OPTIMIZATION OF GREENHOUSE HYDROPONIC LETTUCE PRODUCTION

By

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LIST OF ABBREVIATIONS

Adriana (Ad)
Alkindus (Al)
Amadeus (Am)
Ammonium (NH_4^+)
Black Seeded Simpson (BSS)
Breen (Br)
Buttercrunch (Bc)
Cedar (Ce)
Cherokee (Ch)
Constant flood table (CFT)
Controlled environment agriculture (CEA)
Daily Light Integral (DLI)
Deep Flow Technique (DFT)
Deep water culture (DWC)
Dissolved oxygen (DO)
Dragoon (Dr)
Electrical conductivity (EC)
High pressure sodium (HPS)
Internal Diameter (ID)
Intred (In)
Liter Per Hour (LPH)
Natalia (Nt)
Navara (Nr)
Nevada (Nv)
New Red Fire (NRF)
Nitrate (NO_3^-)
Nitrite (NO_2^-)
No supplemental light (NSL)
Nutrient film technique (NFT)
Photosynthetic photon flux density (PPFD)
Photosynthetically active radiation (PAR)
Potential of Hydrogen (pH)
Red Sails (RS)
Red Salad Bowl (RSB)
Rex (Rx)
Salanova Red Butterhead (SRB)
Salanova Red Oakleaf (SRO)
Salvius (Sl)
Shoot Dry Weight (SDW)
Skyphos (Sk)
Supplemental lighting (SL)
Total electrical energy consumption (EEC)
Truchas (Tr)
Walkmann's Dark Green (WDG)

ABSTRACT

As the world population continues to grow, it will be challenging to manage resources, reduce environmental pollution and maintain growing demand for food production. Controlled environment agriculture (CEA) is a novel solution to reduce freshwater use in agriculture, minimize environmental pollution from agriculture sector, and meet the growing food demand. CEA allows for the year-round cultivation in inhospitable climatic conditions. Hydroponics is a common method of growing crops in CEA, where plants grow in a solution enriched with nutrients and oxygen. The technique significantly reduces water use and fertilizer run-off during production. In the United States, lettuce is one of the most important crops grown using hydroponics.

Hydroponic production uses several methods to grow lettuce including nutrient film technique (NFT) and constant flood table (CFT). Moreover, several cultivars of lettuce are grown in the Midwest. There is a lack of knowledge on whether optimal fertilizer concentrations change depending on the cultivar or hydroponic production system. Little information is known about the suitability of a cultivar to a specific method of hydroponic production. For year-round lettuce production in hydroponics, supplemental lighting (SL) and heating are required in the Midwestern regions of the U.S. The energy requirements for SL and heating can be too costly in winter for some growers to produce crop year-round. In addition to light quantity, spectral composition of light can impact growth. Heating the root zone to produce a micro-climate may be more efficient than heating the entire greenhouse and possibly reduce overall heating costs. However, information on spectral composition of light and the efficacy of root zone heating is unclear, at best. Certain cultivars that can tolerate cold stress can be more suitable in the U.S. Midwest during winter. Lettuce cultivar screening for yield under cooler environments is limited.

A completely customizable hydroponic production system that can aid in conducting research related to above-mentioned issues was built as a part of my Master of Science program. Using this system, 24 popular cultivars from four lettuce groups were evaluated for productivity during summer/fall under different concentrations of fertilizer solution, and in two production methods including NFT and CFT during spring. In addition, yield of all 24 cultivars were evaluated under 10, 15.5 and 21.1 °C in a growth chamber. The eight best performing cultivars from the

summer/fall trial were evaluated during the winter in a greenhouse with the addition of SL and root zone heating with minimal ambient air heating.

Results indicated that the lowest level of electrical conductivity (EC) of the fertilizer solution used (1.3 $dS\cdot m^{-1}$) resulted in highest yield, regardless of cultivar or method of production. Among the 24 cultivars; Red Sails (Leaf), Salvius (Romaine), Cedar (Oakleaf), and Adriana (Butterhead) had the highest yields among each group during summer. Growth chamber study indicated that Dragoon, Adriana, New Fire Red and Red Sails cultivars had higher yields than other cultivars under cooler (10 and 15.5 °C) air temperature conditions. In the winter study, lettuce cultivars did not reach harvestable size even after 40 days of growth without SL and root zone heating. Supplemental light composition significantly affected lettuce growth with higher yield under Purple (with higher proportion of red) than White LED lighting. Commercially acceptable lettuce could be produced using root zone heating. In general, plants grown under CFT yielded higher than those grown under NFT in the winter trial. Among the cultivars, Salvius, Black Seeded Simpson, Cedar, and Red Sails performed better under SL and root zone heating during winter.

CHAPTER 1. SUMMARY OF LITERATURE

1.1 Conventional Agriculture vs. Controlled Environmental Agriculture

Conventional agriculture requires large amounts of resources including farmable land, water, and fertilizer (Barbosa et al., 2015). In 2012, 17% or roughly 340 million acres of the United States land was used for crops while 29% was used for pasture and range (Bigelow, 2017). In regards to Earths total water, 3% is fresh water while only 0.5% of it is available and nearly 70% of that is devoted to agriculture (Kern et al., 2016). Current agricultural practices often waste and pollute our already limited water sources. To minimize this effect, responsible use of fresh water in agriculture is urgent. As the world population rises and weather becomes more extreme, conventional field agriculture becomes less effective in supporting the worlds growing food needs. Controlled environment agriculture (CEA) is a technology-based method to produce environmentally friendly and higher quality crops (Both et al., 1997). CEA could minimize agricultural waste and increase production. Unlike conventional agriculture, CEA can be utilized in any environment without the typical risk factors that go along with farming the land, such as nutrient leaching, erosion, and extreme temperatures (Alshrouf, 2017). Systems that have temperature control and supplemental lighting (SL) can provide food year-round despite the outside conditions. Research has found that hydroponic lettuce production offered roughly 11 times the yield and required 13 times less water when compared to same amount of land in Conventional farming (Barbosa et al., 2015). The use of pesticides is reduced or unnecessary in CEA (Alshrouf, 2017).

In CEA, crops are grown using hydroponics method, which involves growing crops in soilless substrates supplied regularly with a fertilizer solution or completely in a solution of fresh water, essential nutrients, and dissolved oxygen (DO). Leafy greens, especially lettuce, are commonly grown in solution culture. Hydroponics produces higher quality vegetables and results in less waste and environmental pollution when compared to conventionally farmed produce (Barbosa et al., 2015 and Kern et al. 2016). However, hydroponics requires 82 times more energy to produce a crop (Barbosa et al., 2015). The bulk of energy requirements in a hydroponics system come from supplemental lighting during winter, cooling, heating, and water pumps. The large equipment costs require an upfront investment that is difficult for many farmers. In addition, not all crops are profitable in a hydroponic system. Low cost crops that are mass-produced such as corn and soybeans aren't profitable in a hydroponic system. Due to the high-energy requirements and large equipment costs, hydroponics is recommended for high value crops (Hussain et al., 2014).

1.2 Origin of Lettuce (Lactuca sativa L.)

The first recorded instance of lettuce cultivation was in Ancient Egypt where it grew thick stems and long pointed leaves (Ryder, 2002). There were many types of lettuce cultivated and bred throughout Egypt where the stem was mainly consumed. Lettuce began to rise in popularity in Ancient Greece and Rome before spreading to the rest of Europe as both red and green cultivars (Ryder, 2002). It's believed that lettuce was spread to the Americas around the 1400's upon the exploration of the New World. The six edible forms of *Lactuca sativa L*. are stem, butterhead, leaf, romaine, Latin, and crisphead (Ryder, 1999). Lettuce has always been a dietary staple for many cultures but has recently found even more popularity in recent years as a health food high in dietary fiber, vitamin A, vitamin B9, vitamin C, vitamin K, and many phenolic compounds (Mampholo et al., 2016). Increased consumption of lettuce and other leafy greens has been shown to reduce cancer, obesity, and heart diseases (Ryder, 2002; Mampholo et al., 2016).

The nutrient levels and growth requirements are different depending on the type of lettuce. Red leaf lettuce has been found to contain higher levels of bioactive compounds and antioxidants than green leaf lettuce (Mampholo et al., 2016). Oakleaf and leaf lettuce typically matures within 40-50 days, butterhead lettuce matures within 45-55 days, romaine lettuce generally takes 45-60 days to fully mature, and Iceberg can take 60-100 days to mature. They all grow optimally at ambient temperatures around 20 ^{o}C.

1.3 Hydroponic Production Systems

There are various production systems in hydroponic agriculture. However, there is little consensus among the scientific community and growers as to which is more productive. Two of the most popular production systems are nutrient film technique (NFT) and Deep Flow Technique (DFT) (Walters and Currey, 2015 as cited by Fennemen et al. 2013). NFT is characterized by the thin film of water that runs through the NFT channel, where the plants roots will grow into the film but are still largely exposed to air. This system is commercially produced as narrow channels where plants are grown in rows of 3-6 meters long. DFT, also known as deep water culture (DWC), has large volumes of nutrient solution for the roots of the plant to grow into ranging from a few cm to more than a meter. The biggest difference between NFT and DWC is the volume of nutrient solution that plants are exposed to. In DWC, solution covers a larger area of the roots than NFT. A study comparing NFT and DWC yield found that spinach grown under NFT has significantly more yield than DWC, but only in the summer (Ikeda et al., 1995). In addition to NFT and DWC systems, a flood table system can be used to produce crops with 8-15 cm of nutrient solution. Constant flood table (CFT) consists of flood trays with nutrient solution is recycled continuously. Plants in this system will grow in 5-8 cm of water where their roots are completely submerged in nutrient solution. The advantages of this type of system are less nutrient solution to manage, easy dissolved oxygen maintenance, and lower costs of heating the nutrient solution.

A more recent publication that compared NFT and DWC in aquaponics, found that DWC out produced NFT in terms of lettuce yield (Lennard and Leonard, 2006). A study of the relationship between production systems for basil yield found that DFT, where plants grew in 15 cm of water, produced a slightly higher yield than NFT where nutrient solution film was 1 cm. But the study didn’t find the differences significant (Walters and Currey, 2015). The current publications are inconclusive for determining the preferred production systems for hydroponic lettuce production. Lettuce cultivars have been known to show preference for specific production system (Assimakopoulou et al., 2013). In commercial hydroponic greenhouses where multiple cultivars are grown, it is possible that some of these cultivars growth could be optimized in one system over another. Observing lettuce cultivar preference in production system could provide growers with additional information on optimizing their hydroponic systems.

1.4 Nutrient Solution Management Strategies

Electrical conductivity, pH, and oxygen solubility are three important characteristics of a nutrient solution, which needs to be maintained at optimal level for yield and plant quality in a hydroponic system. If electrical conductivity (EC) and pH aren’t monitored then plants are subject to nutrient deficiencies, slow growth or even death. EC is the measure of the salt concentration within a solution and the pH is a measure of the acidity or basicity of solution. The EC and pH of a nutrient solution will change as plants grow because plants selectively obtain their nutrients from the nutrient solution. Oxygen solubility is important in maintaining a nutrient solution and is affected by temperature. When solution temperatures exceed 26 ^{o}C it loses its ability to maintain proper oxygen solubility (Goto et al., 1996). The optimum level of DO in a hydroponic nutrient solution should be around 8 mg·L^{-1} but lettuce yield won't be harmed by DO levels as low as 2 mg·L^{-1} (Goto et al., 1996). Lack of oxygen in the nutrient solution results in an accumulation of organic acids, ethylene, and dissolved carbon dioxide which inhibit the metabolism and growth of the roots (Goto et al., 1996).

Most commercial hydroponic growers maintain a three-tank system where multipart fertilizers and pH buffers are kept separate until they are diluted into the nutrient solution. Fertilizers are kept separated as concentrated stock due to the possibility of precipitation by the salts when mixed at too high of a concentration. Incompatibility between fertilizers can also occur triggering certain elements to become unavailable upon mixing. Many growers choose to manage nutrients based on EC levels by adding either additional fertilizer concentrate or fresh water to maintain EC. Sometimes deficiencies may appear when maintaining EC in a recycled solution. This issue occurs when slower absorbed nutrients accumulate, causing a false EC measurement. Nutrients such as nitrogen, phosphate, and potassium are taken up by the roots quickly while other salts such as calcium are absorbed at a slower rate, accumulating in a recycled solution over time (Bugbee, 2004). Higher salt concentrations have also been found to increase the osmotic stress on the plant and inhibit the uptake of other nutrients (Ding, 2018). Maintaining nutrient concentrations by EC isn't the only method for growers to use, but it is the simplest. Monitoring individual nutrients in a solution is often expensive and only affordable in large-scale hydroponic operations. This method is the most accurate but requires lab equipment and testing. For smaller hydroponic farms, in areas where water is affordable, simply replacing their solution at regular times during growth will ensure optimal EC without sending water samples to a lab. Research by Samarakoon et al., (2006), found that a tank changes every two weeks is enough to maintain nutrient availability as well as reduce osmotic stress in lettuce. Changes in EC level are directly correlated with biomass and metabolite concentration (Abou-Hadid et al., 1996). Several studies have been done on the optimal EC of a solution for production of leafy vegetables. Lettuce EC should be maintained between 1.0 and 2.0 dS·m^{-1} for optimal growth (Brechner and Both, 2013; Gent, 2003). EC levels that are too high can result in osmotic stress for the plant which inhibits nutrient uptake (Ding, 2018).

The charge of nutrients affects the pH of the solution. For example, additional ammonia in a system will decrease the pH of a solution while nitrate will increase it (Zhao and Shen, 2018). The optimal pH of lettuce for a hydroponic solution is between 5.5-6.0 (Gent, 2017; Brechner and Both, 2013). Macronutrient such as nitrogen, phosphorus, and potassium are unavailable at lower pH while micronutrient such as iron, magnesium, and boron are unavailable at higher pH levels. Nutrient deficiencies can appear in a plant when the pH is out of range.

Nitrogen is an essential macronutrient whose availability and concentration affects plant yield (Zhao and Shen, 2018 and Lastra et al., 2009). Plants utilize inorganic forms of nitrogen including Ammonium (NH_4^+) and Nitrate (NO_3^-). NH_4^+ is taken up quickly by the plant but in high concentrations can result in the acidification of the nutrient solution or NH_4^+ toxicity (Borgognone et al., 2013). Higher concentrations of NH_4^+ to NO_3^- result in larger root growth as NH_4^+ is immediately used after uptake while NO_3^-, which is mobile, moves through the plant to where it is needed (Abd-Elmoniem et al., 1996). Fresh weight of plants increased when NO_3^- to NH_4^+ ratios were higher (Borgognone et al., 2013). When leafy greens are grown in higher than necessary EC levels, they begin to store excess nutrients such as NO_3^- in their leaves (Lastra et al., 2009) which can be harmful for human health. Depending on the concentration of fertilizers, high NO_3^- levels can pose a risk to children and the elderly. NO_3^- toxicity affects the ability of the human body to transport oxygen through the blood. Plenty of research has been conducted to find methods to decrease the NO_3^- accumulation in lettuce. Hydroponic growers concerned with NO_3^- levels in leaves should reduce their EC to around 1.2 $dS \cdot m^{-1}$ (Gent, 2003). Cooling lettuce in the last week of growth has been proven to decrease the amount of NO_3^- accumulation in the leaves due to the reduction of NO_3^- uptake in the roots (Gent, 2016). NO_3^- accumulation in lettuce has been found to decrease when plants are grown under long periods of red and blue light due to increased photosynthetic activity and increased carbohydrate synthesis which produced higher concentration of NADPH, Ferredoxin, and Nitrate Reductase which reduces NO_3^- into nitrite (NO_2^-) for protein synthesis (Bian et al., 2016). Red and blue LED lighting showed to be more effective than white LED lights for reducing leaf NO_3^- content through more efficient light use in the NO_3^- metabolism (Bian et al., 2016). In the case of EC monitored nutrient solution, it was found that lower EC levels and higher levels of SL decreased NO_3^- concentration in butterhead lettuce (Sago and Shigemura, 2018). If there is enough SL, safe levels of NO_3^- in the leaves are possible. It is well documented that different lettuce cultivars can differ significantly in NO_3^- accumulation (Escobar-Gutiérrez, 2002).

