

Effects of Nutrient Solution Temperature on Lettuce Grown in a Nutrient Film Technique System

by

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Abstract

Increases in global population coupled with predicted depletion of natural resources has created a push for more intensive and efficient food production. Greenhouse production has been shown to significantly increase water, nutrients, and land use efficiencies over conventional field production. Despite the many advantages of greenhouse vegetable (GHV) production, a lack of wide spread adoption can be attributed to a significant increase in developmental costs and energy needs. Compared to traditional field cropping systems, GHV production requires higher energy inputs, mainly for heating and cooling systems. Although high production is possible with CEA, high energy requirements have limited expansion.

This research examines the effects of heating nutrient solution temperature during cooler months or chilling nutrient solution during warmer months on *Lactuca sativa L.* 'Rex' grown in a nutrient film technique (NFT) system. Fifty lettuce seedlings were transplanted at a uniform size two weeks after germination to a NFT system located at Paterson Greenhouse Complex at Auburn University. Nutrient solution was heated with two Aquarium heaters (Odyssea Aquarium Appliance Co. Ltd) or chilled solution using a thermostat-controlled chiller (Penguin Chillers ½ HP Water Chiller). Size index was measured at 15 and 30 days after transplant and market fresh weight and head fresh weight were measured at termination.

Head fresh weight, market fresh weight and size index were greater for the heated nutrient solution than the unheated, control solution. Head fresh weight increased 182%, MFW

increased 136%, and SI increased 27% at termination. Chilling nutrient solution decreased MFW, HFW, and density but increased SI. Lettuce grown in nutrient solution chilled to the target temperature of 24°C had a 14% lower MFW, 17% lower HFW, 18% lower density and a 2% higher Size index (SI) than those grown in the non-chilled, control solution. Nutrient solution temperature also affected nutrient uptake. Chilling the nutrient solution led to a 24% increase in foliar calcium (Ca) percentage, and increased foliar manganese (Mn), sodium (Na), and zinc (Zn) by 69, 40, and 177%, respectively, but decreased foliar iron by 33%.

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List of Abbreviated Terms

DWC Deep Water Culture

NFT Nutrient Film Technique

CEA Controlled Environment Agriculture

RZT Root Zone Temperature

RDW Root Dry Weight

HAF Horizontal Airflow Fans

L Liter

DAT Days after transplant

Chapter 1

An Introduction and Literature Review

Introduction

Increases in global population coupled with predicted depletion of natural resources has created a push for more intensive and efficient food production. Greenhouse production has been shown to significantly increase water, nutrients, and land use efficiencies over conventional field production. Hydroponics allows for intensive crop production in areas where soil is unfit for agricultural use (Jensen 1999).

Hydroponics is the technique of growing plants by placing the roots in a nutrient solution with or without mechanical root support; however, there is some disagreement as whether aggregate hydroponics, or hydroponics that provides root support, qualifies (Jones 2005). Though the term 'hydroponics' was coined in the 1930's by Dr. W.F. Gericke, the practice of hydroponics has been around for centuries (Jones 2005; Jensen 1999). Research by Julius von Sachs and Wilhem Knop introduced water as a source of media for plant growth (Morgan and O'Hare 1978). Their research focused on the effects of chemical compounds on plant growth, and the use of water as a growth media allowed for easier isolation of compounds. Increasing popularity for hydroponics in both research and production sectors have led to development of various forms of production systems, including Deep Water Culture (DWC) and Nutrient Film Technique (NFT). Hydroponic production offers producers in areas not conducive to field lettuce production the chance to benefit from the 'locally grown' crop movement, and the higher market

prices that come with it (Xu et al 2015). Hydroponic production also comes with the added benefits of decreased threat from soil-borne pests and pathogens, further benefitting producers (Jensen 1999).

Despite the many advantages of greenhouse vegetable (GHV) production, a lack of wide spread adoption can be attributed to a significant increase in developmental costs and energy needs. Compared to traditional field cropping systems, GHV production requires higher energy inputs, mainly for heating and cooling systems. Although high production is possible with CEA, high energy requirements have limited expansion.

U.S. Lettuce Production

Lettuce is a cool season crop that is subject to a number of physiological problems at higher than optimal temperatures, including tip burn, ribbiness, loose heads, rib discoloration, bolting. Lettuce is produced throughout the United States, with California and Arizona accounting for the majority of domestic leaf and head lettuce, and is valued at \$1.9 billion per year (USDA-ERS 2017b). Lettuce is the second most popular vegetable crop in the U.S., behind only potatoes (USDA-ERS 2017b) In 2016, the USDA reports that 281,700 acres of head, leaf, and romaine type lettuce was harvested in the U.S. (USDA-NASS 2016), Greenhouse lettuce production is less expansive than field lettuce production with nearly 40 acres of greenhouse lettuce produced in the U.S. in 2013 (Hickman 2016). High temperatures throughout the southeast limit conventional field production of lettuce. However as the popularity of CEA has

increased, primarily due to technological advances in greenhouse production, producers in the southeast have more opportunities in the hydroponic lettuce market.

Greenhouse Lettuce Production

Due to high summer temperatures and high humidity in the Southeastern U.S., tipburn and bolting are common problems in greenhouse lettuce production. Though tipburn is caused by a calcium deficiency in young leaves, the exact origin of this physiological disorder is unknown (Jenni and Yan 2009; Hartz et al. 2007). High humidity can result in reduced transpiration rates, which, in turn, inhibits the uptake of calcium through mass flow (Wien 1997). Calcium is an immobile nutrient, meaning that the plant cannot relocate it to newly forming tissue. A common symptomatic response Ca deficient plants exhibit is necrotic margins on new leaves and shoot (Wien 1997). Increased growth rates caused by higher temperatures, coupled with reduced transpiration, may also contribute to tipburn due to an inability of the plant to move nutrients to new tissue at a rate fast enough to sustain adequate growth rates, causing necrotic edges to form on the leaves (Wien 1997).

Studies on reducing the effects of tipburn have shown some success. Foliar spray applications have been found to decrease side effects of tipburn in some loose lettuce head types (Corriveau 2012) and strategies to increase transpiration by increasing air movement across the leaves have contributed to reduced severity in tipburn (Lee et al. 2013). While decreasing the effects of tipburn may be useful, the development of tipburn resistant cultivars, such as those

available in crisphead types, would ease production difficulty in the southeast (Jenni and Hayes 2010).

Greenhouse Heating

While many crops thrive in cooler temperatures, heating is still necessary, especially during the winter months as cooler temperatures can increase production times, resulting in increased production costs. The optimum temperature for field lettuce production is 15.6-18.3°C, above 20° C, the lettuce may flower and produce seed (Sanders 2001). To keep greenhouses at the optimum temperature, heating may be required in cooler months. Greenhouses are typically heated using traditional fuel-based technology. A forced air heating unit is a common choice for growers, as it has low upfront costs and is reliable (Sanford 2011). Though an often economic choice, forced air units are often inefficient at uniform heating (McMahon 2012). Horizontal Airflow fans (HAF) are often incorporated to help circulate the heated air across the greenhouse (Ball 1997). Forced air heaters and HAF fans heat the total volume of air in a greenhouse, leading to unnecessary fuel expenditures. Heating costs can consume up to 30% of a producers overall budget and though fuel costs have fluctuated throughout the years, they are on an overall upward trend (Lopez and Runkle 2014; Latimer 2012). Because the cost of heating is so high, many producers choose to lower the set greenhouse temperature, slowing production times and possibly causing producers to miss market dates. More efficient sources of heating greenhouse crops may allow producers to decrease heating and fuel costs.

