SEASONAL AND ROOT-ZONE TEMPERATURE INFLUENCE ON SESQUITERPENE LACTONE AND SUGAR CONCENTRATION IN HYDROPONICALLY-GROWN LETTUCE

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Abstract: Lettuce is one of the most important leafy vegetables in the United States and is subjected to a decrease in edible quality when cultivated in environments with hot temperatures and increased day-length. Specifically, bitter-tasting compounds called sesquiterpene lactones (SLs) begin to accumulate, especially during bolting, or the transition from vegetative to reproductive development. This shift in development is unwanted and necessary to avoid, if possible, and impacts other important indices of edible quality such as sugar concentration and the Sugar:SL ratio. Two studies: A 12-cultivar trial across four harvest seasons and a hydroponic nutrient-solution chilling experiment were investigated. Research showed a significant difference in harvest, cultivar, and their interaction for free and total SLs, sucrose, and Sugar:SL ratio. Plant fresh weight was greatest in Spring, 2020, perhaps due to an increase stage of maturity at harvest, followed by Fall, 2020, Summer, 2021, and Winter, 2021. Total SLs and glucose were highest in Fall, 2020, followed by Spring, 2020, Summer, 2021, and Winter, 2021. Total sugars were the same between harvests, and Winter, 2021 had a significantly lower Sugar:SL ratio than the other three harvests. Romaine and batavian cultivars (excluding ‘Cherokee’) emerge as top candidates to grow during the summer due to higher plant weights, sugar concentrations, and the Sugar:SL ratio, as well as decreased SL concentrations. Salanova® cultivars produced low yields (exception ‘Sweet Crisp Green’) in comparison to traditional varieties, and often had significantly greater levels of sesquiterpene lactones. Chilling with a temperature differential of approximately 8 °C (15 °F) was found to be an effective treatment in the summer to reduce total SL concentration, and increase the Sugar:SL ratio, especially in romaine and Salanova® cultivars. Total sugar concentrations were not significantly different in the summer or winter using chilling. Chilling is a less effective option for winter months; however, overall SL and sugar concentrations were notably low for the Winter, 2021 harvest season.

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CHAPTER I

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a member of the Asteraceae plant family. Formally known as Compositae, this plant family includes other species such as chicory (*Cichorium intybus* L.), endive (*Cichorium endivia* L.), and globe artichoke (*Cynara cardunculus* var. *scolymus* L.) (Bohm et al., 2001). A unique identifier for the Asteraceae family is their distinct flower arrangements. What appears to be a single flower, is in fact a cluster of several smaller flowers, called a head or capitulum (Bohm et al., 2001). While many members of this family are intentionally grown through their reproductive stage, lettuce is not, and is typically harvested at an immature growth stage.

Lettuce is one of the most important leafy vegetables in the United States, ranking highest in leafy vegetable crop production and economic value, and ranking s only to potatoes in per capita consumption (USDA, NASS, 2020). In 2019, head and leaf lettuce types, ranked fifth and seventh in loss-adjusted vegetable availability; both lettuce types ranked s in fresh-market availability (Kantor et al., 2021). These vegetable availability indices are good indicators of a vegetable's importance in the marketplace, as there is a correlation to consumer demand. The consumer demand for lettuce is largely due to its importance as a principle ingredient in many popular food preparations, such as salad and salad mixes, as a "wrap-up vegetable", and in sandwiches

(Assefa, 2018). Over 101,215 ha (250,000 acres) of lettuce is harvested from outdoor production in the United States, annually, with approximately 90% of all commercially-grown lettuce produced in California and Arizona, due to favorable environmental conditions for commercial production (USDA, NASS, 2020).

Lettuce is a cool season crop – daily temperatures of 23 °C (73 °F) and nightly temperatures of 7 °C (45 °F) are optimal for proper development (Smith et al., 2009). An unfavorable growth environment, often due to elevated temperatures, leads to bolting in lettuce, or the physiological shift from vegetative to reproductive growth. In addition to elevated temperatures, long-daylight hours, poor moisture, and hormonal stresses also induce bolting (Hao et al., 2018). This shift in developmental stage is associated with a decrease in edible quality due to an accumulation of bitter-tasting compounds called sesquiterpene lactones (SLs), as well as translocation of sugars from leaves to reproductive tissues (Khan, 2018; Lee and Sugiyama, 2006). Prior to bolting – SLs accumulate in lactifiers, which are closely associated with the vascular tissues of the Asteraceae family, and greater concentrations of SLs occur in higher leaf positions, closer to the shoot apical meristem (Sessa 2000, Seo et al., 2009). When a stem is cut, latex can be seen exuding from these ducts where bitter compounds sequester. During bolting onset, lettuce elongates its flower stalks and the shoot apical meristem begins to swell. This swelling is suggested to occur due to carbohydrate relocation from leaves to stems, causing turgor pressure to increase, rapidly driving water in and expanding the size of the cells (Lee and Sugiyama, 2006). The stalk extends upward, producing flowers and seeds. In order to develop seeds, plants shift resources, i.e. sugars, from other areas of the plant, in particular the leaves, resulting in reduced edible quality.

The shift from vegetative to reproductive growth is a complicated developmental process that is regulated through several biochemical pathways, with recent research elucidating *LsSOC1* as an important gene in heat-induced bolting (Chen et al., 2018). An elevated concentration of the plant hormone auxin plays a critical role, and was found in greater concentrations in a heat-treated group (33/25 °C; day/night) compared to a control group (20/13 °C; day/night) (Hao et al., 2018). Exogenously applied indole-3-acetic acid (IAA), a member of the auxin family led to accelerated bolting within 5 days after treatment, providing further evidence that auxins play a role in the developmental shift form vegetative to reproductive growth.

Sesquiterpene compounds are C-15 terpenoids that naturally occur as hydrocarbons, alcohols, ketones, aldehydes, acids, or lactones (Graziana et al., 2015). Sesquiterpene lactones (SLs) are a large class of sary metabolites with over 500 compounds identified (Sessa et al., 2000). These compounds are thought to be anti-feedants with bitter taste that can protect against herbivory (Sessa et al., 2000). Although SLs contribute to an unpleasant bitter flavor, SLs may also serve as putative anti-tumor, anti-leukaemic, cytotoxic, and anti-microbial sources in the human diet (Price et al., 1990; Tamaki et al., 1995). A subset of SLs called guaninosides, including lactucin, 8-deoxylactuin, and lactucopicrin, are found in members of the Asteraceae family. Lactucin and lactucopicrin are the most abundant SLs in lettuce, and research shows these SLs and their glycoside derivatives are correlated to overall bitterness perception in lettuce, especially when plants are grown past horticultural maturity (Price et al., 1990; Seo et al., 2009). SLs are found in either a “free” or “bound” form. The “free” form is the “base” form – before any chemical groups are added to the compound. The “bound” forms are glycosylated versions of these compounds, meaning glucose is attached. Specifically, the SLs of interest are glycosylated at the C-15 position, giving rise to lactucin-15-glycoside (picriside A), 8-deoxylactuin-15-glycoside (crepidiaside A), and lactucopicrin-15-glycoside (Sessa et al., 2000). While Price et. al (1990) showed glycoside-derivatives demonstrate a greater correlation to bitterness perception compared to their non-glycosylated counterparts, steric hindrance within glycosylated SLs may not directly contribute, but can rapidly have the glucose molecule cleaved, giving rise to additional, non-glycosylated SLs. In order to quantitate “bound” SLs, samples were incubated with cellulase enzyme (*Aspergillus niger*), hydrolyzing and cleaving the glucose molecule, which reverts “bound” SLs back into “free” SLs for the purpose of analytical detection (Price et al., 1990). Because these samples with glucose cleaved are the addition of both “free” and “bound” forms, the nomenclature “total” SLs describe samples treated with cellulase enzyme. In order to determine the amount of SLs in “bound” forms, subtract “free” SLs from “total” SLs, which effectively subtracts a baseline to account for the original amount of “free” SLs in samples. One caveat to quantitating “bound” SLs this way is that natural variability between samples may lead to less accurate results than quantitating “free” and “bound” forms independently.

The nutritional composition of fruits and vegetables depends on several factors including genetics, growth conditions, maturity at harvest, and post-harvest handling. Lettuce is lower in nutritional content compared to other leafy greens, but does provide a good source of water and phytochemicals such as carotenoids, folate, anthocyanin, fatty acids, and phenolics (Kim et al., 2018). Research shows lettuce varieties and cultivars differ significantly in nutritional content, with red cultivars having increased antioxidants and health benefits (Price et al., 1990; Kim et al., 2018). This is beneficial, as visually-aesthetic lettuce cultivars are making a comeback into the marketplace. Any additional vegetable consumption is considered a step in the right direction, as research suggests only a quarter of adults and 7% of children consume the recommended amounts of vegetables each day (Bakke et al., 2018).

The perception of flavor is a combination of several senses. One source defines flavor as being the “sensation arising from the integration or interplay of signals produced as a consequence of sensing smell, taste and irritating stimuli from a food or beverage” (Laing, 1996). Humans often misuse the term “taste” to describe “flavor,” but the two are distinct; taste is only one of several multisensory components representing flavor. Smell also contributes to taste. What humans smell are volatile aromatics released from food, and binding to olfactory receptors in the nose (Axel, 1995). Smells associated with lettuce are miniscule, but taste can be significantly impacted by metabolite composition. With a metabolite composition dominated by sugars, lettuce will promote a sweet taste, while one greater in sesquiterpene lactones (SLs) may impart a bitter one (Chadwick et al., 2016).

