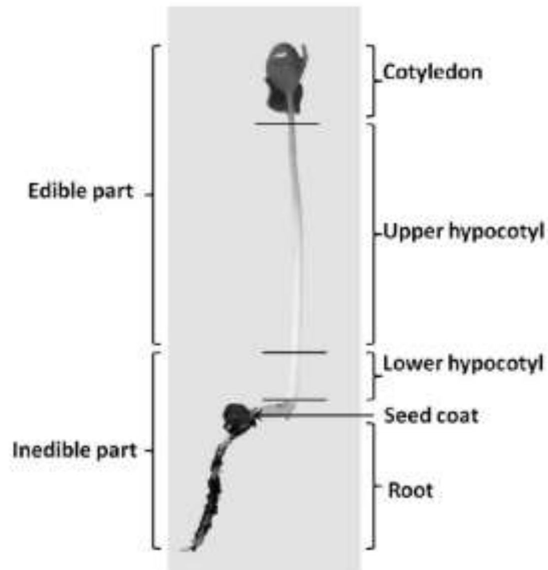

Figure 6. Diagram of a radish microgreen at 7 days old. The upper hypotcotyl and cotyledon sections are the edible parts. (Xiao, et al., 2015).

Literature Review

Before 2010 there were very few articles published on microgreens (Murphy, et al. 2010). In the past several years, many researchers have begun studying and publishing many topics on microgreens, mainly nutritional content, food safety and basic beginner information. Numerous businesses have recently begun selling microgreens and developing innovative marketing schemes, and so there many media articles on successful startups. Most of these growers learned their production processes through trial and error.

Currently, no studies have been published that focus on comparing microgreen varieties grown on hydroponic burlap. As a rising business endeavor and promising solution to malnutrition, growers need more resources to help maximize yield and efficiency of this new exciting crop.

Nutrition

More than 60% of the world's population is malnourished, in both developed and developing countries (White & Broadley, 2009). Compounds such as zinc, iron, selenium, calcium, magnesium and copper are among the most common nutritional deficiencies in humans (Pinto, et al., 2014; White & Broadley, 2009). This problem is completely preventable with proper daily nutrition. Unfortunately, there are many obstacles preventing populations from getting all the essential nutrients in their daily diets. Numerous populations deal with inequality and extreme poverty that cause poor access to food across the globe. Food deserts exist in many urban areas, where shelf life and marketing are important for food sales in local stores.

Hydroponic microgreens are a potential solution to providing nutritious food for those who have a small space and a limited supply of water, since microgreens require less space, fertilizer, and water than growing plants to maturity (Weber, 2017). When comparing microgreens to mature greens, many researchers found that nutrients are up to 40 times more concentrated in microgreens (Cramer, 2017; Pinto, et al., 2014; Warner, 2012; Weber, 2016; Xiao, et al., 2012). Compounds often measured by researchers include ascorbic acid (Vitamin C), carotenoids (Vitamin A compounds), phylloquinone (Vitamin K), tocopherols (Vitamin E) and glucosinolates (Sun, et al., 2015; Xiao, et al., 2012).

When consumed, microgreens are a good source of fiber, vitamins and minerals, and they have valuable antioxidant capabilities that help to protect the body against cancer and cardiovascular disease (Brazaityte et al., 2015; Rice-Evans and Miller, 1995). There is also evidence that microgreens can help regulate body weight and cholesterol levels, preventing many common illnesses (Huang, et al., 2016). However, the health benefits of microgreens can vary with many factors.

The concentration of nutrients can differ among crop species and growing conditions. Some varieties of microgreens contain higher concentrations of certain vitamins and minerals than other varieties (Fig. 7). For example, red cabbage, cilantro, garnet amaranth and green daikon radish have more nutrients than other common varieties (Xiao, et al., 2012).

No single microgreen variety contains the best combination or highest level of nutrients. Rather, some varieties often acquire certain minerals more than others do. For example, basil microgreens take up less nitrogen and potassium than arugula microgreens, but more phosphorus (Bulgari, et al., 2017). Swiss chard microgreens can absorb more potassium, yet less iron than arugula and basil, while calcium concentrations can be the highest in arugula (Bulgari, et al., 2017). Red cabbage microgreens contain the most Vitamin C, but daikon radish has the most Vitamin E (Xiao, et al., 2012). Therefore, consuming a blend of different types of microgreens has more health benefits than only eating one variety.

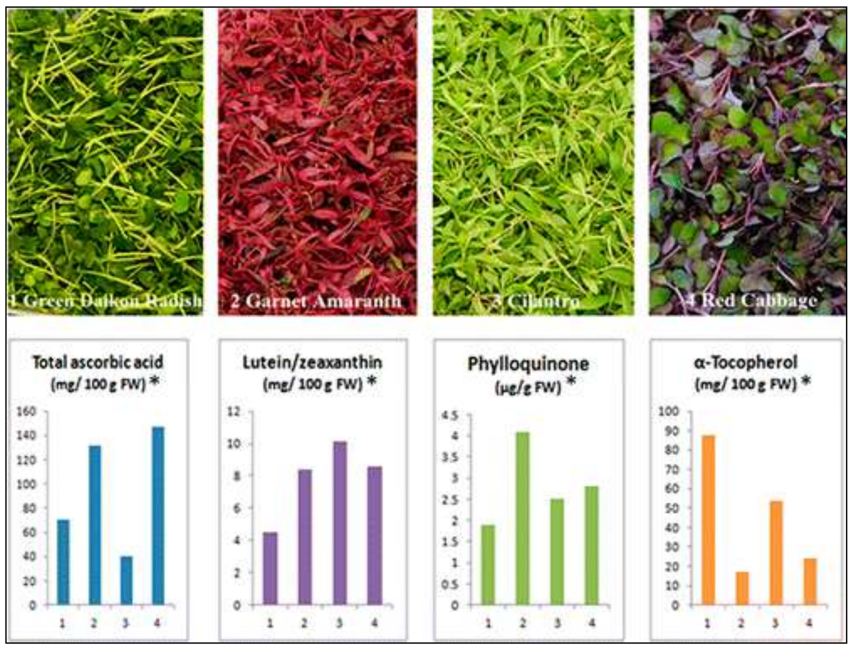

Figure 7. Photos of several microgreen varieties; 1. Green daikon, 2. Garnet amaranth, 3. Cilantro and 4. Red cabbage, with nutritional concentrations of all four varieties to compare their nutrient concentrations (Xiao, et al., 2012).

The content of harmful nitrates is lower in microgreens than in baby and mature greens, especially for Swiss chard and arugula (Bulgari, et al., 2017; Pinto, et al., 2015). The concentration of sugars is lower too (Xiao, et al., 2015b). These characteristics contribute to the health benefits of microgreens because high levels of nitrates and sugars can cause damage to the body (Santamaria, 2006). Microgreens are beginning to gain more attention among urban farmers, chefs and nutritionists. The high nutritional value of microgreens contributes to the economic potential of this new, exciting industry.

Economics

Many businesses are successfully marketing microgreens to local chefs and restaurants (De Bona, 2014; Pirnia, 2015; Urie, 2018,). Microgreens are the future of farming for many urban growers. Many have revived abandoned warehouses or a basement to start their hydroponic production systems (Lam, 2016; Urie, 2018). Other farmers were already running agriculture businesses and decided to switch to microgreens, or add them as a supplemental crop (Bilyj, 2017; GEI Media Inc., 2015). Urban hydroponic microgreen farms are becoming more common. Microgreen farming is the source of successful businesses in Pennsylvania, Ohio, North Carolina, California, Colorado and elsewhere (Bilyj, 2017; De Bona, 2014; Pirnia, 2015; Urie, 2018). Growers can sell them for a premium price; $24 - $48 per lb. is the typical price range (Espiritu, 2016). They are attractive to chefs and are becoming more popular with consumers of all ages. For many business owners, microgreens are the perfect niche market.

Owner and farmer, David Sasuga, runs a microgreen company called Fresh Origins, located in San Marcos, California (Bilyj, 2017). Sasuga had years of experience in the seedling and plug production business. He switched to microgreens and his company is thriving. His innovative idea to market his microgreen products are Herb Crystals, or sugarcoated microgreens. Although they may be less healthy, this technique helps maintain freshness and gives them a dazzling appearance and unique texture. He sells an array of microgreen products directly to chefs and ships the produce for next day arrival. At Fresh Origins, they grow 400 varieties of edible greens and flowers, including unusual varieties, such as pumpkin, nutmeg basil, tangerine, and shamrock. His business has been successful and now employs 375 people (Bilyj, 2017).

A hydroponic farm in Pennsylvania operates inside an abandoned warehouse and grows microgreens on a 16 ft. rack with 6 shelves (Urie, 2018). Seeds are sown on a moistened hemp pad, and then the greens are harvested and shipped with roots intact. The farmers, Charles and Hays, say that microgreens are "healthier and last longer" (Urie, 2018). They sell the produce at wholesale to stores, markets and restaurants.

In Hendersonville, North Carolina, an indoor microgreen farmer, Thomas Meuller, operates Lila's Garden (De Bona, 2014). He sells to restaurants and local retailers, including the local hotels, country clubs and Whole Foods. The 2-oz. packages of microgreens sell for $5 each. The growers use burlap to grow microgreens hydroponically with the addition of LED grow lights and high-quality water. The business is expanding while keeping a local focus.

In Colorado, the owner of Deep Nutrition Farm cuts his microgreens as he sells them at the farmer's markets, for peak flavor and nutrient concentration (Markets, 2018). The grower, Walter Fifer, says that his most popular varieties are sunflower, radish and pea. In Baltimore, a man started a microgreen company in his basement apartment, called City Hydro (Lam, 2016). He now markets grow system equipment and supplies to expand his sales beyond produce. His technique uses coconut coir pads and hand watering. He started selling microgreens to pay his bills, and now he teaches chefs to grow their own.

Overall, the indoor farming technology market, including system kits for hydroponics and microgreens, was estimated to have a value of $25.40 billion in 2017, reaching $40.25 billion in 2020 (Newswire Europe, 2018). This industry is obviously booming. Growing microgreens has other economic concerns

to address, however. It takes a lot of continuous labor, since the turnaround is so quick (Kaiser & Ernst, 2018). The most labor-intensive tasks are the harvest and handling of the final product. The capital cost required can be very high to build a greenhouse or other structure, plus the shelves or tables, the hydroponic plumbing, irrigation equipment, lots of grow media and seeds, etc.
The cost of seeds can be significant. If producers can sell their microgreens for $25 - $50 per lb., they are likely to generate some income (Kaiser & Ernst, 2018). Knowledge of best practices is crucial to success, especially since low yields are typically a limiting feature for the microgreen industry (Bulgari, et al., 2017; Kou et al. 2014; Sun et al. 2015).

Consumers enjoy microgreens for their unique flavor, appearance and delicate texture (Fig. 8). Professional taste testers evaluated six varieties of microgreens, and rated them to measure their sales potential (Xiao, et al., 2015b). The qualities tested for include bitterness, spiciness, sourness, sweetness, texture and appearance. The bull's blood beet microgreens had the highest total rating out of all the varieties sampled. Overall, the evaluation results show that microgreens are generally likable and have good consumer acceptability. Microgreens continue to increase in popularity and demand (Pinto, et al., 2015; Wang & Kniel, 2016).

Figure 8. Harvested microgreens for visual evaluation. A. Bull's blood beet; B. Garnet red amaranth; C. Dijon mustard; D. China rose radish; E. Peppercress; and F. Opal basil (Xiao, et al., 2015b).