Radiant heaters allow producers to heat the air just around the plants through the use of hot water or steam circulated in iron or aluminum pipes throughout the greenhouse (Nelson 2012). Infrared heaters are another option for greenhouse heating. These work by emitting infrared radiation, which is then absorbed and converted to heat (Bakker 1995). The radiation is emitted through heat exchangers suspended over the plant canopy. Infrared heating can reduce fuel costs, however heat exchangers can block light reaching crop canopies (Bakker 1995; Stone and Youngsman 2006). Giving producers the ability to localize heating to the just the root-zone of the plant may allow lower greenhouse set temperatures without affecting plant growth.

Root-zone Heating

Root-zone temperature is easily modified and therefore, can help regulate plant growth while helping reduce overall greenhouse heating costs (Morgan et al. 1980). Sachs and others (1992) reported that root-zone heating helps to warm the plant canopy from below while also heating the roots, reducing overall heating of the greenhouse and therefore reducing energy consumption. Maintaining a root-zone temperature of 22° C was found to maximize growth and mineral uptake in snapdragons (Olberg and Lopez 2017; Hood and Mills 2008). Significant differences in treatments were found with sub-surface, root-zone heating and aerial coverage in lettuce (Bumgarner et al. 2011). Lettuce that was grown under a high tunnel setting with root-zone heating has significantly higher growth rates than those grown with just root-zone heating. Similarly, Boxall (1971) found that heating the soil, and therefore the root-zone, decreased production time of butterhead lettuce by 14-17 days on average in a field setting. The effects of

root-zone heating show that it is the plant temperature, not air temperature, that regulates plant growth (Nelson 2012). Because of this, heating the root-zone and not the air around the plant may be a more efficient heating strategy. Root-zone heating may be a relatively easy to implement strategy in hydroponic production that may increase plant growth rates and decrease energy costs.

Hydroponics offers advantages compared to soil-based systems due to higher control of irrigation and nutrient management. Nutrient levels can be kept constant and in the correct ratios while the constant presence of water essentially eliminates plant water stress and maximizes uniformity (Morgan & R. O'Haire 1978). This level of control may apply to temperature modification as well.

At of $4.18 \text{ J/}^\circ\text{C g}$, water has an approximately 4 times higher heat capacity than air (U.S. Department of the Interior 2016). While water requires more energy to heat per unit, it also loses heat less rapidly than air. As lower temperatures lead to increased production times, increasing the nutrient solution temperature in lieu of greenhouse air temperature, may help keep production times more constant annually, allowing for decreased production costs and increased profit. Thompson and others (1998) reported that production of 'Ostinata' Butterhead lettuce was optimized when nutrient solution was heated to 24°C . Damage and variations were also minimized at that temperature. Economakis and L. Krulj (2001) reported that root-zone heating of 'Osso Grande' strawberries grown in an NFT system increased overall dry weights and percentage of total yield at 25°C . In another study, lettuce grown in a solution heated to either 15°C or 20°C had significantly higher shoot fresh weights than those grown in the unheated

nutrient solution (Economakis and M. Said 2002). They also reported increased water content, increased number of leaves, and decreased root dry weight (RDW) in the heated solution as opposed to the unheated nutrient solution. Similarly, solution heated to 24°C was found to increase the growth rate of chrysanthemums while simultaneously allowing for decreased air temperature (Morgan et al. 1980).

While heating nutrient solution helps to increase hydroponic production in cooler seasons, cooling nutrient solution may help crop production in hotter months, especially in the southeast where high temperatures occur much of the year. Some beneficial results of nutrient solution cooling have been found. Lee and Takakura (1995) reported greater growth of 'Okame' spinach when nutrient solution temperatures were chilled to between 18 and 26 °C. Above 26° C, growth slowed and browning of the roots occurred in nutrient solution at 33 °C. The effects of root-zone cooling have also been studied in Butterhead Lettuce. Ilahi and others (2017) reported significant differences in size and leaf number on butterhead lettuce with root-zone cooling and an increase in incidence of tipburn on those under the non-chilled, control treatments of plants grown in a coir-perlite mixture. Cooling of the root-zone may also affect photosynthetic rate of plants. Jie and Kong (1998) reported that lettuce with root-zone cooling had 50% higher photosynthetic rates than those that were exposed to whole plant hot ambient temperatures. While the effects of root-zone cooling have not been widely studied, previous research suggests that there is cause to further examine its effect.

Research Objectives

Temperature constraints are a major problem faced by hydroponic lettuce producers. Heating and cooling costs can overwhelm a producer's budget and drive the production costs up. By providing producers with alternative greenhouse heating and cooling options, production costs may be reduced, and/or production times may be decreased, allowing for increased profits. Our objectives are to determine the effects of nutrient solution heating in cooler months of hydroponically produced butterhead lettuce in the southeastern U.S. and to determine the effects of nutrient solution cooling in hotter months on hydroponically produced butterhead lettuce in the southeastern U.S.

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Chapter 2

Effects of Heating Nutrient Solution on Hydroponic Lettuce Grown in an NFT System

Introduction

Greenhouse vegetable production offers many advantages compared to traditional soil-based production systems including, among others, greater environmental control, more precise application of fertilizers, elimination of soil-borne pests and diseases (Jensen 1999), and more constant rates of growth (Morgan and O’Haire 1978). Greenhouses are typically heated using traditional fuel-based technologies, using a forced-air heating unit because they are relatively inexpensive, low maintenance, and reliable (Sanford 2011). Since plant temperature, not air temperature, is an important factor influencing plant growth (Nelson 2012), heating the total volume of greenhouse air is inefficient when considering how little of that volume the crop occupies. Heating costs can consume up to 30% of a producer’s overall budget and though fuel costs have fluctuated throughout the years, they are on an overall upward trend (Lopez and Runkle 2014; Latimer 2012). Because the cost of heating is so high, many producers choose to lower the set greenhouse temperature, slowing production times and possibly causing producers to miss market dates. More efficient sources of heating greenhouse crops may allow producers to decrease heating and fuel costs.

Root-zone heating improves plant production in the field (Boxall 1971) and in the greenhouse (Thompson et al. 1998; Economakis and Krulj 2001) and has the added benefit of

potentially reducing production costs in greenhouses (Sachs et al. 1992). Root-zone heating is easily implemented (Morgan et al. 1980) and likely becomes most efficient in heating hydroponic systems due to the high heat capacity of water compared to air.