Human tongues are covered with various types of papillae, denoted by macroscopic, “bumpy” appearances (Bartoshuk, 1993). Of these papillae, fungiform, foliate, and circumvallate papillae house taste buds, which contain clustered taste receptor cells (TRCs) where taste processing begins (Bartoshuk, 1993). Tastants are molecules that activate specific TRCs, which “transmit information via sensory afferent fibers to specific areas in the brain that are involved in taste perception.” (Lee, 2017). While there are variations of stimuli perception around the perimeter of the tongue, this variation is small. Humans can sense stimuli on all portions of the tongue's perimeter, allowing us to move away from the old "tongue-map" ideology. Evidence debunking the "tongue-map," or idea that distributions of taste sensitivities are spatially separated on different perimeters of the tongue, was presented in a study in which patients whose chorda tympani nerve was damaged. Researchers hypothesized this would impair their ability to perceive sweetness, but not only does this not occur, damage to the chorda tympani nerve produces "virtually no change in the subjective taste world," suggesting receptors are not localized to specific areas of the tongue (Bartoshuk, 1993).

Figure 1 shows three of four morphologic subtypes of taste cell receptors that have been identified. The types of taste receptors are - Type I, expressing glial-like cells and detecting saltiness, Type II cells, expressing G-protein coupled receptors (GPCRs) and detecting sweet, bitter, and umami tastes, Type III, detecting sour stimuli, and Type IV (not shown), which are thought to represent progenitor taste cells (Lee, 2017). Generally, sweet, bitter, and umami tastants activate Type II cells by inducing them to release ATP through Pannexin 1 (Panx1) hemi-channels. The extracellular ATP released excites ATP receptors (P2X, P2Y) on sensory nerve fibers and on taste cells, generating a response of flavor perception (Lee, 2017). Sesquiterpene lactones are shown to activate the generalist Type-II taste receptor T2R46 in humans (Brockhoff, 2007). While 25% of the human population experiences bitter-blindness, or an increased concentration threshold for bitterness-perception, researchers believe SLs are not affected by this genetic mutation, because polymorphisms in the T2R38 gene lead to bitter-blindness, not T2R46 (Chadwick et al., 2018).

Not only do absolute concentrations of phytochemicals affect flavor perception, but the ratio in which the phytochemicals are present contribute to the overall perception of flavor. Detection thresholds, or sensitivity, may also play an important role. Detection threshold varies; in one study the bitter compound, quinine, was perceived at 25 uM concentrations while sucrose was perceived at 10,000 uM – a 400-fold difference (Hladik, 1996). SL (lactucin, 8-deoxylactucin, and lactucopicrin) and sugar (glucose, fructose, sucrose) concentrations are over 1000-fold different in most lettuces, with SLs measured on a $ug\cdot g^{-1}$ dry-basis, and sugars measured on a $mg\cdot g^{-1}$ dry-basis. Nonetheless, the lesser concentrated SLs can overpower sugars, with the overall perception of lettuce flavor becoming bitter. One study evaluates the Sugar:SL ratio in lettuce, highlighting the impact the ratio plays on perceived taste (Chadwick et al., 2016). Research shows a greater Sugar:SL leads to increased palpability for consumers.

Another point of consideration, various sugars and SLs are not perceived with equal ratings of sweetness and bitterness –fructose is the sweetest amongst glucose, fructose, and sucrose, while lactucopicrin is the most bitter amongst lactucin, 8-deoxylactucin, and lactucopicrin (Chadwick et al., 2016). The authors note that while sensory perception of sugars is well established, perception of SLs can be contradictory and have not been considered in regard to “tastant mixture suppression”. This thesis project does not include a taste survey, but rather, focuses on metabolite concentrations for two chemical classes of compounds that greatly contribute to the overall flavor perception in lettuce.

Genetics play an important role in heat-tolerance, and over 100 new lettuce varieties are introduced each year (Van Treuren et al., 2018). Not only has the discovery of the gene *NCED4* led to improved thermo-tolerance for germination in warm soils (Huo et al., 2013), certain types such as batavian have been bred to resist bolting, especially compared to traditional market types such as loose-leaf. There are a number of lettuce market types, including loose-leaf, romaine, batavian, butterhead, and Salanova®. Loose-leaf lettuces have loose tops without forming heads, poor heat-tolerance, and are quick to bolt. Romaine market types have a prominent midrib, grow up-right, and have some heat-tolerance. Batavian market types can either be open or closed-headed depending on variety, and are known to have greater heat-tolerances and delayed bolting. Butterhead market types tend to be head-forming lettuces with soft and tender, almost orbicular leaves (Lindqvist, 1960). Salanova® lettuce, a novel-type bred by Rijk Zwaan, include butterhead, sweetcrisp, and oakleaf cultivars, in both green and red colors. Salanova® varieties are touted to grow twice the amount of leaves compared to traditional varieties, suggesting decreased harvest times and requiring less resources for production. Heat-tolerance and production for Salanova® market types are not widely-known.

Lettuce can be cultivated in a greenhouse using hydroponics, or grown in the field. Field grown lettuce has several advantages – less maintenance on the production system, a lower initial investment cost, and lower capital expenses throughout production. Field production systems for lettuce also have several disadvantages – production is based on favorable weather conditions, increased likelihoods of herbivory and disease damage, and slower growth compared to hydroponics (Lei et al., 2021). Growing field lettuce in Oklahoma is a challenge due to elevated temperatures and sporadic temperature fluctuations, due to lettuce's poor germination in warm soils and ease of bolting (Huo et al., 2013).

Hydroponics is a type of soil-less production that delivers nutrients and oxygen directly to the plant's roots. This is beneficial to the plant because less time and energy are spent searching for nutrients, resulting in shorter harvest times and greater yields compared to soil-grown production (Lei et al., 2021). Cultivating lettuce using hydroponics allows for year-long production in a greenhouse, and the ability to modify and manipulate the growing environment. Farmers must decide if the increased resources needed for hydroponic production are economically feasible to their operation. For small farm agriculture wanting to provide produce year-round, the answer can be yes. For this reason, hydroponics is the general approach for farmers wanting to produce lettuce non-seasonally in Oklahoma.

Chilling hydroponic nutrient-solutions during summer months positively impacted growth and delayed the onset of bolting, even in environments with elevated air temperatures (Thompson et al., 1998). In conjunction with research showing that SLs accumulate prior to the onset of bolting, chilling hydroponic nutrient-solutions may also lead to lower SL concentrations, which in turn could increase the crop's edible quality. Nutrient-solution temperature had an effect on °BRIX value, which is a measure of soluble sugar content, with chilled-solution temperatures of 18.3 °C and 21.1 °C having greater °BRIX values than the non-chilled-temperature control group, which ranged from 19.9 °C to 25.5 °C, 23.3 °C to 30.4 °C, and 16.7 °C to 24.7 °C for replications one, two, and three, respectively (Thakulla et al., 2021). The 18.3°C group had a 26% greater °BRIX value than the 21.3 °C group, but plants had less growth and biomass, suggesting a temperature in-between 18.3 - 21.1 °C may be optimum for both °BRIX values and productivity.

There are several hydroponic production systems that can be used to grow produce. Aeroponics and nutrient-film technique (NFT) are common hydroponic systems used for lettuce. An aeroponic system, especially in the case of lettuce production, is sometimes referred to as a vertical farming system. This system is closed-loop, utilizing vertical towers with 45° slotted compartments, and a sump pump that circulates the nutrient-solution to the top of the tower, where the nutrient-solution falls down tubing, and is atomized onto the plant's root system. Droplet sizes get down to 1 micron, and atomizers are categorized as high, medium and low-frequency, and need a particle velocity high enough to penetrate the root hairs (Lakhiar, 2018). According to data generated by NASA, the advantages to this system include better oxygenation of the roots, water reduction up to 98%, fertilizer reduction up to 60%, and reduced usage of growing supplies. This production system was invented by NASA, and one of the biggest suppliers of lettuce in Oklahoma, Scissortail Farms located in Tulsa, implements it.

Nutrient-film technique (NFT) is a closed-loop system in which a thin film of nutrient solution is pumped up from a reservoir into downward sloping channels, where gravity allows the nutrient-solution to pass over the plant's root system, collect back into the reservoir, and re-circulate over the roots again. The nutrient solution is monitored and adjusted for dissolved oxygen (DO), electrical conductivity (EC), and pH. Maintaining the optimal range for each component is critical for proper development of plants grown in hydroponic solutions, because there is no soil to buffer nutrient levels. Poor levels of nutrients result in stunted development, while excessive nutrients result in burn, with research showing that hydroponically-grown plants are at an increased risk for tipburn compared to their soil-grown counterparts (Frantz et al., 2004). Research shows that hydroponic nutrient-solution concentration impacts SL levels, with low EC solutions resulting in greater concentrations of lactucin and lactucopicrin (Seo et al., 2009).

OBJECTIVES

The research presented has two objectives:

1. Evaluate seasonal influence on sesquiterpene lactone and sugar concentration, Sugar:SL ratio, and plant fresh weight for several hydroponically-grown lettuce market types and cultivars.
2. Evaluate nutrient-solution chilling in summer and winter months on sesquiterpene lactone and sugar concentration, Sugar:SL ratio, and plant fresh weight.