A business in microgreens can be easy to start and scale up (Storey, 2017). Even just a few trays in someone's kitchen can be used to test one's skills and can produce enough to send samples to clients (Storey, 2017). When orders are made, trays of microgreens can be delivered in less than 2 weeks. When demand increases, a shelf unit can be used to expand vertically and multiply the grow space on the same floor. Microgreens are practical for growing in cities because they take up less space than most vegetables. Because of their young harvest stage, the plants do not need a lot of root space, headspace, light, water or nutrients, compared to mature vegetables (Cramer, 2017).

The microgreens industry has huge untapped economic potential. Many business articles cited above were published recently, in 2017 and 2018, showing how these businesses are beginning to gain more media attention. Many cities can expect increasing demands for microgreens as people discover the health benefits and fun flavors. The product has already begun to expand beyond specialty chefs and high-end restaurants, as they become more popular in produce sections and farmer's markets (Johnny's, 2017b; Kaiser & Ernst, 2018). Microgreens can satisfy the growing demand of young consumers for fresh and sustainable food.

Sustainability

Microgreens are more sustainable than growing mature vegetables (Cramer, 2017; Weber, 2017). One researcher found that it can take about 328 times more water to grow the same amount of nutrients from mature broccoli than from the microgreens (Weber, 2017). Since they take up less space, time, water and fertilizer, microgreens are more environmentally friendly than traditional farming (Alley, et al., 2017; Cramer, 2017).

The consumption of microgreens in general creates less waste when compared to mature broccoli because consumers typically throw out the stem and leaves when preparing broccoli florets for their meal (Weber, 2017). The addition of hydroponic production methods allow for reuse and containment of wastewater making microgreens more sustainable than conventional agriculture (Cramer, 2017). Drawbacks to growing microgreens include infrastructure, capital and utility costs and the increased amount of labor required.

Growers spend hours sowing seeds and harvesting every week, since microgreens grow so quickly. Harvesting was one of the most time-intensive requirements of the process (Storey, 2017). Some growers harvest by hand, which can take much longer than using electric blades, such as grass or hedge trimmers (Storey, 2017). Other growers simply sell their microgreens still rooted, eliminating the need to harvest, and keeping them fresh during transportation. Hydroponic mats, which are like a fabric that replaces soil or loose hydroponic medium, are best for selling live microgreens to chefs, since dirt or other fine granules are not allowed in the kitchen (Kaiser & Ernst, 2018). Burlap is the most renewable option compared to other hydroponic mats, like those made of plastic fiber, since burlap breaks down quickly when composted.

A concern is that microgreens produce less volume per seed, compared to the same plant grown to maturity. This may seem trivial to the grower, since seeds are a small expense when compared to the combined costs of supplies, utilities, labor and overhead fees. However, no studies have focused on the sustainability of microgreens as it pertains to seed production. Microgreens require a lot of seed. Growing the seed requires large fields, a lot of time, perhaps pesticides and pollution. Seed production is often dismissed when analyzing the environmental influence of microgreens. The ecological impacts of microgreen production is not a popular topic however, while there are many more studies on the microbial safety of eating microgreens.

Food Safety

Raw leafy greens are some of the most susceptible food products when it comes to the transmission of food borne illnesses (Olaimat & Holley, 2012). Every producer of edible greens must be able to uphold clean and food safe methods for the safety of the consumer. Bacterial infection of microgreens can occur at any stage of growth and can be passed on to produce from the seed (Xiao, et al., 2015a). Therefore, seed quality is very important to food safety of microgreens. Water quality is also important and growers must use potable water from either a municipal or well source, and not surface water like ponds, to avoid contamination and illness, especially for microgreens (Kaiser & Ernst, 2018; Moran, 2017).

Food safety for microgreens requires regular sanitation practices and seeds that are clean and pure, such as seeds labeled for microgreens. Companies offer seeds specifically pre-treated, or cleaned, for use as microgreens, which can reduce the risk of a food borne illness outbreak, since the seed coat is often consumed with the microgreens (Johnny's, 2017a). Despite the high level of risk, microgreens have caused many fewer outbreaks of food borne illness when compared to sprouts (Buck, et al., 2003; Reed, et al., 2018; Xiao, et al., 2014). The environmental conditions used to grow sprouts are very humid and dark, plus the root is consumed, causing the higher level of outbreaks. On the other hand, microgreens are grown in lower humidity, with more light, and are harvested so that only the above ground portion is eaten, hence fewer outbreaks.

Microgreens may pose less risks when they are grown hydroponically, since the plastic troughs and soilless medium allow for easier sanitation between crops. Especially if the production system is indoors, in a protected environment, the risk of contamination is lower than unprotected plants grown in soil or in field conditions (Johnny's, 2017; Tripp, 2012). When selling live microgreens to chefs, hydroponic mats, like burlap, are more suited than soil, because any loose medium is not desirable in the kitchen (Kaiser & Ernst, 2018). Despite the increased safety of growing microgreens indoors with hydroponic methods, there is still a risk of contamination.

Many researchers have measured the CFUs (colony forming units) of bacteria present on microgreens grown with different methods. Scientists found higher populations of bacteria on microgreens grown on hydroponic mats than microgreens grown in soil-like medium (Reed, et al., 2018; Xiao, et al., 2015a). Microgreens can pose a threat for food safety no matter how they are grown, but when grown in hydroponics it seems they may pose an even greater risk. Many believe that hydroponics makes for a safer alternative to soil production, but research shows that microgreens may be riskier when grown on hydroponic mats than in a soil medium (Reed, et al., 2018; Xiao, et al., 2015a).

Perhaps the cause of concern for food safety in hydroponic systems is due to the recirculating water. The reuse of water in hydroponic systems reduces the amount of water required, and is more sustainable, since it prevents pollution from run off of fertilizer. Unfortunately, reusing water also allows microbes to spread throughout the system more easily than in soil. For example, researchers have found that a human norovirus pathogen survived in recirculating water of a microgreen hydroponic system, even 12 days after removing the source of the infection (Wang, 2016). Scientists also observed higher *E. coli* O157:H7 populations on the roots of bottom-irrigated microgreens, compared to overhead irrigation (Xiao, et al., 2015a). Hydroponic systems allow for wet conditions that promote bacterial growth and recirculating water can increase the mobility of microbial cells (Xiao, et al., 2015a).

In addition, researchers isolated the norovirus from within the roots and stems, making sanitation of the microgreens after harvest difficult or near impossible (Wang, 2016). The microbes grow along with the microgreen, allowing the infection to become systemic (Reed, et al., 2018). Although hydroponic microgreens may harbor more microbes than soil-grown microgreens, both are still safer than sprouts (Reed, et al., 2018). Proper sanitation is vital to keeping consumers safe, especially with hydroponically grown microgreens.

Today's producers ensure food safety during packaging and transportation using plastics and sanitizers. Commercial applications of peroxyacetic acid, such as Tsunami 100, can be used to sanitize seed surfaces (Moran, 2017). A cheaper option to sanitize seeds is to combine 4 tsp. white vinegar and 4 tsp. food-grade hydrogen peroxide in 1 qt. water and soak the seeds for 10 min (Moran, 2017).

For post-harvest sanitation, Chandra et al. (2012) found a dilution of chlorine in water (100 mg L^{-1}, or 100 ppm) can be used to reduce the risk of food borne illness outbreaks. They also found that a more environmentally friendly option for surface sanitation of microgreens was washing them with a citric acid solution (0.5%) and then spraying with ethanol (50%,) (Chandra, 2012). These options might be very useful to farmers who rely on proper sanitation for the safety and marketability of their microgreen produce. The method of production is also an important factor to growing microgreens that farmers must consider.

Production Methods

Production methods tend to determine the quality and nutritional value of a microgreen product (Bilyj, 2017; Brazaityte et al., 2015; Renna, et al., 2018; Weber, 2016). The method chosen by growers will depend on their conditions, preferences and market. For example, in drier climates, growers may need to increase the humidity level. A 50% relative humidity or higher will produce crispier greens, while low relative humidity (20-30%) can result in softer microgreens that are less desirable (Bright Agrotech, 2018; Storey, 2017). In humid climates, dehumidifiers, fans and lower seed densities may be required to prevent fungal infections.

There are many factors to consider when growing microgreens, including growth medium, irrigation, fertilization, light, harvest method, pest and disease management, climate and environmental conditions, seed density and seed quality. Some researchers have tested for practices to speed the growth of microgreens and increase production rates using seed treatments (Lee & Pill, 2005, Murphy, et al., 2010; Murphy & Pill, 2010). Fertilizer also increased the yield of the beet microgreens when 150 mg L^{-1} of calcium nitrate was used daily (Murphy et al, 2010).

There are many introductory guides published on microgreen production that cover many topics on how to get started growing microgreens (Bright Agrotech, 2018; Johnny's, 2017a, 2017b, 2018; Lynette, 2014; Treadwell, 2016). Many experienced growers recommend that each farmer test and adjust their techniques to find the growing methods that work best for their needs and situation (Bilyj, 2017; Johnny's, 2017a; Storey, 2017). One grower says burlap is his favorite way to grow microgreens (Cramer, 2017). Only a handful of studies compare the different growth mediums for microgreen production.

Growth Medium

Most researchers studying microgreens are using trays with either soil or loose hydroponic medium such as vermiculite, perlite, peat moss, and coconut fiber (Bulgari, et al., 2017; Murphy, et al., 2010; Murphy & Pill, 2010; Pirnia, 2015; Treadwell, et al., 2015; Weber, 2017). Gutierrez (2018) claims that the microgreens industry is split evenly between conventional and hydroponic growing methods. The type of hydroponic medium a grower choses to use does matter. For example, researchers found a 46% increase in yield when using NFT grow mats compared to peat-lite in trays (Murphy, et al., 2010). A few publications actually compare the different types of mediums used to grow microgreens with NFT, such as polyester, hemp, burlap and other fibers (Cramer, 2017; Di Gioia, et al., 2016; Gutierrez, 2018).

Burlap is typically made from 85% jute and 15% kenaf fibers and can also be called jute-kenaf fiber mats (Di Gioia, et al., 2016). These plant materials are easily renewable and are a sustainable alternative compared to substrates made from peat-based mixes or synthetic material (Di Gioia, et al., 2016). Researchers found that microgreens grown in burlap produced greater yields compared to microgreens grown in peat moss or synthetic mats (Di Gioia, et al., 2016).

The fresh yield of burlap-grown microgreens was 13% greater than that of microgreens grown in hydroponic pads made by Sure to Grow® (Di Gioia, et al, 2016). Only one scientific publication was found that addresses the effectiveness of using burlap to grow microgreens (Di Gioia, et al., 2016). Many researchers use loose hydroponic medium, such as vermiculite, to grow microgreens, which is especially useful for Swiss chard and beet, since these seeds germinate better when covered with loose media (Bulgari, et al., 2017; DelValle, 2018; Murphy, et al., 2010). Some commercial growers prefer soil, claiming it produces microgreens with more flavor, better texture and longer shelf life (Cramer, 2017).

In terms of comparing hydroponic and soil methods of growing microgreens, currently available publications focus on nutrient content. For instance, researchers found less protein in microgreens grown in soil, compared to those grown on hydroponic mats (Weber, 2017). However, the compost-grown microgreens contained more elements important for human nutrition, like potassium, and they had more biomass, and fewer microbes (Weber, 2017). The compost used for this experiment was made by students in a Worm Factory vermicompost system. Students used kitchen scraps, like banana peels, and other household waste to generate the worm castings. The banana peels may have been the reason for higher contents of potassium in the compost-grown microgreens compared to those grown in hydroponic systems (Weber, 2017).