Root-zone heating is often used to hasten production times in cooler months. Other studies have reported improvements in plant growth in multiple species and systems. Economakis and Krulj (2001) reported that nutrient solution heated to 25°C increased overall dry weights and percentage of total yield of ‘Osso Grande’ strawberries grown in an NFT system. Heating of the nutrient solution reportedly increased early fruit set and the number of malformed fruits as well. Production of butterhead lettuce was optimized when nutrient solution was heated to 24°C in DWC (Thomson et al 1998). At the 24°C treatment, damage and variations were also minimized.

Nutrient solution heating may lead to overall increased yield, minimized growth variations in some crops, and reduced costs from heating the entire greenhouse. In a study using lettuce, nutrient solution heated to either 15°C or 20°C had higher shoot fresh weights, increased water content, and increased number of leaves, and lower root dry weight than those grown in the unheated nutrient solution (Economakis and Said 2002). The effects of nutrient solution heating on butterhead lettuce grown in a NFT system have not been widely studied. Previous research suggests that nutrient solution heating may be beneficial on butterhead lettuce grown in a NFT system in cooler months. The objective of this research is to determine the effects of nutrient solution heating on butterhead lettuce grown in a NFT system.

Materials and Methods

On 2 November 2016, 16 January 2017, and 21 March 2017 55 *Lactuca sativa* L. ‘Rex’ seeds were sown in 2.54cm x 3.18cm x 3.81cm OASIS[®] horticubes (OASIS[®] Grower Solutions, Kent, Ohio). They were watered to capacity with municipal water, covered in a clear humidity dome, and placed on a greenhouse bench at Paterson Greenhouse Complex in Auburn, AL. After germination, humidity domes were removed and seedlings were fertilized with nutrient solution from municipal water (Auburn, AL) containing 150, 80, 200, 150, and 35 mg L⁻¹ N, P, K, Ca, and Mg, respectively from water-soluble 8N-6.5P-30K (Gramp’s Original hydroponic lettuce fertilizer, Ballinger, TX), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg).

After two weeks, 50 seedlings were transplanted to a nutrient film technique (NFT) system (Farmtek Dyersville, IA) comprised of 10, 10 ft troughs and supported on by a 3m x 3m frame. Five troughs each were fed nutrient solution from one of two 132 L sumps (Rubbermaid Atlanta, GA). Sumps were each filled with 95 L of the same nutrient solution used to fertilize seedlings after germination. One fountain pump in each sump pushed nutrient solution through .635 cm irrigation line and spaghetti tubing delivered 300 mL min⁻¹ nutrient solution to NFT troughs. Five seedlings were placed in every other space in each trough, and empty spaces were covered with blue painters tape and white duct tape to exclude light and reflect heat.

Sump 1 was wrapped with wall insulation (Johns Mansville R13 insulation) and two Aquarium heaters (Odyssey Aquarium Appliance Co. Ltd) set to 24°C were attached with suction cups to opposites sides of the interior of the sump. Sump 2 was not heated or insulated.

Air temperature at plant height and nutrient solution temperature in both sumps was recorded once per hour for the duration of the experiment using WatchDog® B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL). Nutrient solution pH and EC was measured five times weekly using LAQUA Twin pH Meter and LAQUA Twin EC Meter (Spectrum Technologies, Inc., Aurora, IL), respectively.

Though pH was not controlled, water levels were managed in the sump. As EC was measured over $2.0 \text{ mS}\cdot\text{cm}^{-1}$, fresh water was added to bring EC back within a target range of $1.5\text{-}1.9 \text{ mS}\cdot\text{cm}^{-1}$. As nutrient solution levels dropped below 75 L, fresh nutrient solution was added to bring nutrient solution levels back to 95 L.

Size index (SI) ($[\text{height} + \text{widest width} + \text{perpendicular width}]/3$) was measured at 15 DAT and 30 DAT. At 30 DAT, plants were removed from NFT system, with the original OASIS® rootball intact. Roots were trimmed leaving approximately 2.5 cm of roots below the root cube. Lettuce heads were weighed with rootball and remaining roots attached to determine market fresh weight (MFW). All roots, including the original root cube were removed and heads were weighed to determine head fresh weight (HFW). Head fresh weight was divided by SI to determine head density.

After termination, a sunrise/sunset calendar was used to determine the sunrise (day) and sunset (night) times for each day of the experiment. Once the night and day times were determined, the temperature data was sorted according to day and night times to find the day and night temperatures.

The experiment was a randomized complete block design with five blocks containing five subsamples each. A block was considered an experimental unit. Size index, MFW, HFW, and head density were subjected to analysis of variance using PROC GLIMMIX and data was corrected for homogeneity. Block and experimental run were treated as random variables. Significance was determined at the $\alpha=0.05$ level.

Results and Discussion

Average pH was 5.97 and 6.10 for heated and unheated treatments, respectively while EC was 1.72 and 1.83 (Table 2.1) While the heated treatment had a target temperature of 24°C, the actual temperature recorded during the project was slightly higher with the average temperature being 25.3°C during the day and 22.5°C during the night. Greenhouse air temperature averaged the greatest difference in day to night. Greenhouse air temperature DIF (difference between night and day temperature) was averaged at 7.6 with the day temperature averaging 27.6 and night temperature 20.0. DIF was lower for the unheated solution than heated solution while the greatest in DIF was found in the greenhouse air (Table 2.2). Size index increased 30% and 27% at 15 and 30 DAP, respectively. Head fresh weight increased 182%, from 54.6 g to 154.2 g and MFW increased 136% from 75.9 g to 178.8 g. Lettuce grown in nutrient solution heated to the target temperature of 24°C had a higher market fresh weight (MFW), head fresh weights (HFW) and Size index (SI) than those grown in the unheated, control solution (Table 2.3)

Lettuce grown in the heated nutrient solution also had higher head density (SI/HFW) than those in the unheated solution. Head density increased by 122% for lettuce grown using the

heated nutrient solution (Table 2.3). Market fresh weight, HFW, and SI averages are larger for heated nutrient solution than the unheated solution. Higher growth rates brought on by higher temperatures may decrease production times and increase overall production for greenhouse growers. In a similar experiment, production of ‘Ostinata’ Butterhead lettuce was optimized when nutrient solution was heated to 24° C (Thompson et al. 1998). Reduced damage and variations from lettuce grown in the heated treatment was also reported. In another study, nutrient solution was heated to either 15 or 20°C (Economakis and Said 2002). Lettuce grown in the heated nutrient solution in both the 15°C and 20°C treatments had higher shoot fresh weights, increased water content, increased number of leaves, and decreased root dry weight than those grown in the unheated nutrient solution. Heating nutrient solution may decrease production time in cooler months, allowing producers to cut costs.

Conclusions and Future Research

Lettuce grown using nutrient solution heating had a significantly higher MFW, HFW, SI and density than those grow using the unheated nutrient solution. Heating nutrient solution may also hasten production time. While these findings may be beneficial for producers, future research is needed to determine the economic viability of nutrient solution heating in lieu of greenhouse air heating and the rate at which heating nutrient solution hastens production times.