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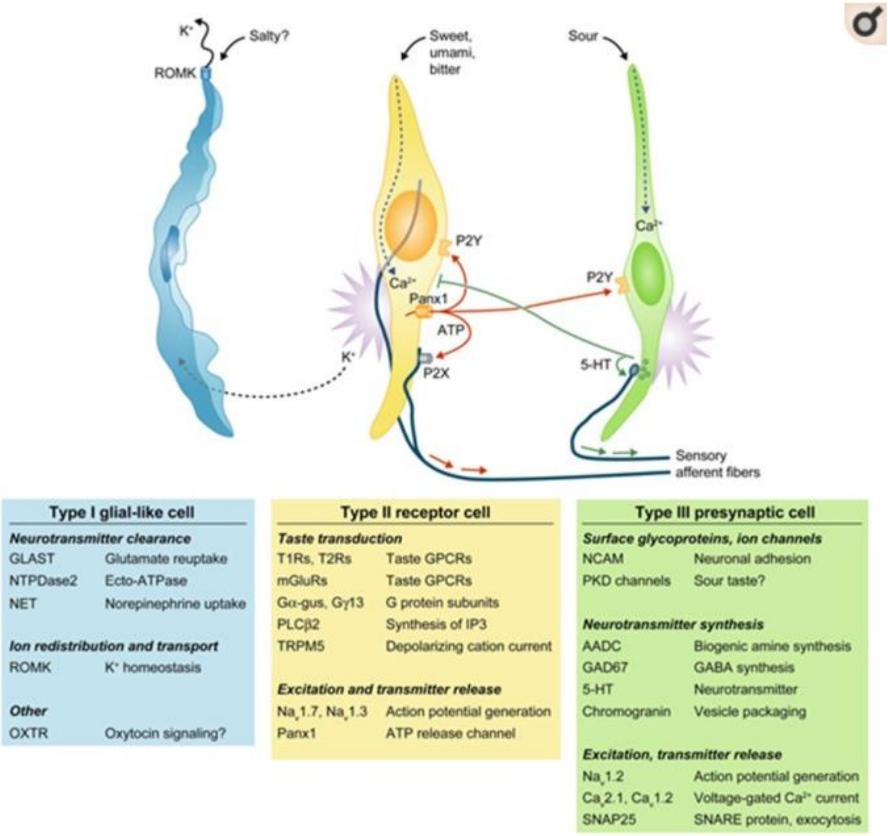
CHAPTER I FIGURES



Type I glial-like cell	
Neurotransmitter clearance	
GLAST	Glutamate reuptake
NTPDase2	Ecto-ATPase
NET	Norepinephrine uptake
Ion redistribution and transport	
ROMK	K^+ homeostasis
Other	
OXTR	Oxytocin signaling?

Type II receptor cell	
Taste transduction	
T1Rs, T2Rs	Taste GPCRs
mGluRs	Taste GPCRs
Gα-gus, Gγ13	G protein subunits
PLCβ2	Synthesis of IP3
TRPM5	Depolarizing cation current
Excitation and transmitter release	
$Na_v1.7$, $Na_v1.3$	Action potential generation
Panx1	ATP release channel

Type III presynaptic cell	
Surface glycoproteins, ion channels	
NCAM	Neuronal adhesion
PKD channels	Sour taste?
Neurotransmitter synthesis	
AADC	Biogenic amine synthesis
GAD67	GABA synthesis
5-HT	Neurotransmitter
Chromogranin	Vesicle packaging
Excitation, transmitter release	
$Na_v1.2$	Action potential generation
$Ca_v2.1$, $Ca_v1.2$	Voltage-gated Ca^{2+} current
SNAP25	SNARE protein, exocytosis

Figure 1. Three of four major classes of taste cells.[Z]

[Z] Adapted from Lee and Owyang (2017).

CHAPTER II

SEASONAL AND ROOT-ZONE TEMPERATURE INFLUENCE ON SESQUITERPENE LACTONE AND SUGAR CONCENTRATION IN HYDROPONICALLY-GROWN LETTUCE

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ABSTRACT

Lettuce is one of the most important leafy vegetables in the United States and is subjected to a decrease in edible quality when cultivated in environments with hot temperatures and increased day-length. Specifically, bitter-tasting compounds called sesquiterpene lactones (SLs) begin to accumulate, especially during bolting, or the transition from vegetative to reproductive development. This shift in development is unwanted and necessary to avoid, if possible, and impacts other important indices of edible quality such as sugar concentration and the Sugar:SL ratio. Two studies: A 12-cultivar trial across four harvest seasons and a hydroponic nutrient-solution chilling experiment were investigated. Research showed a significant difference in harvest, cultivar, and their interaction for free and total SLs, sucrose, and Sugar:SL ratio. Plant fresh weight was greatest in Spring, 2020, perhaps due to an increase stage of maturity at

harvest, followed by Fall, 2020, Early Summer, 2021, and Winter, 2021. Total SLs and glucose were highest in Fall, 2020, followed by Spring, 2020, Early Summer, 2021, and Winter, 2021. Total sugars were the same between harvests, and Winter, 2021 had a significantly lower Sugar:SL ratio than the other three harvests. Romaine and batavian cultivars (excluding ‘Cherokee’) emerge as top candidates to grow during the summer due to higher plant weights, sugar concentrations, and the Sugar:SL ratio, as well as decreased SL concentrations. Salanova® cultivars produced low yields (exception ‘Sweet Crisp Green’) in comparison to traditional varieties, and often had significantly greater levels of sesquiterpene lactones. Chilling with a temperature differential of approximately 8 °C (15 °F) was found to be an effective treatment in the summer to reduce total SL concentration, and increase the Sugar:SL ratio, especially in romaine and Salanova® cultivars. Total sugar concentrations were not significantly different in the summer or winter using chilling. Chilling is a less effective option for winter months; however, overall SL and sugar concentrations were notably low for the Winter, 2021 harvest season.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most important leafy vegetables in the United States (Kim et al., 2018). Belonging to the Asteraceae plant family, lettuce varies in market type and cultivar, with market types including loose-leaf, romaine, butterhead, batavian, and proprietary market types such as Salanova®. Lettuce varieties possess different morphological and genetic traits including open and closed-heads, midrib formation, and degree of heat-tolerance – an important characteristic to possess when grown in hot climates such as Oklahoma (Thakulla et al., 2021). Lettuce heat tolerance has been previously documented, with romaine market types yielding greater plant fresh weights, and specific cultivars such as ‘Nevada’ (batavian) and ‘Parris Island’ (romaine) performing better in hot greenhouses compared to ‘Buttercrunch’ (butterhead), ‘Coastal Star’ (romaine), and ‘Jericho’ (romaine) (Afton, 2018; Holmes et al., 2019).

Lettuce is a cool season crop that performs poorly under heat and light stress (Hao et al., 2018). These stresses cause lettuce to prematurely bolt – the shift from vegetative to reproductive growth. Along with this change is a decrease in edible quality due to accumulation of bitter-tasting compounds called sesquiterpene lactone (SL), as well as translocation of sugars from leaves to reproductive tissues (Khan, 2018; Lee and Sugiyama, 2006). Research shows that lower SL concentrations are correlated to a better perceived taste in lettuce; additionally, sugar concentration and the Sugar:SL ratio interplay and are important metrics to include for quantitative assessment of lettuce edible quality (Chadwick et al., 2016).

Season plays an important role in lettuce production, as environmental factors for growth such as maximum temperature, light intensity and duration, and humidity change depending on the time of year (Sublett et al., 2018). One way to increase consistency between seasons is by growing plants in a greenhouse. While there remain noticeable differences in environmental conditions between seasons in a greenhouse, the differences are less pronounced than outdoors, and mechanical systems can moderate hot summer temperatures (and low winter temperatures) to allow non-seasonal production (Lei et al., 2021).

Research shows that chilling hydroponic nutrient-solutions during summer months positively impacts growth and delays the onset of bolting, even in environments with elevated air temperatures (Thompson et al. 1998). One study showed lettuce grown using chilling was 15% greater for shoot fresh weight than plants grown at non-chilled conditions (21.1 °C for chilled temperatures versus non-chilled temperatures up to 25.5 °C, 30.4 °C, and 24.7 °C for replications one, two, and three, respectively) (Thakulla et al., 2021). °Brix values, or soluble sugar content, showed an increase with a decreasing nutrient-solution temperature, and the authors suggested 21.1 °C was the optimal temperature to minimize root-zone stress from temperatures too high or low, but 18.3 °C showed increased °Brix values possibly due to an induced water stress, impacting osmoregulation, and accumulating sugars, minerals, and amino acids into the cells to maintain turgor pressure. Delayed bolting may reduce sesquiterpene lactone (SL) accumulation and/or increase the Sugar:SL ratio, which could lead to a better perceived flavor. The effects of chilling during the winter are less studied.

This study investigated the extent to which SL, sugar, Sugar:SL ratio and plant weight in hydroponically grown lettuce were influenced by season and cultivars representing different lettuce market types. Hydroponic nutrient-solution chilling was also investigated in two seasons (summer and winter) to assess changes in these variables across cultivars and lettuce market types. The objectives of the study were a) to evaluate SL, sugar, Sugar:SL ratio and plant weight across spring, summer and winter seasons of production, and then b) to determine if chilling the nutrient-solution in summer and winter production seasons could change SL, sugar, Sugar:SL ratio and plant weight for twelve cultivars representing romaine, batavian, butterhead and Salanova® market types of lettuce. The overall goals of our studies were to identify lettuce market types and/or cultivars which grow well and exhibit favorable indices of quality in different production seasons, as well as to develop production practices which improve quality.