There is currently not enough evidence available to growers to make informed decisions on which production methods would work best for their situation. For instance, very few articles compare seed density for all the different varieties. No comparisons were found for the seed density differences of each variety between burlap and other mediums. Currently this detailed comparison is only available for broccoli microgreens, where the results suggested no difference for seed density in soil and hydroponics (Di Gioia, et al., 2017). The density of seed may be an overlooked factor to crop success when growing microgreens.

Seed Density

Each grower typically determines seed density based on their own experimentation by measuring and recording the density, then adjusting sowing rates as needed. Other growers sow microgreen seeds by eye, meaning without any measurements to monitor seed density. Instructions in grower's manuals and company pamphlets are very broad when describing seed density, such as a range of 61 to 122 $g \cdot m^{-2}$ without specifying any variety (Crop King, 2014). In an introductory article, Greens (2018) wrote, "Don't worry about spacing" when describing how to sprinkle microgreen seeds onto the grow medium. The same company, Crop King (2018), and another article claims that "the correct seed density is crucial to the success of growing microgreens". Unfortunately, the author fails to mention the surface area for the reported seed densities, such as per channel, per tray or m^{-2} for the seeding rates that they recommended (Crop King, 2018).

Table 1. Means for seed density and fresh weight (FW) yield from Johnny's Selected Seeds microgreen trial (Johnny's, 2017c).

	Seed Density ($g \cdot m^{-2}$)	FW Yield ($g \cdot m^{-2}$)	Avg. Days to Harvest
Arugula	77.5	2196	14
Basil	50.4	1648	19
Mild Blend	85.2	2526	13.5
Mustard Blend	58.1	2197	13.5
Radish Blend	195	2160	8

Johnny's Selected Seeds (2017c) conducted microgreen trials (Table 1) to provide an estimated range of the seed densities their growers were using. This popular seed company discloses that they received many questions from growers asking how many seeds to sow for microgreens, but these types of questions were difficult to answer (Johnny's, 2017c). Staff in the company designed and conducted an experiment to measure the density of seed and corresponding yield in microgreen trays with soil. Johnny's microgreen trial is the only one of its kind that compares densities for this many varieties, according to current research.

The microgreen yield trial compared 29 microgreen varieties grown during a 3-month period to study volumes of seed density and yield (Johnny's, 2017c). For the experiment, the growers sowed seeds by eye (based on 10-12 seeds per square inch for small seeds and 6-8 seeds per square inch for large seeds) and then weighed the packet to determine the weight of seed that was sown. After harvest, the workers weighed the yields from each tray and published the results (Table 1). The average densities ranged from 50 $g{\cdot}m^{-2}$ for basil to over 200 $g{\cdot}m^{-2}$ for some radish varieties. Average days to harvest (DTH) were also included in their publication, which ranged from 8 to 19 DTH.

Different growth conditions may result in different yields, even when using the same seed density. Traditional methods of mature plant spacing and recommended space between rows are not useful when growing microgreens, since they are not grown to maturity, and typically not in rows. Studies are still very limited for the topic of microgreen seed density. A study that used vermiculite in cell trays reports using densities of 45 $g{\cdot}m^{-2}$ to 242 $g{\cdot}m^{-2}$ (Bulgari, et al., 2017). Other researchers measured microgreen densities in $shoots{\cdot}m^{-2}$ and reported using the same 30,680 $shoots{\cdot}m^{-2}$ for *Brassica rapini* microgreens in each of the growth mediums they tested (Di Gioia, et al., 2016).

With higher densities, growers might expect to have a bigger yield, making up for the cost of seed. However, higher densities can cause fungal infections that reduce the quality and profitability of the microgreens. Many growers agree that if seed density is too high, diseases will occur (Bright Agrotech, 2018; Johnny's 2017a; Storey, 2017; Treadwell, 2016). The economics of seed density are also a concern to growers, considering the high cost of seeds. Therefore, growers may find it helpful to know the exact seed density that can help to maximize profits while using the lowest amount of seed possible.

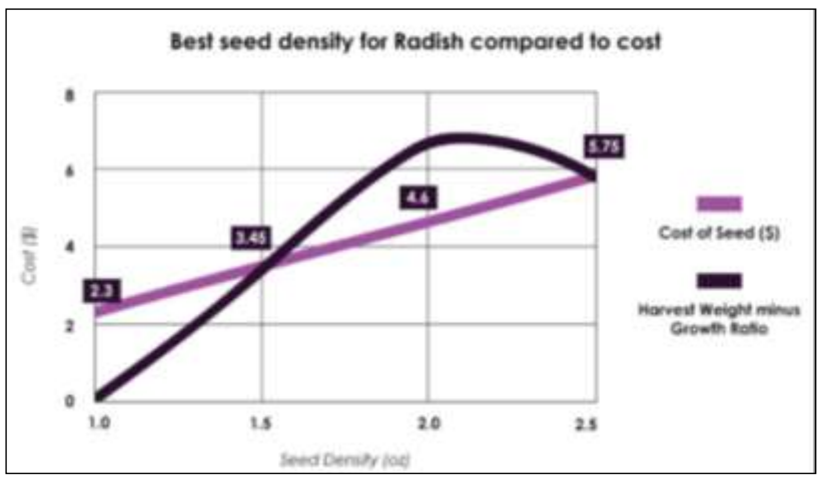

Figure 9. Seed density for radish microgreens compared to cost. Around 2.0 oz. per tray was found to be the best seed density for yield and economics (Storey, 2017).

For example, one company conducted a study to compare seed densities and FW (fresh weight) yields with seed prices. The most economical seed density that was determined for radish microgreens was around 439 $g{\cdot}m^{-2}$ (Storey, 2017). Seed densities higher and lower than 439 $g{\cdot}m^{-2}$ caused a reduction of FW, creating a somewhat parabola shape (Fig. 9). Researchers found that a density of 549 $g{\cdot}m^{-2}$ or higher caused the microgreens to be less profitable because of the reduced yield and increased cost of seed. Growers at Bright Agrotech (2018) were getting yields up to 3053 $g{\cdot}m^{-2}$ from radish using a seed density of 439 $g{\cdot}m^{-2}$, which were much higher than Johnny's results (2017c). The growers at Bright Agrotech (2018) used an enclosed seedling cart to grow their microgreens with flood irrigation on hemp growing pads.

Graphing yield and seed density provided a visual curve from the fluctuations in FW yield as seed densities rise. Storey (2017), from Bright Agrotech, recommended using a seed density of 220 g·m^{-2} for arugula microgreens to allow for the most net profit (Fig. 10). Crop King (2014) says to use 439 g·m^{-2} and Johnny's (2017c) reports using 77 g·m^{-2}. Horticulturists in New Zealand sowed microgreen seeds at densities of 48.5 g·m^{-2} for basil, 242 g·m^{-2} for Swiss chard and 45 g·m^{-2} for arugula (Bulgari, et al., 2017). There is some discrepancy among the published studies on seed density.

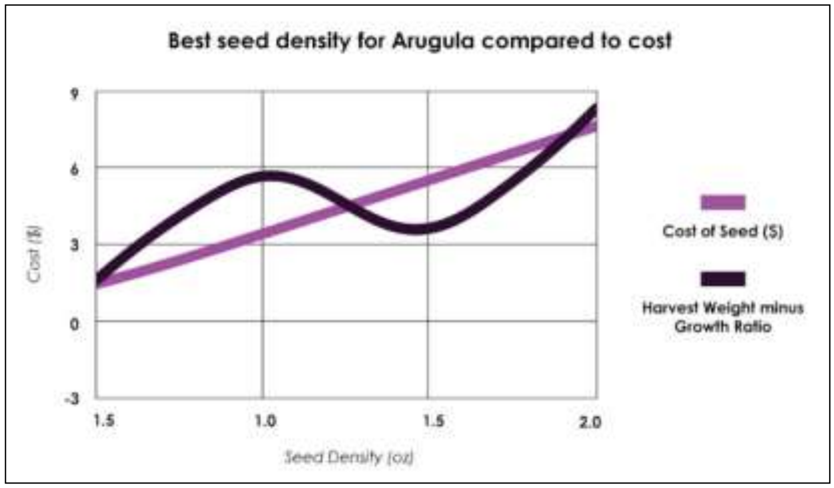

Figure 10. The best seed density for arugula microgreens compared to cost. Even though the highest density produced the most yield, lower densities were more economical when the cost of seed was considered (Storey, 2017).

Sowing densities can vary with several factors, making it difficult to determine standardized densities and the expected yields associated with them. For example, the size of the seed can fluctuate among varieties of the same species or cultivars, but can also change within each seed lot, due to different seed production methods (Bulgari, et al., 2017). The grower may need to adjust their microgreen density with each bag of seeds and for their specific conditions. These topics demonstrate gaps in the knowledge on seed density and yield for microgreen production (Kyriacou, et al., 2016). On the other hand, other production topics are well studied, such as seed treatments.

Seed Treatments

Treating seeds before germinating them may increase the yield and quality of microgreens (Murphy, et al., 2010). For instance, growers can achieve increased seed vigor and growth efficiency by pre-screening seeds to remove lower quality seeds from a batch. This can be done by removing seeds that float, or by pre-germinating to ensure a quicker turnover (Welbaum, 2017).

Scientists have studied a pregermination treatment that resulted in a 25% increase in yield, which involves soaking the seeds in water and vermiculite for 5 days at 20°C (Murphy, et al., 2010). Larger seeds benefit from soaking for 8 hours before germination, or use shorter soak times for smaller seeds (Moran, 2017). Another treatment used is pregermination. One study found that pregerminating beet seeds in vermiculite and water increased fresh weight more than pregermination in a hydrophilic polymer gel (Murphy, et al., 2010). However, when both treatments were combined the greatest fresh weight was achieved (2,450 g·m^{-2}).

Most microgreen growers do not use treated seeds for microgreens. Common conventional seed treatments include pelleting, fungicide or color coating that can have many benefits when growing crops to maturity (Welbaum, 2017). However, for microgreens, the seed coat can end up in the final product and they are very difficult to separate. Any seed coating treatment for conventional crops is not meant to be eaten since the plant grows to maturity before harvest. Other than pre-germination, treated seeds are not used for microgreens.

On the other hand, cleaning and purifying of seeds is not considered a treatment, but a common process for all seed production facilities (Welbaum, 2017). It is important to have clean and pure seeds for microgreen production to prevent the chance of contamination with weed seeds and pathogens. Seed quality and pre-germination treatments could be major factors that determine the quality and quantity of profitable for microgreens. Sanitizing seeds can also prevent plant and human diseases in microgreens, which are major concerns for commercial growers.

Pests and Diseases

Crop failure due to plant diseases and pests can make the product inedible and can ruin a business's profits. Once a channel or tray of microgreens becomes diseased, the produce is no good for consumption or sales, unless the diseased microgreens are isolated and can be removed. Because microgreens grow so quickly there is little to no time for pests to become established. However, this also provides no chance for the grower to treat a disease. Prevention is the only effective method to avoid plant diseases in microgreens. Fungi are a very common problem with microgreens, especially when growers sow seeds too densely, or have very humid conditions, such as that which frequently occurs in hydroponic systems.

Diseases common in microgreens include damping off caused by *Phytophthora* and *Pythium*, plus fungi like *Botrytis*, *Sclerotinia* and *Rhizoctonia* (Kaiser & Ernst, 2018). Air circulation can help to alleviate these issues, also lower seed densities and proper sanitation. Insect pests reported on microgreens include thrips, aphids, flies and fungus gnats, which can spread diseases to the plants. Fungus gnat larva spread *Pythium,* which can then cause root rot and damping off, killing microgreens within a day.

Some varieties are more prone to challenges imposed by diseases, such as Swiss chard, cilantro, beet, amaranth, carrots, scallions and purslane (Moran, 2017). Obtaining high quality seed and/or sanitizing seeds before sowing is a great way to prevent diseases in microgreens (Moran, 2017). In addition, maintaining a clean environment, sanitizing between crops, and using high quality seeds are also imperative to combat diseases in microgreens.

