Improving Romaine Lettuce Production in Greenhouse Hydroponic Systems

By

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List of Abbreviations

NFT	Nutrient Film Technique
DWC	Deep Water Culture
HFW	Head Fresh Weight
MFW	Market Fresh Weight
RDW	Root Dry Weight
DAT	Day after Transplant
SI	Size Index
GA	Gallic Acid
ppm	parts per million
g	gram
h	hour
L	Liters
°C	Degrees Celsius

Chapter I

Literature Review

Rapid increases in worldwide population along with depletion and contamination of limited natural resources has created demand for more effective and intensive food production. Greenhouse production allows greater control of external environmental conditions such as water, light, temperature, and nutrient controls compared to conventional field production. Hydroponics, generally known as a greenhouse production technology, is a technique of growing plants without soil by placing plants in nutrient solution (Jones, 2005; Resh, 2004).

U.S. lettuce production

Lettuce is a cool season vegetable produced throughout the United States, with California and Arizona being the two major production states of leaf and head lettuce. Lettuce is the second most popular vegetable crop in the U.S., behind only potatoes (USDA-ERS, 2017). 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). There are five common lettuce types including Summer Crisp, Butterhead, Cos or Romaine, Leaf, and Crisphead (Ryder, 1999). Field production of lettuce is restricted to a short window of time during the late fall to early spring in the southern U. S. because lettuce is susceptible to a number of temperature-related physiological effects including tipburn, loose head, leaf discoloration and bolting as well as diseases susceptibility. Therefore, greenhouse production can play an important role in growing lettuce through manipulating environmental conditions including temperature, light, and nutrients.

Greenhouse lettuce production

A greenhouse can be defined as “a structure designed for the cultivation of plants to protect against extreme environmental conditions and/or pests (Hickman, 2016).” Greenhouse lettuce production was nearly 1,500 acres in the U.S. in 2013 (Hickman, 2016). Greenhouse production has many advantages over field production such as saving water, saving nutrients, flexibility of growing locations, reducing soil-borne diseases, reducing pesticides and so on. However, one limitation to greenhouse production is increased production cost when compared to conventional production. More energy inputs and costs are needed, especially for cooling and heating costs in adverse weather conditions. Barbosa et al., 2015 found that greenhouse production was 11 ± 1.7 times higher yields but required 82 ± 11 energy costs than traditional production. Even in controlled environmental conditions, growth challenges occur frequently in an unfavorable micro environment which can cost enormous economic loss for growers. For example, high temperature and humidity in the Southeastern U.S. promote tipburn and bolting, two common physiological disorders of greenhouse lettuce, which ruin lettuce market value. Customers would prefer healthier, fresher, and better-tasting lettuce. Growers often have trouble with tipburn and bolting when growing lettuce in greenhouse hydroponic systems in the Southeastern U.S. Greenhouse lettuce now plays an essential role on worldwide lettuce production. Thus finding a way to mitigate tipburn and bolting for greenhouse hydroponics production is a research priority.

Nutrient film techniques and deep water culture system

Greenhouses have enhanced growing certain leafy crops using Nutrient Film Technique (NFT) and Deep Water Culture (DWC) and among others. The NFT system was developed during the late 1960’s by Dr. Allan Cooper at the Glasshouse Crops Research Institute in the U.K. The NFT system uses a thin film of re-circulated water typically flowing down a narrow channel, with plant roots partially in the water film (Morgan, 2003). The Deep Water Culture system is also called floating system which grow plants on floating foam rafts where root grow into the solutions below to absorb nutirents and water. EC and pH are required to be monitored and kept at a certain level for hydroponics systems. The NFT and Deep Water Culture systems are a very popular commercial production systems which do not often perform well in hot summers common to the Southeastern U.S. To improve crop selections and cultural improvements are needed in order to improve production potential in the region.

Lettuce physiological disorders (tipburn and bolting)

Tipburn reduces marketability and in some cases renders lettuce unsellable. It is a common physiological disorder of lettuce, possibly caused by various environmental factors, in field production and greenhouse hydroponics and aeroponics (Holmes et al., 2019). Tipburn is induced by a calcium ion deficiency in the youngest developing leaves of lettuce but is most commonly caused by sub-optimal environmental factors. Calcium is essential for cell membrane and cell wall construction (Saure, 1998). Lack of calcium in the youngest leaves causes membrane failure and cytoplasm leakage leading to tipburn symptoms (Lim and White, 2005). Tipburn is considered a complex physiological disorder controlled by several genes. Modern biological techniques are in progress to better understand the molecular mechanisms (Unoa et al., 2016). Saure (1998) reported that gibberellins (GA) increases the permeability of the cell membrane, leading to tipburn. These endogenous factors inducing tipburn are still in progress in research. External environmental cultural practices greatly influence tipburn. Temperature, light intensity, humidity, nutrient solution concentration, and air flow are possible contributors to tipburn (Swaef et al., 2015). High light intensity and extended photoperiod increase the incidence and severity of tipburn (Saure, 1998; Sago 2016). Previous research has proved that night-time fogging increases humidity that can decrease tipburn but direct application of calcium on leaves does not mitigate tipburn (Corriveau et al., 2012).

Bolting is another physiological disorder associated with temperature stress. It is the transition from the vegetative stage to the reproductive stage with rapid stem elongation and followed by flowering. If the crop is exposed to high temperatures, it grows faster and flowers earlier, but bitter flavors can accumulate (Tudela et al., 2017). High temperature stress upregulated gene *LsGA3ox1* responsible for increasing GA synthesis which promote lettuce bolting (Fukuda et al., 2009). It is significant to mitigate bolting by selecting heat enduring cultivars and applicable cultural practices.

Root-zone solution cooling

Lettuce is a temperate crop, with an optimal temperature ranging from 15-20°C (Qin et al., 2002). Studies have shown that limiting photosynthesis under a high root-zone temperature may cause stomatal closure, reducing carbon dioxide in the intercellular space and chloroplast (He et al., 2001). Stomatal closure causes water deficiency because high root-zone temperature changes the balance of root water uptake and shoot water loss. On the other hand, a poorly formed root system may limit water uptake, which can cause less chlorophyll formation and limits photosynthesis. Crop nutrient deficiencies frequently occur under high temperature conditions (He et al., 2001). In order to overcome heat-related stress, root cooling technology has been used for hydroponic lettuce. Root-zone cooling increases photosynthesis rates and productivity of lettuce plants by 4–5 fold compared to plants growing in warmer temperatures, as cooling prevents photoinhibitory damage (Choong et al., 2013). Thompson and Longans (1998) also found that high temperatures influence the photosynthetic function and the thylakoid membranes. Wolfe (1991) observed a reduction in leaf area for many crops when grown in cooler environments. It is observed that physiological disorders such as tipburn and bolting increase when the temperature is not optimal. In addition, cooler temperature may reduce the incidence of pathogens (Morgan, 2003). Jensen and Malter (1995) also found that a cooler environment dramatically reduced bolting and the incidence of fungus *Pythium aphanidermatum.* It's more expensive to cool the greenhouse ambient temperature than cool down plant root-zone solution temperature. Root-zone cooling may help alleviate temperature associated physiological disorders and diseases.

Increasing horizontal air flow

Tipburn is a severe problem which has been reported to cause nearly 50% loss in greenhouse hydroponics systems (Vanhassel et al., 2015).Ccalcium deficiency is attributed to lettuce tipburn. calcium is an immobile xylem transport macronutrient that is known to contribute to cellular structural integrity. Physiologically, calcium deficiency readily occurs due to limitation of apoplastic pathway, which is highly dominated by the low transpiration rate of young leaves (White and Broadley, 2003). Studies showed that in the plant factory increasing horizontal air flow can decrease tipburn but decreasing air temperature had no effect on tipburn incidence and total tipburn leaves (Lee et al., 2013). Cox and Dearman, 1981 reported that calcium ion in the water absorbed can be transported to non-transpiration leaves in cabbage and strawberry by root-pressure flow. Horizontal air flow can increase the transpiration rate which may increase the calcium concentration in inner leaves by making calcium more evenly distributed. On the other hand, increasing horizontal air flow may decrease the air temperature on the micro-area top of the plants, and this may benefit growth.