1.5 Cultivar Selection for Hydroponic Production

Lettuce and other leafy greens are important due to their high dietary fiber, vitamins A and C and abundant antioxidants (Drewnowski and Gomez-Carneros, 2000). There are many different types of lettuce including Leaf, Romaine, Butterhead, Oakleaf, and Iceberg. Leaf lettuce doesn't form a head and has leaves that come in an assortment of shapes. Romaine lettuce has long tall leaves that form a dense head. Butterhead lettuce forms a cup shaped head and is generally known for its soft textured leaves. Oakleaf lettuce also doesn't form a head and its leaves are lobed much like an oak leaf. Iceberg is the most common lettuce known for its dense heads and crisp texture. Lettuce screenings for phytochemical concentrations found that red leaf lettuce had significantly higher bioactive compounds and antioxidants, while having lower yield than green leaf cultivars (Mampholo et al., 2016). Lettuce taste, appearance and size are also important factors in determining consumer preference (Drewnowski and Gomez-Carneros, 2000; Mampholo et al., 2016). However little published information has been gathered to see how different lettuce cultivars differ in terms of suitability to hydroponic production systems. There are some cultivar screenings done in the warmer western climates that looked at heat tolerance and stress recovery to improve yield (Han et al., 2016) but these studies were limited to germplasm for breeding purposes. Limited published research has grown lettuce to harvest stage and looked at yield performance. By screening for crop productivity in native greenhouse conditions, growers can be more informed as to what lettuce cultivars perform well in Midwest greenhouses. Additionally, the large shift in climate each season stresses the importance of temperature stress tolerance and the opportunity that CEA offers. Increased temperature stress tolerance in cultivars can help reduce costs associated with heating and cooling in greenhouses. Lettuce grown outside of its optimal range will suffer in growth rate and yield (Brechner and Both, 2013). Research on regional suitability among different lettuce types such as butterhead, romaine, cedar, and leaf lettuce in hydroponics may increase grower profits.

1.6 Year-round Production of Lettuce

There are many different factors that play a part during lettuce growth including light, ambient temperature and/or solution temperature, EC of the solution, pH, and dissolved oxygen (DO) concentration (Gent, 2017). Light and temperature play a key role for achieving year-round production, especially during winter when temperatures and photoperiod are at their lowest. The daily average temperature during winter months (Nov. to Feb.) in Indiana is 5.1 ^{o}C, with many nights falling below -4.5°C (iclimate.org). Lettuce is generally considered a cool season crop and can be germinated in temperatures as low as 10 ^{o}C (Grahn et al., 2015). The optimal temperature range for growing lettuce is between 20 and 24 ^{o}C (Brechner and Both, 2013; Gent, 2016). Lettuce germination at suboptimal temperatures exhibit slow and uneven growth with lowered germination rates (Grahn et al., 2015). Leaf growth at lower temperatures exhibits decreased surface area and thicker leaves (Dale, 1965). Temperature has a significant effect on the growth and composition of lettuce. Romaine lettuce conventionally grown in South Carolina in December required 40-60 more days to reach maturity than lettuce sown in August or April (Dufault et al., 2009). Exposure to temperatures below 10 ^{o}C has a significant reduction in polyphenol production which are beneficial to human diets. (Jeong et al., 2015). Plants grown in cold stress increase their leaf thickness and head compactness (Gent, 2016). Screening studies such as those performed by Han et al. (2016) and Grahn et al. (2015), only focused on optimizing lettuce germination under colder temperatures. Whole-plant acclimation to cold stress is a rarely researched topic for lettuce. Screening for cold tolerant, fast growing cultivars of lettuce would prove beneficial to growers by providing insight into which cultivars are suited for winter production.

In the Midwest, light levels in greenhouses rise to values of 20 $mol \cdot m^{-2} \cdot d^{-1}$ or more in the summer and fall to less than 5 $mol \cdot m^{-2} \cdot d^{-1}$ in the winter without supplemental lighting (Korczynski et al., 2002; Light in Greenhouses, 2007). The quality and the quantity of lighting are what determine how well plants grow (Kubota et al., 2016). Light quality refers to the spectral composition of light within the photosynthetically active radiation (PAR), more specifically the number of photons received by the plant within each light color. The peak PAR that plants absorb the most are within the 400-700nm range (Brazaitytė et al., 2006). The quantity of light refers to how many photons of light intercepted by the plant and is generally measured as instant photosynthetic photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$, PPFD) or daily light integral (DLI, $mol \cdot m^{-2} \cdot d^{-1}$).

Increasing PPFD has been shown to increase biomass in lettuce (Zhang et al., 2018 and Fu et al., 2012). DLI (mol·m^{-2}·day^{-1}) is calculated from PPFD (μmol·m^{-2} ·s) as:

$$DLI = \frac{PPFD \times Photoperiod\left(\frac{hours}{day}\right) \times 3600\left(\frac{seconds}{hour}\right)}{1{,}000{,}000}$$ (Zhang et al., 2018).

The average winter DLI is well below the optimal 15-18 mol·m^{-2}·day^{-1} required for optimal lettuce growth (Kang et al., 2013; Both et al., 1997; Zhang et al., 2018). Supplemental lighting during the winter is required for high quality lettuce production. Lettuce grown at suboptimal light quantities have slow growth, lower nutrient quantities, and higher leaf NO_3^- contents (Gent, 2011; Li and Kubota, 2009). By increasing the light quantity with supplemental lighting, growers in the Midwest can produce greenhouse lettuce year-round regardless of outside conditions. Increasing the PPFD was found to increase lettuce growth but eventually efficiency of production declined as lettuce reached maturity and growth rate plateaued (Hiroki et al., 2014). The length of the photoperiod during crop production influences crop yield and phytochemical production (Kang et al., 2013). Lettuce grows optimally at photoperiods with a longer light period (18-20 h) (Hiroki et al., 2014; Kang et al., 2013). Lettuce exposed to a continuous light 24 hours a day resulted in tip burn (Hiroki et al., 2014). Some popular forms of SL include high pressure sodium (HPS) lamps, metal halide lamps, and LED's (Brechner and Both, 2013). These forms of SL vary in energy use, light quality, and intensity. Double-ended HPS and LED have comparable energy use efficiency, but LED put off less heat and can be placed much closer to the plant canopy to decrease light falling outside the plant canopy (Hernández and Kubota, 2015; Nelson and Bugbee, 2014). The customization of light quality by LEDs makes them a favorable method of SL because lettuce yield can by optimized by adjusting the light spectral composition (Brazaityté et al., 2006).

Light spectral composition influences different physiological processes in lettuce (Li and Kubota, 2009) and crop quality can suffer without an optimal spectrum (Kong et al., 2019). Lettuce plants grown in exclusively red LED exhibited lower levels of chlorophyll in the leaves than plants grown in exclusively blue light (Kobayashi et al., 2013). Plants grown under LED lighting without blue light showed larger leaf surface area and lower photosynthesis (Brazaityté et al., 2006) indicative of shade stress. Growth of plants in single spectrum lighting was lower than light sources comprised of multiple spectra (Kobayashi et al., 2013). A combination of different light spectra is necessary for optimization of hydroponic lettuce growth (Lin et al., 2013). A controlled combination of blue, red, and far red light provided as SL may improve the phytochemical content and yield of lettuce plants (Li and Kubota, 2009).

Supplemental lighting has always been considered the key technology to increase crop yield when PPFD was insufficient for healthy crop growth (Kubota et al., 2016). The addition of SL has large initial costs as well as increased electricity usage, which can be expensive for many growers. As technology advances, SL becomes more affordable and efficient (Nelson and Bugbee, 2014). Kong et al. (2019) showed that, for lettuce, spectral composition affects crop growth, with higher proportion of red light in the total light better for lettuce. The cost of electricity is the determining factor as to whether SL is an economical option. One method to decrease SL costs is to supply lighting to plants on electrical grid off hours when electricity rates are lower in some areas. In areas with high-energy costs, this method offers a good way to save money and extend the photoperiod of a crop. In studies using sole source lighting, lettuce grown under R/B LED have higher biomass than other spectrum compositions (Kong et al., 2019). In cases where LED supplemental lighting is added at night with sunlight during the day, the spectrum may have significant effect on plant growth. Few publications have looked at the effects of spectral composition when SL is provided during nighttime. It would be beneficial to compare the effects of different spectra of nighttime SL during winter hydroponic lettuce production.

1.7 Root Zone Heating

Greenhouse energy costs for heating are typically in the range of 10 to 40% of production cost of a crop (Aldrich and Bartok, 1994 as cited by Marsh and Singh, 1994). Heating is important for hydroponic lettuce production in the Midwest to produce crops quickly with higher quality. While fuel cost is a large part of heating costs, the heat requirements of a greenhouse are more affected by how much heat energy is lost through the greenhouse materials (Worley, 2014). The most common method of heating a greenhouse for hydroponic lettuce is by heating the ambient air to the desired temperature in the entire greenhouse. Heating the ambient air of a greenhouse may not be the most efficient method because much of the space heated isn’t utilized by the plant. By heating the root zone, micro-climate around plants can be maintained at an optimal temperature.

There have been publications proposing that root zone heating could be an effective alternative to decrease leaf temperature stress and increase crop yield (Kawasaki et al., 2014 and Sakamoto and Suzuki et al., 2015). Hydroponically grown tomato plants were shown to have significantly higher yields with root zone heating than those grown without heating in temperatures as low as 5.6 ^{o}C (Kawasaki et al., 2014). In floating hydroponic systems, by controlling the temperature of the pond, lettuce plants were able to grow to marketable quality in non-optimal air temperatures (Thompson et al., 1998). Current literature points to this method being more effective in high volume hydroponic systems (Thompson et al, 1998; Kawasaki et al., 2014). This method of producing crops by heating the root systems and allowing ambient temperatures to stay lower may reduce fuel consumption considerably (Kawasaki et al., 2014). Root temperature effects plant morphology, respiration, transpiration, water movement, and nutrient uptake (Gent, 2016). Heating the root zone in NFT systems is considered an economic alternative to heating the ambient shoot temperature (Jensen, 1999). By decreasing the ambient temperature of the greenhouse and increasing the temperature of the nutrient solution it should be possible to increase lettuce growth as well as save money. There is little published information that compares efficacy of root zone heating under NFT and CFT production systems and for different cultivars.

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CHAPTER 2. CONSTRUCTION OF CUSTOM HYDROPONIC SYSTEM

2.1 Greenhouse Setting

All experiments were performed in the Horticulture and Landscape Architecture Plant Growth Facility at Purdue University in West Lafayette, Indiana. The individual greenhouse where all experiments were performed was an A-frame greenhouse. The base of the greenhouse is 9.14 m wide by 12.2 m long, the floor to gutter is 4 m tall, and the gutter to roof peak is 2.1 m tall. The ambient air temperature and light intensity of the greenhouse were remotely controlled by a PRIVA climate control system (PRIVA INC, Camarillo, California). Heating was provided by circulating hot air through radiators on each wall of the greenhouse and cooling was achieved by 7m long, 1.5 m wide evaporative cooling pads with a flow rate of 3.8 $m^3 \cdot s^{-1}$. Air was drawn through the greenhouse with three fans (DC wall slant fan, ACME Engineering and Manufacturing Corporation, Muskogee, Oklahoma) with 1 hp engines installed in the back of the greenhouse at an estimated 7,079 $L \cdot s^{-1}$ each. Experiments were performed in a 111.5 m^2 area on four benches that measured 7.62 m long, 1.5 m wide, and standing 1.1 m tall. The surface of the benches was mesh metal sheets. Hydroponic production systems were set up on the metal sheets while the nutrient solution reservoirs were placed directly underneath the benches.

2.2 Supports and Lighting Fixtures

On each bench, PVC supports were built using 8.9 cm diameter PVC pipes for the supplemental lighting. There were two separate structures, dimensions of each are 2.43 m long x 1.4 m wide x 1.06 m high. To support the weight of the light fixtures, four additional 1.06 m long supports were added to the structure by T-clips ((8.9 cm), JM Eagle, Los Angeles). On top of the structure, horizontal pipes were fitted using (8.9 cm) T-clips to support the lamps. LED lights were hung at four places using adjustable chains (#16 x 10' (3.05 m) Jack Single Chain, SecureLine®) fastened to horizontal pipes. Chain length was adjusted to ensure that photosynthetic photon flux density incident on plants was uniform across different experimental units. Finished assembly is shown in Figure 2.1.

Figure 2.1: Image of supplemental lighting and their PVC supports in the greenhouse

Two types of LED based SL that differed in spectral composition were used in the study. Model RAY22-I-1-06-N5 lights were obtained from Fluence Bioengineering in Austin, Texas. The dimensions were 55.9 cm long. Of the 32 supplemental LED light units used, sixteen were full spectrum PhysioSpec IndoorTM spectra. This lighting treatment was referred to as warm white lighting. The other lighting treatment used Modified PhysioSpec with blue and red wavelengths and were referred to as warm purple for our experiments. Each light required 85 W of input power. Figure 2.2 shows the spectral distribution and PPFD intensity heat map of both SL treatments. By measuring the PPFD using a line quantum sensor (MQ – 301, Apogee Instruments, Logan, U.T.) below light fixtures, the height of LED fixtures above plants was fixed so that the average PPFD in a light treatment was 145 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

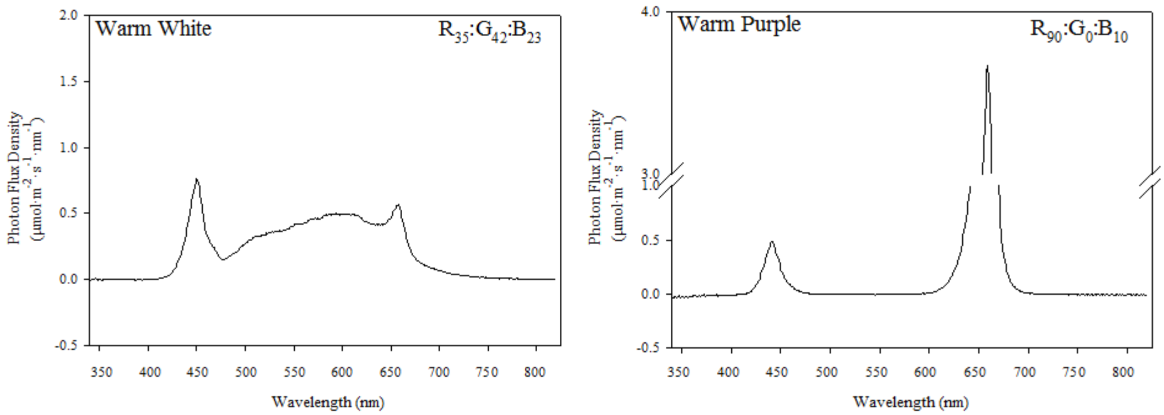

Figure 2.2: Spectral composition of warm white LED (left) and spectral composition of warm purple LED (right) from Fluence Bioengineering. Spectral scans were taken off the surface of the production surface with a spectroradiometer.