MATERIALS AND METHODS

2.1 Reagents and Chemicals

High-performance liquid chromatography (HPLC) water was sourced at a conductivity of 18.2 megohm (D-4641, Barnstead E-Pure Ultrapure Water Purification System, Thermo Scientific, Waltham, MA, USA). BSTFA plus 1% trimethylsilyl (TMS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). HPLC grade methanol (≥ 99%) was purchased from EMD Millipore Corporation (Billerica, MA). HPLC grade acetonitrile (≥ 99%) was purchased from Spectrum Chemical (Gardena, CA). Dichloromethane (≥ 99.5%) and Isopropanol (≥ 99.5%) were purchased from Pharmco-Aaper (Brookfield, CT). Ethyl acetate (≥ 99.5%), dimethylformamide (≥ 99.8%), formic acid (≥ 98%), D-(+)-glucose (≥ 99.5%), D-(−)-fructose (≥ 99%), sucrose (≥ 99.5%), the internal standard, inositol (≥ 99%) and the internal standard santonin (≥ 99%) were purchased from Sigma-Aldrich (St. Louis, MO). Lactucin (≥ 95%) and lactucopicrin (≥ 95%) standards were purchased from Extra Synthase (Cedex, France), and 8-deoxylactucin (≥ 95%) was purchased from Analyticon Discovery (Potsdam, Germany).

2.2 Plant Material

Twelve cultivars of lettuce, encompassing romaine, butterhead, batavian, and Salanova® market types were grown for this study. Seeds were purchased from Johnny's Seed Company (Winslow, Maine, USA) as pelleted or non-pelleted dependent upon commercial availability of the pelleted form. Lettuce market type, cultivar, and seed form (pelleted or non-pelleted) information are included in Table 1. Seedlings were germinated in 1.5 cm^3 Oasis cubes (Oasis Grower Solutions, Kent, OH) at a density of 1 plant per cube, on a mist bench in two locations: Oklahoma State University Horticulture Research Greenhouse facility adjacent to campus and the Greenhouse Learning Center on the Oklahoma State University Campus (Stillwater, OK, USA). Mist bench emitters were turned on in 10 min intervals for a duration of 5 ss. Miracle Gro (200 ppm solution of 24N-4.8P-9.6K (Scotts Miracle-Gro Company, Marysville, OH, USA) was applied in a single application 2 weeks after placement on the mist bench to increase rooting. Seedlings were held on the mist bench for 4 weeks prior to transferring into hydroponic culture.

2.3 Hydroponic Culture

Seedlings were transplanted into Hydrocycle Pro NFT tables (Growers Supply, Dyersville, IA, USA) in a randomized complete block design (RCBD) with five plants per cultivar per replication, and four replications over time in Spring, 2020, Fall, 2020, early Summer, 2021, and Winter, 2021 for the season evaluation study on dates denoted in Table 2. Two tables were used and each hydroponic table included 10 troughs, with 18 planting-holes per trough (180 planting-holes per table). Each trough measured 10 cm wide x 5 cm deep x 900 cm long, with 20 cm spacing between planting-holes. The tables had a decline in slope of approximately 3% between the inlet and drainage ends. Hydroponic solutions within each 150 L table reservoir were initially started at 1.0 mS EC using fertilizer (Jack's Hydroponic Special 5N-12P-26K; JR Peters Inc., Allentown, PA, USA) and at pH 6.0 using pH down (General Hydroponics, Santa Rosa, CA, USA). Electrical conductivity (EC) was increased to 2.0 mS over the course of 2 weeks (0.5 mS/wk). Solutions were then maintained at 2.0 mS by monitoring and adjusting daily. EC and pH were measured using a dual EC/pH meter (HI 9831-6, Hanna Instruments, Woonsocket, RI, USA). Flowrate of nutrient solution was 1500 $L \cdot hr^{-1}$ for each table. Dissolved oxygen was maintained between 8 to 14 ppm using an aquarium air pump (Hydrofarm, Active Aqua AAPA15L, Petaluma, CA, USA). Temperature and humidity readings were recorded using a TR-7Ui multi-data logger (T&D, Matsumoto-City, Japan) and is reported in Table 3.

2.4 Hydroponic-Solution Chilling Study

A hydroponic nutrient-solution chilling study was conducted with a subset of lettuce cultivars utilized for the season evaluation study (Table 1**)** in parallel with the early summer and winter plantings in 2021 (Table 2). The nutrient solution, maintained in a separate reservoir under the same conditions as indicated for the season evaluation study, was chilled using a flow-through TK-2000 aquarium chiller (TEKO) set at 18.3 °C (65 °F). Actual nutrient-solution temperatures for chilled and non-chilled treatments were monitored using a Digi-Sense K-type thermocouple probe, located in the nutrient solution within the hydroponic trough and downstream of the plants (Cole-Parmer, Model 20250-02, Vernon Hills, IL, USA). Readings were taken every 10 min.

2.5 Lettuce Harvesting and Processing

At harvest, the three (of five) best performing lettuce plants, separated by replication, with adhering roots attached to the Oasis cube, were lifted out of the hydroponic trough, placed into a labeled bag, transferred to laboratory facilities at the Noble Research Center on the Oklahoma State University campus and held in a cold room at 2 °C prior to processing. During processing lettuce plants were cut at the Oasis cube and the cube and roots were discarded. Damaged leaves were removed and shoot fresh weights for each lettuce head was recorded. Samples were washed, head cores removed, and a final weight was taken. Samples were secured in cheesecloth, placed into a freezer bag, and held in a walk-in freezer at -20 °C prior to freeze-drying. Samples were freeze-dried using a Harvest Right freeze-dryer (HRFD-PLrg-SS, Harvest Right, North Salt Lake, UT, USA) with a final shelf temperature of 21.1 °C (70 °F) for approximately 100 h. After completion of drying, lyophilized samples were weighed and ground into 120 mL brown bottles through a 1mm screen using a UDY Cyclone Mill (UDY Corporation, Fort Collins, CO, USA). Immediately after grinding, duplicate samples of approximately ~150 mg were weighed for each lettuce sample to undergo moisture content analysis. Samples were placed into an oven at 80 °C for 48 h. Moisture content of freeze-dried samples was determined by weight loss and calculated as a percent. The remainder of the sample was utilized immediately for sugar and SL extraction and analysis as described below.

2.6 Sugar Extractions

Sugar extraction and preparation for analysis was done according to Maness (2010) and Davies (1988), with some modifications. Approximately 200 mg of freeze-dried sample materials was accurately weighed into duplicate 2-dram vials. Samples were extracted with 2 mL of 95% ethanol by boiling under reflux at 85 °C for 20 min, with mixing every 5 min, using a digital dry block heater (Isotemp, Fisher Scientific, Waltham, MA, USA). After extraction, samples were centrifuged for 15 min at 3,000 g_n using a SpeedVac® centrifuge (SPD-121P, Thermo-Savant, Waltham, MA, USA) and filtered using Whatman 41 filter paper (Cole-Parmer, Vernon Hills, IL, USA) into 10 mL volumetric flasks. Samples were re-extracted three additional times, and the combined supernatants were brought to volume after rinsing the filter paper three times with 95% ethanol. Sample solutions were then transferred and stored in securely capped brown bottles.

Duplicate 300 µL aliquots were placed into 2 dram vials and 100 µg of inositol was added as internal standard to each sample. Samples were dried overnight in a Speed Vac. To remove contaminants, 250 mg of a MB-1 ion-exchange resin (UCW3600, Purolite, Philadelphia, PA, USA) and a micro stir-bar were added to each sample. Deionized H_2O (1 mL) was added, and samples were stirred on a multi-stir plate (Cole-Parmer, Vernon Hills, IL) for 2 h. Samples were then centrifuged for 10 min at 3,000 g_n and the supernatant was decanted into a new vial. The supernatant was dried using a

SpeedVac and placed into a desiccator overnight with lids loosened. N, O-Bistrifluoroacetamide (BSTFA) + 1% TMS (50 µL) was added, samples were vortexed for 30 ss and incubated at room temp for 1hr. Dimethylformamide (100 µL) was added, samples were vortexed for 30 s and incubated for another hour at room temperature prior to analysis as described below. Samples appeared to be stable for at least 6 h after addition of DMF. Multiple samples were prepared for morning injection and new batches were prepared for afternoon injection onto a Gas-Chromatograph (GC)

2.7 Sugar Analysis by Gas-Chromatography (GC)

Sugars were quantitated by injection onto a Varian 3400 GC (Agilent Technologies, Santa Clara, CA). Samples were vortexed for 30 s and 0.5 µL was injected onto a DB-5 capillary column (30 m column length, 0.25 mm diameter, 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA) equipped with a splitless injector held at 260 °C. The column temperature was initially held at 140 °C for 2 min, followed by a ramp of 20 °C/min until reaching 280 °C and held for 9 min. Peaks were detected using a flame ionization detector (FID) held at 300 °C. Chromatographic data from the FID signal was collected using Dionex Peak Net (Dionex Corporation, Sunnyvale, CA) software. Sugars were identified according to co-elution with authentic standards and quantitated using inositol as internal standard.