Lettuce antioxidant contents

Romaine lettuce is a very popular type of lettuce which contains healthy compounds with high nutritional value. Mineral nutrients and bioactive compounds, such as vitamin C, total phenolic content, and carotenoids, have significant benefits in human health. Previous studies have reported the antioxidant activities and phenolic content components in lettuce (Kim, et al., 2016). Romaine lettuce is reported to be relatively rich in potassium, sodium, and iron. Green romaine lettuce is also a good source of vitamin C that is essential for the immunization and antioxidant functions (Altunkaya et al., 2009). Flavonoids and phenolic contents are sensitive to environmental factors. Reactive Oxygen Species (ROS) production usually increases with biotic and abiotic stress. Previous study showed that removing calcium from leaves exacerbated tipburn symptoms, increased superoxide presence and oxidative damage, and increased SOD activity (Carassay et al., 2012). In addition, less tipburn happens to red leaf lettuce than green lettuce. Thus, concentration of phenolic content in tipburn developmental processes are significant to be analyzed. Moreover, the physiological relationship between antioxidants and calcium deficiency are essential to investigate. Phenolic compounds, carotenoids as well as flavonoids have been shown to protect against oxidative stress, inflammation, diabetes, and cardiovascular diseases (Kim, et al., 2016). Previous studies indicated that total phenolic compounds and antioxidant contents relate closely to genetics and cultural practices (Liu et al., 2007). Red leaf lettuce usually contains more phenolic content and flavonoids than green leaf lettuce (Perez-Lopez et al., 2018, Liu et al., 2007,). Outer leaves have more phenolic contents than inner leaves possibly because of positive impact of light intensity. Lettuce grown in outdoor fields have more flavanol compounds than lettuce grown in tunnels due to the higher solar radiation capacity (Liu et al., 2007, Baslam et al., 2013). High temperature and cooling stress often increase the antioxidant activity. Therefore, the oxidative contents in lettuce leaves are supposed to increase. On the other hand, under chill and heat stress, cultivars produce different amount of secondary metabolite contents.

Research Objectives

High temperature and humidity cause many problems in greenhouse Romaine lettuce production in the Southeastern U.S. It is important to select the best cultivars that can perform well in the hot summer time. Objective #1 is to select high-performing Romaine lettuce cultivars when using root-zone nutrient solution cooling in greenhouse nutrient film technique. Objective #2 is to increase horizontal air flow to eliminate physiology diseases like tip burn and bolting. Objective #3 is to know the postharvest nutritional value of different cultivars and find some healthy, popular and marketable cultivars for greenhouse production in the southeastern U.S.

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Chapter II

Effects of Root-zone Cooling on Growth and Quality of Romaine Lettuce Cultivars Grown in a Nutrient Film Technique System

Abstract

Due to the high temperature and humidity in the Southeastern U.S. in summer, lettuce grows too fast in the greenhouse hydroponic system. Tipburn and bolting occur severely, damaging lettuce market value. Previous studies have been concentrating on alleviating lettuce tipburn and bolting but have not made great improvements. Root-zone solution cooling to 18°C was investigated to slow down lettuce growth. Six Romaine lettuce cultivars and tipburn sensitive ‘Bambi’ lettuce were grown in the greenhouse Nutrient Film Technique system. Size index was measured at 7 DAT, 14DAT, 21DAT and 30 DAT. Head fresh weight and dry weight were measured at 30DAT. Chlorophyll content, tipburn and bolting incidence were recorded. The results showed that Root-zone cooling significantly slowed down growth and reduced head fresh weight except for ‘Bambi’ lettuce. Root-zone cooling had no influence on tipburn but alleviated bolting. Cultivar ‘Dragoon’ and ‘Breen’ were less susceptible to bolting but ‘Intred’ and ‘Bambi’ were more susceptible to bolting. Cultivar, inner and outer leaf locations and root-zone solution temperature all affected lettuce total phenolic content contents. In conclusion, root-zone cooling significantly slowed down lettuce growth. Tipburn can’t be mitigated by root-zone solution cooling but bolting can be alleviated in the Southeastern U.S. Lettuce total phenolic content is determined by genetic background and stressful environmental conditions.

Introduction

Lettuce is a cool season vegetable typically grown in low temperatures and low light (Frantz et al., 2004). According to the USDA-ERS, 2017, lettuce is the second most popular vegetable in the U.S., behind only potato. Greenhouse lettuce production was nearly 1,500 acres in the U.S. in 2013 (Hickman, 2016), the vast majority of which was grown hydroponically. Especially, in the Southeast U.S., small hydroponic businesses are growing rapidly. However, greenhouse production relies on a good integration of multiple factors including temperature, light, humidity, CO_2, and fertility. Typically, in summer, temperature and humidity are high in greenhouses. Lettuce is susceptible to physiological disorders such as tipburn and bolting which lead to significant yield reduction and economic loss. Tipburn is a physiological disorder which often occurs near harvest and results in necrosis of young growing leaf margins (Uno et al., 2016). According to previous tipburn prevention studies, several environmental and physiological factors such as light intensity, photoperiod, temperature, relative humidity, growth rate, and cultivar are associated with tipburn development (Sago et al., 2016; Frantz et al., 2004; Saure, 1998). Since the effects of these multiple environmental factors are not independent, not a single pure cause has been found responsible for tipburn. Wissemeier and Zuhlke, (2002) found that rapid plant growth led to decreased calcium concentrations in leaves and an increased risk of tipburn. Slowing lettuce growth rate to let calcium efficiently be transported to growing points may be one way to mitigate tipburn. Root-zone cooling may be an effective way to slow lettuce growth. On the other hand, under environmental stresses, plants generated reactive oxygen species which can be decreased by plant secondary metabolites. Researcher found that low temperature help plants accumulate more antioxidants (Kalisz et al., 2016). These antioxidants are supposed to be important in preventing physiological disorders like tipburn. Bolting is another physiological disorder with rapid stem elongation and followed by flowering. If the crop is exposed to high temperatures, it grows faster and flowers earlier and bitter flavors can accumulate (Tudela et al., 2017). Plant antioxidants such as total phenolic content and flavonoids are important in improving the quality of fruit and vegetable and stress resistance. Increase in the total concentration of phenols in various species could help to reduce

ROS (Edreva, 2005; Oh et al., 2009; Samuolienė et al., 2012). Flavonoids and phenolic and other antioxidants can improve the quality of vegetables (Oh et al., 2009; Samuolienė et al., 2012; Sgherri et al., 2017).

Materials and Methods

Six Romaine lettuce (*Lactuca sativa* L.) cultivars 'Dragoon', 'Breen', 'Truchas', 'Intred', 'Thinker', and 'Newham', and one Bibb lettuce cultivar, Bambi' seeds were selected based on customer and growers' preferences then purchased from Johnny's Seeds (Johnny's Selected Seeds, Winslow, ME). On 14 March 2019, 8 April 2019, and 11 May 2019, lettuce seeds were sown and grown for two weeks in OASIS® Horticubes (OASIS® Grower Solutions, Kent, Ohio) (2.54 cm ×3.18 cm × 3.81 cm) on a greenhouse bench in Auburn, AL. Seedlings were fertilized with 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) daily for two weeks before being transplanted to NFT system (NFT 8-4, Cropking Lodi, OH) Eight troughs were supplied with nutrient solution from a 102-L cooled sump (BLUE HAWK™ Atlanta, GA). Eight troughs were supplied with nutrient solution from a 102-L non-cooled sump (BLUE HAWK™ Atlanta, GA). One fountain pump in each sump supplied nutrient solution through a 0.6-cm irrigation line delivering 300 $mL\cdot min^{-1}$ nutrient solution to the NFT troughs. Seven seedlings were placed in every trough (2.4 m x 18 cm) and were spaced 20 cm center-to-center. Six Romaine lettuce (*Lactuca sativa* L.) cultivars 'Dragoon', 'Breen', 'Truchas', 'Intred', 'Thinker', and 'Newham', and one Bibb lettuce cultivar, 'Bambi' were randomly assigned to each trough. Nutrient solution in one sump was chilled to 18 °C, and the other sump was not chilled and was allowed to equilibrate with the ambient temperature inside the greenhouse. Second pump (Smartpond Premium Pond Pump 330 GPH, Smartpond® West

Palm Beach, Florida) was placed in chilled sump which constantly supplied nutrient solution to a thermostat-controlled chiller (Penguin Chillers ½ HP Water Chiller, Temptek®, Greenwood, IN). Located adjacent to the sump on a greenhouse bench. Air temperature at plant height and in the nutrient solution in all sumps was recorded once per hour for the duration of the experiment using temperature data loggers (HOBO®, Onset Computer Corporation, Burlington, VT). Nutrient solution pH and EC were measured daily using HANNA® Instruments Model HI 9813-6 pH and EC meter (City, State) and, size index (SI) ([height + widest width + perpendicular width]/3) was recorded weekly. When nutrient solution levels decreased / declined below half level and EC was above 2.0 mS/cm, fresh nutrient solution was added to restore nutrient solution levels t. At 30 DAT, plants were removed from the NFT system with the original OASIS® (ASIS® Grower Solutions, Kent, Ohio) root ball intact. Roots were trimmed leaving approximately 2.5 cm of roots below the root cube. All roots, including the original root cube, were removed and heads were weighed to determine head fresh weight (HFW). The experiment was a randomized split plot design. The whole plot factor was nutrient solution temperature while the subplot factor was cultivar. Each experimental unit was a trough. A unit was repeated 8 times at the same greenhouse. Three experimental runs were conducted from 18 March 2019 to 22 June 2019. At 7 DAT, 14 DAT, 21 DAT and 30 DAT, lettuce size index (SI) was recorded. At 30 DAT, lettuce head fresh weight (HFW) was recorded. Three random samples from each trough were selected to measure leaf temperature using the infrared thermometer (VWR® 36934−178, Vernon Hills, IL) Chlorophyll Meter (Minolta® SPAD 502, Strong, ME) was used to measure chlorophyll.