2.3 Production Systems Assembly

A custom production system was built using nutrient solution reservoirs (Active Aqua 76 liter Premium White Reservoir, Petaluma, California), submersible pumps (530 Liter Per Hour (LPH), Total Pond, West Palm Beach, Florida), LED lights (see light section above), modified flood table (31 cm x 122 cm x 10 cm black ebb and flow tray, Botanicare, Vancouver, Washington), and custom length NFT channels (13 mm Crop King INC, Lodi, Ohio). Figure 2.3 show the connections and measurements for the custom production systems used. The pumps were connected to power by surge protected power strips, which were in turn connected to GFCI protected extension cords. Each fountain pump in a reservoir fed nutrient solution through black vinyl tubing (13 mm OD, Crop King INC). The flood table was converted into a CFT system by recycling nutrient solution continuously.

The CFT system used was a modified ebb and flow table. On the outlet of the CFT system two extension (25 mm ebb and flow extension fittings, Botanicare) were connected to the barbed fittings to raise the water level to 5.08 cm before flowing back to the reservoir. The endcap (40 mm x 100 mm end cap with spout, Crop King INC) of the NFT channel was connected to the 1.27 cm black tubing, which led nutrient solution back to the reservoir. A single plastic sheet (black corrugated plastic sheeting, Meyers Plastics, Lafayette, Indiana) covered CFT tray. The area of a CFT tray was 0.45 m^2 and each tray held 16 plants. Holes on the plastic sheet held net cups (5.08 cm, General Hydroponics, Chico, California) for placing lettuce plants. The growing area of NFT channels was 0.4 m^2 and 16 plants were grown on two channels.

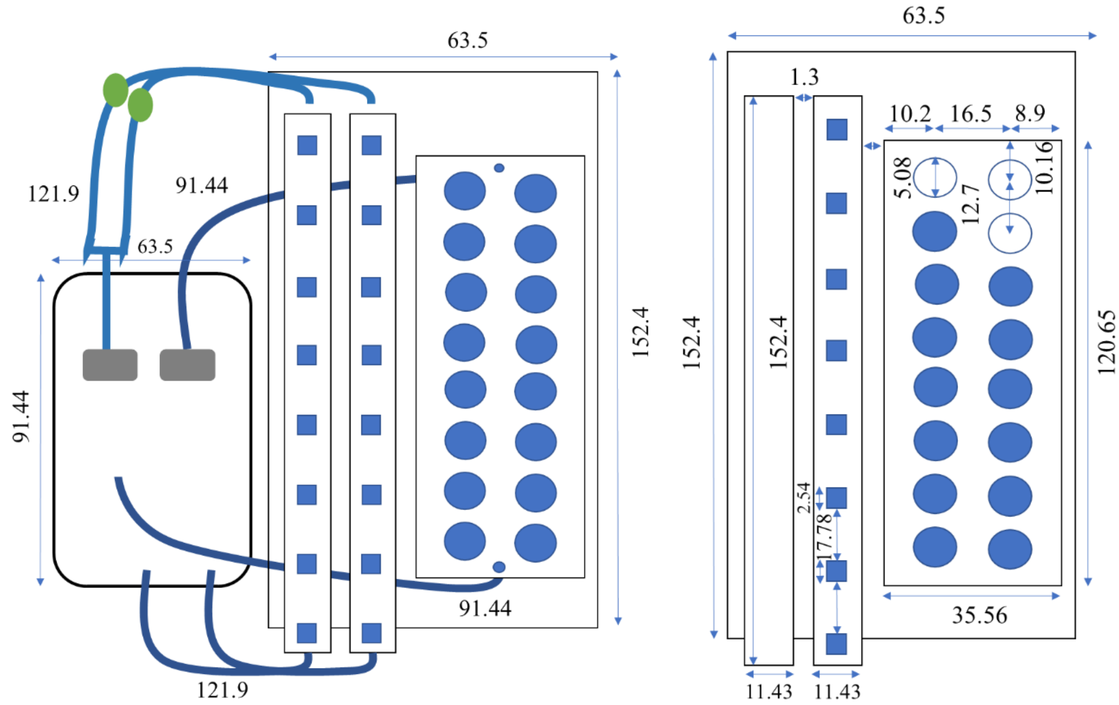

Figure 2.3: Representative diagram of the arrangement, spacing between plants (cm), and lengths of tubing (cm) from system to reservoir.

To control the flow rate of the nutrient solution, the black tubing was cut into two pieces and connected to a single valve (13 mm Green Back in-line valve, Botanicare). The valve was connected to the fitting (13 mm Ebb and Flow Barbed fitting, Botanicare) on the CFT system or directly into the channel for the NFT system. CFT tables were set to a flow rate of 6 liters per minute and NFT systems received a flow rate of one liter per minute. A single pump supplied water to two NFT channels or a CFT tray. Production systems were horizontally laid next to each other with 13 mm of space in between each system. Nutrient solution was continuously circulated through the PS. The fertilizer solution was mixed to the desired EC for each reservoir by a Dosatron (D14MZ2, Dosatron International, Clearwater, Florida) shown in figure 2.4. EC probe (HI9811–0; Hanna Instruments, Smithfield, RI) and pH probes (pH sensor SI600 s/n 21668, Spectrum Technologies, Aurora, Illinois) were used twice every week to ensure that the EC and pH values were within the targeted range. Recycled nutrient solution was discarded and reservoirs were refilled once per week with fresh nutrient solution.

Figure 2.4: Picture of Dosatron which was assembled to mix together fertilizers at accurate concentrations.

2.4 Data Collection

The environmental data in the greenhouse was continuously monitored using sensors connected to a datalogger (CR1000X-NA-ST-W, Campbell Scientific, Logan, Utah) and multiplexer (AM16/32B-ST-SW multiplexer, Campbell Scientific). The datalogger in figure 2.5 was programmed to measure sensors every minute and calculate hourly averages. Ambient air temperature was measured with temperature probes (ST-100-L-15 Soil temperature probe 15 meters, Apogee Instruments, Logan, Utah) shown in figure 2.7. Ambient light levels were measured continuously using quantum sensors (SQ-500-L-15 Full Spectrum Quantum sensors 15 meters long, Apogee Instruments). There were three quantum sensors and three temperature sensors on each bench with one sensor per light treatment. Ambient light and temperature levels were measured continuously by connecting sensors to the datalogger. In addition, spectral composition of LED lights was measured using a Spectroradiometer (SS-110, Apogee Instruments, Logan, Utah). The spectral composition of LED lights was measured at nighttime whereas spectral composition of light received by plants in the no SL treatment was measured in the morning on a cloud-free day above the plants. Electrical conductivity and temperature of the nutrient solution in the reservoirs were monitored with 16 individual sensors (ECH20 Soil Moisture Sensor 5TE, Meter Group, Pullman, WA) shown in figure 2.7. For the winter study, nutrient solution temperature was monitored by additional soil temperature probes connected to an additional CR1000 data logger.

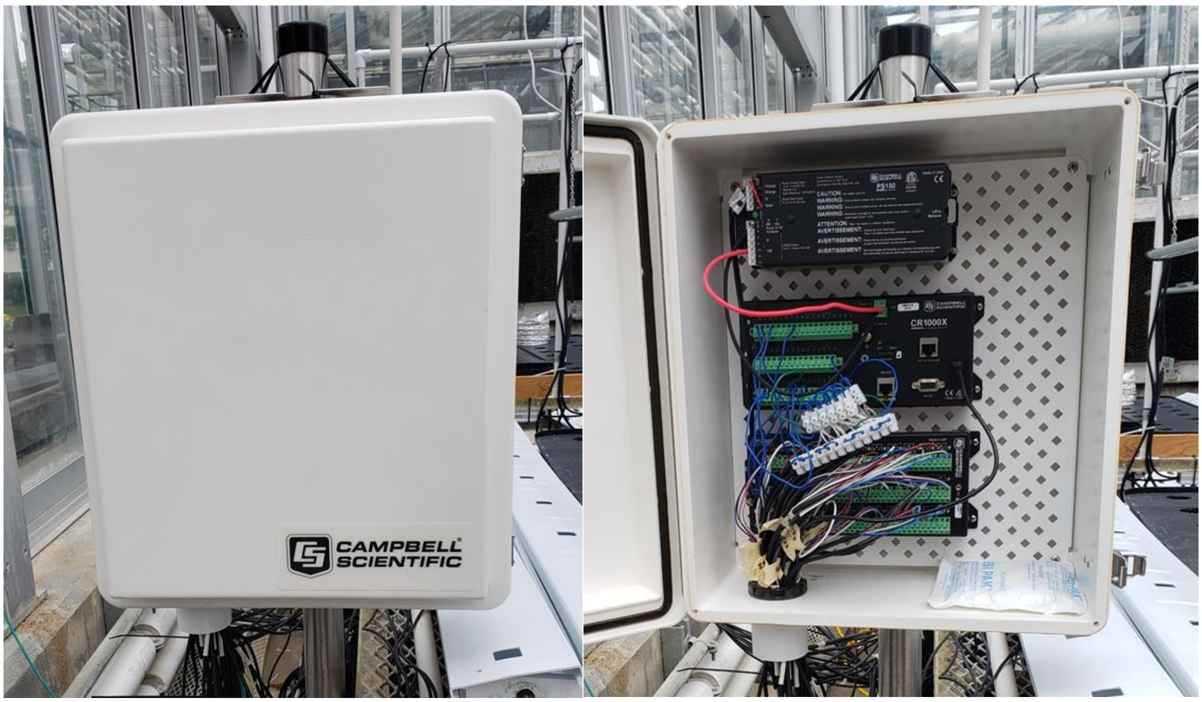

Figure 2.5: Image of the datalogger case (left) and inside with wiring (right)

Figure 2.6: Image of the quantum sensor used to measure light intensity

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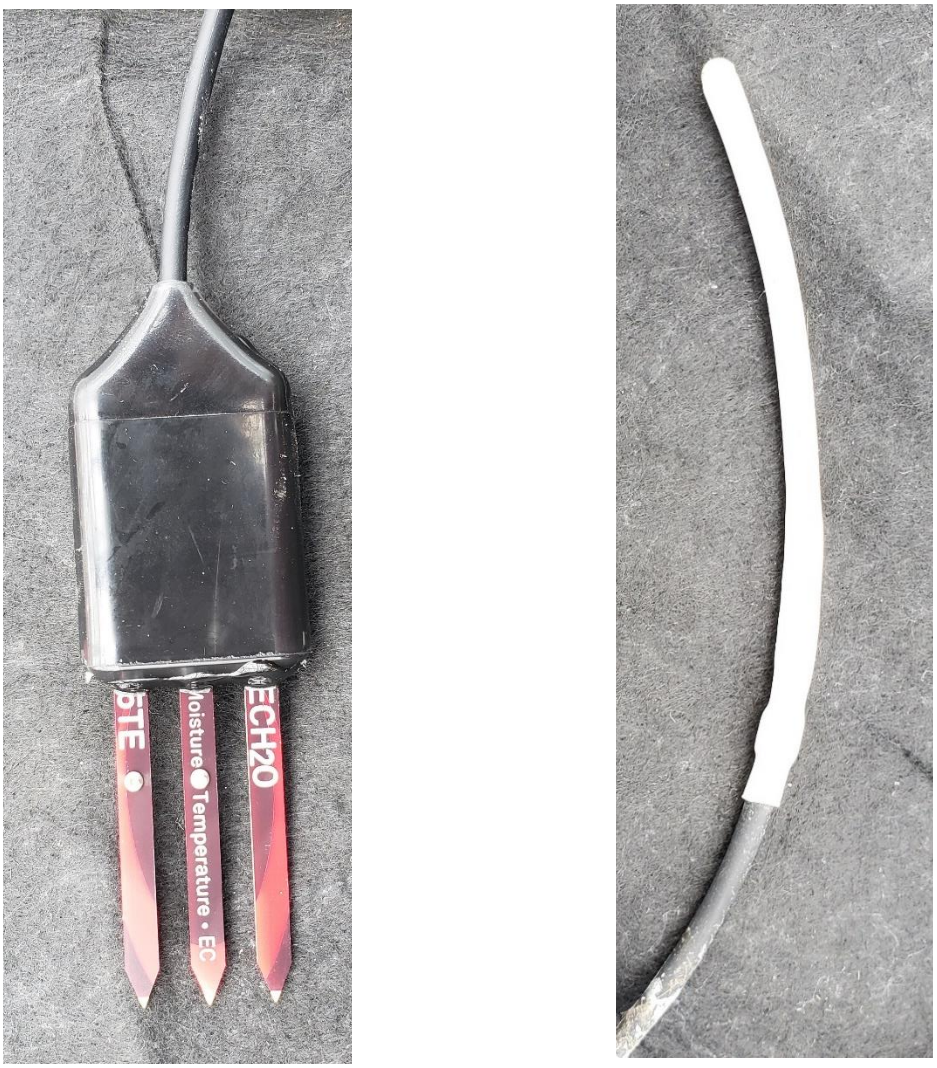

Figure 2.7: Image of the ECHO20 EC sensors (left) used to monitor nutrient solution and the temperature sensors (right) used to monitor nutrient solution temperature and ambient air temperature

CHAPTER 3. SCREENING LETTUCE CULTIVARS FOR PRODUCTIVITY AND COLD TOLERANCE

3.1 Introduction

Hydroponics is a popular method of production for many greenhouse growers. Research shows that hydroponic production of leafy greens produces 11 times greater yield and uses 13 times less water than conventional farming (Barbosa et al., 2015). It has been shown that hydroponically produced leafy greens are higher quality than conventionally grown greens (Kern et al., 2016). Hydroponic systems produce 35% less food waste than conventional farming produces. On the downside, hydroponics requires 82 times more energy than a conventionally produced crop (Barbosa et al., 2015). Despite growing popularity of hydroponic technologies there is still much to be studied about this rising industry.

3.2 Hydroponic Production Systems

There are many types of hydroponic production systems that have been developed within the last fifty years. Two common methods are nutrient film technique (NFT) and deep water culture (DWC). NFT commonly uses a thin film of nutrient solution that runs down a channel where roots are in contact with nutrient solution and air. DWC requires a much higher volume of nutrient solution. The plants root system will be completely submerged in a couple of centimeters to a few meters of water. Constant flood table (CFT) is a production system like DWC but plants roots are submerged in nutrient solution which is 5-8 cm deep and requires less nutrient solution to manage. Literature comparing different systems has yet to reach a consensus as to which production system produces the highest yield. Spinach grown in both NFT and DWC showed significantly higher yield in NFT (Ikeda et al., 1995), while basil was shown to not be significantly different in either system (Walters and Currey, 2006). With multiple studies coming to different conclusions, it is largely undecided whether hydroponic lettuce will produce higher yields in NFT or DWC. Since there are so many different types of lettuce cultivars such as romaine, butterhead, leaf, and oakleaf, it would be beneficial to compare production systems to see if cultivars have a certain production system preference.