2.8 Sesquiterpene Lactones (SLs) Extrraction

A modified procedure from Ferioli and D'Antuono (2012) was used to extract SLs. Approximately 200 mg of freeze-dried plant material was accurately weighed in duplicate for free and for bound SL determinations of each sample. Prior to extraction of the quadruplicate samples, 20 µg of santonin was added as internal standard and 3 mL of extraction solvent [MeOH, H_2O (4:1, v/v) + 2% - Formic Acid] was then added. The samples were mixed for 15 s using a Vortex Genie stirrer (Scientific Industries, Bohemia, New York) set at maximum speed and incubated at 60 °C for 30 min, with stirring every 10 min. After incubation, the samples were centrifuged at 3,000 g_n for 20 min using a Speed Vac centrifuge. The supernatant was transferred into a separate vial, and the extraction was repeated. Due to cloudiness, the combined supernatants were re-centrifuged, decanted into a clean vial, and dried in a Speed Vac Centrifuge overnight (SVC-100H, Savant, Farmingdale, NY, USA).

2.9 Enzymatic Cleavage for Determination of Bound SLs

The dried quadruplicate samples were reconstituted into deionized H_2O (3 mL) using vortex stirring for 20 s. For bound SL determination, Cellulase enzyme (*Aspergillus niger*, 25 mg, 1.1 units/mg, Sigma-Aldrich) was added to one duplicate set of the samples, and both free and bound duplicate sets of samples were vortexed and incubated at 40 °C for 2 h. SLs were recovered into 2 mL of ethyl acetate with vortexing for 15 s, and samples were centrifuged at 3,000 g_n in a Speed Vac centrifuge for 10 min to accommodate phase separation. The upper ethyl acetate phase was recovered and the ethyl acetate SL recovery process was repeated twice more. Combined ethyl acetate phases were evaporated to dryness for 3 h using a Speed Vac. The residues for both free and bound samples were re-dissolved into 1 mL of methanol and overlaid with 5 mL of dichloromethane to be further processed as described below.

2.10 Purification of SLs using Solid-Phase Extraction (SPE)

Both free and total SL containing fractions were processed by SPE according to Ferioli and D'Antuono (2012) using Extract-Clean silica columns (2.8 mL reservoir/500 mg silica sorbent; Alltech Associates Inc., Deerfield, IL, USA). The columns were preconditioned with 6 mL of dichloromethane/isopropanol (1:1, v/v) and equilibrated with 6 mL of dichloromethane. Samples were gravity fed through the columns and the eluate was dried for 2 h using a Speed Vac. Columns were reconditioned with 6 mL of dichloromethane/ethyl acetate (3:2, v/v) and the eluate was discarded.

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2.11 Quantitation of SLs using High-Performance Liquid Chromatography (HPLC)

Prior to HPLC injection, samples were dissolved in 1 mL HPLC H_2O/MeOH (1:1, v/v), vortexed until the pellet was re-dissolved, and filtered using a stainless steel Millipore Filter apparatus (Millipore Corportation, Billerica, MA) with a 0.45 µm Nylon 66 filter (Supelco, Bellefonte, PA) and a Whatman 41 (Whatman International, Maidstone, England) pre-filter. HPLC analyses were performed using a Thermo-Dionex Ultimate-3000 (ThermoFisher Scientific, Waltman, MA, USA) gradient pump and PDA-

1 diode array detector. Sesquiterpene lactones (SLs) were detected at 264 nm, and injection volumes were set for 10 µL. Samples were injected using a Thermo-Dionex Ultimate 3000 autosampler (ThermoFisher Scientific, Waltman, MA, USA). The system employed a Kinetex (5µm) XBC18 [250 x 4.6 mm] column equipped with a C18 [4 x 3.0 mm] pre-column with cartridges placed in a Security Guard apparatus (Phenomenex, Torrance, CA). Flow rate was set to 1.0 mL min^{-1} and elution solvents were 10% and 55% acetonitrile in HPLC H_2O, for Solvent A and Solvent B, respectively. An eluent gradient program of 48 min was established: 100% solvent A for 5 min, followed by a linear gradient to 85% solvent B by 35 min, and then to 100% solvent B at 36 min. Solvent B was held at 100% for 8 min, and then returned to 100% solvent A over 1 min. Initial conditions of 100% solvent A were held for 3 min prior to the next injection. Chromatograms were analyzed using the chromatography data system Chromeleon 7 (Thermo-Dionex, Waltman, MA). SLs were identified according to co-elution with authentic standards and quantitated relative to santonin as internal standard.

2.12 Data Analysis

Data was analyzed with SAS 9.4 (SAS Inc., Cary, NC, USA) using the PROC GLIMMIX procedure. The season evaluation study included harvest season, cultivar, and the harvest season x cultivar interaction in four replications, while the hydroponic nutrient-solution chilling study evaluated differences between non-chilled and chilled nutrient-solution temperature treatments during both a summer and winter season. When appropriate, differences among treatment means were determined using Tukey's LSD (p-

≤ 0.05) for the season evaluation study. An f-test (sliceby function) was utilized to determine significance ($p \leq 0.1$) between nutrient-solution temperature treatments for the hydroponic nutrient-solution chilling study, with an adjustment to account for unequal variances between temperature treatments.

RESULTS

Season Evaluation Study - Harvest Season and Cultivar Effect on Lettuce Quality Indices

All three free and total SLs, sucrose and the Sugar:SL ratio exhibited significant harvest season x cultivar interactions (Table 4). Significant differences were only observed for free lactucin in Summer, 2021, with butterhead 'Buttercrunch' showing the greatest concentration (Table 5). Batavian ‘Cherokee' showed the most abundant free 8-deoxylactucin levels regardless of harvest season. Lactucopicrin was the most abundant SL in free and bound forms across all harvests and cultivars, with the occasional exception of batavian ‘Cherokee’ which showed exceptionally high 8-deoxylactucin. Generally, romaine market types displayed less total SL concentrations, while Salanova® displayed greater total SL concentrations. Significant differences were only observed for sucrose in Summer, 2021, with butterhead ‘Nancy' showing the greatest concentrations. The Sugar:SL ratio was significantly different in Summer, 2021 and Winter, 2021 harvest seasons, with batavian 'Nevada' showing the greatest numerical Sugar:SL ratio during Summer, 2021 and romaine 'Jericho' showing the greatest in Winter, 2021.

Significant main effects within harvest season were noted for bound SL concentrations of 8-deoxylactucin and lactucopicrin, fructose, glucose, total sugars and plant fresh weight (Table 4). Bound SL concentrations were almost a magnitude lower than their free counterparts (Tables 5, 6). Bound 8-deoxylactucin was greatest in spring, 2020 and least in Fall, 2020 and winter, 2021 (Table 6). Bound lactucopicrin was greatest in spring, 2020 and Summer, 2021, and was least in Winter, 2021. Fructose, glucose and total sugar was greater in summer harvest seasons than in spring. Fructose was greatest for both summer harvest seasons (Fall, 2020, Summer, 2021) and least in spring, 2020, and winter, 2021. Glucose was greatest in Summer, 2021, followed by Fall, 2021, then spring, 2020 and winter, 2021 which showed similar concentrations. Total sugar was greatest in Summer, 2021 and least in spring, 2020. Plant fresh weight was greatest for spring, 2020 and Fall, 2020, and least in Summer, 2021 and Winter, 2021.

Significant main effects within cultivar were noted for bound 8-deoxylactucin, fructose and plant fresh weight (Table 4). Bound 8-deoxylactucin was greatest for 'Cherokee' (Table 7). Fructose concentrations were highest in two batavian cultivars ('Nevada' and 'Sierra'), and lowest in Salanova® 'Butter Red'. Plant fresh weight was numerically greatest for romaine and batavian market types, with romaine 'Jericho' showing the greatest plant weight and Salanova® 'Butter Red' and 'Butter Green' showing the least. Notably ‘Summer Crisp Green’ was the only Salanova® cultivar studied which fell into the upper statistical grouping.

Nutrient-Solution Chilling Study – Nutrient-Solution Chilling and Cultivar Effects on Quality Indices

Thermocouple data for nutrient-solution temperatures are provided in Figure 2 (Summer, 2021) and Figure 3 (Winter, 2021). For Summer, 2021, the non-chilled treatment mean and standard deviation was 29.6 ± 2.7 °C and chilled mean and standard deviation treatment was 22.1 ± 2.1 °C, accounting for an average 7.5 ± 1.2 °C temperature differential. For Winter, 2021, the non-chilled treatment mean and standard deviation was 17.7 ± 3.8 °C, and the chilled treatment mean and standard deviation was 17.3 ± 1.7 °C, with a temperature differential of only 1.9 ± 1.7 °C. Due to the unequal experimental variance caused by differences in hydroponic nutrient-solution temperatures between the seasons, data was analyzed by season.