Light

Another factor that affects microgreen yields is the amount of sunlight during growth. Despite the quick turnover for microgreens, light can be a substantial factor for many variables. Too much light has been found to lower the concentration of carotenoids and chlorophyll in mizuna and mustard microgreens, even under different types of light and wavelength ratios, such as far red, green, red and blue (Craver, et al., 2017). This could be due to photodegredation of pigment molecules, or dilution caused by increased water content (Craver, et al. 2017). Other researchers found that higher light intensities around 315 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased chlorophyll content, but also decreased the concentration of nutrients and reduced the leaf area of kohlrabi microgreens (Gerovac, et al. 2016). Lower light levels around 105 $\mu mol \cdot m^{-2} \cdot s^{-1}$ caused an increase in nutrient content (Gerovac, et al. 2016). However, ideal light ranges can differ between species, varieties and even between seed lots of the same variety (Brazaityte et al., 2015; Craver, et al., 2017).

The concentrations of carotenoids and other compounds can be influenced by the amount of light and fertilizer provided during microgreen growth (Craver, et al., 2017; Gerovac, et al., 2016). LED (light-emitting diode) grow lights can function as a sole source of light for growing microgreens in stacked shelves to save space (Craver, et al., 2017; Gerovac, et al., 2016). Some varieties may require more light to maximize yield. It is important for growers to know how much light they need for their microgreens, especially when growing in a stacked hydroponic rack, where many shelves are shadowed from the sun.

Post-Harvest Handling

Another important factor for growing and selling microgreens is the shelf life and storage of the final product. Techniques for post-harvest handling are being developed to increase the shelf life of microgreens (Kou, et al., 2014). Kou and other researchers (2014) discovered many better storage qualities when they added supplemental calcium to broccoli microgreens. They found that spraying the microgreens with 10 mM calcium chloride daily before harvest increased the product's quality and decreased microbial populations during storage (Kou, et al., 2014).

Other effects of calcium spray treatments included higher concentrations of beneficial antioxidants, such as superoxide-dismutase (SOD) and peroxidase (POX) activities which are associated with decreasing the expression of genes associated with senescence, allowing for longer shelf life (Kou, et al., 2014). These powerful antioxidants also protect plants from oxidative stress (Caverzan, et al., 2015). However, too much calcium when growing microgreens can also be harmful. For instance, the 20 mM calcium solution caused the cotyledons to turn yellow during storage. Not all fertilizers promoted better quality microgreens, since treatments with magnesium caused shorter microgreens compared to using just water (Kou, et al., 2014).

Some containers may work better than others for storing microgreens. Researchers found that polyethylene bags performed better than polypropylene bags, when storing microgreens for 9 days in a refrigerator (Chandra, 2012). Bags with micro-perforations are available for sale online to use as food-grade storage of microgreens (Johnny's, 2018). Even when growers sell their microgreens still rooted, a food-safe container will be required to get the product to the customer.

Education

A review of microgreens would not be complete without referencing to its potential for education. Many people have not heard of microgreens or they are unaware of the huge health benefits they provide. Teachers can use microgreens as an education tool for topics like nutrition, sustainable agriculture, science, mathematics and more. In addition, microgreens provide a hands-on component that students can do in a couple weeks. Professors are bringing microgreen seeds and trays into their classrooms to teach these topics to all ages, from pre-school to post-graduate, and for many different applications. Weber (2017) developed a classroom curriculum based on growing microgreens to teach quantitative analysis to undergraduates. Microgreens are so much easier to grow in a classroom compared to mature vegetables. Growing and analyzing microgreens in the classroom provides students with a hands-on learning experience that applies to real world issues, such as the environmental impacts of agriculture, nutrition, health and food security (Weber, 2017).

Materials and Methods

Setting

Figure 11. The Presidents Park Greenhouse interior, view from room two. The microgreen rack in room 3 shown on the other side of the wall. Photo taken by Evan Cantwell.

The GMU greenhouse was a free-standing A-frame structure (54 ft. x 24 ft.) (Fig. 11). The greenhouse was built on GMU's campus in 2010 (Jaderloon Co, Inc., Columbia, SC). The glazing for the greenhouse was made of double-walled polycarbonate panels. The interior was equipped with HAF (horizontal airflow fans), automatic heaters and evaporative coolers that were all controlled by the automatic StepUp Controller (Wadsworth Control Systems, Arvada, CO). Dual-purpose shade curtains were used on the ceiling in the summer and winter to help regulate temperatures as needed. The curtains provided shade during summer to assist with cooling and were used in winter to trap the heat in and reduce heating costs.

Temperatures fluctuated within each day and season, but typically ranged from 50° (nighttime in winter) to 85°F (daytime in summer). Relative humidity levels (%RH) were not controlled, so the %RH fluctuated from 20% to 90% and in summer reached 100% for sustained periods.

System

The microgreens were grown hydroponically in a microgreen rack that was purchased in 2015 (Crop King Lodi, Ohio). The entire rack covered an area of about 3.7 x 1.2 m and stood 2 m high. The rack contained four shelves each lined with four rectangular PVC troughs or channels which were 0.25 m wide, 3.7 m long and 5 cm tall (Fig. 12). The shelves of channels have 0.46 m of space between them to allow ambient light to reach the plants. These channels were used to hold a single layer of burlap (1 mm thick) for growing microgreens hydroponically. The burlap (Crop King, Lodi, OH) was purchased in rolls 0.25 m wide to fit in the hydroponic channels of the microgreen rack.

This hydroponic technique of sub-irrigation and soilless medium is called NFT (nutrient film technique). Underneath the microgreen rack is a 302.8 L stock tank (Rubbermaid, Atlanta, GA) was filled with filtered municipal water using a drinking water quality hose. The municipal water was filtered to remove the chloramine used in the tap water. A float valve, attached to the stock tank, maintained the water level at approximately 160.3 L, adding about 7.5 L of fresh water each day. Fertilizers were prepared by mixing stock solutions from two powdered fertilizers and using it as a nutrient solution. The fertilizer solutions were made using 37.9 $mg \cdot ml^{-1}$ Hydro-Gro Leafy fertilizer (Crop King) labeled as concentrate tank A and 51.3 $mg \cdot ml^{-1}$ $CaNO_3$ fertilizer for tank B.

These two solutions were then measured and added to the irrigation water to achieve an electrical conductivity (EC) of 1.2 $ds \cdot m^{-1}$, or approximately 2.5 kg from concentrate tank A and 2.0 kg from tank B. The fertilizers were added after the tank was cleaned and filled with fresh water, which typically occurred every week or two as needed. The nutrient concentration was not automatically monitored, so the fertilizer content of the stock tank decreased overtime. The EC dropped to 0.8 ds·m^{-1} after 5 days and to 0.6 ds·m^{-1} after 8 days. The source water had an EC of 0.3 to 0.4 ds·m^{-1} and a neutral pH. Automatic control of the stock water's pH was achieved using BlueLab's automatic pH Controller and with a diluted solution of sulfuric acid (Interstate Batteries, Dallas, TX) to maintain a pH of 5.8.

The microgreens were irrigated with the treated water from the stock tank using a submersible water pump with 1/8 hp and 1900 gph (Little Giant). The water ran through a 1.3 cm tube from the pump up along the structure's edge and along each shelf of the microgreen rack. Attached to the tube were adapters to make corners and provide water flow to each shelf. Each channel was equipped with 3 mm tubes attached with *vari-flow* valves (Crop King) to the main tubing, to provide drip irrigation or full flow to the microgreens and NFT medium.

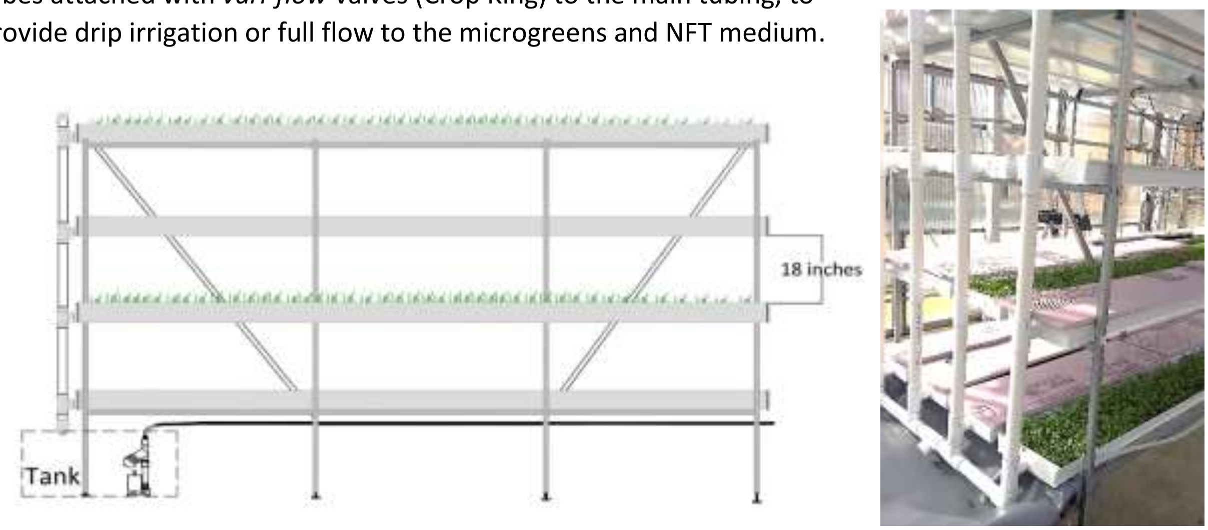

Figure 12. The hydroponic microgreen rack system, sold by Crop King. Image on left shows the design specifics (Crop King, 2014). The image on the right shows the rack in the greenhouse with purple foam coversto keep the seeds dark during germination.

There were tubes at the top of each channel and extra tubes at the one-thirds and two-thirds length of each channel as well. These additional tubes were added because without them, the fertilizer was not evenly distributed, and the microgreens tended to be larger at the top compared to the microgreens in the rest of the channel. By having three water outlets along each channel, the fertilizer is evenly distributed.

The irrigation scheduling was monitored automatically using a digital timer attached to the pump's outlet. The timer allowed for 28 settings that provided on and off controls. The frequency and duration of the irrigation events were changed with the seasons to avoid overwatering and drying out. The first irrigation event was set between 6:00 am and 7:00 am, while the last irrigation event for the day was set between 4:00 pm and 7:00 pm, depending on the time of sundown. The water turned on every 1-2 hours as needed to keep the burlap moist and to prevent wilting.

Each watering event lasted about 2- 5 minutes. The frequency and duration were higher during the middle of the day, to provide water every hour or half-hour. In the morning and evening, the irrigation was only needed every 2-3 hours. The frequency and duration of the water were also changed with the seasons and depending on plant stress. If there were frequent flooding or fungal issues in the microgreens, the irrigation schedule or flow of water coming out of the tubes were changed to fix the problem from happening again.

For disease control, beneficial biostimulants and biopesticides were regularly added to the irrigation water every week or twice a week. Common pests included fungus gnats (*Sciara hemerobioides)*, thrips (*Frankliniella tritici)*, *Pythium* root rot, *Botrytis cinerea* mold, and other unknown fungi that afflicted the microgreens, particularly in summer. The living products used regularly for prevention were Hydrogaurd by Botanicare (*Bacillus* bacteria), Rootshield WP+ by Bioworks (*Trichoderma* spp.), Orca by Plant Success (diverse mycorrhizae and bacteria blend), beneficial nematodes (*Steinernema feltiae)* by Organic Control Inc*.,* and Mosquito Bits by Summit (*Bacillus thuringiensis* var *israeliensis*).