3.3 Nutrient Solution Management Strategies

In a hydroponic production system, plants receive their nutrients from the hydroponic nutrient solution. Many commercial hydroponic growers manage nutrient solution with a three-part fertilizer system where a combination of stock tanks maintains the appropriate nutrient levels. This method is popular because some fertilizers have the tendency to precipitate when they are mixed at two high of a concentration. Growers inject fertilizers into fresh water at low concentrations to keep all elements available for their crop. In large scale hydroponic systems, growers may send the nutrient solution to either a lab for testing or purchase expensive equipment to test the concentration of individual elements. Test results will assist growers in nutrient solution management. Buying sensors or sending the necessary samples to a lab is expensive, so many growers rely on simple methods to monitor their nutrient solution.

By measuring the electrical conductivity (EC) of a nutrient solution, growers can have a general idea of how much fertilizer they are supplying to their plants. Using EC measurements is a useful and fast method to estimate the amount of salts within a nutrient solution. This is a widely used metric for hydroponic growers. Many different cultivars of lettuce exist on the market today that differ in size, growth rate, and type. It is possible that the required EC for optimal growth can vary by the cultivar. Optimal EC may be also affected by the PS due to differences in volume of solution used and proportion of roots submerged in water. The general finding for hydroponic lettuce show that an EC of 1.0 and 2.0 $dS \cdot m^{-1}$ are best for optimal growth (Brechner and Both, 2013; Gent, 2016). High EC has been correlated with osmotic stress and EC changes are directly correlated to biomass and metabolite concentration (Abou-Hadid et al., 2016). Often experiments to find the optimal EC levels only contain a few lettuce cultivars. Including large number of cultivars in experiments is necessary to generalize optimal EC for hydroponic lettuce production.

3.4 Cultivar Selection for Hydroponic Production

As consumer interest in health and nutritious leafy greens increases, the market for locally sourced produce becomes more profitable. Lettuce and other leafy greens are high in dietary fiber and abundant in vitamins A, C, and antioxidants (Drewnowski and Gomez-Carneros, 2000). Red leaf lettuce gets its appearance from its high concentration of anthocyanin's. These lettuce cultivars contain more bioactive compounds and antioxidants important for human health than green leaf cultivars (Mampholo et al., 2016). Lettuce taste, appearance and size are also important factors in determining consumer preference (Drewnowski and Gomez-Carneros, 2000; Mampholo et al., 2016). However little published information has been gathered to see how different lettuce cultivars differ in terms of hydroponic suitability, yield, and popularity. There are some cultivar screenings done in the warmer western climates that looked at heat tolerance and stress recovery to improve yield (Han et al., 2016) but these studies were limited to germplasm for breeding purposes. Cultivar screening for yield is limited. There is a lack of lettuce cultivar screening data for the Midwest region. By screening for lettuce productivity, growers can be more informed as to what lettuce cultivars perform well in Midwest climates.

3.5 Cold Temperature Stress

Greenhouse hydroponics allow for year-round production of leafy greens like lettuce in the Midwest. However, heating the greenhouse to an optimal 21.1 ^{o}C is required for maximizing productivity during the winter in the Midwest. Heating costs for maintaining a 30.5 m × 9.2 m greenhouse at 21.1 ^{o}C can vary from \$ 90 to 110 per day, when outside air temperature is -1.1 ^{o}C (avg. Midwest winter temperature). Thus, heating a greenhouse during the winter can be expensive and affect profits in hydroponic production. One way to reduce heating associated costs is to grow lettuce varieties that can tolerate low temperature conditions. A cold tolerant variety can produce 'relatively' more yield under cooler air temperature conditions than a cold-sensitive variety. This means, growers can maintain a lower air temperature in the greenhouse during the winter using cold-tolerant varieties and reduce heating costs without a large impact on lettuce yield. There are many different types of hydroponic lettuce varieties, but little information is available about the cold tolerance level of varieties. This information can be quite valuable to hydroponic growers for maximizing profits during winter.

Cold Temperature has a significant effect on the growth and composition of lettuce (Gent, 2016). Lettuce grown outside of its optimal range will suffer in growth rate and final yield (Brechner and Both, 2013). Romaine lettuce conventionally grown in South Carolina in December required 40-60 days more to reach maturity than lettuce sown in August or April (Dufault et al., 2009). Lettuce grown in cooler temperatures has been shown to have 50% higher concentration of sugar and 40% lower NO_3^- concentrations (Gent, 2016). Exposure to temperatures below 10 ^{o}C has a significant reduction in polyphenol production, which are beneficial to human diets (Jeong et al., 2015). Plants grown in cold stress increase their leaf thickness and head compactness (Gent, 2016). Screening studies such as those performed by Han et al. (2016) and Grahn et al. (2015), only focused on optimizing lettuce germination under colder temperatures. Screening cultivars that yield higher under cold conditions would prove beneficial to growers by providing insight into which cultivars are suited for winter production.

The objectives of the study are to (i). Conduct a lettuce varietal screen for productivity under different EC levels and using CFT and NFT production systems and (ii). Test different cultivars for cold tolerance under mild and moderate levels of cold stress

3.6 Materials and Methods

Two screening experiments were conducted in this study. First experiment was intended to study the interaction between EC levels and production systems on the productivity of several cultivars of lettuce in a greenhouse during summer/fall. In the second experiment, different cultivars of lettuce were tested for growth under optimal, mild and moderate levels of cold stress in a growth chamber.

Experiment I.

The experiment was performed in a greenhouse during August of 2018. The average ambient temperature within the greenhouse during the experiment was 24.5 (± 0.39)/ 22.5 (± 0.35) ^{o}C during day/night with a maximum temperature of 29.2 ^{o}C and a minimum temperature of 16.7 ^{o}C (Figure 3.1). The PRIVA system within the greenhouse was set to maintain the ambient temperature of the greenhouse between 26.7/18.3 ^{o}C day/night (Figure 3.1). However, the temperature often exceeded what the evaporative cooling pads was able to maintain so we relied on solar shades in order to reduce the temperature. When temperatures exceeded 32.2 ^{o}C within the greenhouse the shade cloths would cover the greenhouse in order to block additional light and maintain temperatures. This resulted in the uncharacteristically low DLI for the experiment (Figure 3.1). The average levels of light per day were 10.5 (± 0.79) mol·m^{-2}·d^{-1}. The minimum DLI received within the greenhouse was 4 (± 0.69) mol·m^{-2}·d^{-1} and the maximum was 15.4 (± 1.11).

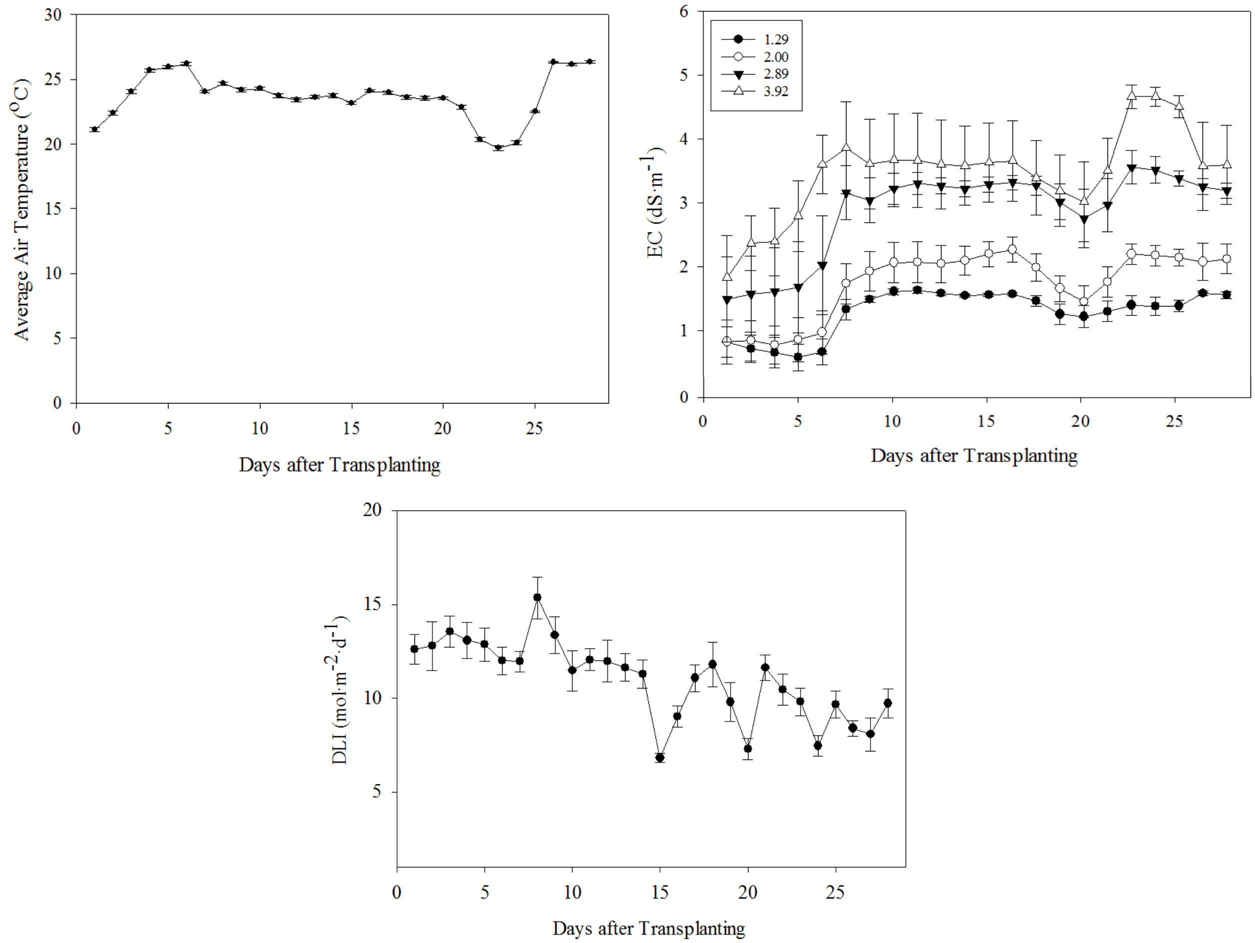

Figure 3.1: Environmental data on different days during the experiment: Average air temperature (top left), electrical conductivity of the nutrient solution in different treatments (bottom), and daily light integral (top right) are shown. Note that EC of the nutrient solution is not adjusted for EC of irrigation water.

Figure 3.2: Experimental set up of the greenhouse across all treatments and replications.

The experiment was laid-out as a split-split plot design. Figure 3.2 shows the four replications of our experiment. It also shows the different levels of the experimental design. At the main split there were four nutrient solution treatments, where EC level was maintained at 1.3, 2.0, 2.9, or 3.9 dS m^{-1} (110, 205, 325, and 460 ppm N). At the second level of the experiment were two different production systems where a single reservoir would supply nutrient solution to one CFT system and two NFT channels. At the final level, there were 24 different lettuce cultivars that were compared. Red and green cultivars belonging to main groups of lettuce including butterhead, romaine, leaf, and oakleaf lettuce were grown.

The EC levels in different treatments included EC of tap water (0.72 $dS{\cdot}m^{-1}$). The EC levels varied on different days of the experiment due to differences in evaporation rate and plant uptake. Excluding the EC of tap water, the EC of fertilizer solution supplied was approximately 0.6, 1.3, 2.2 and 3.2 $dS{\cdot}m^{-1}$ for EC treatments of 1.3, 2.0, 2.9 and 3.9 $dS{\cdot}m^{-1}$, respectively. The nutrient solution EC levels were maintained twice a week by adding fresh water or additional nutrient solution. The EC level maintained was not including the already existing EC of the tap water. The pH was maintained within a range of 5.5-6.5 by adding 85% sulfuric acid when needed. There was a total of 48 NFT and 48 CFT systems used in different treatments. A single NFT or CFT system holds 16 lettuce plants. Cultivars of lettuce were chosen by contacting local hydroponic growers and seed companies for recommendations. Cultivars were selected on the following criteria: type (Leaf, Butterhead, Oakleaf, and Romaine), color (red or green), hydroponic suitability, days to harvest, and popularity amongst growers. Seeds were purchased from Johnny's Selected Seeds and Paramount Seeds. Chosen cultivars are shown in Table 3.1.

Table 3.1: Above are the different popular cultivars chosen with their color and type.

Cultivar (*Lactuca sativa L.*)	**Type**	**Color**
Adriana (Ad)	Butterhead	Green
Buttercrunch (Bc)	Butterhead	Green
Rex (Rx)	Butterhead	Green
Natalia (Nt)	Butterhead	Green
Alkindus (Al)	Butterhead	Red
Salanova Red Butterhead (SRB)	Butterhead	Red
Skyphos (Sk)	Butterhead	Red
Walkmann's Dark Green (WDG)	Leaf	Green
Black Seeded Simpson (BSS)	Leaf	Green
Nevada (Nv)	Leaf	Green
Red Sails (RS)	Leaf	Red
Cherokee (Ch)	Leaf	Red
New Red Fire (NRF)	Leaf	Red
Cedar (Ce)	Oakleaf	Green
Salanova Green Oakleaf (SGO)	Oakleaf	Green
Navara (Nr)	Oakleaf	Red
Salanova Red Oakleaf (SRO)	Oakleaf	Red
Red Salad Bowl (RSB)	Oakleaf	Red
Salvius (Sl)	Romaine	Green
Dragoon (Dr)	Romaine	Green
Amadeus (Am)	Romaine	Green
Breen (Br)	Romaine	Red
Intred (In)	Romaine	Red
Truchas (Tr)	Romaine	Red

Seeds were sown in rockwool cubes and transplanted after 7 days after sowing into the hydroponic system. Lettuce was harvested after 28 days of growth in different treatments. Nutrient solutions were managed twice a week by adding fresh water or fertilizer as needed and a full tank change after 14 days of growth. Data was constantly recorded by a data logger (CR1000X-NA-ST-W, Campbell Scientific, Logan, Utah). Light, EC, and temperature of the ambient air were continuously monitored by quantum sensors (SQ-500-L-15 Full Spectrum Quantum sensors, Apogee Instruments), dielectric sensors (5TE, Meter Group, Pullman, WA) and thermistors (ST-100-L-15 Soil temperature probe, Apogee Instruments, Logan, Utah), respectively. At harvest, the shoots from all plants belonging to a cultivar in a given PS, EC treatment and replication were cut placed in separate bags and then dried in an oven until they no longer lost more than one percent of water weight per day. Dry weights were then measured and analyzed according to a split-plot model using statistical analysis software (SAS, Cary, North Carolina) to determine significance. Means were separated using Tukey's honestly significant difference (HSD). A P-value ≤ 0.05 was considered significant for all statistical comparisons.

Experiment II:

This study was performed inside two growth chambers (Model E15, Conviron, North Branch, Minnisota). Seeds were germinated in rockwool cubes at 21.1 ^{o}C for seven days before transplantation into a custom CFT production system (Figure 3.3). Lettuce roots were submerged in roughly 5 cm of nutrient solution that was continuously recirculated from reservoir to trays using submersible pump (Aquanique, 190 LPH). Germinated seedlings were placed into 2.5 cm net cups and placed inside in CFT production system. Seedlings belonging to 24 lettuce cultivars (Table 3.1) were grown under three different ambient air temperatures (10, 15.6, and 21.1 ^{o}C). Lettuce plants were grown using a nutrient solution with an EC of 1.8 $dS \cdot m^{-1}$ (includes EC of irrigation water) and a pH level of 5.5-6.5. The nutrient solution was replaced weekly. Plants received approximately 10.4 $mol \cdot m^{-2} \cdot d^{-1}$ of light during a 16-hour photoperiod for the duration of the experiment. Plants were grown under fluorescent lights. After the 28 days, plants were harvested to determine fresh and dry weights. Experiment was laid-out in a split plot model with air temperature as the main plot and cultivar as the split plot. There were four replications of the main plot in the experiment. Data were analyzed using general linear model of statistical analysis software (SAS, Cary, NC) with appropriate error terms for whole- and split-plots. Means were separated using Tukey's honestly significant difference (HSD) with $P \leq 0.05$ considered statistically significant.