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Table 2.1. Effects on pH and EC of heating nutrient solution to a target temperature of 24°C in a recirculating nutrient film technique systems used to grow *Lactuca sativa L.* ‘Rex’ in a 30-d experiment

Nutrient Solution	pH ^z	EC ^y
Heated	5.97	1.72
Control	6.10	1.83

^zpH was measured using LAQUA pH twin meter

^yEC was measured using LAQUA EC twin meter

Table 2.2. Mean temperatures for nutrient solution heating in a NFT System averaged across all runs in a 30-d experiment

Treatment ^z	Target Temperature	Actual Temperature ^y		DIF ^x
		Day	Night	
Heated	24	25.3	22.4	2.9
Control	Unspecified	23.8	21.1	2.7
Greenhouse ^w	Unspecified	27.6	20	7.6

^z Treatment is defined as heated nutrient solution using height aquarium heaters or unheated

^y Temperature recorded using WatchDog® B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL) and reported in °C

^xDIF is equal to day temperature – night temperature

^w Greenhouse temperature recorded was the ambient air temperature at plant height

Table 2.3. Effects of heating nutrient solution to a target temperature of 24°C on size index, market fresh weight, head fresh weight, and density of *Lactuca sativa* L. ‘Rex’ in a 30-d experiment

Treatment ^z	MFW ^y	HFW ^x	SI ^w		Density ^v
			14DAT	28 DAT	
Heated	178.8	154.2	11.7	24.06	6.41
Unheated	75.9	54.6	9	18.92	2.89
<i>Significance</i>	<i><.0001</i>	<i><.0001</i>	<i><.0001</i>	<i><.0001</i>	<i><.0001</i>

^zTreatment is defined as heated nutrient solution using aquarium heaters or unheated

^y Market Fresh weight is equal to Head Fresh Weight + rootball with 1 inch of root and is reported in grams

^x Head Fresh Weight is reported in grams

^w Size Index is measured as (Height + widest width + width perpendicular)/3 and reports in centimeters

^vDensity= (HFW/ SI at 28 DAT)

Fig. 2.1 Effects of nutrient solution temperature heated to a target temperature of 24°C on market fresh weight (g) and head fresh weight (g) of *Lactuca sativa L.* 'Rex' grown in a NFT system in a 30-d experiment

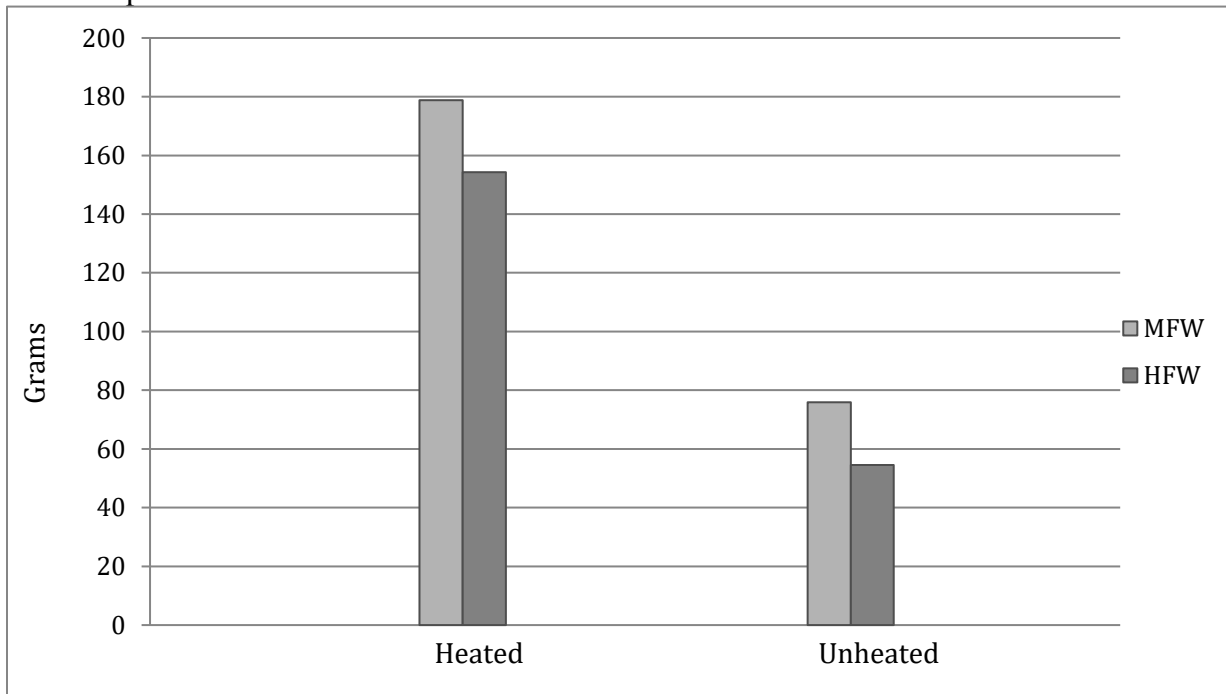


Fig. 2.2 Density of *Lactuca sativa* L. 'Rex' grown in nutrient solution heated to a target temperature of 24°C and non-heated nutrient solution grown in a NFT in a 30-d experiment