Three samples of each treatment and cultivar were dried in a heating oven (Grieve® SC-350) at 75.5 °C for three days and weighed to determine head dry weight (HDW).

Tipburn of 30 DAT mature lettuce was evaluated with a rating scale. Ratings of light, moderate, and severe indicated that tipburn occurred on fewer than five leaves, between 5 and 10 leaves, and more than ten leaves, respectively. Counts of each rating were recorded and analyzed in SAS program using multinomial probability distribution. Bolting of 30 DAT mature lettuce was evaluated with bolted and unbolted according to stem elongation. Obvious stem elongation was recorded as bolted and not obvious stem elongation was recorded as unbolted. Counts were recorded and analyzed in SAS program using multinomial probability distribution.

Samples utilized for total phenolic assay was harvested after 30 DAT. All lettuce samples were divided into inner and outer parts, frozen by liquid nitrogen, packed with paper bags and kept in -80°C refrigerator. Following storage in -80ºC samples were immediately freeze dried in order to enhance phenolic analysis and determination. Preliminary trials revealed total phenolic extraction process was quantitatively more efficient following lyophilization and compared to fresh frozen samples. In addition, final dry weight expression was consistently higher than fresh weight determinations and similar to reported values (Baslam et al., 2013; Lopez et al., 2014; Liu et al., 2013). Harvest Right freeze dryer (Harvest Right® scientific freeze dryer, medium size, Salt Lake City, Utah) was used to freeze dry all lettuce samples. For each of the three runs totaling eighty-four samples including outer and inner leaves of three samples of each seven cultivars for cooling and non-cooling treatments (2 locations * 7 cultivars * 2 treatments * 3 replicates = 84 samples) were freeze dried. Freeze dry instructions were followed by the harvest right freeze dryer manual. Dry samples were sealed in Ziploc bags and stored and kept from moisture for future analyzing.

Total phenolic compounds were extracted according to Wiecznska and Cavoski (2018). Freeze-dried lettuce 0.25g sample was extracted with 20ml of methanol: water: acetic acid (85: 15: 0.5) solvent. The solution was vortexed for 30 s and sonicated for 5 min, then incubated at room temperature for 20 min and vortexed again for another 30s. The tubes were centrifuged at 10000 rpm for 10 minutes. Supernatants were decanted and stored at -80°C for further analysis.

Total phenolic contents were assayed according to Folin-Ciocalteu method (Singleton & Rossi,1965). Each 40µl extract was added to 200µl Folin-Ciocalteu reagent and added 1.5ml HPLC grade milli-Q water. Tubes were vortexed and incubated for 2.0 minutes at room temperature. 160 µl of 7.5% sodium bicarbonate solution was added and incubated samples at 40°C water bath for 30 minutes. Absorbance of samples were read at 765nm against a blank (0 µl GA standard).

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was completely randomized and experimental runs (months) were treated as a random variable. For 30 DAT SI, HFW, chlorophyll, dry weight and tip burn, the treatment design was 2-way with cultivar and treatment. Tip burn was analyzed using the multinomial probability distribution. Least square means comparisons among cultivars was determined using the simulated method. Differences between treatments were determined using F-tests. The experimental design for size index was 3-way with cultivar, treatment, and days after treatment (DAT). Linear and quadratic trends were examined over DAT using qualitative-quantitative model regressions. The treatment design for concentration (mg/100gDW) was 3-way with treatment, cultivar, and location. All significances were at $\alpha=0.05$.

Results

Root-zone Solution temperature

Nutrient solution temperature for the cooled nutrient solution treatment (NS_C) was relatively constant at 18°C for 30 days (Figure 2.1), while nutrient solution temperature for the not-cooled nutrient solution treatment (NS_{NC}) was variable from 19°C to 30 °C (Figure 2.2).

Plant temperature

Lettuce leaf temperature showed no significant difference based on nutrient solution temperature treatment (NS_T) (P=0.0526, Table 2.6) but was different for each experimental run (From March to June, P<0.001, Table 2.6).

Growth (Size Index, Head Fresh Weight, Head Dry Weight)

A significant quadratic trend was observed for size index and time for all cultivars except for cultivar ‘Thinker’ (P<0.0001, Table 2.5) in which lettuce grew slowly in the first 14 DAT, then faster from 14 DAT to 21 DAT and turned to slower from 21 DAT to 30 DAT. Significant linear trend was found between size index and time for cultivar ‘Thinker’(P<0.0001). Lettuce grown in NS_C were smaller than those grown in NS_{NC} (Table 2.4). Cultivars ‘Dragoon’, ‘Newham’ and ‘Bambi’ had the largest size index at 7DAT and 14DAT, and cultivar ‘Newham’ had the largest size index at 21DAT and 30DAT. Cultivar ‘Intred’ had the smallest size index at 21DAT and 30DAT.

At 30 DAT, there was a significant interaction between cultivar and nutrient solution temperature treatment (NS_T) for size index (P=0.0002, Table 2.1). For cultivar ‘Thinker’, NS_C led to a lower size index (22.3) than non-cooled nutrient solution treatment (NS_{NC}) (24.2), but for cultivar ‘Bambi’, NS_C led to a higher size index (22.3) than NS_{NC} (20.5). For both NS_C and NS_{NC}, cultivar ‘Newham’ had the highest size index, while cultivars ‘Intred’ and ‘Bambi’ had the lowest size index.

There was also significant interaction between cultivar and NS_T for head fresh weight (HFW) (P=0.0006, table 2.1) in which ‘Bambi’ was the only cultivar whose HFW was not influenced by NS_T. All other cultivars had higher HFW in the NS_{NC} treatment than in the NS_C treatment. Cultivars ‘Dragoon’ and ‘Newham’ had the highest HFW, while cultivars ‘Breen’, ‘Truchas’, and ‘Intred’ had the lowest HFW.

Head dry weight (HDW) was lower for all cultivars grown in NS_C compared to NS_{NC} (P=0.0123, Table 2.7). Cultivars ‘Newham’ and ‘Dragoon’ had the highest HDW under NS_{NC} treatment at 7.0g and

6.4g, respectively, while cultivars 'Breen', 'Truchas', and 'Intred' had the lowest HDW of 3.4g, 3.7g and 3.1g, respectively (Table 2.2).

Chlorophyll

There was a significant interaction of chlorophyll between cultivars and NS_T. For red leaf cultivar 'Truchas', NS_C increased leaf chlorophyll compared to NS_{NC}. However, for green leaf cultivar 'Newham', NS_C decreased leaf chlorophyll compared to NS_{NC}. For the cultivars that were not significantly influenced by NS_T, 'Dragoon'(green), 'Newham'(green), 'Intred'(red), and 'Bambi'(green) had higher leaf chlorophyll than the other cultivars (Table 2.2).

Total Phenolic content

There was a three-way interaction (P=0.0028) of total phenolic content content among cultivar, leaf location and root-zone solution temperature. 'Breen' 'Truchas' and 'Intred' lettuce outer leaves of non-cooling root-zone solution temperature contained higher total phenolic content when compared to other cultivars within leaf locations and root-zone solution temperatures.

Tipburn

There was no difference of tipburn incidence between cooling and non-cooling root-zone solution temperature. When harvesting, tipburn sensitive lettuce 'Bambi' was observed tipburn at the same rate level (Figure 2.4)

Bolting

There was no plant bolted in the first two runs when greenhouse temperature is lower. Plants started bolting in May. There was a significant difference (P=0.0047) of bolting incidence between cooling and non-cooling root-zone solution temperature. When cooling the root-zone solution, all eight plants were all not bolted. But when root-zone solution was not cooled, two plants were bolted, and six plants were not bolted (Table 2.4). Study showed that stem extension was positively correlated with high temperature. We observed that ‘Bambi’ and ‘Intred’ lettuce were bolted in non-cooling root zone solution but not bolted in cooling root-zone solution (Figure 2.7).