The ingredients were weighed according to the label instructions and combined in a sanitized bucket of filtered water to create a stock solution of beneficial microbes. The solid ingredients of the Mosquito Bits and nematodes were added to a filter bag and agitated to dissolve into the stock solution. and directly to the NFT channels after diluting

Operating Procedures

Figure 13. The top shelf of the microgreen rack (Crop King, Lodi, Ohio). The burlap laid in the channel on left contains basil seeds. Mature basil microgreens shown in the channel on the right.

With the help of volunteers, interns and staff, the microgreens were harvested and sown every week. To maintain food safety throughout the growing and harvesting processes, standard operating procedures were implemented and taught to the workers and volunteers. The food safety protocols included hand washing upon entry, sanitizing surfaces before use, and wearing nitrile gloves and hairnets during harvest. A diluted bleach solution (~100 ppm) was used to sanitize objects by either soaking or wiping them clean.

Reusable cloth towels for sanitation were received every 2 weeks through a delivery service (Cintas Corporation, Cincinnati, Ohio). After wiping, the surfaces stayed wet until they air dried. Each channel was wiped clean with a towel soaked in diluted bleach after harvest and before sowing.

The burlap was prepared by cutting it to lengths about 1.3 m, and counting 3 strips for each channel. Then the burlap was then soaked in diluted bleach for several minutes. Then it was rinsed and placed in pH-balanced water (pH 5.8).

The wet burlap strips were placed in the channels by flattening them along the bottom surface of each channel. The short edges were folded under to hide loose strings and any other lose burlap strings were removed or cut, so they were not caught in the shears during harvest. The burlap strip at the bottom of the channel was lifted up enough to cover the drainage spout, so the seeds were not washed away down the drainpipe into the stock tank.

Records were kept of each channel sown using printed logs. Each channel had an assigned number, to keep track of each harvest. The date, variety and seed density were recorded for each channel. The seed density used for each crop was determined by observations from past yields and quality, not based on experimental design, allowing for multiple years of data.

Table 2. The seed densities used most often during the experiment. Shown in converted units for the reader's convenience.

	Seed Density Used Most Often			
	$g \cdot m^{-2}$	$g \cdot ft^{-2}$	Oz./Tray	Lbs. per Ch.
Arugula	39.1	3.63	0.18	0.08
Basil	39.0	3.62	0.18	0.08
Carrot	48.8	4.53	0.22	0.10
Mild Blend	48.8	4.53	0.22	0.10
Mustard Blend	48.8	4.53	0.22	0.10
Radish Blend	102.5	9.52	0.47	0.21

Decisions were based on whether there were any fungal infections or overcrowding in a variety during the week before. If overcrowding was suspected, a lower seed density was used for the next week.

The seed were weighed in cups and recorded for each channel on the microgreen rack. Seed densities ranged from 19.5 $g \cdot m^{-2}$ to 129.4 $g \cdot m^{-2}$, depending on the variety (Table 2). After weighing and recording, the seeds were sprinkled evenly by hand onto the burlap surface inside in the channel (Fig. 13). Every square cm of burlap was covered with several seeds to keep the density even. Then the seeds were hydrated using a mist of filtered water from the hose.

Figure 14. Radish on burlap, hanging for harvest.

The channels were then covered to provide darkness for good seed germination. To achieve this, purple insulation foam boards were cut into 0.25 m wide panels. The foam insulation boards were 25.4 mm thick, opaque, waterproof, lightweight, sturdy and reusable. The seeds remained covered until they were fully emerged and about 3-5 cm tall, or about 3-5 days. Harvesting occurred every Tuesday, typically after the microgreens were 13-27 days old. At maturity, the microgreen roots are fully embedded into the burlap, which together create a strip of microgreens.

When ready for harvest, each strip of microgreens was lifted by the corners and picked up gently to be removed from the rack. Then they were folded in half and placed on a wooden beam to hang (Fig. 14). The wood was held up on the microgreen rack at one end and on the greenhouse wall at the other end, to keep it securely placed over the walkway. The excess water was allowed to drain from the microgreens for a couple minutes as they hung.

Figure 15. Quick and efficient harvest of microgreens by vertically hanging the burlap strips and using cordless shears to cut the microgreens.

The microgreens were not left hanging more than 5-10 minutes before beginning harvest, because the burlap dried out quickly and the microgreens wilted. This was especially true in hot, dry weather. To begin harvest, one or two wide, flat bins were placed on a table directly under the hanging microgreens. Cordless electric shears (Black and Decker) were used to cut the greens from the burlap (Fig. 15).

The cordless grass shears were made of several double-edged blades. The sharp edges rubbed against each other to cut like scissors, grabbing the microgreen's hypocotyl (stem) from both sides, for a clean cut. This technique worked extremely well and was very efficient. The microgreens fell off the burlap cleanly and then were gathered in the bins below. To see videos of the harvest in action, watch the introductory video (George Mason University, 2018), linked in the appendix. After harvest, the remaining roots and burlap were composted in indoor vermicomposting bins, located in the prep room of the greenhouse.

Three *Worm Factory* 360 compost bins (The Squirm Firm) and red wriggler worms (Uncle Jim's Worm Farm) were used to compost the burlap and roots after harvest. The multi-tier bins allowed the burlap to break down in 4-12 weeks. Once fully composted, the finished worm castings were used in the gardens on campus as a soil amendment and the red wriggler worms were returned to the bins.

The worm castings could not be used in the hydroponic system because of Sodexo's food safety guidelines. Either way, the renewable aspect of burlap was more sustainable than using other hydroponic growth mediums that did not break down as quickly. In addition, burlap allowed for a source of organic fertilizer for the gardens on campus and provided another educational component to the program.

Seeds & Varieties

The varieties of microgreens grown for this study included a mustard blend, made with cultivars of *Brassica juncea* (mustard) such as Garnet Giant, Golden Frill, Green Wave and Barbarossa. The mild blend includes different types of *Brassica oleraceae* (cabbage) such as Red Russian Kale, Mizuna, Red Pac Choi, Broccoli, Vates Collard, Tatsoi and Purple Kohlrabi.

The other blends were created with different types of *Raphanus sativus* (radish) including Hong Vit (purple), Red Arrow (pink), Red Rambo (indigo) and Daikon (white). Non-blended varieties included *Daucus carota* (carrot), *Eruca sativa* (arugula), and *Ocimum basilicum* (basil), specifically the variety Italian Long Leaf.

The seeds used were labeled specifically for use as microgreens (Johnny's Selected Seeds, Fairfield, Maine), which likely means that the company cleans the seeds thoroughly. This helped to ensure the seeds were free from contaminants, like weed seeds, dirt and pathogens, for increased food safety. The seeds were stored in their original plastic and/or paper bags inside a cabinet in the prep room of the greenhouse, until they were moved to the fridge in sealed plastic bags, for storage that helped to maintain seed vigor (at 40°F). Some of the seeds used were over 2 years old.

Data Collection

Data collected included date sown, channel number, crop variety and seed density used for each channel. When ready to harvest, observations were made such as the appearance of density, the size of true leaves and details of any disease symptoms. Once the harvests were collected they were weighed and recorded as FW per channel.

To measure the impact of light exposure on FW yield, the channel numbers on the top shelf and south-facing shelves were considered full sun, since they were fully exposed to the sunlight. The channels located underneath or behind the sunny channels were shaded and had less light exposure. Light levels were measured using a photosynthetically active radiation (PAR) light sensor (SQ-110, Apogee) connected to a data logger (Agriculture Sensor v2.0 Waspmote). For this example, PAR measurements were made every 20 minutes from October 27, 2016 to February 2, 2017.

The nighttime PAR levels were below zero (-4.12 µmols·m^{-2}·sec^{-1}). This is assumed to be an error caused by improper calibration. To make up for this, the data were adjusted by adding 4.12 µmols·m^{-2}·sec^{-1} to the PAR readings. Light levels below 2 µmols·m^{-2}·sec^{-1} were removed from the calculations for average light intensity to omit nighttime measurements. Excluding the low nighttime readings removed the influence of light caused by passing cars. The adjusted mean light intensity during the hours of sunlight for the sampled time period was 44.23 µmols·m^{-2}·sec^{-1} (n= 2,986) with a maximum of 517.74 µmols·m^{-2}·sec^{-1}. Light exposure of the shady channels was measured for five days in December 2018 every 20 seconds. The mean daytime light intensity of the shady channels was not measured due to lack of equipment and technical support.

Analysis Methodology

Data analysis were performed with Excel and JMP Pro software. Some of the data were removed if a channel's yield failed due to drying out or had uncertain or missing data. Out of 4 years of data, only the last two years were usable from March 2016 to July 2018, since the first 23 months from did not have reliable recordings. To find the surface area of the channels in Crop King's microgreen rack kit, the length and width were multiplied to get 10 ft^2. The seed densities were calculated by dividing the weight of seeds sown in each channel by the surface area of the channel. To compare the data to other studies, the units had to be converted from the square-footage of 1.389 ft^2 was used to convert the data to g·m^{-2} from the units of a standard tray, which was 254 mm by 508 mm (10x20-inch).

The means, medians, modes and standard deviations were calculated for each variety using Excel. Graphs were created in Excel using the fresh weight (FW) yield on the Y axis and seed density on the x-axis. Trendlines were fitted to the scatter plots. A growth ratio was calculated by dividing the FW yield from the seed density. This provides a non-biased way to compare the variable response without the influence of the different seed densities used. The growth ratio was used to measure the impact of days to harvest (DTH), light exposure and season on FW yield. Scatter plots were made using the growth ratio on the Y-axis and one of the variables on the X-axis. Cubic polynomial trendlines were added to the scatter plots.

The seasons were based on the equinox and solstice days (March 21, June 21, Sept. 21 and Dec. 21 marked the first days of the next season). For the statistical analyses, JMP Pro software was used to calculate p-values in paired ANOVA tests. Some data points had to be omitted for these analytical tests, due to their single sample size.

Hypotheses

The hypothesis was that yield would change with seed density, and that as seed density increases, the yield will initially increase, then it will plateau and begin to decline. The assumption was that a mid-range of densities would produce the greatest FW. In addition, each variety required a different seed density to achieve maximum FW. The FW was also predicted to change along with the seasons, due to temperature and light fluctuations.

Also tested were the number of days to harvest (DTH), since this was predicted to influence FW as well. Too young or too old can reduce potential yield. These parameters, if found to be significant, may provide insight to the knowledge base of growing microgreens, and practices for using hydroponic systems and burlap.

Results

The mean FW yield among all the varieties was 636 g·m^{-2}, ranging from a minimum of 79 g·m^{-2} to a maximum of 2,165 g·m^{-2} (n=446). Based on the means of FW yield for each variety, the most successful variety was the radish blend, with a mean FW yield of 1,153 g·m^{-2} (Table 3). However, each radish microgreen was about double the size of the other varieties, as were the seeds, so it makes sense that it produces the most weight comparatively.

In order to discover which of the varieties was the most productive the growth ratio was used to measure the growth per g of seed. The variety that produced the highest mean growth ratio was basil. In other words, basil microgreens grew 14.3 times their mass from germination to harvest. The least productive variety was carrot, only yielding 7.8 times the original mass of the seeds. This makes sense as carrot microgreens were very thin and narrow, compared to the thick canopy that basil creates, or the wide juicy stems from the radish microgreens.