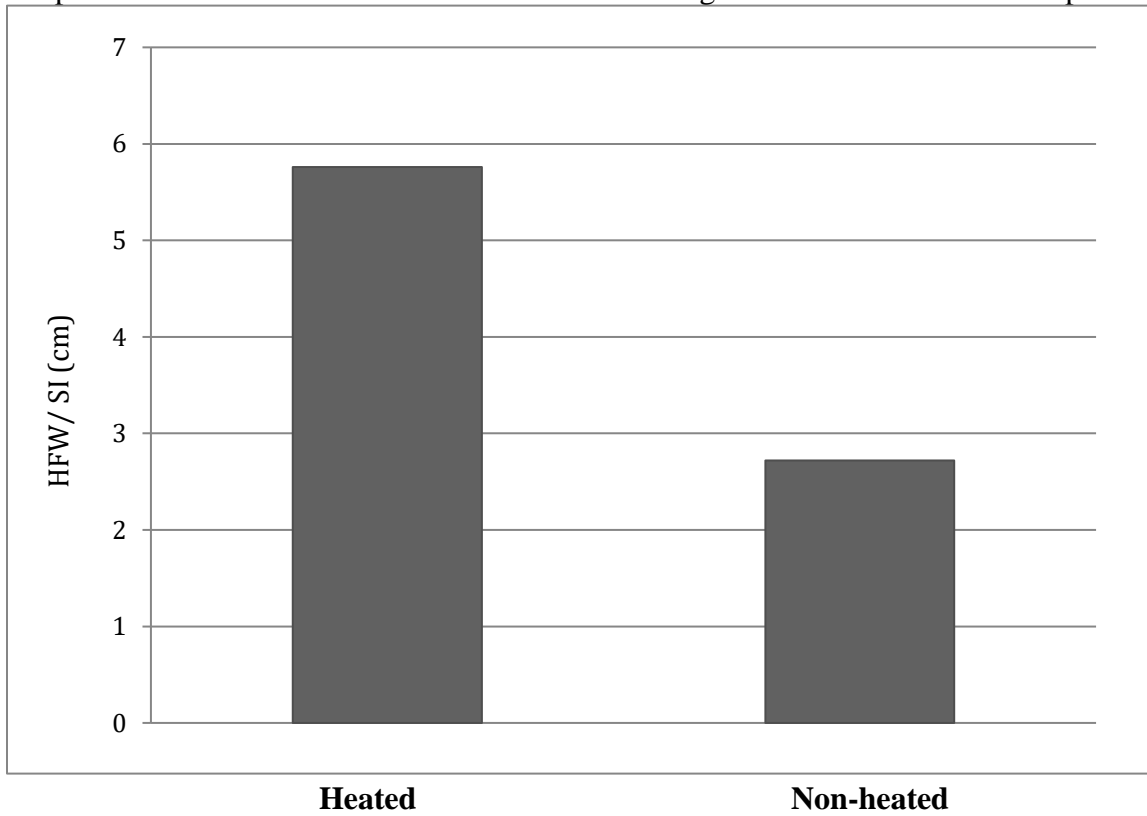
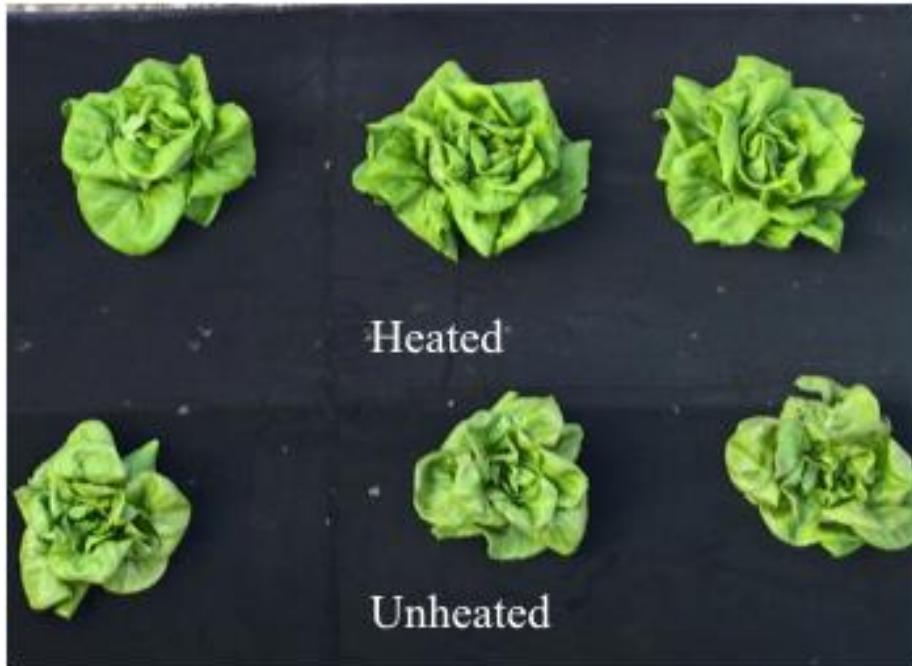


Fig. 2.3 Lettuce (*Lactuca sativa* L. 'Rex') heads grown in a nutrient film technique system in either heated (24°C) or unheated nutrient solution for 30-d



Chapter 3

Effects of Chilling Nutrient Solution on Hydroponic Lettuce Grown in a NFT System

Introduction

Consumer demand for fresh vegetables has increased in recent years, causing a need for increase greenhouse production. Regions such as the southeast, experience high temperatures throughout much of the year. Lettuce is a cool season crop that suffers from physiological problems at above optimal temperatures and may benefit from root-zone cooling in warmer months. Optimizing root zone temperature (RZT) may allow more crops to be grown in areas in which they are not traditionally suited for. Using hydroponics offers many advantages in plant production. The overall control that comes with hydroponics may apply to nutrient solution temperature modification as well.

Greenhouse vegetable production, which is often synonymous with hydroponics in the U.S., offers many advantages over traditional field production. Nutrient solution production allows for a more constant rate of growth, as it eliminates any irregularities due to irrigation (Morgan and O’Haire 1978). Roots are kept in constant contact with the nutrient solution, meaning that water is constantly available to the plants. It also may allow for more precise temperature control of the root-zone. This is important, as it is the plant temperature, not air temperature, that is the regulating factor in plant growth (Nelson 2012). The ability to cool the nutrient solution temperature may help reduce damages, such as tipburn. Increased growth rates caused by high temperatures are a possible cause of tipburn. Though tipburn is caused by a

calcium deficiency in young leaves, the factors affecting tipburn occurrence aren't widely understood (Jenni and Yan 2009; Hartz et al. 2007).

While the effects of root-zone have not been widely studied, there is evidence that root-zone cooling may be beneficial in warmer climates. Illahi and others (2017) reported differences in butterhead lettuce grown in a coir-perlite mixture with nutrient solution cooling. Significant increase in size index and leaf number were reported on butterhead lettuce grown with root-zone cooling as was an increase in incidence of tipburn on those grown under the non-chilled nutrient solution treatment. Effects of nutrient solution cooling have been studied on other crops as well. Increased growth of 'Okame' spinach was reported when nutrient solution temperatures were chilled to between 18 and 26 °C (Lee et al 1995). Above this temperature range, growth slowed and browning of the roots occurred.

Materials and Methods

On 23 June 2017 and 24 August 2017 55 and 110 *Lactuca sativa* L. 'Rex' seeds were sown in 2.54cm x 3.18cm x 3.81cm OASIS[®] horticubes (OASIS[®] Grower Solutions, Kent, Ohio). They were watered to capacity with municipal water, covered in a clear humidity dome, and placed on a greenhouse bench at Paterson Greenhouse Complex in Auburn, AL. After germination, humidity domes were removed and seedlings were fertilized with nutrient solution from municipal water (Auburn, AL) containing 150, 80, 200, 150, and 35 mg L⁻¹ N, P, K, Ca, and Mg, respectively from water-soluble 8N-6.5P-30K (Gramp's Original hydroponic lettuce fertilizer, Ballinger, TX), calcium nitrate (15.5N-0P-0K), and magnesium sulfate (10% Mg).

After two weeks, 50 and 100 seedlings were transplanted to a nutrient film technique (NFT) system (Farmtek Dyersville, IA) comprised of 10, 10 ft troughs and supported on by a 3m x 3m frame. Five troughs each were fed nutrient solution from one of two 132 L sumps (Rubbermaid Atlanta, GA). Sumps were each filled with 95 L of the same nutrient solution used to fertilize seedlings after germination. One fountain pump in each sump pushed nutrient solution through 0.635 cm irrigation line and spaghetti tubing delivered 300 mL min⁻¹ nutrient solution to NFT troughs. Five seedlings were placed in every other space in each trough, and empty spaces were covered with blue painters tape and white duct tape to exclude light and reflect heat.