Discussion

Shoot fresh weight of root-zone cooling to 20°C was 3.5x higher than ambient temperature in the results of Qin et al., 2002. Root-zone temperature at 24°C had higher dry mass than 17°C and 31°C (Thompson et al., 1998). Ilahi el al. (2017) found root zone cooling significantly increased shoot dry weight. Sun el al. 2016 also found root-zone cooling increased plant shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, total plant fresh weight and total plant dry weight. Our result was different that root-zone cooling decreased fresh weight probably because the total root-zone cooling time is longer and the ambient air temperature is higher.

Even the P-value (P=0.0526) is not statistically significant for plant temperature of NS_{C} and NS_{NC}. There was a potential trend to be significant. It was obvious that leaf temperature was more dependent on greenhouse ambient air temperature rather than NS_{T}. Plant temperature increased with ambient air temperature from March to June. A-RZT chlorophyll content was lower than root-zone cooling solution (He et al., 2001). Our results like ‘Truchas’ lettuce chlorophyll content was increased by NS_{C}. It is more determinant by cultivars in our study since most cultivars showed no changes of chlorophyll under NS_{C} and ‘Newham’ lettuce decreased chlorophyll.

Study showed that stem extension was positively correlated with high temperature. Lettuce shoot fresh weight was 46% higher when air temperature was constant at 13°C than those grown at 25°C (Al-said et al., 2018). Our results corresponded with this study that NS_{C} mitigated bolting in June. Bolting was not observed with NS_{C} and NS_{NC} when temperature was lower in March and April, both NS_{C} and NS_{NC} lettuce were not bolted. Tipburn was determined by various environmental factors such as temperature, light intensity, humidity and so on. Lee et al. (2013) demonstrated that decreasing temperature had no influence on tipburn incidence. Our results supported it that there was no tipburn differences observed from sensitive lettuce 'Bambi' of NS_C and NS_{NC}.

Phenolic compounds and carotenoids are reported to protect against oxidative stress, inflammation, diabetes, and cardiovascular diseases (Kim, et al., 2016). Our results indicate that total phenolic content was affected by selected cultivar, NS_T, and leaf location. Baslam et al. (2013) reported that lettuce phenolic content is dependent on lettuce types and cultivar. 'Maravilla' inner leaves contained higher phenolic content when compared outer leaves. 'Cogollos' and 'Batavia' showed no differences of inner and outer leaves for phenolic content. Lopez et al. (2014) reported that Romaine lettuce contained higher phenolic content when compared to Little Gem lettuce. Liu et al., (2007) also reported that total phenolic content is influenced by cultivar choice, color and maturity at harvest. Our total phenolic results are in agreement coincide concerning red leaf lettuce contain higher total phenolic content when compared to green leaf lettuce with previous(Baslam et al., 2013; Lopez et al., 2014; Liu et al., 2007) reports that red leaf lettuce had higher phenolic content than green leaf lettuce. In addition, outer leaf lettuce tipically have had higher phenolic content than inner leaf lettuce and NS_C had higher phenolic content than NS_{NC}. Tipburn leaves consumed more total phenolic content than no tipburn leaves for higher antioxidant activities. It is the same that lettuce in NS_{NC} had more antioxidant activities which used more total phenolic content. On the other hand, lettuce harvest in March had lower phenolic content than in June which indicated that phenolic content was also determined by environment conditions such as light intensity, humidity and so on. It is reported that the highest temperature and light intensity of the day had the highest total phenolic content to protect against the ROS (Wang and Zheng, 2001). If high temperature last for a long time, lettuce in the Southeastern U.S. may have higher phenolic content and antioxidant activities.

Conclusions

In the Southeastern U.S., root-zone cooling slowed down lettuce growth rate, and reduced head fresh and dry weights but did not alleviate tipburn. Bolting was alleviated by nutrient solution cooling. Cultivars ‘Dragoon’ and ‘Thinker’ were resistant to tipburn but ‘Bambi’ and ‘Intred’ were very susceptible to tipburn. Cultivars ‘Dragoon’, ‘Breen’ and ‘Thinker’ performed well in greenhouse hydroponic production experiments. Future research, should focus on optimal environmental conditions to reduce physiological disoders (ie, tipburn, bolting, enzymatic browing) which reduce consumer demand and consumption of lettuce.

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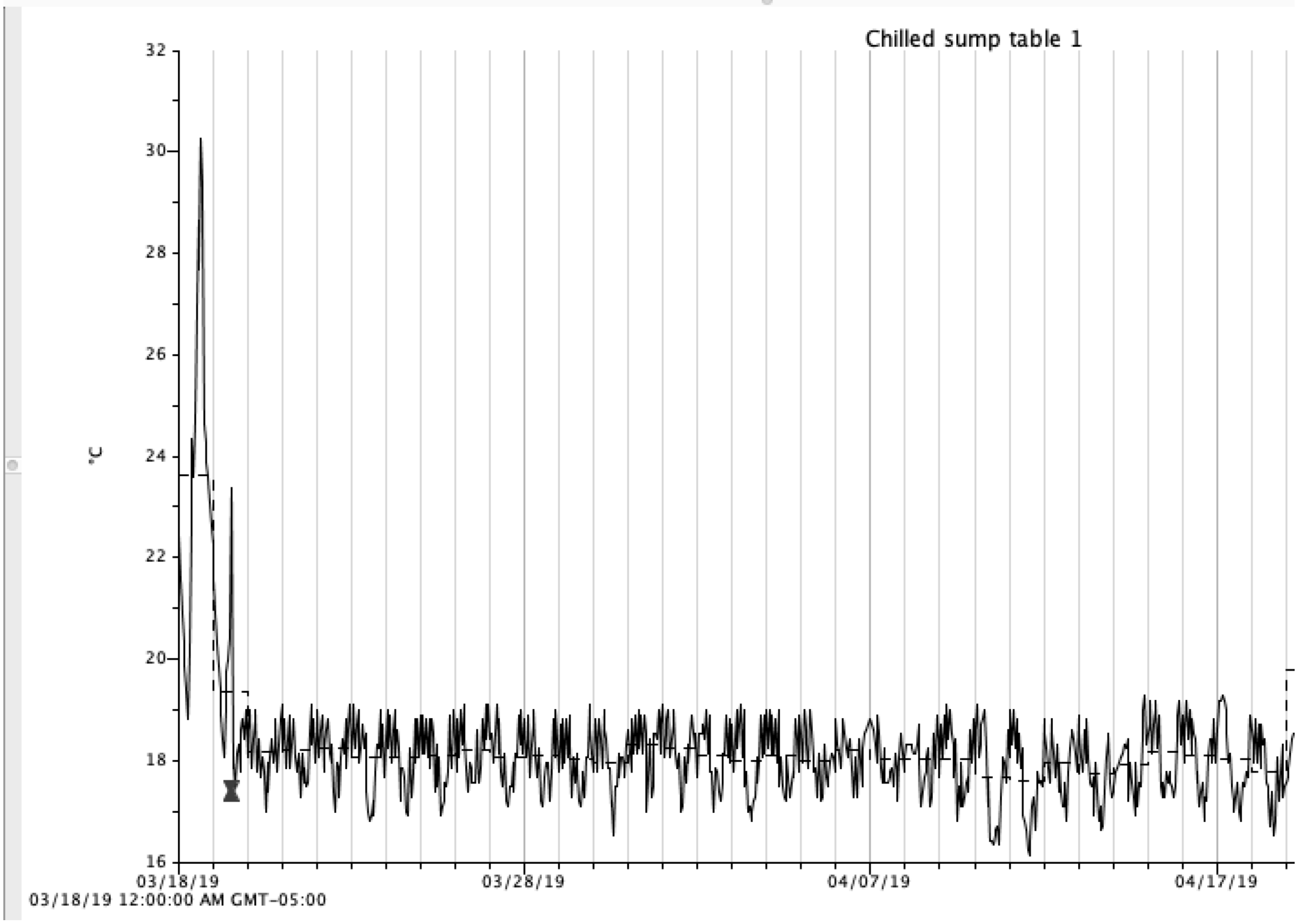
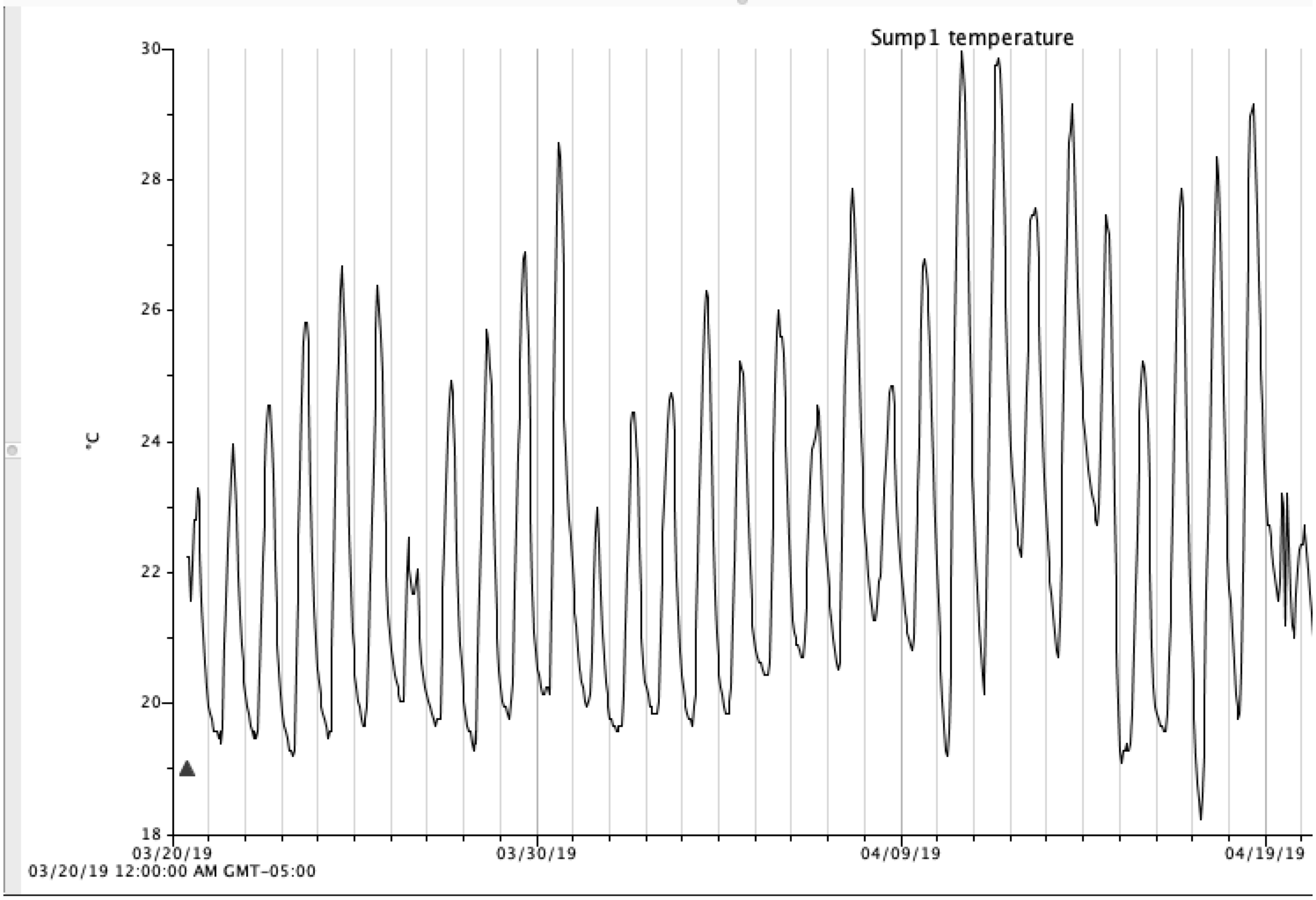
Figure 2.1.