Table 3. Means ± standard deviations of FW yield (g·m^{-2}) and means of seed density (g·m^{-2}) The sample size (n) shows the number of harvests recorded for each variety. Growth ratio was yield divided by seed

	Mean	n	Seed Density	Growth Ratio
Arugula	491 (±146)	94	39.1	11.6
Basil	574 (±172)	41	39.0	14.3
Carrot	443 (±183)	42	48.8	7.8
Mild Blend	435 (±171)	63	48.8	8.1
Mustard Blend	613 (±241)	126	48.8	12.0
Radish Blend	1153 (±473)	80	102.5	10.9

The variance among the growth ratios of each variety were significantly different (p-value <0.0001). This means that the varieties were different in terms of their productiveness, or relative FW yield. The remaining results show that each variety was influenced by the variables differently, including seed density, days to harvest (DTH) and light exposure. In other words, the microgreen varieties did not have the same reaction to each of the variables.

Seed Density and Yield

The seed densities of all the varieties tested had a mean density of 59.2 g·m^{-2}, ranging from 19.5 g·m^{-2} to 129.4 g·m^{-2}. Graphing FW yield as y and seed density as x reveals little to no correlation. The varieties whose FW yields were significantly different at each seed density, were basil, carrot, and the blends of mild and radish (Table 4). This means that FW yield does not stay the same at each seed density.

Table 4. Mean ± standard deviations (g·m^{-2}) for seed densities of each variety. FW for each density were subjected to ANOVA.

	Seed Density Mean	P-value
Arugula	42.9 (±6.0)	0.5883
Basil	41.0 (±7.8)	**0.0012**
Carrot	57.8 (±8.8)	**0.0221**
Mild Blend	55.7 (±16.8)	**0.0055**
Mustard Blend	51.5 (±5.7)	0.4319
Radish Blend	91.2 (±18.7)	**0.0075**

Basil and the radish blend appear to be the most affected by seed density. Arugula and mustard blend were not

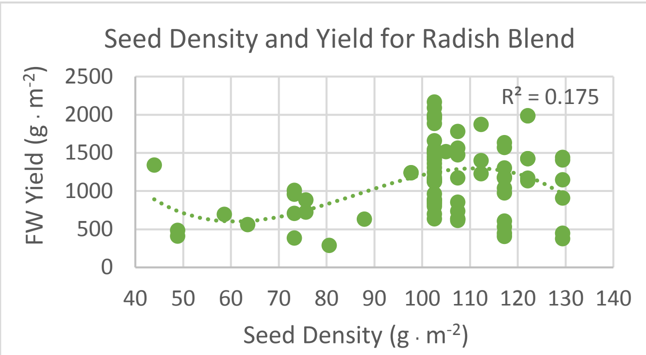

Figure 16. Seed density and yield for radish blend microgreens.

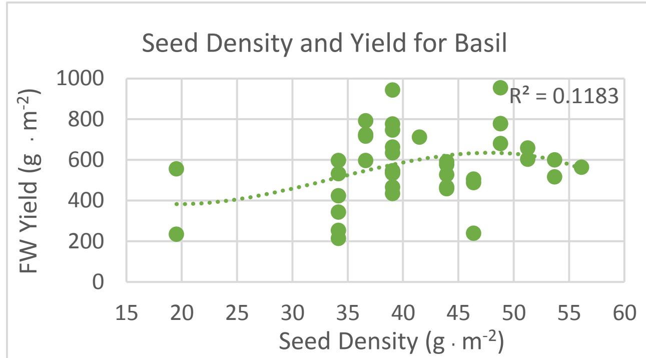

Figure 17. Seed density and yield for basil microgreens.

influenced by changing seed densities. The plotted graphs provide a cubic polynomial curve that was fit to the FW yields on the scatter plot, to represent the influence of seed density on yield.

The curve for radish blend shows an increase in FW yield once the seed densities were in a range of 100 g·m^{-2} to 125 g·m^{-2} (Fig. 16). At seed densities below 90 g·m^{-2} the FW yield of the radish blend tends to decrease. This suggests that higher densities can produce greater FW to some degree. However, the R^2 value (0.175) reveals that there is little to no correlation.

Basil also had significant results for the variance in yields at each seed density (Table 4). Basil microgreens seemed to produce the most yield at a density around 37 and 49 g·m^{-2}, but there was so much variation in the data that no correlation could be made (R^2 = 0.1183) (Fig. 17). For instance, in the middle of this upper range in seed density there was an unexpected decrease in FW yield between the seed densities 40 and 47 g·m^{-2}. Other growth factors may have affected the yield of the basil to produce this erratic pattern.

The FW yields for carrot did not adhere to the predicted pattern at all. Instead, the trendline showed that yields increased at the lower and higher seed densities and decreased in the middle range of densities (Fig. 18). It appeared that the most suitable seed densities for carrot

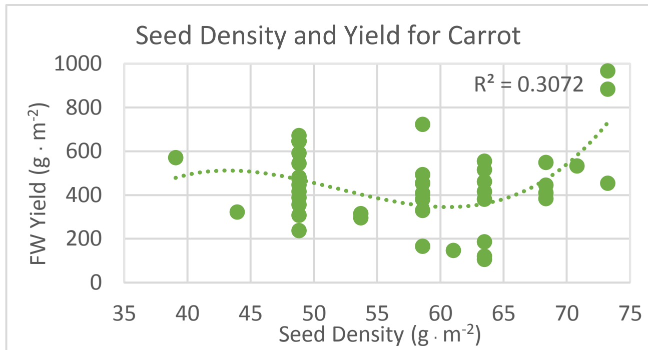

Figure 18. Seed density and yield for carrot microgreens.

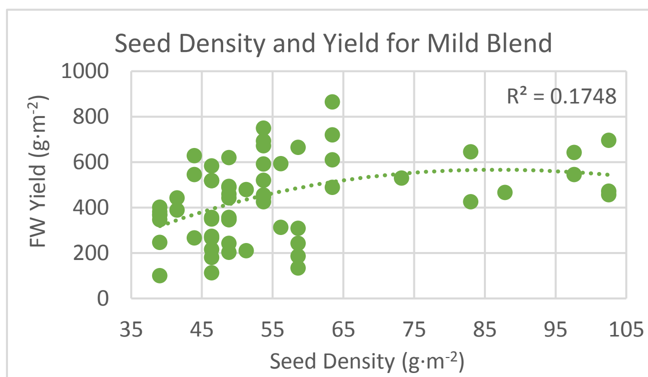

Figure 19. Seed density and yield for mild blend microgreens.

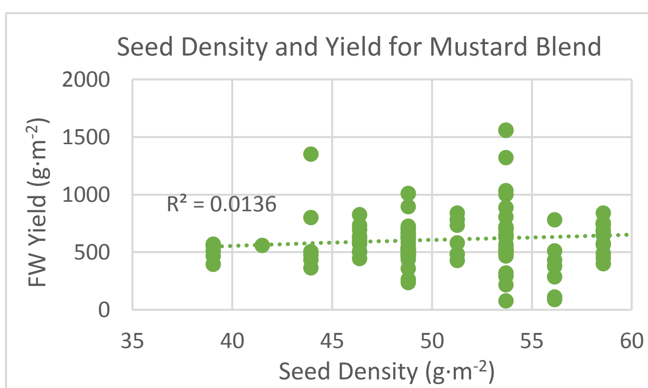

Figure 20. Seed density and yield for mustard blend microgreens.

were around 68 g·m^{-2} and 73 g·m^{-2}. However, at these maximum seed densities there were high frequencies of fungal disease occurring in carrot. Despite increased yields at these densities, the fungal infections lowered the quality of the microgreens.

There was a significant difference (p-value=0.0221) among the yields of carrot for each seed density tested. In addition, the trendline was better fit to the datapoints than those found in the other varieties, however, it was not enough to suggest correlation (R^2 = 0.3072).

The mild blend of microgreens produced greater yields at the higher seed densities tested from 63 g·m^{-2} to 102 g·m^{-2}. At densities lower than 60 g·m^{-2} the FW yields tended to decrease (Fig. 19). This suggests that greater yields could be obtained from mild blend when using higher seed densities. However, there was too much variation in the data to infer a connection between density and yield (R^2 = 0.1748).

The mustard blend had similar yields at every density tested (Fig. 20). There was no correlation between FW yield and seed density for the mustard blend (R^2 = 0.0136). Even the lowest and highest seed densities produced similar yields. This suggests that mustard microgreens were not influenced by seed density. Perhaps other factors were more likely to affect their growth.

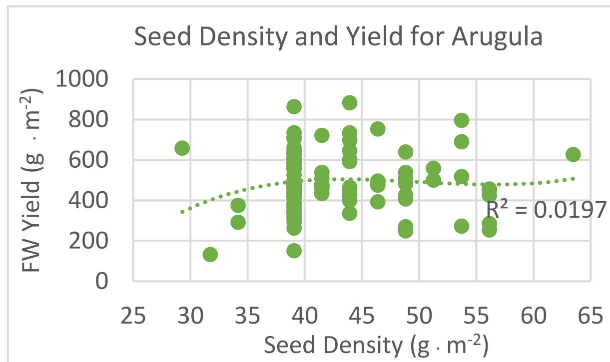

Figure 21. Seed density and yield for arugula microgreens.

Arugula also had no correlation. The bell curve that was expected was not found in arugula (Fig. 21). This suggests that seed density did not affect the FW yields of arugula. A different growth pattern was found in all the highest FW yields achieved from different densities. Almost all of the top 15 FW yields of arugula were harvested in cool seasons from different years, like October, November, February and March. This pattern implied that season and temperature may affect arugula more than seed density.

Most of the FW yield results from this study were much smaller than the yields obtained by another grower using soil in a greenhouse (Johnny's, 2017c). For example, Johnny's (2017c) highest mean FW yield of arugula was 2,197 g·m^{-2}. This was four times the weight achieved in this study, which was 490 g·m^{-2} (Table 5). The growers at Johnny's (2017c) attained consistently higher yields from growing microgreens in trays filled with soil on a table in a greenhouse. The difference suggested that microgreens were capable of greater yields and increased growth ratios when grown in soil and in higher light levels. However, Johnny's (2017c) did not include any data

Table 5. Mean FW yields (g·m^{-2}) compared to Johnny's microgreen trail (2017c) using unstacked soil in trays.

			Johnny's (2017c)	
	FW Yield	**Growth Ratio**	**FW Yield**	**Growth Ratio**
Arugula	490.6	11.6	2197	24.6
Basil	573.9	14.0	1648	32.7
Carrot	443.4	7.8	-	-
Mild Blend	435.5	8.1	2526	29.64
Mustard Blend	613.0	12.0	2197	37.8
Radish Blend	1145.2	8.0	2160	11.1

for light levels, fertilizer or even for carrot microgreens.

There were significant differences in the seed densities recommended by other researchers compared to the seed densities used in this study (Table 6). The growers at Johnny's (2017c) used almost twice as much seed per area for the radish blend (195 g·m^{-2}) and a range of 10-40% more seed for the other varieties. Researchers from Bright Agrotech (2017; Storey, 2017) recommended even higher seed densities from 220 g·m^{-2} for arugula to 439 g·m^{-2} for radish. These growers used a hydroponic seedling cart with temperature controls and supplemental lighting. These conditions may have allowed for higher seed densities without any issues. On the other hand, higher densities require more seed, which increases costs and could be less economical.

Bulgari, et al., (2017) used a seed density of 45 g·m^{-2} for arugula, which was very similar to the average seed density used in this study (43 g·m^{-2}). They used vermiculite and hydroponics to grow their microgreens in high light levels using temperature controls only during the germination stage (Bulgari, et al., 2017).

Table 6. Mean seed densities (g·m^{-2}) compared to other studies on microgreens. Johnny's (2017c) used soil in trays, Bright Agrotech (2018) used a temperature controlled hydroponic seedling cart with hemp grow pads, Bulgari et al., (2017) used vermiculite in cell trays on a floating hydroponic system. Crop King (2018) did not specify if densities were per channel or per tray, or calculations were made based on per tray.