Summer, 2021 – Nutrient-Solution Chilling Study

Within the cultivar x nutrient-solution temperature treatment interaction, significant differences were observed for free lactucin, free and bound 8-deoxylactucin, total SLs, fructose, sucrose, Sugar:SL ratio, and plant fresh weight (Table 8). Romaine ‘Coastal Star' and butterhead 'Nancy' showed a greater concentration of free lactucin when the hydroponic nutrient-solution was chilled whereas Salanova® 'Butter Red' and 'Butter Green' showed less concentration when the hydroponic nutrient-solution was chilled (Table 9). Batavian ‘Cherokee' exhibited greater concentrations of both free and bound 8-deoxylactucin versus all other cultivars and across both nutrient-solution temperature treatments. Total SLs were significantly reduced when the hydroponic nutrient-solution was chilled for all romaine (Parris Island, 'Jericho', 'Coastal Star') and Salanova® ('Butter Red', 'Butter Green', 'Sweet Crisp Green') cultivars. Romaine cultivars ‘Parris Island' and 'Coastal Star' showed a significantly greater concentration of fructose in chilled samples. Butterhead ‘Nancy' and batavian 'Nevada' showed less sucrose when the hydroponic nutrient-solution was chilled. All romaine cultivars (‘Parris Island’, 'Jericho', 'Coastal Star') and one Salanova® cultivar ('Sweet Crisp Green') exhibited greater Sugar:SL ratios when the hydroponic nutrient-solution was chilled. Butterhead ‘Nancy' and Salanova® 'Sweet Crisp Green' had significantly greater plant fresh when the hydroponic nutrient-solution was chilled.

Significant main effects within cultivar were noted for free and bound lactucopicrin, and total sugar (Table 8). Salanova® ‘Butter Red' and 'Butter Green' showed the greatest concentration of free lactucopicrin, while Batavian 'Nevada' and 'Cherokee' showed the least. All romaine cultivars ('Parris Island', 'Jericho', 'Coastal Star’), Salanova® 'Sweet Crisp Green' and batavian 'Nancy' showed low levels of free lactucopicrin. Batavian ‘Cherokee' showed the least bound lactucopicrin concentration, while Salanova® 'Butter Red’ showed the greatest. Batavian ‘Nevada' showed greater total sugar concentration than batavian 'Cherokee' and Salanova® 'Butter Red'. The other cultivars were not significantly different from either group.

Significant main effects across nutrient-solution chilling treatments were noted for free and bound lactucopicrin, and bound lactucin (Table 8). The non-chilled treatment showed significantly greater concentrations for all three main effects variables (Table 11).

Winter, 2021 – Nutrient-Solution Chilling Study

Within the cultivar x nutrient-solution temperature treatment interaction, significant differences were observed for free 8-deoxylactucin and lactucopicrin, bound lactucin, and total SLs (Table 12). Romaine ‘Coastal Star’, batavian ‘Cherokee’, and Salanova® ‘Butter Green’ and ‘Sweet Crisp Green’ showed a greater concentration of free 8-deoxylactucin when the hydroponic nutrient-solution was chilled (Table 13). Romaine ‘Parris Island’, in addition to all Salanova® cultivars (‘Butter Red’, ‘Butter Green’, and ‘Sweet Crisp Green’) showed greater concentrations of free lactucopicrin when the hydroponic nutrient-solution was chilled; all of these cultivars also showed greatest concentrations of total SLs except for Salanova® ‘Butter Green’ when the hydroponic nutrient-solution was chilled. Batavian ‘Nevada’ and ‘Cherokee’ showed lower concentrations of bound lactucin when the hydroponic nutrient-solution was chilled.

Significant main effects within cultivar were noted for free lactucin, bound 8-deoxylactucin and lactucopicrin, fructose, glucose, total sugar, and plant fresh weight (Table 12). Several cultivars shared statistical groupings for free lactucin, with Salanova® ‘Butter Green’ showing a higher concentration than batavian ‘Nevada’ and romaine ‘Jericho’ (Table 13). Bound 8-deoxylactucin was not noted in the three romaine cultivars (‘Parris Island’, ‘Jericho’, and ‘Coastal Star’) and two of the Salanova® cultivars (‘Butter Red’ and ‘Summer Crisp Green’) and was otherwise not different among the remaining four cultivars. Bound lactucopicrin was greater in Salanova® ‘Butter Red’ than in romaine cultivars ‘Parris Island’ and ‘Jericho’ and batavian ‘Cherokee’. Slanova ‘Butter Red’ exhibited lower fructose concentration than the romaine cultivars (‘Parris Island’, ‘Jericho’, and ‘Coastal Star’) and Salanova® ‘Summer Crisp Green’, while Salanova® ‘Butter Green’ and butterhead ‘Nancy’ exhited lower glucose concentration than the romaine cultivars (‘Parris Island’, ‘Jericho’, and ‘Coastal Star’), the batavian cultivars (‘Nevada’ and ‘Cherokee’) and Salanova® ‘Summer Crisp Green’. For total sugars romaine ‘Coastal Star’ exhibited greater concentration than butterhead ‘Nancy’ and Salanova® ‘Butter Red’ and Butter Green’. Romaine ‘Jericho’ had the greater plant fresh weight, than all cultivars except romaine ‘Coastal Star’.

Significant main effects within the hydroponic nutrient-solution treatment were noted for sucrose and total sugar (Table 12). The non-chilled treatment showed greater concentrations of sucrose (Table 15). Total sugar ($p = 0.089$) was determined to be significant at the designated p-value ($p \leq 0.1$), although the pairwise comparison returned with non-significant means separations.

DISCUSSION

Seasonal Evaluation Study - Harvest Season and Cultivar Effect on Quality Indices

Most studies involving hydroponic lettuce have reported plant growth as a primary outcome of their work (Lei et al., 2021; Sharma et al., 2018; Thakulla et al., 2021) but only a few papers have established a seasonal influence on hydroponic lettuce growth (Djidonou and Leskovar, 2019; Fallova et al., 2009). Djidonou and Leskovar (2019) observed greater head weight, as a product of higher rate of leaf appearance, in hydroponically-grown lettuce in the spring versus the fall and winter growing seasons. We found that head weight was greater in both spring and fall production seasons versus winter (Table 6). We also noted a substantial decrease in head weight during summer, with head weights equivalent to winter lettuce. Water temperature is a critical environmental factor in hydroponic grow environments, along with air temperature and light duration, as each affect photosynthesis, respiration, and ultimately, plant growth (VanDerZanden, 2008; Zobayed et al., 2005). Unregulated water temperatures trend higher in summer and lower in winter in greenhouse production environments; too high or too low nutrient-solution temperature may stress the plants and lead to poor plant growth due to inadequate nutrient uptake (Nxawe et al., 2009; Thakulla et al., 2021), leading to a low growth rate (Djidonou and Leskovar, 2019). Lettuce is a long-day plant that undesirably flowers when exposed to light durations exceeding 12 hours per day, but insufficient light durations can occur in the winter and lead to slow plant growth (VanderZanden, 2008). Cultivars, as a main effect, showed differences in plant fresh weight, highlighting the importance cultivar selection plays in maximizing plant growth, especially when cultivating in more limited growing spaces such as greenhouses.

Romaine market types were among the largest, which agrees with previous research (Afton, 2018; Table 8). Afton (2018) showed that romaine market types were heaviest compared to butterhead, crisphead, and leaf market types. Our data suggests most romaine and butterhead lettuce heads were equivalent in weight, although romaine ‘Jericho’ did yield more than butterhead ‘Nancy’ (Table 7). Afton (2018) also noted differences in weight between cultivars within a market type, indicating that while lettuce yield may be loosely categorized by market type, substantial differences in performance of individual cultivars must be considered in making production decisions based on yield.

A significant cultivar x harvest season interaction for all free SLs, total SLs and the Sugar:SL ratio, but not for total sugars (Table 4), may indicate that any seasonal influence on putative lettuce flavor across cultivars depended more on SL concentrations than on total sugars for the lettuce market types and cultivars we studied. According to Chadwick et al. (2016), a high Sugar:SL ratio for lettuce correlates to less bitter perception by taste panelists and higher overall acceptance of the lettuce. Significant differences in the Sugar:SL ratio occurred between cultivars in Summer, 2021 and Winter, 2021, with Winter, 2021 exhibiting greater ratios (presumably less bitter flavor) than the Summer, 2021 harvest (Table 5). Total SL concentrations were about three times lower in Winter, 2021 for most cultivars while total sugar concentrations across cultivars were less than two times lower in the Winter, 2021 versus the Summer, 2021 harvest seasons (Table 6). This data further supports our finding that SL concentration of lettuce may exert a larger influence on the Sugar:SL ratio than sugar concentration, which was not directly investigated in previous research (Chadwick et al., 2016). Some caution in this finding may also be warranted when individual sugar concentrations are considered; plants from Summer,2021 harvest season were significantly greater in fructose compared to Winter, 2021, which may serve to modulate bitter perception indicated by the greater Sugar:SL ratio in the Winter, 2021 production season (Table 5). Fructose is arguably the most important sugar for flavor perception, as research shows it is perceived to be sweeter than glucose and sucrose (Chadwick et al., 2016).

Romaine and batavian (with the exception of 'Cherokee') cultivars consistently performed in lower statistical groupings for the concentration of the most abundant SL found in the lettuce samples, lactucopicrin (Table 5). Since lactucopicrin predominated in concentration among the other SLs evaluated in this study, with the exception of 'Chreokee,' in which 8-deoxylactucin predominated, a lower abundance may decrease the putative perception of bitterness. Seo et al. (2009) found that lactucopicrin was also the major SL in Korean lettuces, and using a bitternes score concluded that lactucopicrin exerted the greatest influence on lettuce bitter off-flavor. Seeing a significant interaction between harvest season and cultivar for free lactucopricrin and total SLs, suggests an interplay for SL production based on differing environmental factors such as temperature and daylength (Hao et al., 2018).