30 days temperature of cooling nutrient solution.

Figure 2.2.



30 days temperature of non-cooling nutrient solution.

Table 2.1 Size index, head fresh weight of various lettuce cultivars grown in either cooled or not-cooled nutrient solution in a nutrient film technique hydroponic system in a greenhouse

Cultivar	Size Index[Z]		Head Fresh Weight	
	Cooled	Not-Cooled	Cooled	Not-Cooled
Dragoon	23.6b[y]NS[x]	24.6ab	135.2aB	185.8aA
Breen	21.9bcNS	23.2b	64.1bcB	91.4cA
Truchas	22.3bcNS	22.8b	65.4bcB	88.5cA
Newham	25.6aNS	26.1a	135.5aB	189.5aA
Intred	21.5cNS	20.3c	57.2cNS	68.7c
Thinker	22.3bcB	24.2abA	83.9bB	134.4bA
Bambi	22.3bcA	20.5cB	112.2aB	132.6bA

[Z] Size Index (SI) was measured ([height + widest width + perpendicular width]/3) at 30 days after transplant.

[Y] Least squares means comparisons among cultivars (lower case in columns) for each treatment using the simulated method at $P < 0.05$.

[X] Least squares means comparisons between treatments (upper case in rows) for each cultivar using F-tests at $P < 0.05$.

Table 2.2. SPAD index and head dry weight of various lettuce cultivars grown in either cooled or not-cooled nutrient solution in a nutrient film technique hydroponic system in a greenhouse

Cultivar	Chlorophyll Content.[Z]		Head Dry Weight
	Cooled	Not-Cooled	
Dragoon	36.8ab[Y]NS[X]	36.3a	6.4ab
Breen	33.7bcNS	31.2b	3.4de
Truchas	36.9abA	31.2bB	3.7de
Newham	35.0abB	38.7aA	7.0a
Intred	39.3aNS	37.3a	3.1e
Thinker	29.8cNS	29.5b	4.8cd
Bambi	39.2aNS	40.4a	5.3bc

[Z] Chlorophyll Meter (Minolta□ SPAD 502) was used to measure chlorophyll.

[Y] Least squares means comparisons among cultivars (lower case in columns) for each treatment using the simulated method at $P < 0.05$.

[X] Least squares means comparisons between treatments (upper case in rows) for each cultivar using F-tests at $P < 0.05$.

Table 2.3. Total phenolic content of leaf tissue as affected by root-zone cooling and non-cooling temperatures for seven Romaine lettuce cultivars[z]

	Total Phenolic content			
Cultivar	Cooling		Non-cooling	
	Inner	Outer	Inner	Outer
Dragoon	562.4ab[y]NS[x]ns[w]	551.2cns	419.9cNS	479.3c
Breen	1073.4aNSa	1013.4abns	422.5bcBb	1329.3abA
Truchas	546.7abcBb	1336.5aAb	916.0abBa	1875.4aAa
Newham	384.0bcBb	610.5bcAb	677.1bcBa	941.1bAa
Intred	745.0abBb	1204.7aAb	1222.6aBa	1682.1aAa
Thinker	475.2bcNSns	558.2ans	431.8bcNS	431.8c
Bambi	329.6cBns	504.3cAa	438.8bcNS	372.6cb

[z] The treatment (cooling and non-cooling) by location (inner and outer leaves) by cultivar interaction was significant at $P < 0.05$.

[y] Least square means comparisons among cultivars (lower case in columns) using the simulated method at $P < 0.05$.

[x] Least squares means comparisons between locations (inner and outer, upper case in rows)

[w] Between treatments (lower case underline in rows) using F-tests at $P < 0.05$.

Table 2.4 Size index of time for Romaine lettuce cultivars^{Z}

Cultivar	Size Index				
	7DAT	14DAT	21DAT	30DAT	Sign.^{Y}
Dragoon	8.4ab^{X}	13.7ab	20.1b	24.1b	Q***
Breen	6.4c	11.9c	17.7de	22.6cd	Q***
Truchas	6.9c	12.4c	18.7cd	22.6cd	Q***
Newham	9.0a	14.9a	22.1a	25.8a	Q***
Intred	7.6bc	12.8bc	17.3e	20.9e	Q***
Thinker	6.9c	11.6c	18.0cde	23.3bc	L***
Bambi	9.1a	14.0ab	19.1bc	21.4de	Q***

^{Z}Size Index (SI) was measured ([height + widest width + perpendicular width]/3) weekly.

^{Y}Significant (Sign.) linear (L) or quadratic (Q) trends using model regressions at $P < 0.001$ (***).

^{X}Least squares means comparisons among cultivars (lower case in columns) for each time using the simulated method at $P < 0.05$.

Table 2.5. Size index of time for root-zone nutrient solution cooling and non-cooling[z]

Time	Cooling	Non-cooling
7 DAT	7.0B[y]	8.5A
14 DAT	12.2B	13.8A
21 DAT	18.4B	19.6A
30 DAT	22.8NS	23.1
Sign.[x]	Q***	Q***

[z] The treatment (cooling and non-cooling) by time (DAT) interaction was significant at $P < 0.05$.

[y] Least squares means comparisons between root-zone solution temperature (upper case in rows) for each cultivar using F-tests at $P < 0.05$. NS = not significant.

[x] Significant (Sign.) quadratic (Q) trends using model regressions at $P < 0.001$ (***). Chill, root-zone temperature is 18°C Non-chill, root-zone temperature is greenhouse ambient temperature.