Figure 3.3: Shown is the CFT production system above the reservoir filled with nutrient solution which was placed within a growth chamber to maintain temperature.

3.7 Results

Experiment 1

There was a significant interaction between production system and at EC on shoot dry weight. Although there were no differences in dry weight between production systems at any given EC level, decrease in dry weight was more pronounced under NFT than CFT with increasing EC level. Lettuce plants grew optimally at EC levels between 1.3 and 2.0 $dS \cdot m^{-1}$. These results correspond with the general findings with research by Samarakoon et al., 2006; Wortman, 2015. Lettuce dry weight at 1.3 and 2.0 $dS \cdot m^{-1}$ are statistically similar while being significantly larger than two higher EC treatments. This is could be attributed to osmotic stress caused by high salt concentrations according to research findings (Ding, 2018). The high concentration of fertilizer salts in the nutrient solution prevent the uptake of water which inhibits growth over time. At such high electrical conductivities, plants are also in contact with high levels of nutrients that can potentially cause toxicity symptoms. Nutrient toxicity from high EC is generally associated with marginal necrosis, decreased growth rate, and high nutrient concentrations blocking the absorption of other nutrients (McCauley, 2011).

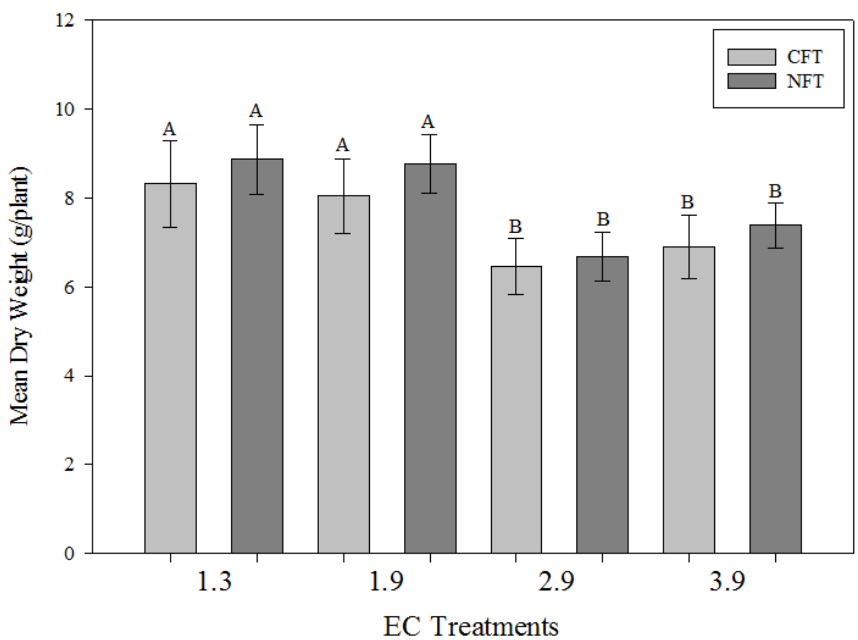

Figure 3.4: Mean dry weight at NFT and CFT production system and electrical conductivity treatments. Graphs show significance at PS level (P≤0.05) and at EC level (P≤0.001). Bars represent interactive LSMeans (average of all 24 cultivars) and error bars represent standard error of mean. Means with different letters are significantly different (P≤0.05).

There was a significant interaction between production system and cultivar on shoot dry weight (Fig. 3.5). Generally, lettuce dry weight was higher when plants were grown in an NFT system, but some cultivars had significantly higher dry weight in CFT system. There are six cultivars that showed significantly higher shoot dry weight in CFT than NFT including Adriana, Navara, New Red Fire, Red Sails, Salanova Green Oakleaf, and Truchas. Within each production system each cultivar was ranked using shoot dry weight data. Lettuce cultivars are generally similarly ranked regardless of production system. Higher ranked cultivars tend to be either Leaf lettuce or Romaine cultivars. Red colored cultivars tended to be lower on the rankings and it may be due to their higher anthocyanin production, which has been correlated to lower fresh weight production (Mampholo et al., 2016). Among different lettuce cultivars, the highest performing cultivars from each type included Red Sails (Leaf), Salvius (Romaine), Cedar (Oakleaf), and Adriana (Butterhead).

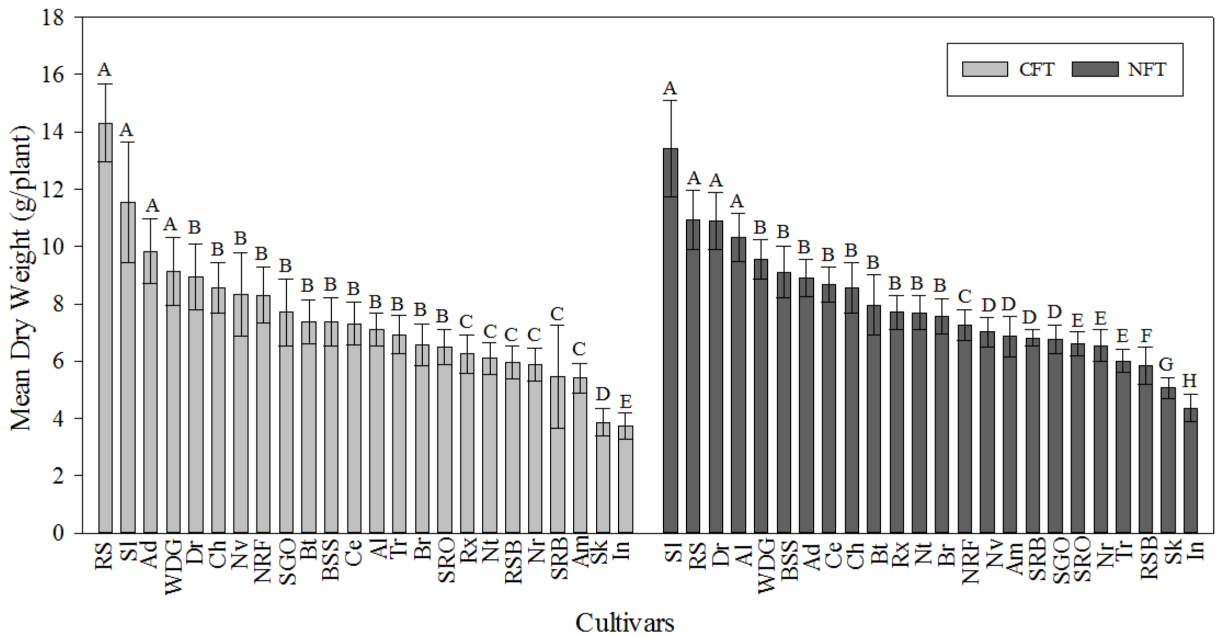

Figure 3.5: Significant interaction between cultivar and PS (P≤0.04) on shoot dry weight ($g \cdot plant^{-1}$) of lettuce. Cultivars within CFT production system (left) and NFT production system (right). Bars represent interactive LSMeans (average of all EC) and error bars represent standard error of mean. Means with different letters are significantly different (P≤0.05).

Experiment 2

There was a significant interaction between temperature treatment and cultivar on fresh weight (Table 3.2). Fresh weight differences among cultivars increased along with increasing temperature treatments. Some cultivars performed better at lower temperatures than others. Fresh weight of cultivars such as Dragoon and New Red Fire was significantly higher than other cultivars at moderately cold temperature (10 ^{o}C) treatment. Dragoon, Adriana, and Waldmann's Dark Green lettuce cultivars had significantly higher fresh weight at milder temperature (15.5 ^{o}C) treatment.

While Salvius, Waldmann's Dark Green, and Adriana were the highest producers at optimal conditions (21.1 ^{o}C). It is evident that the size of plants belonging to Adriana and Waldmann's Dark Green in the optimal treatment (21.1 ^{o}C) generally correlated to their ability to produce higher shoot fresh weight under suboptimal treatments. Dragoon and New Red Fire have the highest fresh weight in the colder treatments but when the temperature increases these cultivars are no longer top producers of shoot fresh weight. This would suggest that Dragoon and New Red Fire have higher true cold tolerance than cultivars such as Adriana or Salvius. New Fire Red is one of few cultivars promoted to perform well in cold temperatures (Fruition Seeds, 2019). As Dragoon was the most cold tolerant, it is possible that its greater head compactness protects the meristem against the cold temperatures as a form of insulation. Butterhead and Oakleaf cultivars grow wide and short keeping their apical meristem exposed to the ambient air for much longer than romaine cultivars. Plants were significantly affected by the different temperature treatments such as previous literature has confirmed (Dufault et al., 2009; Gent, 2016). Across all cultivars, plants grown in higher temperature treatments grew better than those grown in lower temperatures. Plants grown at moderately cold temperature treatment did not reach commercial size after 28 days of growth. This indicates that growers should at least maintain 15.5 ^{o}C during production, however, growth is optimal at 21.1 ^{o}C.

Table 3.2: Comparison of average shoot fresh weight (g·plant^{-1}) of 24 cultivars of lettuce under three growth period temperatures of 10, 15.5, and 21.1 ^{o}C (P≤0.01). Values represent interactive LSMeans (FW g·plant^{-1}) and values in parenthesis are standard error. Means with different letters are significantly different (P≤0.05) within a temperature treatment.

Cold Tolerance Cultivar Fresh Weights			
Cultivars	**Temperature (^{o}C)**		
	10	**15.5**	**21.1**
Adriana	4.3(1.60) B	52.5(19.81) A	150.6(9.32) A
Buttercrunch	3.5(1.75) B	33.6(6.18) B	63.4(5.59) D
Natalia	2.5(1.16) B	26.7(9.13) B	29.2(2.58) E
Rex	3.5(1.16) B	31.2(2.83) B	23.3(5.21) F
Black Seeded Simpson	2.0(0.58) B	39.5(13.39) B	127.5(6.02) A
Nevada	3.4(1.97) B	28.2(3.51) B	40.8(0.85) E
Waldmann's Dark Green	3.1(1.61) B	42.3(5.76) B	129.2(18.48) A
Cedar	2.9(0.9) B	31.4(3.32) B	86.4(3.85) C
Salanova Green Oakleaf	1.9(0.54) B	12.4(3.85) C	37.6(4.94) E
Amadeus	2.7(1.28) B	11.6(4.86) C	27.4(7.10) E
Dragoon	5.9(2.57) A	53.0(3.48) A	67.3(6.41) D
Salvius	3.9(1.16) B	28.1(13.54) B	130.7(8.89) A
Alkindus	1.9(0.54) B	20.2(3.7) B	40.4(3.24) E
Salanova Red Butterhead	2.1(0.93) B	14.7(2.14) C	18.4(2.52) G
Skyphos	3.4(1.44) B	18.0(4.68) C	31.9(1.89) E
Cherokee	4.0(1.47) B	17.5(2.51) C	31.2(4.42) E
New Red Fire	5.5(2.24) A	27.2(5.22) B	91.6(2.61) B
Red Sails	4.3(1.61) B	53.4(10.23) A	88.5(3.05) B
Red Salad Bowl	2.5(0.66) B	16.6(2.14) C	31.6(1.83) E
Navara	2.7(1.11) B	11.1(4.55) C	28.3(2.17) E
Salanova Red Oakleaf	0.7(0.01) C	15.1(2.78) C	20.7(4.5) F
Breen	2.5(0.66) B	19.9(2.26) C	95.6(26.54) B
Intred	2.4(1.05) B	8.5(1.53) D	19.9(2.59) G
Truchas	2.2(0.91) B	16.3(3.17) C	25.0(1.06) E

3.8 Conclusion

In conclusion, our experimental results suggest that growing plants in a nutrient solution with EC of 1-1.5 $dS \cdot m^{-1}$ will yield the largest lettuce crops. Decrease in growth can be higher under NFT than CFT system. NFT produced slightly higher dry weights, but there is a cultivar preference for CFT with top performing cultivars Red Sails and Salvius. The cold tolerance study indicated that the optimal temperature is 21.1 ^{o}C. The cold tolerance study showed that most of lettuce cultivars are sensitive to cold, but some have significantly different cold tolerance. Only New Red Fire and Dragoon cultivars appear to have some level of tolerance to cold among cultivars tested in our experiment.

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CHAPTER 4. NIGHTTIME SUPPLEMENTAL LIGHTING AND HEATED HYDROPONIC SOLUTION EFFECTS ON THE GROWTH OF DIFFERENT LETTUCE CULTIVARS IN NUTRIENT FILM AND CONSTANT FLOOD TABLE TECHNIQUES

4.1 Abstract

Hydroponic lettuce production in greenhouses is becoming popular in the U.S. Information on the effects of different light spectra provided at nighttime, root zone heating, different production systems and cultivars on lettuce productivity is limited. This information can be used to optimize winter greenhouse hydroponic lettuce production in the Midwestern parts of the U.S. The objective of this study is to evaluate the interactive effect of nighttime supplemental lighting, nutrient solution temperature, and production system on the growth of different lettuce cultivars in a greenhouse maintained at suboptimal air temperature. The treatments were laid-out in a split-split-split plot design. Whole-plots comprised of three light treatments [no supplemental lighting (4.2 $mol \cdot m^{-2} \cdot d^{-1}$), purple LED supplemental lighting (R_{90}:G_{0}:B_{10}; 13 $mol \cdot m^{-2} \cdot d^{-1}$), and warm white LED supplemental lighting (R_{35}:G_{42}:B_{23}; 12.6 $mol \cdot m^{-2} \cdot d^{-1}$)], each with suboptimal and optimal solution temperature treatments [unheated (12.6 °C) and heated (18.8 °C)]. Further, each solution temperature treatment contained two production systems [nutrient film technique (NFT) and constant flood table (CFT)]. Red and green lettuce (*Lactuca sativa*) cultivars belonged to leaf (Red Sails and Black Seeded Simpson), butterhead (Alkindus and Adriana), oakleaf (Navara and Cedar) and romaine (Breen and Salvius) groups were grown in each production system. The greenhouse air temperature was maintained at 15.6/10 (13.4 ± 0.17) °C (day/night). Results indicated shoot dry weight of lettuce was lowest when supplemental lighting and heating were absent. Under these conditions, plants did not reach marketable size at harvest and there were no effects of production system on the yield of a cultivar. However, large differences in shoot dry weight were observed among treatments that included supplemental lighting and/or heating. Regardless of cultivar, shoot dry weight of lettuce was higher under CFT than NFT system in both heated and unheated solution treatments when supplemental lighting was provided to plants. The differences between CFT and NFT were larger under purple compared to white LEDs. In general, effects of production system or solution temperature on the yield of a cultivar were more pronounced under purple than white LED lights. Cultivars like Adriana, Cedar, Red Sails and Salvius responded more to root zone

heating and produced higher yields in CFT than NFT. Shoot water content, a major factor affecting economic yield in lettuce, was highest when heated solution was provided to plants. In the absence of root zone heating, plants grown in CFT had higher shoot water content compared to those grown in NFT. Adding supplemental lighting and root zone heating increased energy use efficiency ($g \cdot KWH^{-1}$) of lettuce yield, especially under purple supplemental lighting. It is recommend LED supplemental lights with higher proportion of red light (i.e., purple lights), root zone heating, CFT production system and fast-growing cultivars like Adriana, Cedar, Red Sails, and Salvius for winter hydroponic production in greenhouses.