Sump 1 was wrapped with wall insulation (Johns Mansville R13 insulation). A second pump (Smartpond Premium Pond Pump 330 GPH, Smartpond[®] West Palm Beach Florida) was placed in sump 1 and constantly pumped nutrient solution through a thermostat-controlled chiller (Penguin Chillers ½ HP Water Chiller) located adjacent to the sump on a greenhouse bench. Sump 2 was not or chilled. Air temperature at plant height and nutrient solution temperature in both sumps was recorded once per hour for the duration of the experiment using WatchDog[®] B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL). Nutrient solution pH and EC were measured five times weekly using LAQUA Twin pH Meter and Size index (SI) ($[\text{height} + \text{widest width} + \text{perpendicular width}]/3$) at 15 DAT and 30 DAT.

Though pH was not controlled, water levels were managed in the sump. As EC was measured over 2.0 mcSm, fresh water was added to bring EC back within the target range. As nutrient solution levels dropped below 75 L, fresh nutrient solution was added to bring nutrient solution levels back to 95 L.

At 30 DAT, plants were removed from NFT system, with the original OASIS® rootball intact. Roots were trimmed leaving approximately 2.5 cm of roots below the root cube. Lettuce heads were weighed with rootball and remaining roots attached to determine market fresh weight (MFW). All roots, including the original root cube were removed and heads were weighed to determine head fresh weight (HFW). Head fresh weight was divided by SI to determine head density. All roots, including the original root cube were removed and heads were weighed to determine head fresh weight (HFW). Bags containing root systems were dried for 72 hours in a forced air dryer and weighed to determine root dry weight (RDW). Heads were dried for 7-d in a forced air dryer before being analyzed for nutrient content. After termination, a sunrise/sunset calendar was used to find the sunrise (day) and sunset (night) times for each day of the experiment. Once the night and day times were determined, the temperature data was sorted according to day and night times to find the day and night temperatures.

The experiment was a randomized complete block design with five blocks containing five and ten subsamples in the first and second experimental period, respectively. A block was considered an experimental unit. Size index, MFW, HFW, and head density were subjected to analysis of variance using PROC GLIMMIX and data was corrected for homogeneity. Block and experimental run were treated as random variables. Significance was determined at the $\alpha=0.05$ level.

Results and Discussion

The chilled nutrient solution pH averaged 5.98 and 5.75 for the control. The EC for the chilled nutrient solution averaged 1.74 and 1.8 for the control (Table 3.1). The nutrient solution was chilled to a target temperature of 24°C. Greenhouse air temperature DIF (difference between night and day temperature) was largest at 6.5 with the average day temperature recorded 29.8°C and night temperature 23.3°C. The chilled nutrient solution DIF was smallest at 0.3 with the average day temperature recorded 22.3°C and night 22.0°C and DIF for the control was 1.8 with the day temperature averaging 26.6°C and night temperature 24.8°C.

Lettuce grown in nutrient solution chilled to the target temperature of 24°C had a 14% lower market fresh weight (MFW), 17% lower head fresh weights (HFW) and a 2% higher Size index (SI) than those grown in the non-chilled, control solution (Table 3.6). These differences may be attributed to the lower temperature of the nutrient solution. Market fresh weight and HFW are larger for non-chilled nutrient solution than chilled but the size index was larger for the chilled nutrient solution than the non-chilled nutrient solution. Although SI was higher for the chilled treatment, head density was 18% higher for the non-chilled treatment with the non-chilled average 7.2 and 5.9 for the chilled solution.

Chilling the nutrient solution led to a 24% increase in foliar calcium (Ca) percentage (Table 3.7) Chilling the nutrient solution also increased foliar manganese (Mn), sodium (Na), and zinc (Zn) by 69, 40, and 177% respectively, but decreased foliar iron by 33%. Although differences in nutrient uptake were detected, deficiency symptoms were not observed for manganese or zinc in either treatment, most likely due to the fact that although differences

existed between treatments, foliar concentrations of each of these nutrients were within sufficiency ranges reported by Bryson and others (2014). Foliar concentrations of iron were below the sufficiency ranges for greenhouse produced butterhead lettuce, but were within sufficiency ranges for field produced butterhead lettuce. Deficiency symptoms were not observed. Although no sufficiency range was reported for sodium, uptake of sodium increased as nutrient solution was chilled.

Calcium levels and tipburn occurrence differed across treatments and runs (Table 3.8). In run 1, foliar Ca did not differ between nutrient solution treatment, however tipburn occurrence was significant. Tipburn was not present in run 1 for the chilled nutrient solution, but was present on every plant for the non-chilled solution. Tipburn was quantified according to a modified USDA rating scale where 1= no tipburn, 2 = widest tipburn spot less than 6.4 mm, 3 = widest spot less than 12.7 mm, 4 = widest spot less than 25.5 mm or larger. Average tipburn rating was 2.68 for the non-chilled plants. In run 2, calcium levels were significant across treatments. Average foliar calcium percent was 1.38% for lettuce grown using chilled nutrient solution. There was a 41% decrease in foliar Ca for plants grown in the non-chilled solution. Though there were differences between treatments in foliar calcium percentages, tipburn occurrence was not significant. There was no occurrence of tipburn in plants in either treatment.

This experiment was conducted in a greenhouse, in which the average day temperature was 29°C during the course of the experiment. A shade cloth (55% shade) was placed over the roof of the greenhouse before run 2, decreasing light intensity. Though the MFW and HFW were lower for plants grown using chilled nutrient solution, calcium levels increased and tipburn

incidence decreased. In a similar study, nutrient solution cooling had a significant effect on size index and leaf number on butterhead lettuce grown in a coir-perlite mixture and an increase in incidence of tipburn was reported on those grown with the non-chilled treatment.

Conclusions and Future Research

Lettuce grown in the chilled nutrient solution had lower MFW, HFW, and density than those grown in the control nutrient solution, however; lettuce grown using the chilled nutrient solution had a higher SI. The higher SI and lower density show the lettuce produced a looser head than those grown in the control nutrient solution. This shows that the chilled nutrient solution may slow production times. Lettuce grown in the chilled nutrient solution had an average 45% less tipburn occurrence than those grown in the control solution. While this research shows some possible advantages to chilling nutrient solution, further research is needed to determine its economic and practical viability. Further research should look at the impact of light intensity on the formation of tipburn in butterhead lettuce and the economic viability of chilling the nutrient solution.