Table 2.6. Plant Temperature of three months and Cooling and Non-cooling Root-zone solution[Z]

ANOVA	Pr>F
Run[Y]	<0.0001
NST[X]	0.0526

[Z] Three random samples from each trough were selected to measure leaf temperature using the infrared thermometer (VWR□)

[Y] Three runs experiments were conducted as run1(March 2019-April 2019), run 2 (April 2019-May 2019) and run 3 (May 2019-June 2019).

[X] The experiment treatment factor is nutrient solution temperature as cooling to 18 °C and non-cooling as greenhouse ambient temperature.

Table 2.7. Romaine lettuce dry weight and bolting incidence of root-zone nutrient solution cooling and non-cooling[z]

Treatment	DW	Not bolted	Bolted
Cooling	4.6$\underline{\text{b}}$[y]	8a[x]A[w]	0bB
No-cooling	5.1$\underline{\text{a}}$	6bA	2aB

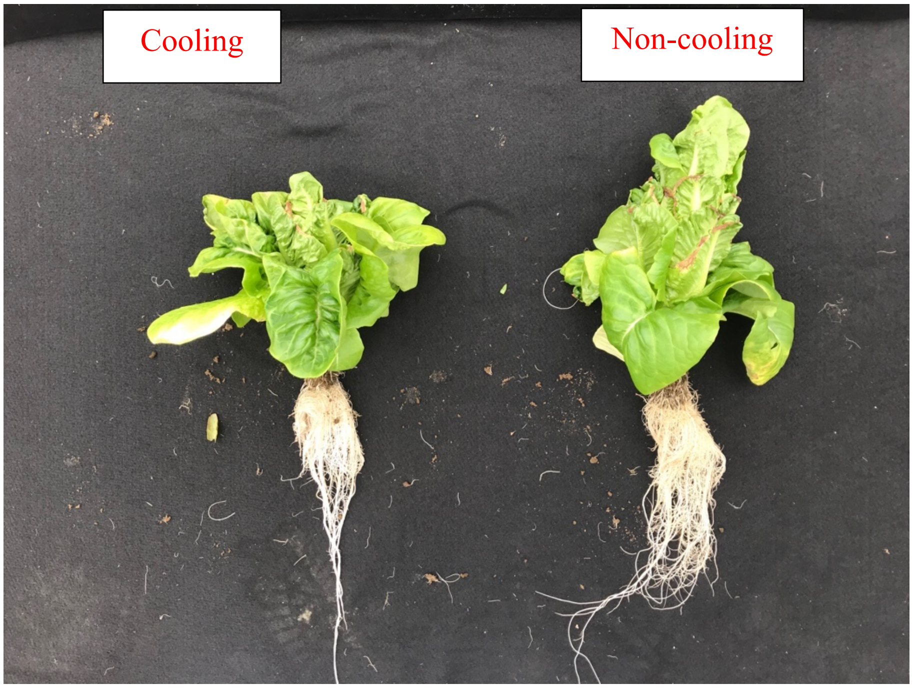
[z] The treatment by bolting interaction was significant at $P < 0.05$.

[y] Least square means comparison between treatments (lower case underline in columns) using the main effect F-test at $P < 0.05$.

[x] Least square means comparisons between treatments between bolting (lower case on rows) using F-teste at $P < 0.05$.

[w] Least square means comparisons between treatments (upper case in columns) using F-teste at $P < 0.05$. DW, dry weight.

Fig. 2.3.



‘Bambi’ lettuce grown in cooling or non-cooling root-zone solution in nutrient film techniques system for 30 days.

Fig. 2.4.

‘Bambi’ lettuce grown in cooling (18°C) and non-cooling root-zone solution in nutrient film techniques system for 30 days.

Chapter III

Eliminating Tipburn by Increasing Horizontal Air Flow Speed in Deep Water Culture System

Abstract

Lettuce grows fast in greenhouse hydroponic system due to the high temperature and humidity in the Southeastern U.S. in summer. Tipburn is a severe physiological disorder with margin necrosis in young lettuce leaves because of calcium ion deficiency. Physiologically, Ca^{2+} deficiency readily occurs due to limitation of apoplastic pathway, which is highly dominated by the low transpiration rate of young leaves. Increasing horizontal air flow speed was investigated to speed up transpiration rate. Tipburn sensitive lettuce cultivar 'Bambi' was grown to trail the effects of air flow speed of 70m min^{-1}, 140m min^{-1} and 210m min^{-1} in the Deep Water Culture system. Tipburn lettuce was counted according to light, moderate and severe levels. Linear trend of increasing light to decreasing severe with increasing air flow speed using model regression at P ChiSq < 0.001 (***) was observed. Lettuce grown at 140m min^{-1} had the best marketable appearance. Head fresh weight were quadratically changed with increasing air flow speed. The highest value was at 70m min^{-1} air flow speed. 30 DAT size index and root dry weight were linearly decreasing with increasing air flow speed. Stomatal conductance was significantly higher at 9:00AM than 11:00AM and higher in inner leaves than outer leaves. Inner leaves and outer leaves chlorophyll content was not affected by different air flow speeds. In conclusion, increasing horizontal air flow speed can mitigate lettuce tipburn for a very sensitive cultivar 'Bambi'.

Introduction

Lettuce is a cool season vegetable typically grown in low temperatures and low light (Frantz et al., 2004). According to the USDA-ERS, 2017, lettuce is the second most popular vegetable in the U.S., behind only potato. Greenhouse lettuce production was nearly 1,500 acres in the U.S. in 2013 (Hickman, 2016), most of which was grown hydroponically. In the Southeast U.S., small hydroponic businesses are growing rapidly. Greenhouse production relies on a good integration of multiple environmental factors including temperature, light, humidity, CO_2, and nutrition input. Typically, in summer, temperature and humidity are high in greenhouses. Lettuce is susceptible to physiological disorders such as tipburn and bolting which lead to significant yield reduction and economic loss. Tipburn is a physiological disorder that occurs near harvest with necrosis of young growing leave margins (Uno et al., 2016). Several environmental and physiological factors such as light intensity, photoperiod, temperature, relative humidity, growth rate and cultivar have been shown to be associated with tipburn development (Sago et al., 2016; Frantz et al., 2004; Saure, 1998). Since the effects of these multiple environmental factors are not independent, not a single pure reason has been found responsible for tipburn. Most researchers found it is related to calcium deficiency of the young growing tips. Physiologically, Ca^{2+} deficiency readily occurs due to limitation of the apoplastic pathway, which is highly dominated by the low transpiration rate of young leaves (White and Broadley, 2003). Increasing the transpiration rate ultimately to enhance the apoplastic calcium transport may help eliminate tipburn. Studies in the plant factory enviroments increasing horizontal air flow can decrease tipburn; however, decreasing air temperature had no effect on tipburn incidence and total tipburn leaves (Lee et al., 2013). Research on cabbage showed the calcium ion can be transported to non-transpiration leaves (Cox and Dearman, 1981). Horizontal air flow could increase the transpiration rate which may increase the calcium concentration in inner leaves by making calcium more evenly distributed. On the other hand, increasing horizontal air flow could decrease the air temperature on the micro-area top of the plants, and this may slow the growth rate. Plant antioxidants such as total phenolic content and flavonoids are important in improving the quality of fruit and vegetable and stress resistance. Increase in the total concentration of phenols in various species could help to reduce ROS (Edreva, 2005; Oh et al., 2009; Samuolienė et al., 2012). Flavonoids and phenolic and other antioxidants can improve the quality of vegetables (Oh et al., 2009; Samuolienė et al., 2012; Sgherri et al., 2017). We conducted an experiment to determine the effects of horizontal air speed in a greenhouse on tipburn incidence, growth rate, biomass production, and total phenolic content content of a tipburn-sensitive Bibb lettuce cultivar 'Bambi'.