Seasonal effects influenced SLs across the board, but some cultivars, especially ‘Butter Red’ and ‘Butter Green’ within the Salanova® market class, appear to be effected greater by increased stress associated with the summer heat and longer day-lengths. Very little is known about putative flavor characteristics for the Salanova® lettuce market class. We did note substantially higher free and total SLs, and corresponding lower Sugar:SL ratio in the “Butter” Salanova® cultivars compared to other market types (Table 5), which might indicate a greater propensity towards bitter flavor perception for these cultivars. Alternatively, the “Summer Crisp” Salanova® cultivars exhibited slightly higher (but not significantly) Sugar:SL ratios compared to the “Butter” cultivars, perhaps indicating that in certain environments there may be differences in bitter flavor perception among the Salanova® cultivars.

Summer, 2021 – Nutrient-Solution Chilling Study

Previous research, although conducted without the use of harvest season repetition, suggested that the use of nutrient-solution chilling may increase sugar concentration in lettuce (Thakula et al., 2021), as well as increase head size in summer production (Thompson et al., 1998). From our data generated in Summer, 2021, chilling appeared to increase indices of quality in lettuce by reducing total sesquiterpene lactone concentration across most cultivars, and increasing the Sugar:SL ratio for select cultivars (Table 9). Romaine and Salanova® market types showed the greatest total SL reduction using chilling, with corresponding increases in Sugar:SL ratio for all romaine and ‘Summer Crisp Green’ Salanova® cultivars. Increasing fructose could be important to increasing overall palatability, as studies show fructose is perceived to be sweeter than the other soluble sugars (Chadwick et al., 2016). Romaine ‘Jericho’ and Salanova® ‘Sweet Crisp Green’ had the highest plant fresh weight, which was an interesting finding as most Salanova® cultivars typically performed in the lowest statistical grouping for plant fresh weight (Tables 5, 9). Through the combination of decreased total SL concentration, increased plant fresh weight, and greater fructose concentrations, our research suggests the romaine market types, especially cultivars ‘Parris Island’ and ‘Coastal Star’, benefits the most from chilling during the summer. Batavian ‘Nevada’ emerged as top candidate to grow in the summer due to significantly less free lactucopicrin concentrations and greater total sugar concentrations (Table 10). Salanova® was benefitted by a reduction in total SL concentration, but the comparatively lower plant fresh weight for the “Butter” cultivars may make them less attractive as a summertime greenhouse crop.

Batavian ‘Cherokee’ was the only cultivar that displayed an increase in total SLs when the nutrient-solution was chilled during the summer trial, mostly due to an increase in 8-deoxylactucin concentration (Table 9). This cultivar exhibited abnormally high levels of 8-deoxylactucin in both free (Table 5) and bound forms (Table 7) in the season evaluation study as well as both nutrient-solution chilling seasons (Tables 9 and 13). Our data agrees with previous studies showing 8-deoxylactucin levels are low or devoid in several lettuce cultivars (Price et al., 1990; Tamaki et al., 1995). An observation we noted is batavian ‘Cherokee’ was phenotypically one of the darkest (red) lettuces in our study, with research showing colored lettuce often ranking higher in bitterness compared to green lettuce due to higher SL concentrations (Chadwick et al., 2016). This supports evidence from our study in which we showed SLs were numerically high for ‘Cherokee’ compared to other cultivars throughout the various harvest seasons. To further this evidence. another study noted struggling with bolting and bitterness when growing ‘Cherokee’ in a summer variety trial (Beebout et al., 2019). Other studies show lower bitterness correlation tied to 8-deoxylactucin compared to lactucin and lactucopicrin (Price et al., 1990; Seo et al., 2009), suggesting compounds besides SLs, such as anthocyanins and phenolics, may significantly impact the overall bitterness perception, especially in darker lettuces (Bunning et al., 2010; Chadwick et al., 2016).

Winter, 2021 – Nutrient-Solution Chilling Study

Data generated in Winter, 2021 yielded interesting information regarding nutrient-solution chilling – not only did nutrient-solution chilling not increase the indices of flavor measured in this study (SLs and sugars), nutrient-solution chilling could potentially be detrimental as free and total SLs showed an increase in concentration for some cultivars in the chilled hydroponic nutrient-solution treatments (Table 13). The chilled hydroponic nutrient-solution treatment also resulting in lower sucrose concentrations across cultivars (Table 15). Sesquiterpene lactones and sugars were notably lower in winter for both chilled and non-chilled hydroponic nutrient-solution treatments compared to summer; therefore, differences in flavor perception are likely to be less pronounced between the two seasons of production. Additionally, plant fresh weight was low in winter (Table 14) even though plants remained in the hydroponic growing system seven days longer than in other harvest seasons (Table 2), supporting research from Djidonou and Leskovar (2019) showing time until harvest maturity (affected by plant growth rate) is season dependent, taking longer in the winter to mature compared to spring. When looking at nutrient-solution temperature data for this season, the chilling treatment reduced water temperature fluctuation throughout the day, reducing the range of temperatures subjected to the plants (Figure 3). The non-chilled solution reached a maximum temperature that was likely not high enough to warrant chilling in the winter, as research shows there is an optimum temperature for growth, and exceeding the lower temperature range can result in less plant fresh weight (Thakulla et al., 2021). A more effective treatment would likely be heating hydroponic nutrient-solutions in the winter, as another study shows plants grown in hydroponic nutrient-solutions with elevated water temperatures (28 °C) had greater fresh plant weight after 8 weeks compared to plants grown in an ambient temperature nutrient-solution (10 °C) during the winter for spinach (Nxawe, et al., 2009).

Conclusions

Overall, romaine and batavian market types (excluding ‘Cherokee’) generally appeared to be the best performers in the summer (greatest plant fresh weight, least SL concentration, greater Sugar:SL ratio), with butterhead cultivars performing similarly in spring, and differences in flavor likely being less pronounced in winter due to lower concentrations of SLs and sugars. Growers want to select cultivars that yield high plant fresh weight, as this leads to a more cost-effective harvest, but also must account for flavor. Lettuce with a lower SL concentration and/or higher sugar concentration suggests less perceived bitterness, which consumers often wish to avoid, and a greater Sugar:SL ratio has been shown to increase palatability in lettuce, as sweetness from sugars can mask bitterness from SLs (Chadwick et al., 2016). Due to greater plant fresh weight, low SL concentrations, and greater Sugar:SL ratio, our recommendation based on this research is to grow romaine and batavian market types (exception, ‘Cherokee’) during the summer months in hydroponic crop culture. Cultivar selection within market type is critical since cultivar performance varies within market type (Afton, 2018).

Hydroponic-solution chilling proved to be an effective way to potentially increase edible quality of some lettuce cultivars grown in the summer through reduced SL concentrations and an increased Sugar:SL ratio. Romaine cultivars may have benefitted most from chilling, as all cultivars ‘Parris Island’, ‘Jericho’, and ‘Coastal Star’ exhibited reduced total SLs and increased Sugar:SL ratios. Salanova® market types also exhibited a reduction in total SLs when using chilling, but generally had low plant fresh weight, and numerically high SL concentrations. Due to the numerically high SL concentrations in the “Butter” Salanova® cultivars, nutrient-solution chilling appears to be critical for summer production if planning on cultivating Salanova® lettuce. Chilling the hydroponic nutrient-solution appeared to be less effective, or even detrimental, to the edible quality of lettuce when used in the winter. Alternatively, research shows heating hydroponic nutrient-solutions may be more beneficial, as previously documented for spinach (Nxawe et al., 2009).

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CHAPTER II TABLES

Table 1. Lettuce market type, cultivar^{Z}, and seed form (pelleted or non-pelleted).

Type	Cultivar	Seed Form^{Y}
Romaine	‘Parris Island’^{*X}	NP
	‘Jericho’*	NP
	‘Coastal Star’*	P
Butterhead	‘Buttercrunch’	NP
	‘Nancy’*	P
Batavian	‘Nevada’*	P
	‘Cherokee’*	P
	Sierra	NP
Salanova®	‘Butter Red’*	P
	‘Butter Green’*	P
	‘Sweet Crisp Red’	P
	‘Sweet Crisp’ Green’*	P

^{Z}Seeds were purchased from Johnny’s Seed Company (Winslow, Maine, USA).
^{Y}Seed Form - NP(Non-Pelleted), P(Pelleted).
^{X*}Indicates cultivars used in hydroponic nutrient-solution chilling study

Table 2. Seeding, transplanting, harvest date, and total days elapsed for lettuce growth during each season

	Seeding Date	Transplanting Date	Harvest Date	Total Days Elapsed[Z]
Spring, 2020	4-Mar-2020	30-Mar-2020 (26)[Y]	4-May-2020 (35)	61
Fall, 2020	29-Jul-2020	27-Aug-2020 (29)	28-Sep-2020 (32)	61
Early Summer, 2021[X]	2-Jun-2021	7-July-2021 (35)	2-Aug-2021 (26)	61
Winter, 2021	14-Oct-2021	12-Nov-2021 (29)	20-Dec-2021 (38)	67

[Z] Total days elapsed since seeding date

[Y] Numbers in parentheses denote elapsed time since previous stage

[X] Early Summer, 2021 and Winter, 2021 plantings included chilled and non-chilled nutrient-solution temperature treatments

Table 3. Temperature and humidity greenhouse data for Spring, 2020, Fall, 2020, Early Summer, 2021, and Winter, 2021

	Temperature (°C)	**% Humidity**
	Spring, 2020	
Mean	22.8	18.7
Standard Deviation	3.5	7.5
Maximum	38.5	51
Minimum	14.7	6
	Fall, 2020	
Mean	26.9	63.7
Standard Deviation	5.7	18.9
Maximum	38.9	97
Minimum	12.4	10
	Early Summer, 2021	
Mean	28.0	74.3
Standard Deviation	4.0	13.3
Maximum	42.3	97
Minimum	21.2	38
	Winter, 2021	
Mean	21.3	38.2
Standard Deviation	3.4	15.7
Maximum	33.8	91
Minimum	9.5	11

Table 4. Effects of harvest season and cultivar on sesquiterpene lactone and sugar content, and plant fresh weight, of lettuce grown for the season evaluation study in NFT hydroponics systems.