	Mean Seed Density	Johnny's (2017c)	Bright Agrotech (2018)	Bulgari, et al. (2017)	Crop King (2018)
Arugula	43 (n=94)	76 (n=3)	220	45 (n=3)	154
Basil	41 (n=41)	50 (n=4)	-	50 (n=3)	220
Carrot	58 (n=42)	-	-	-	-
Mild Blend	56 (n=63)	85 (n=5)	300	-	220
Mustard Blend	52 (n=126)	58 (n=6)	-	-	154
Radish Blend	103 (n=80)	195 (n=5)	439	-	-

The FW yield obtained for arugula from Bulgari, et al., (2017) was around 1,600 g·m^{-2} which was still much higher than the average 490.6 g·m^{-2} FW yield recorded for the current study, but lower than the yield reported by Johnny's (2017c), which was 2197 g·m^{-2} FW. Similar densities were found among two different researchers for basil microgreens, which was 50 g·m^{-2} reported by both Bulgari, et al., (2017) and Johnny's (2017c). This density was fairly similar to the 41 g·m^{-2} seed density used in this study for basil.

The seed densities from Crop King (2018) were assumed to be measured in ounces per tray, because the article did not specify. Since the growers at Crop King (2018) were using the same microgreen rack and burlap system to grow their microgreens, assumedly, these densities should be closest to those used in this study. The densities reported by Crop King (2018) were similar to those reported by Bright Agrotech, but were much higher than densities used in Johnny's trials (2017c), and in scientific research by Bulgari, et al., (2017). Unfortunately, the growers at Bright Agrotech (2017) and Crop King (2018) did not report any FW yields associated with their recommended densities. The diverse densities and yields among the studies suggest that the most suitable methods for growing microgreens can differ greatly depending on growth medium, light levels and other factors.

Days to Harvest

The number of days allowed until harvest can have an influence on yield, perhaps more than other factors. Days to harvest (DTH) in this study were restricted to the workers' weekly schedule, causing a preference for 13 and 20 DTH. Carrot and the mustard blend produced greater yields the longer they grew. The highest mean growth ratio generated by carrot was 10.15 g·m^{-2} at 27 days (Fig. 22). The data suggest that carrot FW increases as it grows from 3 weeks to 4 weeks. However, there were no significant differences in the ratios of carrot at each DTH (p-value=0.686). The only microgreen variety that had significant results for DTH was the mustard blend.

The mustard blend produced significantly different growth ratios at each DTH tested (p-value <0.0021). The growth ratio achieved at 20 DTH (mean = 17.67 g m^{-2}) was higher than the growth ratios achieved at 11-15 DTH (mean = 11.56 g m^{-2}). This suggests that growing mustard microgreens for about 3 weeks compared to 2 weeks may help to maximize yield.

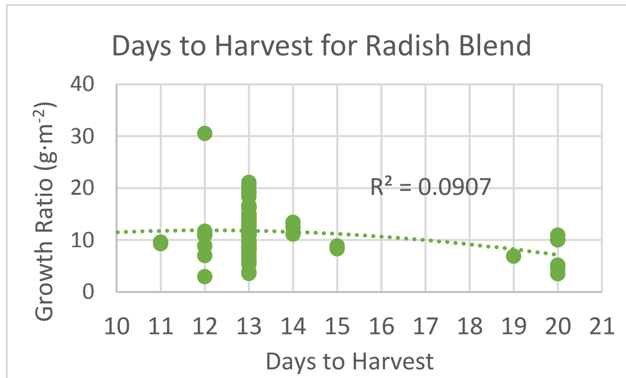

Figure 23. Days to harvest for radish blend, using the growth ratio (yield divided by seed density). Each dot represents a single harvest from one channel.

DTH had the opposite effect on the radish blend. Higher growth ratios were higher from radish blends at a range of 10-15 DTH compared to 19-20 DTH (Fig. 23). Radish blends produced more per seed when harvested young. Other growers using soil and high light levels were able to harvest radish microgreens at only 8.5 days on average, while almost doubling the mean yield obtained compared to the current study (Johnny's, 2017c). The radish microgreens grown by Johnny's Selected Seeds (2017c) were harvested 4.5 days sooner on average than the mean DTH for the radish blend in the current study.

The only two varieties with similar means for DTH in the current study and Johnny's (2017c) were the mild blend and arugula (Table 7). The other growers achieved more FW yield in a shorter amount of time possibly by using higher seed densities. Johnny's (2017c) used 195 g·m^{-2} seed which was almost twice the density used in the current study. Fewer DTH allow for more crop turnovers over time. If growers can get double the yield in less time, more profits were possible. However, using twice as much seed could be costly.

Table 7. Mean days to harvest (DTH) compared to mean DTH used in Johnny's microgreen trials (Johnny's, 2017c). DTH values for each variety from the two studies were compared using ANOVA.

	DTH	P-Value	Johnny's (2017c)
Arugula	16	**0.010**	14
Basil	24	0.174	19
Carrot	20	0.685	-
Mild Blend	14	0.232	13.5
Mustard Blend	13	**0.003**	13.5
Radish Blend	13	0.053	8.5

The potential FW yield per year was greater when harvesting radish at 8.5 days than harvesting at 13 days, even if the yield per harvest was the same. If higher densities were required to achieve the shorter crop turnover, more money would be spent on seed. Calculations show that the quicker turn would allow for greater revenue per year, despite the increased cost of seed. Faster crop cycles can produce more microgreens overtime, meaning more net profit, even with double the seed density. However, seed density may not be the only factor allowing for higher profits. The environmental conditions used by Johnny's (2017c), like greater light levels and a soil medium, could have affected the growth rate as well. If space and sunlight were not an issue for a grower, they should utilize full sun to increase the growth rate of their microgreens, and thus increase their annual revenue potential.

Season and Sun Exposure

Table 8. Mean growth ratios for sunny and shady channels.

	Sunny	Shady	P-Value
Arugula	12.2 (n=31)	11.3 (n=63)	0.324
Basil	14.7 (n=20)	12.5 (n=21)	0.016
Carrot	9.2 (n=22)	6.4 (n=20)	0.048
Mild Blend	8.4 (n=19)	7.9 (n=44)	0.583
Mustard Blend	12.8 (n=58)	11.3 (n=68)	0.058
Radish Blend	10.7 (n=34)	11.7 (n=46)	0.340

The results demonstrate that most of the varieties produced different mean yields based on the level of sun exposure (Table 8). Most of the varieties performed better in the sunny channels compared to the shady channels. The only variety whose yields were significantly different in each light exposure was basil. Grown in full sun, basil produced significantly more fresh weight (p-value= 0.016) compared to basil yields grown in shady conditions. Another variety that produced higher yields under full sun was carrot, which was slightly significant (p-value= 0.048). The only variety that had higher FW yields when grown in the shade was the radish blend.

The radish blends of microgreens only performed slightly better, on average, in the shady channels. However, there was no significant difference. Even though greater yields were obtained from growing radish blends in the shady channels, more often, the yields were very close (Fig. 22). This demonstrates that radish microgreens may be equally productive when grown under low and high light intensities.

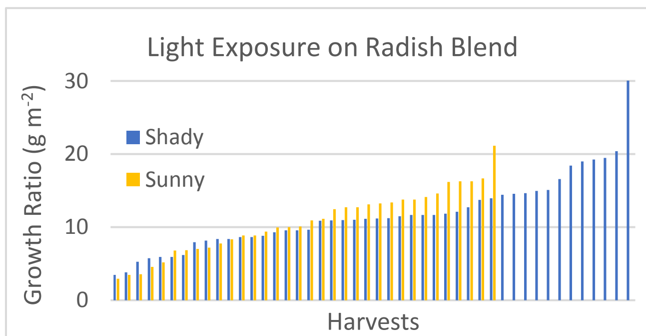

Figure 24. Fresh weight yields from each harvest of the radish blend, for sunny (yellow) and shady (blue) channels.

Some varieties had obvious differences between FW yields in the sunny and shady channels, but were not significant. For example, the mild blend produced greater mean growth ratios in high light levels, but the growth ratios from lower light levels were similar in range (Fig. 24). These patterns show support for the concept that microgreens can be grow in shady conditions, without sacrificing too much yield.

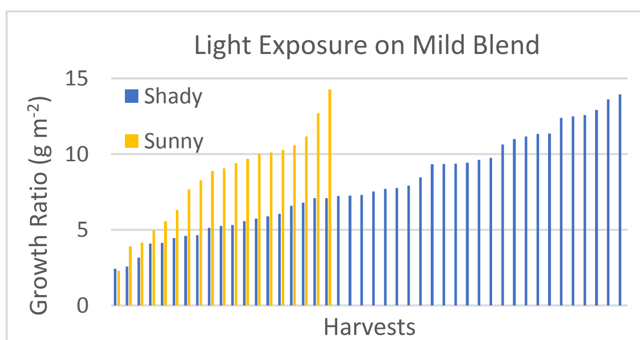

Figure 25. Fresh weight yields from each harvest of the mild blend, for sunny (yellow) and shady (blue) channels.

Microgreens can also be influenced by temperature, which was based on the seasons for comparison. The analysis for seasonal trends shows that growth ratios were not the same throughout the year. Higher yields were attained from the mild blend in spring than any other season (Fig. 25). Arugula grew much better in the winter than in the spring and summer. Basil grows best in the spring and summer, and its yields decreased abruptly in the fall. The winter basil crop failed, thus no data.

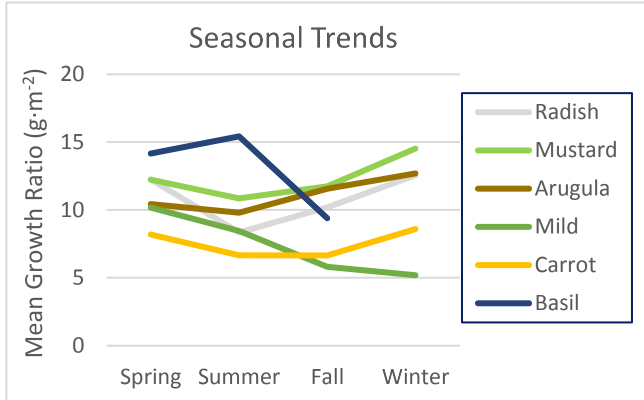

Figure 26. Mean growth ratios per season.

The growth ratios changes in each variety throughout the year were likely caused by seasonal temperature fluctuations in the greenhouse (50 - 85°F). For example, when temperatures dropped in autumn, basil yields declined (Fig. 26).

Some brassicas, like mustard and arugula were difficult to grow during the spring and summer months, but they thrive in the cooler days of fall and winter. This may be because brassicas were cool season crops. In addition, fungal diseases that thrive in warm and humid conditions were not a concern in the cooler seasons. The radish blend yields significantly less FW in the summer compared to other seasons (p-value =0.0002).

The growth ratios at each season were significantly different for the blends of mild, radish and mustard microgreens (p-values = 0.0136, 0.0002 and <0.0001, respectively). The seasons did not significantly influence FW yield for arugula or carrot, which contradicts the current assumptions. Growing arugula in the summer was often avoided since it always grew very thin and was low quality. Basil was not significant due to lack of yield records in the winter. There was an assumption that all brassica varieties grew better in cooler temperatures. However, the mild blend generated greater mean growth ratios in the spring and summer surprisingly. In addition, the mild blend had its lowest growth ratios in the fall and winter. Other factors may have influenced these yields to misrepresent seasonal results, because temperatures should be similar in spring and fall. Many of the varieties had higher yields in either spring or fall but not both.