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Table 3.1. Effects on pH and EC of chilling nutrient solution to a target temperature of 24C in a recirculating nutrient film technique systems used to grow *Lactuca sativa L.* ‘Rex’ in a 30-d experiment

Nutrient Solution	pH ^z	EC ^y
Chilled	5.98	1.74
Control	5.75	1.8

^z pH was measured using LAQUA pH twin meter

^y EC was measured using LAQUA EC twin meter

Table 3.2. Mean temperatures for nutrient solution chilling in a nutrient film technique system 7 July 2017

Treatment ^z	Target Temperature	Actual Temperature ^y		DIF ^x
		Day	Night	
Chilled	24	18.9	18.3	0.6
Control	Unspecified	28.3	25.9	2.4
Greenhouse ^w	Unspecified	29.2	24.7	4.5

^z Treatment is defined as chilled nutrient solution using penguin chiller or non-chilled

^y Temperature recorded using WatchDog® B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL) and is reported in °C

^x DIF is equal to day temperature – night temperature

^w Greenhouse temperature recorded was the ambient air temperature at plant height

Table 3.3. Mean temperatures for nutrient solution chilling in a nutrient film technique system 7 September 2017

Treatment ^z	Target Temperature	Actual Temperature ^y		DIF ^x
		Day	Night	
Chilled	24	23.9	23.7	0.2
Control	Unspecified	25.4	23.9	1.5
Greenhouse ^w	Unspecified	29	22.5	6.5

^z Treatment is defined as chilled nutrient solution using penguin chiller or non-chilled

^y Temperature recorded using WatchDog® B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL) and is reported in °C

^x DIF is equal to day temperature – night temperature

^w Greenhouse temperature recorded was the ambient air temperature at plant height

Table 3.4. Mean temperatures for nutrient solution chilling in a nutrient film technique system 7 July 2017 and 7 September 2017

Treatment ^z	Target Temperature	Actual Temperature ^y		DIF ^x
		Day	Night	
Chilled	24	22.3	22	0.3
Control	Unspecified	26.6	24.8	1.8
Greenhouse ^w	Unspecified	29.8	23.3	6.5

^z Treatment is defined as chilled nutrient solution using penguin chiller or non-chilled

^y Temperature recorded using WatchDog® B-Series Button Loggers (Spectrum Technologies, Inc. Aurora, IL) and is reported in °C

^x DIF is equal to day temperature – night temperature

^w Greenhouse temperature recorded was the ambient air temperature at plant height

Table 3.5. Effects of nutrient solution cooling to a target temperature of 24°C on size index, market fresh weight, and head fresh weight of *Lactuca sativa* L. ‘Rex’ in a 30-d experiment

Treatment ^z	MFW ^y	HFW ^x	SI ^w		RDW ^v	Density ^u
			21DAT	28DAT		
Chilled	129.9	113.1	21.6	19.14	0.333	5.91
Non-chilled	151.4	135.6	19	18.79	0.356	7.217
<i>Significance</i>	<i>0.0207</i>	<i>0.0143</i>	<i>NS</i>	<i>NS</i>	<i><0.001</i>	<i><0.001</i>

^z Treatment is defined as Chilled or Non-chilled

^y Market Fresh weight is equal to Head Fresh Weight + rootball with 1 inch of root and is reported in grams

^x Head Fresh Weight is reported in grams

^w Size Index is measured as (Height + Width 1+Width 2)/3 in centimeters

^v Root Dry Weight is the roots – (rootball+ 1 inch of root) and is reported in grams

^u Density= (HFW/ 28 DAP SI)

Table 3.6. Effects of chilling nutrient solution to a target temperature of 24°C on foliar calcium, iron, manganese, sodium, and zinc on *Lactuca sativa* L. ‘Rex’ grown in a nutrient film technique system in a 30-d experiment

Treatment ^z	Calcium ^y (%)	Iron ppm	Manganese	Sodium ^x	Zinc
Chilled	1.23	64.91	283.44	1682.07	212.49
Non-chilled	0.99	97.11	167.81	1200.94	76.71
<i>Significance</i>	<i>0.0018</i>	<i>0.0018</i>	<i><.0001</i>	<i><.0001</i>	<i><.0001</i>
Sufficiency Range ^w	0.80-1.20	168-223	55-110	-	33-196

^z Treatment is defined as chilled nutrient solution using penguin chiller or non-chilled

^yNutrients were analyzed using ICP analysis

^xNo sufficiency range is given for sodium as it is not an essential element

^w Reported ranges from Jones , J. B., Jr. , G. M Bryson, H. A. Mills, and D. N. Sasseville. 2014. Plant Analysis Handbook III: a guide to sampling, preparation, analysis and interpretation for agronomic and horticultural crops. Athens, GA: Micro-Macro Publishing.

Table 3.7. Effects of chilling nutrient solution to a target temperature of 24°C on foliar calcium and tipburn occurrence in *Lactuca sativa* L. Rex grown in a nutrient film technique system in a 30-d experiment

Treatment ^z	7-Jul-17		7-Aug-17	
	Calcium(%) ^y	Tipburn ^w	Calcium(%)	Tipburn
Chilled	0.89	1	1.38	1
Non-chilled	0.89	2.68	0.81	1
<i>Significance</i>	<i>NS</i>	<i><0.0001</i>	<i><0.0001</i>	<i>NS</i>

^z Treatment is defined as chilled nutrient solution using penguin chiller or non-chilled

^y Calcium levels are presented in foliar percentages

^x Tipburn is measured according to a modified version of the USDA Lettuce Statistics in which 1= no tipburn,

2 = widest tipburn spot less than 6.4 mm, 3 = widest spot less than 12.7 mm, 4 = widest spot less than 25.5 mm or larger.

Fig. 3.1. Effects of nutrient solution chilled to a target temperature of 24°C on market fresh weight (g) and head fresh weight (g) of *Lactuca sativa* L. 'Rex' grown in a nutrient film technique system in a 30-d experiment

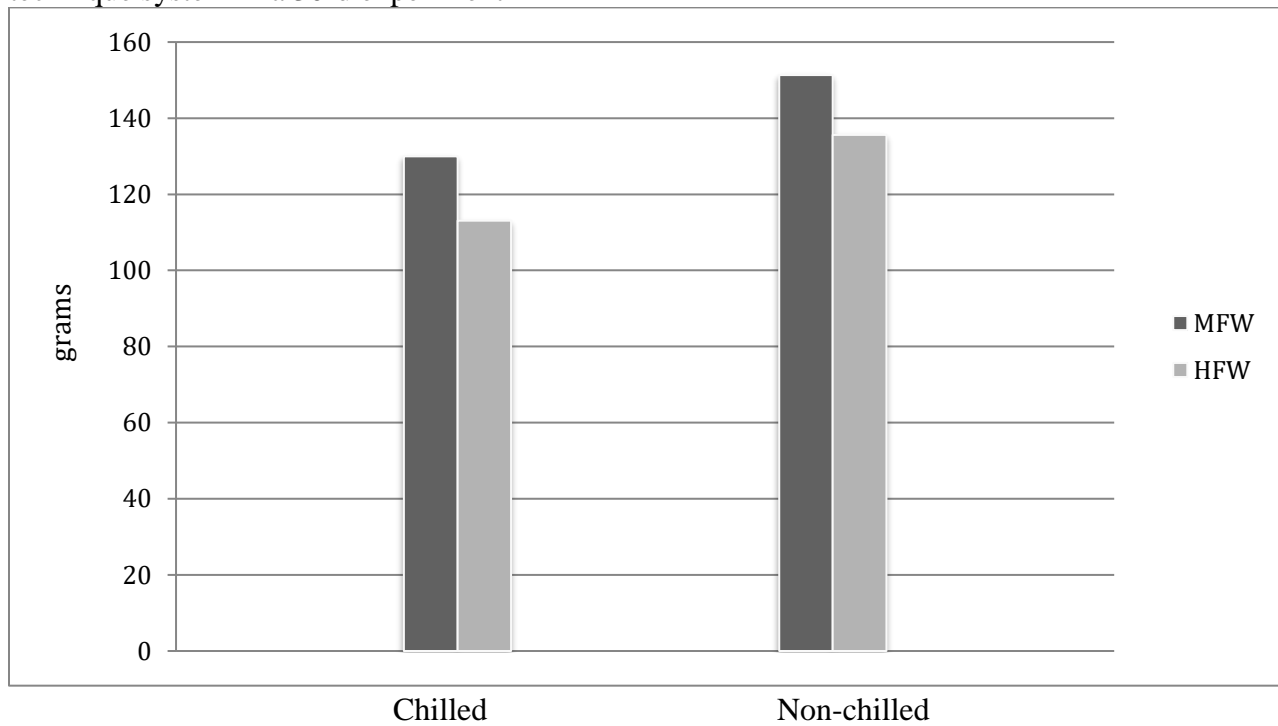


Fig. 3.2. Effects of nutrient solution chilled to a target temperature of 24°C on root dry weight (g) of *Lactuca sativa L.* 'Rex' grown in a nutrient film technique system in a 30-d experiment