4.2 Introduction

Outdoor field production is not possible during winter in the Midwestern parts of the U.S due to cold temperatures, shorter photoperiods and lower light levels. Controlled environment agriculture (CEA) involves growing lettuce year-round under managed environments in facilities like greenhouses using supplemental lighting and artificial heating. Hydroponics is one of the methods of growing lettuce in CEA. Common techniques of hydroponic production include Nutrient Film Technique (NFT), Deep Water Culture (DWC) and Constant Flood Table (CFT). Leafy greens are a popular choice for greenhouse hydroponics with lettuce being the most popular species (Gent, 2017).

4.2.1 Supplemental Lighting

Addition of supplemental lighting and heating during winter can enable lettuce production but also significantly increase operational costs. Greenhouse energy costs associated with heating and lighting account for 10 to 40% of production costs (Aldrich and Bartock, (1994) as cited by Marsh and Singh, 1994). Hydroponically produced lettuce required a much greater energy input than that of conventionally produced field lettuce (Barbosa et al., 2015). Several cultivars of lettuce belonging to leaf, oak leaf, butter head and romaine groups are commonly grown in hydroponics. Lettuce cultivars are known to show preference for specific production system (Assimakopoulou et al., 2013). Optimizing supplemental lighting, heating, production system and cultivar for winter production is crucial to improve productivity and profits during winter production. Available information, especially on the interaction among these factors, is limited.

Both light quantity and spectral composition determine the quality of a crop (Kubota et al., 2016; Kong et al., 2019). Optimal level of light intensity for lettuce is 12-15 $mol \cdot m^{-2} \cdot d^{-1}$ (Kang et al., 2013; Both et al., 1997; Zhang et al., 2018), which is well above the levels received inside a greenhouse during winter in Midwestern parts of the U.S (Korczynski et al., 2002; Light in Greenhouses, 2007). Thus, supplemental lighting is necessary in greenhouses to produce high-quality hydroponic lettuce during winter in the Midwestern parts of the U.S. Increasing light intensity has been shown to increase biomass in lettuce (Zhang et al., 2018; Fu et al., 2012). When light levels are too low, lettuce plants have a slower growth rate and accumulate lower level of nutrients and higher levels of NO_3^- in leaves (Gent, 2011; Li and Kubota, 2009). Light spectral composition has a significant effect on the physiology and metabolite production of lettuce and a combination of blue, red, and far red light has been known to improve phytochemical content (Li and Kubota, 2009). Recently, Kong et al. (2019) concluded that a red to blue ratio of 4.5 resulted in an optimal spectrum for lettuce cultivars. Supplemental lighting added during the day only increased the intensity of lighting, while spectrum of supplemental light provided during the day had little to no effect on lettuce growth (Ouzounis et al., 2015). This may be due to confounding effects of solar spectrum on plants. Nighttime supplemental light intensity has been shown to have the same effectiveness as that of daytime (Tewolde et al., 2016). Therefore, studying the effect on lettuce growth under different nighttime SL spectral compositions could provide more insight for how growers should select LED lights. In addition, limited information is available on the growth effects of different light spectra provided at night on lettuce cultivars.

4.2.2 Temperature Effects on Lettuce

Temperature has a considerable effect on the growth and quality of hydroponic lettuce. Although lettuce is a cool season crop, the optimal temperature range for lettuce growth is between 20 and 24 ^{o}C (Gent, 2016; Brechner and Both, 2013). Cold temperature reduces leaf area and increases leaf thickness in lettuce (Dale, 1965). Low temperature also results in slower and uneven growth (Grahn et al., 2015). Lettuce grown at suboptimal winter temperatures can take 40-60 days longer to reach maturity than lettuce grown over the summer (Dufault et al., 2009). Root temperature was found to affect plant morphology, respiration, transpiration, water movement, and nutrient uptake (Gent, 2016). Generally, greenhouse heating during winter involves maintaining air temperature within a desirable range. An alternative to conventional heating is root zone heating, which involves maintaining optimal solution temperature despite sub-optimal air temperatures. Controlling the temperature of the nutrient solution produced marketable lettuce plants under non-optimal air temperatures (Thompson et al., 1998). Heating the root zone was indicated as an economic alternative to heating the ambient shoot temperature in lettuce production (Jensen, 1999). As solution volume varies among different production systems used in hydroponics, it is unclear whether root zone heating has similar effects on lettuce produced in different production systems.

4.2.3 Comparison of Production Systems

Findings from studies that compared hydroponic production systems are inconclusive. A study that compared NFT and DWC in aquaponics, found that DWC out produced NFT in terms of lettuce yield (Lennard and Leonard, 2006). In another study, spinach grown under NFT had significantly more yield than DWC, but only in the summer (Ikeda et al., 1995). For basil, DWC produced a slightly higher yield than NFT, but differences were not significant (Walters and Currey, 2015). Compared to DWC, it is relatively easier to manage nutrient and oxygen levels of the solution in a CFT system due to smaller volume of solution. However, there are limited studies that compared NFT and CFT production systems. Faster lettuce growth is preferred in any season, but more so in winter. Longer crop cycles increase operational cost per cycle and reduce overall profits. Therefore, cultivars that grow faster under colder conditions are preferred for winter. Information on lettuce cultivar evaluations for winter suitability is limited. Cultivar screening studies are limited to germination rates under cold conditions (Han et al. 2016 and Grahn et al. 2015).

Keeping the above described issues in mind, the objective set for this research was to study the interactive effects of nighttime supplemental lighting with differences in spectral composition, root zone heating, and production systems on the growth of different lettuce cultivars in a greenhouse maintained at suboptimal conditions generally observed during winter. Our goal is to develop recommendations on optimal light spectra, root zone heating, production systems, and cultivars for winter greenhouse hydroponic production for Midwestern parts of the U.S.

4.3 Materials and Methods

Plant materials. Eight red and green cultivars of lettuce (*Lactuca sativa* L.) belonging to leaf (*cv* Red Sails and Black Seeded Simpson), butter head (*cv* Alkindus and Adriana), oak leaf (*cv* Navara and Cedar) and romaine (*cv* Breen and Salvius) groups were grown in the study. Cultivars were selected based on their yield from a previous trial (data not shown). Shown in Figure 4.1, experiments were conducted in a climate-controlled greenhouse at Purdue University, West Lafayette, Indiana during the months of January and February of 2019. The ambient temperature of the greenhouse was maintained at 15(± 0.19)/10 (± 0.15) ^{o}C (day/night). The average DLI from sunlight was 4.2 (± 0.49) mol·m^{-2}·d^{-1}. Greenhouse surface area was 111.5 m^{2}. Seeds were transplanted 10 days after sowing and plants were grown for 40 days in the experiment.

Figure 4.1: Shown is our experimental design within a single light treatment within a replication where heated and unheated hydroponic systems, CFT and NFT production systems, and different cultivars are randomized.

Nutrient solution preparation. The solution was prepared using 5-11-26 (Peters Professional, ICL Specialty Fertilizers, Tel Aviv, Israel) and 15.5-0-0 calcium nitrate (YaraLiva, Yara North America, Tampa, FL). A fertilizer injector (D14MZ2, Dosatron International, Clearwater, FL) was used to mix nutrient stock solution with water to a desired strength. The outlet of the fertilizer injector was connected to a water-hose that delivered nutrient solution to reservoirs (see below). The fertilizer stock solutions were mixed with acidified tap water with an existing electrical conductivity (EC) of 0.7 $dS \cdot m^{-1}$. The measured EC of the nutrient solution supplied to plants was 1.0 ± 0.24 $dS \cdot m^{-1}$ for the first two weeks and after the first tank change, EC was raised to 1.7 ± 0.2 $dS \cdot m^{-1}$ until harvest. The pH of the nutrient solution was maintained 5.5-6.5 by adding 85% sulfuric acid to the nutrient solution as irrigation water pH was higher than 7.5. The EC of the nutrient solution was adjusted by adding fresh water or concentrated fertilizer twice a week. After second and fourth week of the experiment, the nutrient solution was replaced with a fresh solution. Sensors for EC (HI9811–0; Hanna Instruments, Smithfield, RI) and pH (pH sensor SI600 s/n 21668, Spectrum Technologies, Aurora, IL) were used to ensure that the EC and pH values were within the targeted range. In addition, EC sensors (5TE, Meter Group, Pullman, WA) connected to a datalogger (CR1000x, Campbell Sci., Logan, UT) measured EC of the nutrient solution in reservoirs in real-time. Nutrient solution was continuously recycled during the experiment.

Production systems. Custom hydroponic production (Figure 4.1) systems were set up on greenhouse benches (7.62 m x 1.5 m x 1.1 m). They were built using nutrient solution reservoirs (Active Aqua 76 liter Premium White Reservoir, Petaluma, CA), submersible pumps (530 LPH, Total Pond, West Palm Beach, FL), CFT trays (0.31 m x 1.22 m x 0.10 m; Botanicare, Vancouver, WA), and NFT channels (1.52 m x 0.12 cm x 0.04 cm; Crop King INC, Lodi, Ohio). A pair of channels consisted of one NFT unit and a tray consisted of a CFT unit. There were two pumps in each reservoir, one for each production system. Black vinyl tubing (1.3 cm Internal Diameter (ID), Crop King Inc.) was used to connect pumps to the production system and drain nutrient solution back to the reservoirs. Flow valves (1.3 cm; Green Back in-line valve, Botanicare) were used to control the flow rate of the nutrient solution in the inlet tubing. The CFT and NFT systems were set to a flow rate of approximately 6 and 1 $L \cdot min^{-1}$, respectively. NFT channels were raised by 0.15 m on the inlet compared to outlet end for nutrient solution to flow downwards along the gradient. An extension fitting (5 cm, Botanicare) was inserted to the outlet end of the CFT tray to allow water to rise approximately 3.8 cm in the trays before draining. The area of CFT (one tray) and NFT (two channels) units were 0.45 and 0.40 m^2, respectively. Each unit of a production system supported 16 plants. Plants were spaced 0.18 m x 0.13 m in the NFT and 0.17 m x 0.13 m in the CFT system. They were grown in rockwool cubes (Grodan, Roermond, The Netherlands) which were inserted through holes on the NFT covers to sit on the channels or placed in net pots (5.08 cm diameter, General Hydroponics, Chico, CA) that snugly fit in to holes made on CFT lids. Production systems were spaced closely at 1.3 cm apart from each other.

Supplemental lighting for plants and heating nutrient solution. Two types of LED supplemental lights (Fluence Bioengineering, Austin, TX) with similar light intensity output but different spectral composition was used in the experiment (Figure 4.2). Four lights were grouped together as a unit. The lights were 0.56 m long and spaced 0.46 m apart. The LED light bars were hung 0.61 to 0.91 m above plants so that the intensity was similar (145 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Supplemental lighting was provided between 4 pm to 6 am for a total of 14 hours per day. Nutrient solution was heated using an aquarium heater (200 W, FS-218, Freesea, Amazon.com). The heaters were placed at the bottom of the reservoirs and the thermostat was set to maximum output (200 W). This helped in keeping heaters on continuously and reduced fluctuations in solution temperature during different days.

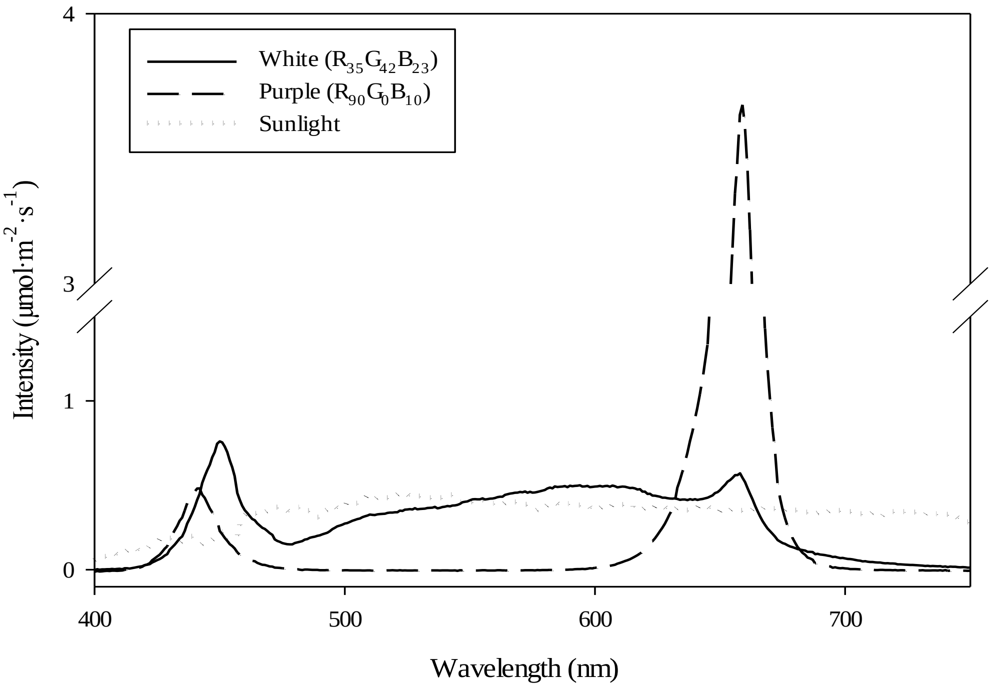

Figure 4.2: Spectral signature curves for two LED supplemental lights used in the study. Numbers indicate peak wavelengths for blue and red wavebands. Numbers indicate peak wavelengths for blue and red wavelengths.

Treatments and experimental design. The experiment was set up as a split-split-split plot design with four replications. Replications were laid perpendicular to the temperature gradient inside the greenhouse. The whole-plots included three supplemental light treatments, i.e., no supplemental light (NSL), purple and white supplemental lights. Plants in the NSL treatment received 4.2 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ of sunlight, whereas those in purple (R_{90}:G_0:B_{10}) and white (R_{35}:G_{42}:B_{23}) treatments received an additional 7.3 $mol{\cdot}m^{-2}{\cdot}d^{-1}$ of supplemental light of different spectra at nighttime. Photoperiod was between 10-11 h in the NSL treatment and 24 h in the PSL and WSL treatments. Light (SQ-110 SS, Apogee Instruments, Logan, UT) and temperature sensors (ST 110, Apogee Instruments) were connected to a datalogger (CR1000x, Campbell Sci.) to continuously measured incident light intensity and air temperature, respectively in each light treatment. Each whole-plot comprised an area of 1.5 m x 2.5 m on a bench. Within each whole-plot, there were two nutrient solution temperature treatments as the first split-plots, i.e., heated and unheated treatments. A temperature sensor (ST 110, Apogee Instruments) connected to a datalogger (CR1000x, Campbell Sci.) continuously measured nutrient solution temperature in reservoirs. The experiment was laid-out as a split-split-split-plot design with light treatment as whole plot, solution temperature as first split, production system as second split and cultivar as a third split. There were four replications of the whole plot (N = 384). The average solution temperature in the heated and unheated treatments were 18.8 (± 0.42) and 12.6 (± 0.37) ºC, respectively. Each solution temperature treatment comprised of a reservoir that supplied solution to two units each of NFT (i.e., four channels) and CFT (i.e., two trays) production systems. Each production system further supported eight cultivars (i.e., third split plot) with four plants per cultivar and four cultivars in a unit. Therefore, two units of each production system were needed to grow eight cultivars. Altogether, there were 1536 plants (4 replications × 3 supplemental light treatments × 2 solution temperature treatments × 2 production systems × 8 cultivars × 4 plants per cultivar) in the experiment. Whole and split plots were randomly allotted in the experiment.