Discussion

Each microgreen variety was influenced by the variables differently. Basil grew better in warm, sunny conditions, and the other varieties performed better in cooler conditions and were not significantly influenced by lower light levels. The results suggest that brassica varieties, including arugula and blends of mild, mustard and radish, perform better in moderate to cool temperatures, and do not need high light levels. These brassica varieties can produce a suitable yield in the shady conditions of the lower shelves on the microgreen rack. Growers can maximize their harvest by growing basil in the exposed, sunny channels of the rack, and placing other varieties, like brassicas, in the shaded channels. This suggests that using the hydroponic microgreen rack allows growers to save on space without sacrificing yield. This correlation may have been made in error, and it's possible that most microgreen varieties could produce higher yields under supplemental lighting.

Other studies reported a 34% increase in dry weight for brassica microgreens under higher light intensities (315 $\mu mol \cdot m^{-2} \cdot s^{-1}$) using LED grow lights with wavelength settings at $R_{84}:FR_7:B_9$ (Gerovac, et al., 2016). Growers at Johnny's (2017c) had higher growth rates using a set-up of soil trays on tables in the greenhouse, which must have fewer shaded areas than the microgreen rack's stacked shelves. On the other hand, more light can cause shorter microgreens, which can make harvesting more difficult (DelValle, 2018; Gerovac, et al., 2016).

In the current study, the radish blend produced higher mean growth ratios when grown in the shade than when grown in the sun. This could possibly be caused by stretching of the hypocotyl caused by the plants' reactions to lower light levels. The elongated hypocotyl allow for increased fresh weight after harvest. Responses to high or low light levels could differ among microgreen varieties and more research is needed to see if supplemental lighting is recommended to quicken crop turnovers and increase annual profits.

Basil microgreens were the second-best performer of the six varieties tested based on growth ratio. The lowest growth ratio mean was produced by the mild blend. The mustard blend had the second best growth ratio. The brassicas in the mild and mustard blends behaved differently, which was not expected. The mustard blend could be more tolerant of the hydroponic conditions compared to the kale, kohlrabi and cabbage in the mild blend. The mizuna in the mild blend seemed to grow faster and always had larger true leaves compared to the kale and red cabbage in the blend. This suggests that perhaps it is better to grow each type of microgreen separately and then blend them together, so some crops can be left to grow more days as needed (Crop King, 2014).

The days to harvest (DTH) significantly influenced the yield of the mustard blend microgreens, suggesting that higher FW yields can be attained if mustard microgreens were left to grow for about 3 weeks, compared to two. However, faster growth rates allow for more crop turnovers and revenue potential per year.

Seed density was not correlated to yield for any of the varieties tested, especially for arugula and mustard blend. Arugula was the only variety that did not seem influenced by any of the factors. Contrary to these findings were other studies that found greater fresh weights in arugula microgreens when using higher seed densities of 55 g m^{-2} compared to lower sowing rates (Murphy & Pill, 2010). Basil and carrot had significantly different yields at each seed density tested, but there was no evidence to suggest a direct correlation, as hypothesized. This shows that using lower amounts of seed may be more economical. On the other hand, comparing the data to Johnny's (2017c) showed that using more seed and faster growth rates can increase revenue potential overtime.

The data show how most of the harvests were not reaching the highest potential yields, despite using the most successful seed density. These results suggest that yields can be improved by manipulating factors other than seed density. Fertilization regimes could be a substantial factor to increase yield and growth rate (Murphy & Pill, 2010). In addition, pre-germinating seeds in vermiculite before sowing could help to increase yield more than fertilizer or other factors (Lee & Pill, 2005; Murphy & Pill, 2010; Murphy, et al., 2010). Greater light intensities may help to increase growth rate as well. Additional research is needed to study the effects of different fertilization methods, and seed pre-germination techniques on the many varieties of microgreens.

Limitations

Thus far, fresh weight has been the only variable measured to quantify the effects of days to harvest, season and seed density on microgreen growth. However, yield was not always the best indicator of success, because the weight of microgreens does not consider the quality of the product. The influence of quality on microgreens was not analyzed in this study and should be addressed in future research. The lack of consideration for quality influenced the results for carrot seed density. The highest yielding carrot channel was at a seed density of 73.2 $g \cdot m^{-2}$. However, the microgreens from that batch contained some mold and dead plant parts, even though it had a high fresh weight. Plant diseases were often the cause for low quality. Prevention of disease may be a very important factor for growing high quality microgreens.

Disease incidence in microgreens has been attributed to high seed density (Kaiser & Ernst, 2018). When microgreens were too close to one another, there was not enough air circulation, and this caused conditions for mold to thrive (Johnny's, 2017a). If the seeds were sown too dense, moisture was trapped among the microgreens, causing fungal disease. Many fungal pathogens affected our microgreens when the seed density was high. Fungi was observed on the leaves, stems, on and under the burlap surface at some points during production. The impact of disease on yield or quality was not analyzed for this study, but should be considered in future studies.

Disease was observed often in the brassica microgreens when the seeds were improperly stored. After storing the seeds in a cabinet for several months, a decrease in seed vigor was perceived. Once the seeds were stored in the fridge, the radish and mild blends seemed to grow faster and healthier. Seeds should be stored in dry and cool conditions to improve overall growth, germination and seedling vigor (Welbaum, 2017). Seeds like basil, though, appeared to be unaffected by high temperature storage. Seed storage could be an important factor to take into consideration when planning production techniques.

Irrigation type and scheduling can also impact quality and yield of microgreens. Dessication and overwatering were common in the NFT system because of the thin layer of growth medium. During rainy days, any automatic irrigation settings could cause overwatering, but during sunny days the microgreens could dry out with the same irrigation methods. These days of dryness and high humidity can increase the stress on the plants and encourage disease, affecting the yield and quality drastically.

Irrigation and humidity may be major factors that can be finely tuned to produce reliable yields. Perhaps the use of moisture sensors linked to controllers for the irrigation can be used to avoid overwatering and drying as the weather changes each day. Or hand watering several times a day, if feasible, can help to monitor moisture levels.

More research is needed to see how automatic sensing equipment could assist with irrigation, and whether this would significantly increase yields. More data collection is needed to analyze the influence of disease and the factors that encourage disease. Another topic that needs more study is the influence of fertilizer on the rate of microgreen growth and fresh yield.

It can take years of trial and error to determine the best methods for a grower's unique conditions. This publication can guide the grower and researcher in making decisions about which varieties to grow and which factors to focus on to maximize their yield from each variety.

The errors that occurred while conducting this research were worth mentioning. Due to the reliance on volunteers' free labor, the data collected was not always consistent. For example, the length of burlap varied within 0 to 203 mm, due to the imprecise preparation methods, and this difference was not recorded. The fluctuating burlap lengths effect the surface area of grow space, possibly skewing the density and yield measurements.

Another discrepancy was that some channel numbers were missing from our written records, and thus not available for those channels. Since it was impossible to match the yield with the measured seed density and other records for some channels, not all harvests were utilized in the analysis.

In addition, other influences on the outcomes may have been caused when volunteers sowed seeds unevenly or forgot to add fertilizer. However, the data presented here were still useful for estimating the influences on yield, for the recorded parameters. In addition, the data were collected over several years and provide insights from over 400 channels harvested. Despite the inconsistencies caused by the nature of having volunteers and other errors, the data provide a basis for further research on microgreen production methods.

Recommendations

Diversity in microgreen varieties is best for maximizing nutritional intake and is more attractive to consumers (Bulgari, et al., 2017, Xiao, et al., 2012). The diverse crops used for microgreen varieties require different conditions to maximize yield. For example, basil microgreens need full sun and warm temperatures to maximize their growth rate and yield potential. When growing in vertical hydroponic channels basil should be placed either on the top shelf of the grow rack or under supplemental lights to provide enough light intensity. Underneath the top shelf, growers can place radish microgreen varieties, since they grew better on average in the shade.

The varieties can also be grown seasonally to maximize yield. The results showed that basil and mild blends produced their highest average FW in the spring and summer while arugula, radish and mustard blends had the greatest FW during winter. Seed density did not correlate with yield, even though there were many varieties that had significant differences in FW yields at each seed density. Sowing seeds too dense was found to cause disease and lower the quality of the final product as other researchers confirmed (Bright Agrotech, 2018; Johnny's 2017a; Storey, 2017; Treadwell, 2016).

The recommended seed densities for each variety are shown in Table 8. Radish blends had the highest yields at a seed density range of 100 $g \cdot m^{-2}$ to 125 $g \cdot m^{-2}$. Basil microgreens seemed to produce the most yield at a density around 37 and 49 $g \cdot m^{-2}$. The most suitable seed densities for carrot were between 48 $g \cdot m^{-2}$ to 73 $g \cdot m^{-2}$, but at higher densities they can be prone to fungal infections. Similar yields were achieved from mild blends when densities ranged from 41 $g \cdot m^{-2}$ to 102 $g \cdot m^{-2}$. Mustard blends were mainly sown at 43 $g \cdot m^{-2}$ and 58 $g \cdot m^{-2}$ without any major fluctuations in average yield.

Table 8. Recommended seed density ranges ($g \cdot m^{-2}$) for hydroponic microgreen production on burlap.

	Recommended Range of Seed Densities	
Arugula	39	53
Basil	37	49
Carrot	48	73
Mild Blend	41	102
Mustard Blend	43	58
Radish Blend	100	125

Some researchers recommended lower densities than those presented here (Greens, 2018) while other growers used much higher densities (Johnny's, 2017c). Higher densities may be more suited to soil growing methods with higher light intensities and faster turnovers. The hydroponic rack system may require lower seed densities and causes slower turnovers due to low light levels.

Using burlap in hydroponic microgreens racks can maximize a grower's space and reduce waste. Since burlap can be renewed quickly through composting, it is more sustainable than other mediums such as perlite and synthetic grow mats that must be disposed of after use (Di Gioia, et al., 2016). In addition, microgreens grown on NFT mats were found to be more productive than those grown in loose hydroponic mediums (Murphy, et al., 2010). However, higher counts of yeast and mold were also found in burlap compared to synthetic mats and other growth mediums (Di Gioia, et al, 2016). Therefore, disease prevention is an important part of a successful burlap production system.

A first step to prevent disease is to sanitize the burlap and equipment before use. It is advised to monitor the seed density, and temperature ranges for each variety, to decrease the incidence of plant diseases. Fungal infections can be avoided by growing microgreens with lower seed densities. Storing seeds in cool and dry conditions can also help prevent disease, and increases germination percentage, especially for seeds of the brassica varieties. Proper air circulation can help to prevent fungal infections in microgreens as well. It is recommended to adjust the irrigation cycles for the hydroponic system along with the seasons to avoid wilting and overwatering.

Hydroponic microgreen production on burlap is a viable option for growers seeking to maximize their yield in a small space and increase the sustainability of their production system. Awareness of the diverse needs for the different varieties of microgreens can help to maximize yield and raise consumer interest. More research on the influence of light intensity, fertilizer and pre-germination techniques are needed to find more ways to maximize yield. Growers can use these insights to guide their efforts to increase revenue and save on space while creating nutritious food.

References

Instrumentation

- Apogee PAR light sensor, SQ-110, attached to the Waspmote Agriculture Sensor v2.0.
- Scale purchased from Webrestaurant, manufactured by Tor Rey, legal for trade.

Supporting Documents

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Appendices

List of Presentations and Features

City Farmer News. 2017. Featured: Meet the budding hydroponic farmer feeding George Mason University in Fairfax, Virginia. http://cityfarmer.info/meet-the-budding-hydroponic-farmer-feeding-george-mason-university-in-fairfax-virginia/

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