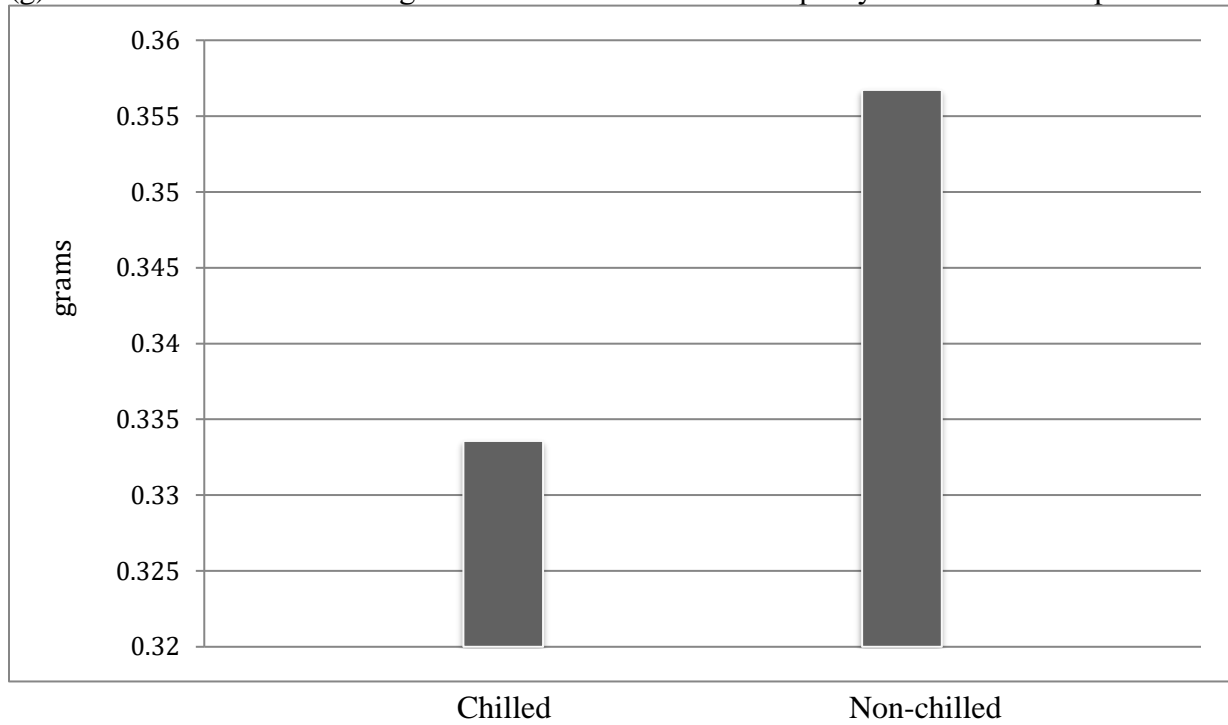


Fig 3.3. Effects of nutrient solution chilled to a target temperature of 24°C on density of *Lactuca sativa L.* 'Rex' grown in a nutrient film technique system in a 30-d experiment

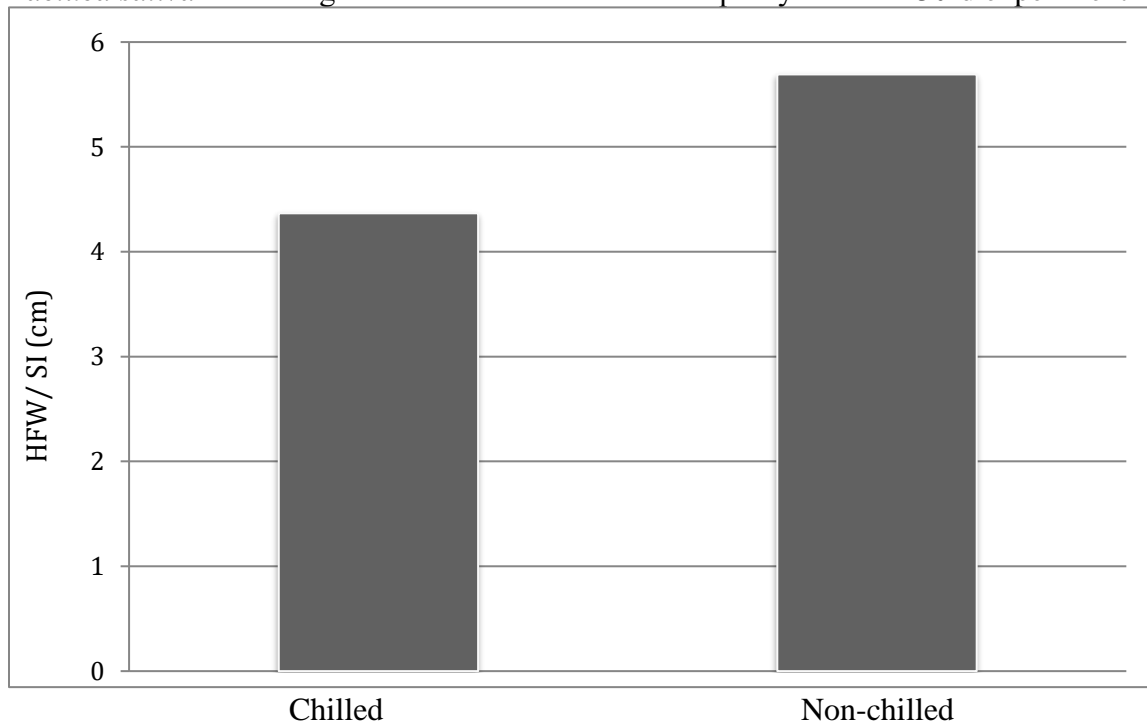


Fig. 3.4. Tipburn present in *Lactuca sativa* L. 'Rex' grown in non-chilled nutrient solution in a nutrient film technique system in a 30-d experiment



Fig. 3.5. Lettuce (*Lactuca sativa* L. 'Rex') heads grown in nutrient solution chilled to a target temperature of 24°C (top) and non-chilled nutrient solution (bottom) grown in a nutrient film technique system in a 30-d experiment