Measurements and calculations. At the end of the experiment, shoots from a group of four plants belonging to a cultivar in an experimental unit were harvested and their fresh weights determined. Plants were placed in bags and dried in a forced air oven maintained at 80 ºC to determine dry weights.

Electrical energy use of LED lights, pumps and heaters were measured separately using a meter (P3 P4400 Kill-A-Watt electricity usage monitor, P3 International, New York, NY).

The electrical energy consumed by supplemental lights was calculated as follows:

$$EEC_{SL} = \frac{W \times 16 \times 40}{1000 \times 0.929}$$

Where, EEC_{SL} is total electrical energy consumption by supplemental lights in a square meter area during the experiment ($KWh \cdot m^{-2}$). W is power consumption in watts, 16 is hours of SL per day, 40 is duration of experiment in days, dividing by 1000 converts W to KW, and 0.929 is area in m^2 illuminated by a lamp.

The electrical energy consumed by pumps was calculated as follows:

$$EEC_P = \frac{W \times 24 \times 40}{1000 \times A}$$

Where, EEC_P is total electrical energy consumption (EEC_P) by pumps in a square meter area during the experiment ($KWh \cdot m^{-2}$). W is power consumption in watts, 24 is hours of nutrient solution circulation per day, 40 is duration of experiment in days, dividing by 1000 converts W to KW, and A is area of two NFT and CFT systems (0.40 and 0.45 m^2, respectively).

Heated nutrient solution was pumped to two CFT trays and four NFT channels from one reservoir at a time. Because the volume of water that flows through CFT was more than that of NFT system, energy consumption can be higher under CFT *per se* than NFT system. To measure heating costs separately for both systems, four NFT channels and two CFT trays were connected to separate reservoirs and energy consumption was measured in each reservoir for a period of 24 h. This was repeated three times for 24-hour periods to calculate average energy consumption for each system. The average electrical consumption for four NFT channels and two CFT trays was 2.35 and 3.17 KWh per day, respectively. This indicates that 42.5 and 57.5% of total energy use was consumed by NFT and CFT systems, respectively. The electrical energy consumed by heaters was calculated separately for NFT and CFT systems as follows:

$$EEC_{NFT} = \frac{W \times 24 \times 40}{1000 \times 0.40} \times 0.425$$

$$EEC_{CFT} = \frac{W \times 24 \times 40}{1000 \times 0.45} \times 0.575$$

where, EEC_{NFT} and EEC_{CFT} refer to total electrical energy consumption in a square meter area during the experiment ($KWh \cdot m^{-2}$) in the NFT and CFT systems, respectively, W is power consumption in watts, 24 hours is the duration of heater usage per day, 40 is the duration of experiment in days, dividing by 1000 converts W to KW, 0.40 and 0.45 represent area in m^2 of NFT and CFT systems, and 0.425 and 0.575 are the proportions of total energy used by NFT and CFT system, respectively

Energy ($BTU \cdot hr^{-1}$) needed to maintain ambient air temperature inside the greenhouse was calculated as follows:

$$Q = U \times A \times \Delta T$$

Where, Q is heat required ($J \cdot s^{-1}$), U is overall heat transfer co-efficient [$J \cdot (m^2 \cdot s \cdot °C)^{-1}$] for glass and curtain wall (5.4), A is the surface area of the greenhouse (209.03 m^2) and ΔT is difference between average inside (13.7 °C) and outside (-3.8 °C) air temperature. Heat requirement was expressed as $KJ \cdot hr^{-1}$ by multiplying with 3.6 (3600 $s \cdot hr^{-1}$ × 0.001 $J \cdot KJ^{-1}$). From this, equivalent electrical energy requirement for greenhouse heating per m^2 area during the experiment (EEC_A, $KWh \cdot m^{-2}$) was calculated as follows:

$$EEC_A = \frac{Q \times 24 \times 40}{3600 \times 111.5}$$

where, Q is heat required (KJ·hr^{-1}), 24 is the duration in hours of heater usage per day, 40 is the duration of experiment in days, dividing by 3600 converts KJ to KWh and 111.5 is area of greenhouse in m^2,

Total electrical energy consumption (EEC, KWh·m^{-2}) was calculated as the sum of different types of energy used in a given treatment. EEC_P and EEC_A were included in all treatments whereas EEC_{SL}, EEC_{NFT} and EEC_{CFT} depended on the treatment.

In order to adjust all values to m^2, shoot dry weight (SDW) was calculated as the average sum of four plants (D) within an experimental unit multiplied by the number of plants within a m^2 of each system (NFT = 41 plants and CFT = 44 plants).

$$SDW \cdot \mathrm{m}^{-2} = D \times (NFT\ or\ CFT)$$

From these, energy use efficiency [EUE, g·(KWh)$^{-1}$] was calculated as the ratio of Shoot Dry Weight (SDW) and EEC as follows:

$$EUE = \frac{SDW \cdot \mathrm{m}^{-2}}{EEC}$$

Statistical analyses. Data were analyzed based on split-split-split plot design with appropriate error terms for whole and split plots using PROC GLM procedure of statistical analysis software (SAS, ver. 9.1, Cary, NC). Means were separated using Tukey's HSD with $P \leq 0.05$ considered significant.

4.4 Results and Discussion

Environmental conditions: DLI on average was 4.2 mol·m^{-2}·d^{-1} and varied between 3 to 10.2 mol·m^{-2}·d^{-1} during the day, among treatments. At night, PPFD in the purple and white LED treatments added an average of 7.2 mol·m^{-2}·d^{-1} (Fig. 4.3). Air temperature on average was 15.6/10 °C (day/night) while minimum and maximum air temperature varied between 13.2 to 22.4 °C (day) and 9 to 14.2 °C (night), respectively during different days of the experiment. Over the course of the experiment, average solution temperature in the heated and unheated treatment were 18.8 °C and 12.6 °C respectively. Nutrient solution temperature ranged between 12.1 to 22.5 °C (heated) and 9.8 to 17 °C (unheated). Electrical conductivity of the nutrient solution ranged around 1 dS·m^{-1} for the first two weeks and then 1.7 dS·m^{-1} after the first tank change.

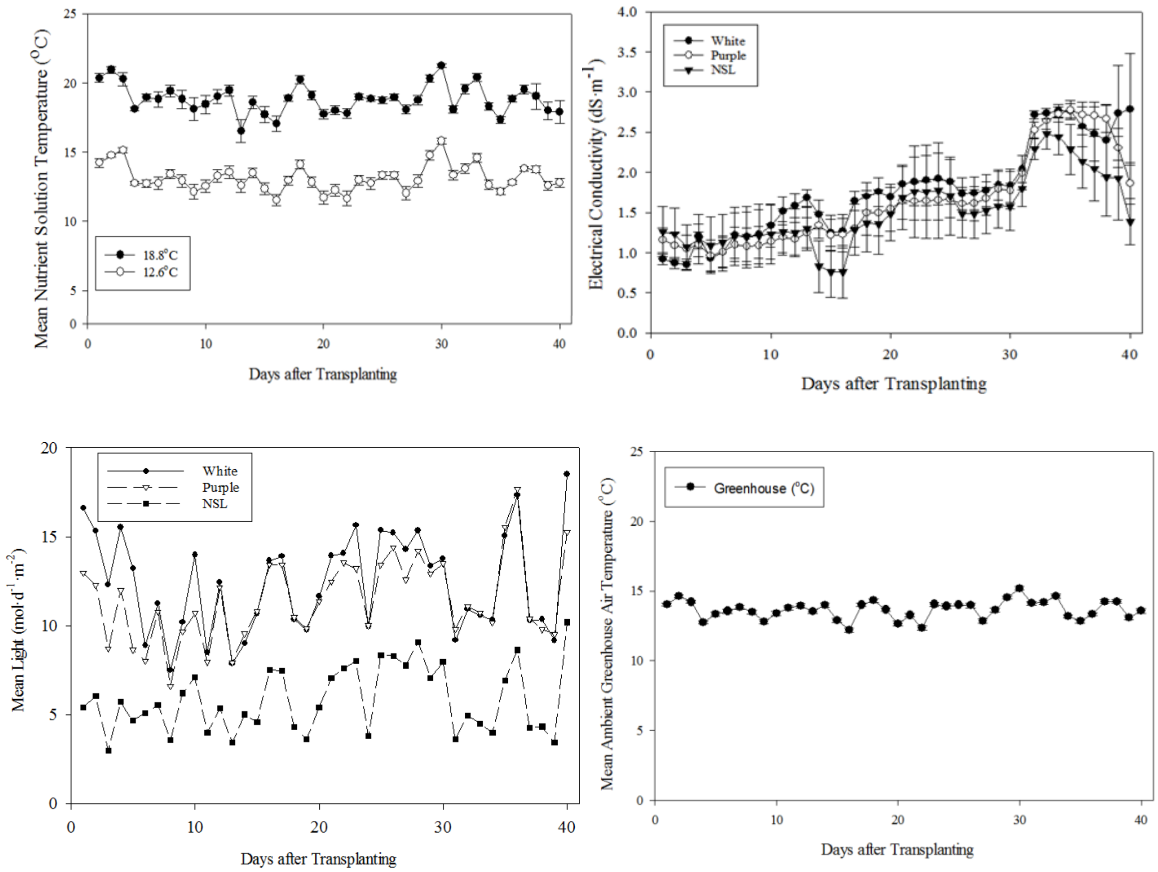

Figure 4.3. Pictured above are graphs displaying ambient air temperature (°C), EC (dS·m^{-1}), nutrient solution temperatures (°C), and PPFD (mol·m^{-2}·d^{-1}) over the course of the experiment.

Shoot dry weight. The three-way interaction between supplemental lighting, solution temperature and production system were significant for SDW. Across all cultivars, lettuce SDW was higher under CFT than NFT system under both heated and unheated nutrient solution treatments only when supplemental lighting was provided to plants (Fig. 4.4). However, there were no differences in shoot dry weight between two production systems under both solution temperature treatments when no supplemental lighting was provided to plants (Fig. 4.4). One major difference between two production systems is the amount of water and nutrients available to roots, especially when plants are small. Rockwool cubes, in which seedlings are grown, are completely submerged in the nutrient solution enriched with oxygen and nutrients in CFT. In case of NFT, nutrient solution rises up by capillarity as rockwool cubes are placed on a thin nutrient film. Due to the lower volume of nutrient solution contacting the medium, fertilizer salts are more likely to accumulate at the top through capillary action (Cox, 2001). Thus, limiting initial growth through salt stress in NFT especially at seedling stage when root growth is restricted to inside of the rockwool cubes. However, plants grown in NFT can uptake water adequately when roots grow out of rockwool cubes and reach to the bottom of the channel. Our observation is that it may take up to a week after transplanting for roots to grow to the bottom of the channel. The suspected lag in the capillary action of water and nutrient uptake in NFT system may have resulted in slower plant growth. Another factor that may have caused differences between the production systems could be the air temperature inside NFT channels. Post experiment measurements (data not shown), showed the channel air temperature was close to the ambient temperature of the greenhouse. In treatments with heated nutrient solution heating, the air temperature was only slightly warmer. As most of the root system in NFT is exposed to cooler channel air, the effects of sub-optimal air temperature can have relatively larger effect on plant growth in NFT than CFT. The difference in plant growth between production systems likely became larger under supplemental lighting treatments. In the absence of supplemental lighting, plants received only a third of the optimal DLI for lettuce (12-15 $mol \cdot m^{-2} \cdot d^{-1}$) (Kang et al., 2013; Both et al., 1997; Zhang et al., 2018). Thus, advantages described above for CFT over NFT were not apparent under low light conditions or in the absence of supplemental lighting.

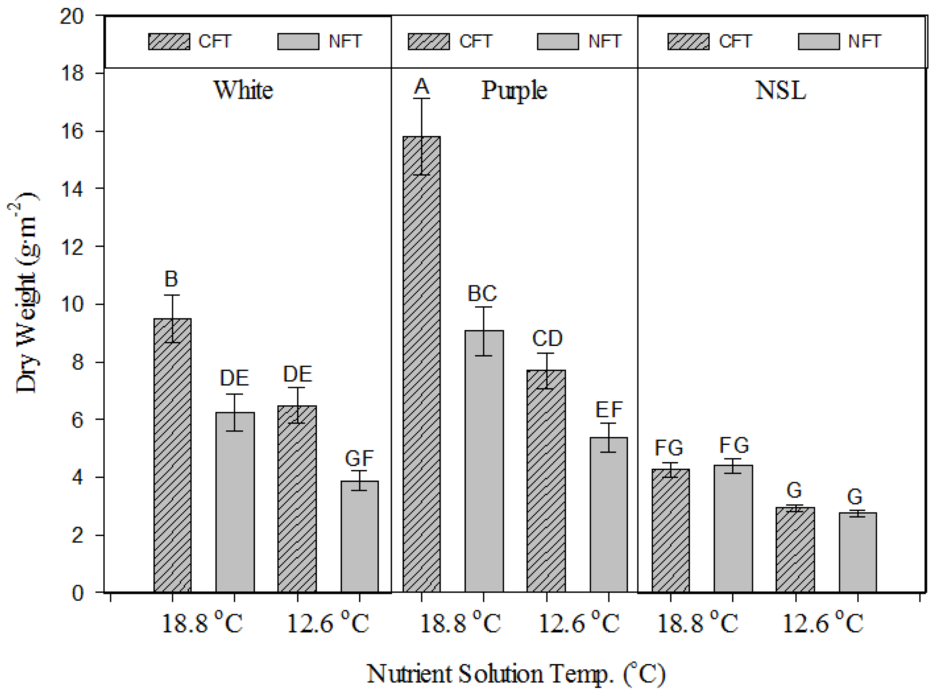

Figure 4.4: Three-way interaction between supplemental lighting (White, Purple, and no supplemental lighting (NSL), nutrient solution temperature (18.8 °C; 12.6 °C) and production system (CFT; NFT) on average shoot dry weight ($g{\cdot}m^{-2}$) ($P \leq 0.001$). Bars represent interactive LSMeans (average of all eight cultivars) and error bars represent standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

Interestingly, plants grown under purple LED and supplied with heated nutrient solution in a CFT system produced largest SDW across all cultivars (Fig. 4.4). In addition, plants under both CFT and NFT systems were larger under purple than white LEDs when heated nutrient solution was supplied (Fig. 4.4). These results clearly indicate that purple LED (R_{90}:G_0:B_{10}) with higher proportion of red light was superior to white LED with lower proportion of red light (R_{35}:G_{42}:B_{23}) across all cultivars and both production systems when heated nutrient solution was provided to plants. Recently Kong et al. (2019) found similar responses for lettuce cultivars grown under indoor production. In their study, sole source lighting with red: blue ratio of 4:1 affected shoot dry weight of lettuce cultivars while there was no effect of green light. They further indicated that higher proportion of green light reduced the proportion of red light in commercial LED fixtures. Moreover, studies indicated that higher proportion of blue light (>15%) reduced vegetative growth of lettuce (Zhang et al., 2018). Collectively, lower proportion of red, higher proportion of green and blue likely resulted in lower SDW of lettuce cultivars under white compared to purple LEDs in our study.