Materials and Methods

On 10 March 2019, 12 April 2019 and 21 May 2019, 'Bambi' lettuce seeds (Johnny's Selected Seeds, Winslow, ME) were sown and grown for two weeks in OASIS® horticubes (OASIS® Grower Solutions, Kent, Ohio) (2.54 cm ×3.18 cm × 3.81 cm) until seedlings had two true leaves in a greenhouse at Auburn University (32° N, 85 W). Seedlings were fertilized with 150, 80, 200, 150, and 35 mg·L-1 N, P, K, Ca, and Mg nutrient solution daily for two weeks before being transplanted to one of eight deep water culture (DWC) containers. Containers were 38-L heavy plastic boxes (58cm*38*cm*20cm; AKRO-MILS® Multi load Tote-42.5-L capacity, Akron, Ohio), with polystyrene rafts floating on nutrient solution, totaling 48 circular holes, each measuring 2.2 cm in diameter, were evenly drilled into eight Styrofoam boards (2.54-cm thick, R5 Unfaced Polystyrene Foam Board Insulation, Kingspan Insulation, Atlanta, Georgia), which had been cut to fit the dimensions of each box. Each box was supplied with 30 L min^{-1} of air pushed through eight air stones using one of two pumps (Hailea ACO-9730 Air Pump, Guangdong, China). Three box fans (Lasko® Philadelphia, PA) were used to provide horizontal air flow at speeds of 70 m min^{-1}, 140 m min^{-1} and 210 m min^{-1}. Fans were placed at plant height and arranged 200cm-240cm, 320-360cm, and 440-480cm from plants to provide the correct air speeds. A handheld digital anemometer (HOLDPEAK® 866B-WM) was used to measure air speed and calibrate the treatments. Control air speed was measured as 10 m min^{-1}. Nutrient solution levels were maintained using either raw water or makeup nutrient solution to maintain an electrical conductivity range of 1.7 to 2.0 mS cm^{-1}. The experiment was a completely randomized design with 12 replications at each treatment level. A single lettuce plant was considered an experimental unit.

At 7 DAT, 14 DAT, 21 DAT and 30 DAT, a size index (SI) was measured as [(height + widest width + perpendicular width)/3]. At 30 DAT, lettuce heads including the original root ball and 2.5-in of roots were weighed to determine a market fresh weight. All roots were then removed, and heads were weighed to determine head fresh weight (HFW). Roots were dried in a forced-air oven at 77 °C and weighed to determine root dry weight (RDW).

At 30 DAT tipburn was quantified by assigning a rating of light, moderate, or severe to lettuce heads having fewer than 5, between 5 and 10, or more than 10, leaves showing tipburn symptoms, respectively. Stomatal conductance was measured using an SC-1 leaf porometer (ICT International®) at 9:00AM 27DAT, 11:00AM 28DAT, 9:00 AM 29 DAT, and 11:00AM 30 DAT in April (Run 2) and May (Run 3). A chlorophyll meter (Minolta® SPAD 502) was used to estimate chlorophyll content at 30 DAT for inner and outer leaves in April (Run 1), May (Run 2), and June (Run 3).

Leaf samples were freeze-dried with Harvest Right® freeze dryer (Harvest Right® scientific freeze dryer, medium size, Salt Lake City, Utah) after harvest. For each of the three runs totaling 48 samples including three samples of two locations (outer and inner leaves) and two leaf areas (margin and central) for four treatments (2 locations * 2 areas * 4 treatments * 3 replicates = 48 samples) were freeze dried. Each lettuce sample was cut into inner and outer parts. Inner and outer leaves were cut into margin and central leaf areas, frozen by liquid nitrogen, packed with paper bags and kept in -80°C refrigerator. Freeze dry instructions were followed by the harvest right freeze dryer manual. Dry samples were sealed in Ziploc bags and stored and kept from moisture for future analysis.

Total phenolic compounds are extracted using methods described by Wiecznska and Cavoski, (2018). Freeze-dried lettuce samples (0.25 g) were extracted with 20 ml of methanol:water:acetic acid (85:15:0.5) solvent. The solution was vortexed for 30 s and sonicated for 5 min, then incubated at room temperature for 20 min and vortexed again for another 30s. The tubes were centrifuged at 10,000 rpm for 10 minutes. Supernatants were decanted and stored at -80°C for further analysis. Total phenolic contents were assayed according to Folin-Ciocalteu method (Singleton & Rossi,1965). Each 40-µl extract was added to 200 µl Folin-Ciocalteu reagent and added 1.5 ml HPLC grade milli-Q water. Tubes were vortexed and incubated for 2.0 minutes at room temperature. 160 µl of 7.5% sodium bicarbonate solution was added and incubated samples at 40°C water bath for 30 minutes. Absorbance of samples were read at 750nm against a blank (0 µl GA standard).

An analysis of variance was performed on all responses using PROC GLIMMIX in SAS version 9.4 (SAS Institute, Cary, NC). The experimental design was completely randomized and experimental runs (months) were treated as a random variable. For DAT30SI, MFW, HFW, RDW, outer chlorophyll, inner chlorophyll, and rating, the treatment design was 1-way with treatment. Ratings were analyzed using the multinomial probability distribution. Presented are counts of ratings for each treatment. Linear and quadratic trends over treatments were examined using model regressions. For stomatal conductance and size index, the treatment design was 2-way with treatment and time. Differences in the two times for stomatal conductance were with main effect F-tests. For concentration (mg/100gDW), the treatment design was 3-way with treatment, locations (outer and inner leaves), and area (margin and central leaf area). Differences in the two location and area were determined using F-tests. All significances were at $\alpha=0.05$.

Results

Growth (Size Index, Market Fresh Weigh, Head Fresh Weight and Root Dry Weight)

30 DAT size index was influenced by increasing horizontal airflow speed as a linear relationship at $P<0.01$. When wind speed increased from 0m min^{-1}(control) to 210 m min^{-1}, size index decreased from 19 cm to 18.3 cm (Table 3.1). The same decreasing linear relations ($p<0.01$) between root dry weight and horizontal air flow speed. When it increased from 0m min^{-1} (control) to 210 m min^{-1}, root dry weight decreased from 0.448g to 0.381g(Table 3.1). Market fresh weight was changed by air speed as a quadratic trend at $p<0.01$. When increasing wind speed from 0m min-1to 70 m min^{-1}, the market fresh weight increased to the highest 159.8g then decreased to 135.1g Table (3.1). When increasing wind speed to 210 m min^{-1}. Quadratic relation at $P<0.05$ between head fresh weight and windspeed was found too. When increasing wind speed from 0 m $min^{-1}$to 70 m min^{-1}, the market fresh weight increased to the highest 142.3g then decreased to 119.4g when increasing wind speed to 210 m min^{-1}.

Stomatal Conductance

For three runs from March to June, lettuce stomatal conductance was different between outer leaves and inner leaves. Stomatal conductance of inner leaves was 910.68 mmol m^{-2} s^{-1} which was higher than out leaves with stomatal conductance 606.89 mmol m^{-2} s^{-1}. It changed at different times of the day. Stomatal conductance at 9:00AM 844.56 mmol m^{-2} s^{-1} was higher than 11:00 AM 673.01 mmol m^{-2} s^{-1} (Table 3.3) For central leaf area, inner leaves stomatal conductance was higher than outer leaves. Stomatal conductance in the early morning (9: 00 AM) was higher than closer to noon (11:00 AM).

Tipburn

Light, moderate, and severe tipburn index was rated and the number of each level was counted and analyzed using the multinomial probability distribution in SAS. There was significant difference ($P<0.0001$) of tipburn incidence of different air flow speeds. Linear trend of increasing light to decreasing severe with increasing air flow speed using model regression at P ChiSq < 0.001 (***) was observed (Table 3.1). Lettuce in control group first began tipburn as early as 21DAT in April, 18DAT in May and 15 DAT in June. 210m min^{-1} air flow speed had the lowest tipburn incidence.

There was no significant difference found in chlorophyll content in inner leaves and outer leaves for different airflow speeds.

Total Phenolic content

There was a two-way significant interaction between air-flow x leaf location. Also, significant interactions were found between leaf locations (inner and outer) and different leaf areas (central and margin) (Table 3.3).

For each air flow speed, the total phenolic content concentration was higher in outer(older) leaves than inner(newer) leaves. When air flow speed was 140m min^{-1}, both out and in leaf total phenolic content was the highest(Table 3.1).

In general, outer leaves (older) contain higher total phenolic content. However, there was not different between central and marginal leaf areas. Among inner leaf area (newer), marginal areas contain higher total phenolic content when compared to central areas (Table 3.3).

Discussion

Tipburn was supposed to be caused by high temperature promoting lettuce growth rate which attributed to limitation of calcium in young leaves (Cox et al., 1976). A study showed that frequent foliar applications of Ca as 90 mg L^{-1}calcium ion significantly decreased tipburn leaves and percent leaf area with tipburn, and increased calcium in young leaves (Corriveau et al., 2012) Decreasing air temperature was also conducted without help to mitigate tipburn and which may cost big investment to production lettuce in hot weather (Lee et al., 2013). High humidity and high light intensity are also possible factors attributed to tipburn. It is proved that high relative humidity under dark conditions reduced the transpiration rate of young tissues which may provide a direction to mitigate tipburn (Islam et al., 2004). Our results corresponded well with previous increasing air flow speed to mitigate tipburn researches. In Lee et al., 2013, three horizontal air flow speeds as 0.28 (Low), 0.55 (Medium), and 1.04 $m \cdot s^{-1}$ (High) were applied to lettuce grown in plant factory. Tipburn was rated according to the indices (0, no symptom; 1, initiation of visible symptoms; 2, initiation of internal breakdown on 2 leaves; 3, internal breakdown on 3 leaves; 4, internal breakdown on most of the inner leaves; 5, severe internal breakdown). Their results showed that tipburn was efficiently reduced at 0.28 $m \cdot s^{-1}$(Low). Vertical air flow was also implemented to lettuce inner leaves. It proved that all day air flow was more effective than day or night air flow, but the grow rate was slower. (Goto and Takakura, 1992). Shibata et al. (2003) demonstrated that increasing vertical air flow speed to 0.7 $m \cdot s^{-1}$increased lettuce yield by 30% compared to increasing the horizontal air flow. Increasing vertical and horizontal air flow speed methods can enhance the transpiration rate which help calcium ion been transported to young growing leaves to prevent lettuce tipburn. When air flow speed was increased, lettuce size index and root dry weight were decreased which was supported by (Lee et al., 2013). When air flow speed was at 70 m min^{-1} and 140 m min^{-1}, lettuce had the highest biomass, and more condense than others. But Lee, et al., 2013 found that control had higher leaf weight than increasing air flow speed.

It is indicated that stomata open help increase stomatal conductance, photosynthesis and the transpiration rates are potentially higher. In our results, Stomatal conductance was higher in the morning when light intensity was lower than at noon when light intensity was higher. It was higher in tipburn leaves when compared to leaves without tipburn. These results were supported by Barcena et al. (2019) that stomatal conductance was higher in inner leaves than outer leaves. But it didn't show that stomatal conductance was higher at noon (13h) than in the morning (10h) or later afternoon (17h). Not only inner and outer leaf locations, chlorophyll content is also dependent on lettuce types Baslam et al. (2013)

showed that ‘Batavia’ and ‘Maravilla’ lettuce chlorophyll had higher chlorophyll (a+b) in inner leaves than in outer leaves, but ‘Cogollo’ type lettuce had no difference of chlorophyll contents of inner and outer leaves.

Phenolic content has been recognized as the most important protective compounds against stresses through antioxidant activities scavenging radicals. Baslam et al. (2013) found lettuce phenolic contents is more dependent on cultivar than leaf position. ‘Maravilla’ inner leaves had higher phenolic contents than outer leaves. But our result was different that outer leaves had higher phenolic content than the inner leaves. It was indicated that tipburn leaves had lower total phenolic content than not tipburn leaves which is reasonable because tipburn leaves experienced more and higher antioxidant activities. Total phenolic content was used more in tipburn leaves than outer leaves.

Conclusions

Increasing horizontal air flow speed can mitigate lettuce tipburn for sensitive cultivar ‘Bambi’ in the Southeastern U.S. When air speed was at 140m min^{-1}, lettuce had the best marketable appearance. Increasing air flow speed didn’t change lettuce total phenolic content. Lettuce outer leaves had higher total phenolic content than inner leaves. For tipburn leaves, the margin area had higher total phenolic content than central area. Phenolic content is more determined by stressful environmental conditions. It seems that stresses stimuli antioxidant activities which needs a big amount of phenolic content. In the future research, to study and mitigate tipburn, it’s necessary to develop the methods for accurately identify and quantify tipburn leaves physiological contents changes and tipburn symptoms at certain stages.

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Table 3.1. ‘Bambi lettuce’ growth index, total phenolic content under increasing horizontal air flow speed

Air flow (m min^{-1})	30DAT SI (cm)	MFW(g)	HFW(g)	RDW(g)	Total Phenolic content	
					Outer	Inner
0	19	151.9	136.9	0.448	629.7a^{Y}	570.7b
70	18.9	159.8	142.3	0.400	584.0a	493.8b
140	19.3	156.2	137.1	0.368	726.3a	573.4b
210	18.3	135.1	119.4	0.381	613.0a	556.5b
Sign.^{Z}	L**	Q**	Q*	L**	NS^{X}	NS

^{Z}Significant linear (L) trend using model regression at $P < 0.01$ (**), Significant quadratic (Q) trend using model regression at $P < 0.01$ (**), significant quadratic (Q) trend using model regression at $P < 0.05$ (*). DAT, day of transplant, SI, size index, MFW, market fresh weigh, HFW, head fresh weight, RDW, root dry weight.

^{Y}Least squares means comparisons between out and in leaves using F-tests at $P < 0.05$.

^{X}Non-significant (Sign.) trends using model regressions at $P < 0.05$.

Table 3.2. ‘Bambi lettuce’ tipburn incidence of increasing horizontal air flow speed

Air flow (m min^{-1})	Tipburn Incidence		
	Light	Moderate	Severe
0	1	3	32
70	6	17	13
140	7	23	6
210	28	5	0
Sign.[Z]			L***

[Z] Significant (Sign.) linear (L) trend of increasing Light to decreasing Severe with increasing air flow speed using model regression at P ChiSq < 0.001 (***). Counts of ratings for each treatment. Analysis used the multinomial probability distribution.

Table 3.3 Stomatal conductance of ‘Bambi’ lettuce measured on outer and inner leaves at different times of a day

		Stomatal Conductance (mmol m^{-2} s^{-1})
Location	Outer leaf	606.9b[Z]
	Inner leaf	910.7a
Time	9:00AM	844.6A[Y]
	11:00AM	673.0B

[Z] Least squares means comparisons between out and inner leaves with the same lowercase letters are statistically different using the main effect F-test at $P < 0.05$.

[Y] Least squares means comparisons between 9:00AM and 11:00AM with the same uppercase letters are statististically different using the main effect F-test at $P < 0.05$. SC, stomatal conductance.

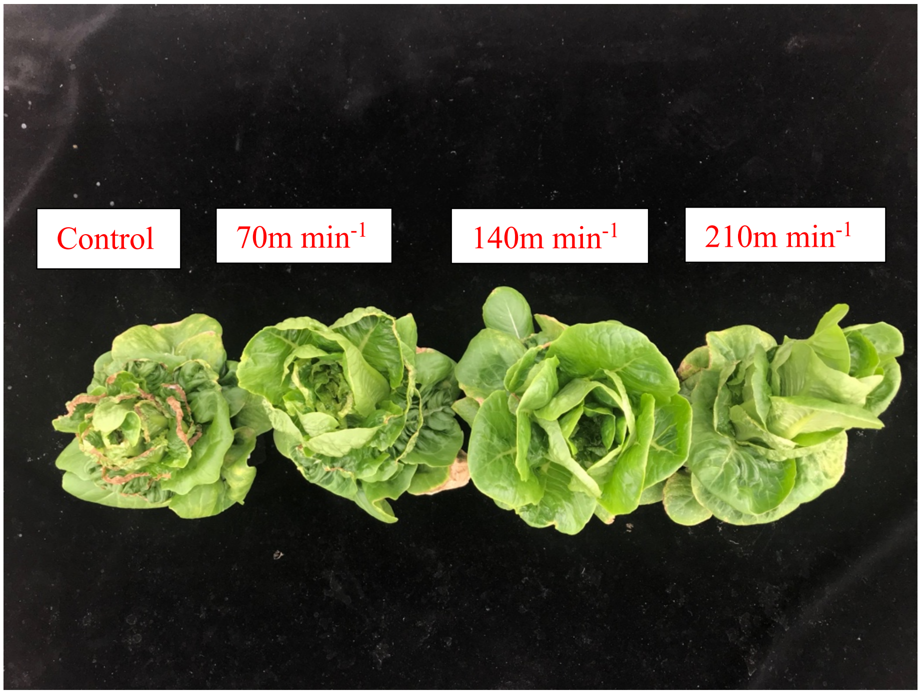
Table 3.4. Total phenolic content of ‘Bambi’ lettuce measured on central and margin leaf area of new and old leaves[Z]

Location	Outer	Inner
Central	624.96nsA[Y]	481.96bB
Margin	651.51A	615.25aB

[Z] Lettuce were divided into inner and outer leaves and each leaf was cut to margin and central areas to measure total phenolic content

[Y] Least squares means comparisons between central and margin (lower case in columns) and between out and in (upper case in rows) using F-tests at $P < 0.05$. ns = not significant.

Fig. 3.1.



‘Bambi’ lettuce grown in different horizontal air flow speeds in deep water culture system for 30 days.

Fig. 3.2.

‘Bambi’ lettuce grown at Control horizontal air flow speed in deep water culture system for 30 days.

Fig. 3.3.

‘Bambi’ lettuce grown at Treatment 1 horizontal air flow speeds (140m min^{-1}) in deep water culture system for 30 days.