Adding heated nutrient solution did not result in differences between two production systems across all cultivars when supplemental lighting was not provided. However, adding supplemental light did result in differences between two production systems and across all cultivars, even when unheated solutions were provided to plants (Fig. 4.4). Above results indicate that addition of supplemental lighting is more important than addition of heated nutrient solution during winter production. This finding can be important under resource-limited situations. As expected, lowest shoot dry weight was observed in both production systems in the absence of supplemental lighting and heated nutrient solution.

The interaction between supplemental lighting, solution temperature and cultivar was also significant for SDW (Table 4.1). There were no differences in SDW of cultivars between heated and unheated solution treatments under no supplemental lighting. Two cultivars (Adriana and Salvius) had significantly higher SDW under heated compared to unheated nutrient solution under white LED. All cultivars had significantly higher SDW under heated compared to unheated solution treatment when grown under purple LED. These results indicate that heated nutrient solution is most effective under purple than white LEDs or no supplemental lights, likely because of benefits of purple lighting described above.

Table 4.1: Three-way interaction between supplemental lighting (White, Purple, and no supplemental lighting (NSL), nutrient solution temperature (18.8 °C; 12.6 °C) and cultivars (Adriana (Ad), Alkindus (Al), Black Seeded Simpson (BSS), Breen (Br), Cedar (Ce), Navara (Nv), Red Sails (RS), and Salvius (Sl)) on average shoot dry weight ($g \cdot m^{-2}$) of eight lettuce cultivars ($P \leq 0.0271$). Values represent interactive LSMeans (average of all eight cultivars) and values in parentheses are standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

Light	**Heat (°C)**	**Cultivar**							
		Ad	**Al**	**BS**	**Br**	**Ce**	**Na**	**RS**	**Sa**
White	18.8	12.2 (1.74) A	6.2 (1.74) AB	8.9 (0.98) B	3.0 (0.81) B	7.6 (1.54) B	5.7 (1.30) B	8.3 (0.88) B	11.1 (1.65) AB
	12.6	5.0 (1.26) BC	4.8(1.28) BC	5.8(1.11) BCD	3.0(0.69) B	6.8(1.14) B	4.6(1.06) BC	4.8(0.74) B	6.4(1.61) CD
Purple	18.8	13.8(1.80) A	8.6(1.83) A	15.5(2.43) A	8.1(0.59) A	15.1(1.78) A	8.9(1.50) A	15.3(1.94) A	14.3(2.38) A
	12.6	7.5(1.34) B	4.9(0.88) BC	7.3(0.89) CB	4.1(0.46) B	7.7(1.06) B	4.8(0.57) BC	7.4(1.59) B	8.4(1.46) BC
NSL	18.8	4.8(0.42) BC	3.9(0.30) BC	3.5(0.63) CD	2.9(0.24) B	4.5(0.39) B	4.8(0.38) BC	5.2(0.08) B	5.1(0.35) CD
	12.6	3.0(0.54) C	2.7(0.42) C	2.8(0.42) D	2.4(0.27) B	3.0(0.41) B	2.9(0.12) C	2.9(0.67) B	3.0(0.18) D

The interaction between supplemental lighting, production system and cultivar was also significant for SDW (Table 4.2). There were no differences in SDW of cultivars between two production systems under no supplemental lighting. Three cultivars (Adriana, Alkindus and Salvius) had significantly higher SDW under CFT compared to NFT under white LED. Whereas several cultivars had significantly higher SDW in CFT than NFT treatment when grown under purple LED. These results indicate that cultivar specificity to production system is affected by light spectral composition. The advantages described above for CFT compared to NFT are realized when spectral composition is optimal, ie., higher red proportion in the total light and blue lower than 15%.

Table 4.2: Three-way interaction between lighting treatment (White, Purple, and no supplemental lighting (NSL), production system (CFT; NFT) and cultivars (Adriana (Ad), Alkindus (Al), Black Seeded Simpson (BSS), Breen (Br), Cedar (Ce), Navara (Nv), Red Sails (RS), and Salvius (Sl)) on average shoot fresh weight ($g \cdot m^{-2}$) ($P \leq 0.009$). Each value represents interactive LSMeans (average of each cultivar) and values in parentheses are standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

Light	**PS**	**Cultivar**							
		Ad	**Al**	**BS**	**Br**	**Ce**	**Na**	**RS**	**Sa**
White	CFT	10.9(2.07) AB	7.1(0.81) AB	8.4(1.17) B	3.6(0.27) B	8.6(0.71) B	6.6(0.92) AB	7.2(1.37) BC	11.4(1.57) A
	NFT	6.3(1.68) CD	3.9(0.83) C	6.3(0.33) BC	2.4(1.28) B	5.8(0.51) BC	3.8(0.52) B	5.9(1.27) BC	6.1(1.52) B
Purple	CFT	13.2(1.96) A	8.8(1.77) A	14.8(2.57) A	5.8(1.4) A	13.5(2.18) A	8.1(1.73) A	14.5(2.21) A	15.2(2.16) A
	NFT	8.2(1.55) BC	4.7(0.73) BC	8.1(1.23) B	6.4(1.45) A	9.2(1.64) AB	5.6(1.04) AB	8.2(1.82) B	7.5(1.08) B
NSL	CFT	3.6(0.98) C	3.4(0.73) C	3.0(0.21) C	2.7(0.49) B	3.8(0.77) C	3.9(1.02) B	4.3(0.97) BC	4.0(1.00) B
	NFT	4.1(0.92) C	3.2(0.79) C	2.5(0.51) C	3.2(0.78) B	3.8(0.95) C	3.7(0.95) B	3.9(1.17) C	4.1(1.05) B

Energy use efficiency. EUE was higher in CFT than NFT in both solution temperature treatments under purple lighting (Fig. 4.7). Under white lighting, EUE was higher in CFT than NFT only in the heated solution treatment. There were no differences in EUE between CFT and NFT systems in both solution temperature treatments when supplemental lighting was not added. These results indicate the SDW differences among treatments had larger impact on EUE than energy consumption differences. Like our findings, Kong et al., (2019) also indicated that SDW had larger effect on EUE than total energy consumption during growth. In addition, their results indicate that EUE of lettuce linearly increased with increasing percentage of red light in the total light output from supplemental lighting. Our results agree with those of Kong et al., (2019) that EUE was higher under purple lighting with higher percentage of red light than white supplemental lighting (Fig. 4.4 and 4.7). Higher EUE under CFT than NFT was likely due to higher SDW under CFT, due to reasons described above. Plants grown under purple lighting and heated solution treatment consumed 44% (541 vs 374 KWh) more electrical energy but had two-fold higher EUE than those grown in the absence of supplemental lighting and heated nutrient solution treatments. This indicates that it can be more cost effective to add additional energy costs to production, as opposed to growing plants under sub-optimal light and temperature conditions.

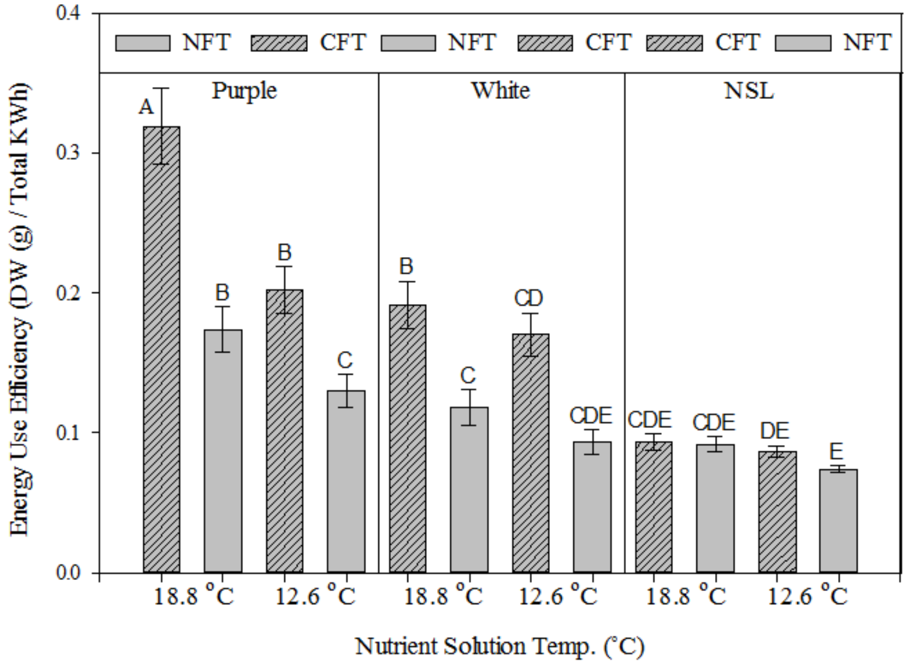

Figure 4.5: Three-way interaction between lighting treatment (White, Purple, and no supplemental lighting (NSL), production system (CFT; NFT) and temperature treatment (Heated (18.8 ºC) and unheated nutrient solution (12.6 ºC)) on average EUE (g·KWh^{-1}) ($P \leq 0.014$). Each value represents interactive EUE (average of each cultivar divided by total KWh^{-1} used by NS heating, ambient heating, light, and pumps) and values in parentheses are standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

In addition, interactive effects of light, production system and cultivar (Table 4.3) or light, solution temperature and cultivar (Table 4.4) on EUE were significant. In general, EUE in most of the cultivars was higher under CFT than NFT under both supplemental lighting treatments whereas no differences were observed between two production systems when supplemental lighting was absent (Table 4.3). Also, EUE in most of the cultivars was higher under heated solution treatment compared to unheated treatment only under purple lighting (Table 4.4). Among cultivars, Salvius, Red Sails, Cedar, Black Seeded Simpson and Adriana had higher EUE than other cultivars. These results indicate that addition of heat to nutrient solution can improve EUE mostly under purple lighting, mainly due to increased SDW under purple than white supplemental lighting.

Table 4.3: Three-way interaction between lighting treatment (White, Purple, and no supplemental lighting (NSL), production system (CFT; NFT) and cultivars (Adriana (Ad), Alkindus (Al), Black Seeded Simpson (BSS), Breen (Br), Cedar (Ce), Navara (Nv), Red Sails (RS), and Salvius (Sl)) on average EUE ($g \cdot KWh^{-1}$) ($P \leq 0.0001$). Each value represents interactive EUE (average of each cultivar divided by total KWh^{-1} used by NS heating, ambient heating, light, and pumps) and values in parentheses are standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

Light	**PS**	**Cultivars**							
		Ad	**Al**	**BSS**	**Br**	**Ce**	**Nv**	**RS**	**Sl**
NSL	CFT	0.09(0.009) C	0.09(0.006) B	0.08(0.009) C	0.07(0.005) C	0.09(0.011) C	0.1(0.009) BC	0.11(0.011) B	0.1(0.011) B
	NFT	0.09(0.009) C	0.07(0.006) B	0.08(0.006) C	0.06(0.004) C	0.09(0.006) C	0.09(0.007) BC	0.09(0.012) B	0.1(0.012) B
Purple	CFT	0.3(0.052) A	0.2(0.025) A	0.32(0.062) A	0.13(0.022) AB	0.3(0.049) A	0.18(0.028) A	0.32(0.04) A	0.34(0.053) A
	NFT	0.17(0.038) BC	0.1(0.014) B	0.17(0.041) B	0.13(0.025) A	0.19(0.026) B	0.12(0.023) ABC	0.17(0.031) B	0.16(0.028) B
White	CFT	0.24(0.037) AB	0.16(0.022) A	0.19(0.028) B	0.08(0.013) BC	0.2(0.031) B	0.15(0.022) AB	0.16(0.031) B	0.26(0.034) A
	NFT	0.13(0.028) C	0.08(0.011) B	0.13(0.024) BC	0.05(0.009) C	0.12(0.023) BC	0.08(0.018) C	0.12(0.021) B	0.13(0.023) B

Table 4.4: Three-way interaction between lighting treatment (White, Purple, and no supplemental lighting (NSL), hydroponic solution temperature (18.8 ºC; 12.6 ºC), and cultivars (Adriana (Ad), Alkindus (Al), Black Seeded Simpson (BSS), Breen (Br), Cedar (Ce), Navara (Nv), Red Sails (RS), Salvius (Sl)) on average EUE (g·KWh^{-1}) ($P \leq 0.014$). Each value represents interactive EUE (average of each cultivar divided by total KWh^{-1} used by NS heating, ambient heating, light, and pumps) and values in parentheses represent standard error of mean. Means with different letters are significantly different ($P \leq 0.05$).

Light	**Heat (ºC)**	**Cultivars**							
		Ad	**Al**	**BSS**	**Br**	**Ce**	**Nv**	**RS**	**Sl**
NSL	18.8	0.1(0.009) C	0.08(0.008) B	0.07(0.006) C	0.06(0.004) B	0.1(0.008) C	0.1(0.008) B	0.11(0.013) BC	0.11(0.014) C
	12.6	0.08(0.006) C	0.08(0.004) B	0.08(0.009) C	0.07(0.005) B	0.09(0.01) C	0.08(0.006) B	0.08(0.008) C	0.09(0.006) C
Purple	18.8	0.27(0.059) A	0.17(0.031) A	0.31(0.071) A	0.16(0.027) A	0.3(0.044) A	0.18(0.029) A	0.3(0.047) A	0.28(0.065) A
	12.6	0.19(0.036) AB	0.13(0.019) AB	0.19(0.033) B	0.1(0.013) B	0.19(0.034) B	0.12(0.022) AB	0.19(0.034) B	0.22(0.038) AB
White	18.8	0.24(0.034) A	0.12(0.018) AB	0.17(0.027) B	0.06(0.013) B	0.14(0.027) BC	0.11(0.022) AB	0.16(0.031) BC	0.22(0.036) AB
	12.6	0.13(0.031) BC	0.12(0.027) AB	0.15(0.03) BC	0.08(0.011) B	0.17(0.034) BC	0.12(0.026) AB	0.12(0.021) BC	0.16(0.038) BC

4.5 Conclusion

In conclusion, heating and supplemental lighting is expensive yet it is required inputs to produce a lettuce crop during winter in the Midwestern regions of the U.S. It was determined that light spectral composition of supplemental light can have a significant effect on lettuce growth when provided at nighttime. LED fixtures with a spectral composition of R_{90}:G_{0}:B_{10} (purple) resulted in increased lettuce growth compared to that with a composition of R_{35}:G_{42}:B_{23} (white). Moreover, root zone heating using heated nutrient solution was more effective under purple compared to white LED lights. Lettuce growth was also higher under CFT than NFT system, in both solution temperature treatments when supplemental lighting was provided at nighttime. Cultivars, especially Black Seeded Simpson, Cedar, Red Sails and Salvius, consistently showed differences in SDW between production systems or solution temperature treatments under purple compared to white LED lighting. Fast growing cultivars in a CFT or similar system provided with root zone heating in purple LED supplemental lighting at nighttime are recommended for winter greenhouse hydroponic lettuce production in Midwestern latitudes.

4.6 References

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