		Harvest Season^{Z}	**Cultivar**^{Y}	**Harvest Season x Cultivar**
Free SL	Lactucin	***^{X}	**	*
	8-Deoxylactucin	***	***	***
	Lactucopicrin	***	***	**
Bound SL	Lactucin	NS	NS	NS
	8-Deoxylactucin	*	***	NS
	Lactucopicrin	**	NS	NS
Total SL	Total SLs	***	***	*
Soluble Sugars	Fructose	***	*	NS
	Glucose	***	NS	NS
	Sucrose	***	***	*
Total Sugar	Total sugar	***	NS	NS
	Sugar:SL ratio	***	***	**
	Plant fresh wt.	***	***	NS

[Z] Harvest seasons included Spring, 2020, Fall, 2020, Early Summer, 2021 and Winter, 2021.

[Y] Twelve cultivars in this study included 'Jericho', 'Coastal Star', 'Parris Island', 'Buttercrunch', 'Nancy', 'Nevada', 'Cherokee', 'Sierra', 'Butter Red', 'Butter Green', 'Sweet Crisp Red', 'Sweet Crisp Green'

[X] Indicates non-significant (NS) at * $p > 0.05$, or significant at * $p \leq 0.05$, ** $p \leq 0.001$, or *** $p \leq 0.0001$.

Table 5. Means, standard error, and mean separations for significant interactions between cultivar (four market types) and harvest season (Spring, 2020, Fall, 2020, Early Summer, 2021, Winter, 2021) for the season evaluation study in NFT hydroponic systems.

		SL concentration (µg·g^{-1})				**Sugar concentration (mg·g^{-1})**	
Type	**Cultivar**	**Free Lactucin**	**Free 8-Deoxy-lactucin**	**Free Lactuco-picrin**	**Total SL**	**Sucrose**	**Sugar:SL Ratio**
				Spring, 2020			
R[Z]	PI[Y]	30±12[X]	0±0 b[W]	72±4 abc	115±17 ab	4±1	519±119
	JER	9±5	0±0 b	31±12 c	46±17 b	5±1	1219±642
	CS	28±1	5±1 b	44±10 bc	104±20 ab	8±2	1111±242
BH	BC	17±4	10±3 b	51±18 bc	96±29 ab	13±7	1497±831
	NAN	17±7	1±0 b	112±19 ab	124±18 ab	11±2	527±212
BV	NEV	8±0	9±2 b	49±9 bc	80±12 ab	8±2	1022±306
	CHR	9±2	78±14 a	46±4 bc	162±16 a	5±2	287±82
	SIE	8±1	11±2 b	63±5 bc	100±3 ab	7±3	762±275
S	BR	18±2	1±0 b	105±9 abc	154±12 a	9±5	582±303
	BG	NA[V]	NA	NA	NA	NA	NA
	SCR	21±10	0±0 b	76±48 abc	112±32 ab	9±3	742±229
	SCG	23±10	1±0 b	131±11 a	146±21 a	6±4	618±265
				Fall, 2020			
R	PI	5±2	0±0 c	41±9 cd	50±11 b	8±5	2392±969
	JER	21±14	0±0 c	31±6 d	67±31 ab	5±1	2175±1053
	CS	18±5	2±1 c	48±8 bcd	79±19 ab	6±2	1905±767
BH	BC	14±5	2±2 c	40±8 d	62±12 b	5±2	1643±273
	NAN	18±9	1±1 c	93±19 abc	118±24 ab	6±1	1116±262
BV	NEV	11±6	4±1 c	41±4 cd	66±11 ab	9±2	2387±884
	CHR	7±2	46±1 a	49±7 bcd	123±6 ab	5±1	1050±384
	SIE	6±3	3±0 c	37±3 d	51±4 b	9±3	3202±1290
S	BR	29±8	0±0 c	94±15 ab	129±23 ab	4±0	479±29
	BG	41±29	12±2 b	130±20 a	194±46 a	5±1	520±79
	SCR	25±12	0±0 c	48±7 bcd	137±66 ab	5±1	941±276
	SCG	24±4	1±1 c	103±9 a	136±8 ab	4±1	675±147
				Early Summer, 2021			
R	PI	11±0 ef	0±0 c	93±11 c	118±11 c	16±2 c	1091±278 ab
	JER	36±4 bc	1±1 c	97±11 bc	144±11 bc	23±3 bc	1052±142 ab
	CS	13±1 def	2±1 c	76±6 c	108±7 c	23±4 bc	1439±381 ab
BH	BC	53±5 a	14±1 b	108±9 abc	202±25 abc	16±1 c	576±74 b

	NAN	17±6 def	1±1 c	106±3 bc	167±10 abc	53±8 a	1068±107 ab
BV	NEV	13±3 def	7±1 bc	74±9 c	115±8 c	40±8 ab	2004±339 ab
	CHR	7±4 f	50±8 a	58±7 c	141±20 bc	22±0 bc	889±79 b
	SIE	11±2 ef	8±1 bc	75±5 c	113±4 c	39±5 abc	1519±164 ab
S	BR	44±7 ab	0±0 c	174±18 a	254±31 a	16±2 c	504±120 b
	BG	27±2 cd	1±1 c	164±34 ab	224±44 ab	31±5 bc	799±229 b
	SCR	26±3 cde	0±0 c	99±11 bc	134±13 bc	29±5 bc	1190±147 ab
	SCG	20±2 cdef	0±0 c	86±8 c	124±13 c	28±2 bc	1194±60 ab
Winter, 2021							
R	PI	3±1	1±1 b	20±4 abc	25±5 bc	36±2	4357±1049 ab
	JER	2±1	0±0 b	7±1 c	12±1 c	43±3	8966±517 a
	CS	4±1	0±0 b	11±1 bc	22±3 bc	34±3	5696±854 ab
BH	BC	2±1	0±0 b	10±1 c	18±1 bc	33±6	5239±1345 ab
	NAN	10±6	1±1 b	29±2 ab	44±9 ab	39±9	1858±564 b
BV	NEV	2±1	1±0 b	14±3 ab	28±8 bc	43±4	4991±1131 ab
	CHR	12±4	11±0 a	9±1 c	44±3 ab	36±6	2986±556 b
	SIE	3±1	2±2 b	16±3 abc	24±4 bc	46±6	5088±1474 ab
S	BR	10±4	0±0 b	24±2 abc	45±7 ab	30±7	1431±202 b
	BG	22±11	1±1 b	30±9 a	61±7a	38±5	1356±124 b
	SCR	16±11	1±1 b	14±3 bc	37±9 abc	37±7	3261±970 b
	SCG	5±1	0±0 b	21±6 abc	29±7 bc	28±5	3989±1598 b

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.

[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.

[X] Values without an adjacent lowercase letter indicate non-significant effects for the variable.

[W] Means (n=6) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.05$).

[V] NA - Not Analyzed.

Table 6. Means, standard error, and mean separations for significant main effects within harvest season (Spring, 2020, Fall, 2020, Early Summer, 2021, Winter, 2021) for the season evaluation study in NFT hydroponic systems.

	SL concentration (µg·g^{-1})		Sugar concentration (mg·g^{-1})			
Harvest Season	**Bound 8-Deoxylactucin**	**Bound Lactucopicrin**	**Fructose**	**Glucose**	**Total sugar**	**Plant fresh wt. (g)**
Spring, 2020^{Z}	3±1 a^{Y}	15±2 a	25±3 b	35±3 c	67±6 c	121±9 a
Fall, 2020	2±0 b	9±4 ab	43±5 a	57±4 b	106±9 b	116±8 a
Early Summer, 2021	2±1 ab	17±2 a	45±3 a	76±2 a	149±7 a	58±5 b
Winter, 2021	1±1 b	4±1 b	21±2 b	35±1 c	92±4 b	40±2 b

[Z] Spring, 2020 means do not include 'Butter Green' (n=45)

[Y] Means (n=48) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.05$).

Table 7. Means, standard error, and mean separations for significant main effects within cultivar (four market types) for the season evaluation study in NFT hydroponic systems.

		SL concentration ($\mu g \cdot g^{-1}$)	**Sugar concentration ($mg \cdot g^{-1}$)**	
Type	**Cultivar**	**Bound 8-Deoxylactucin**	**Fructose**	**Plant fresh wt. (g)**
R[Z]	PI[Y]	0±0 b[X]	37±8 ab	94±14 ab
	JER	0±0 b	33±7 ab	123±21 a
	CS	1±1 b	37±4 ab	90±14 abc
BH	BC	2±1 b	34±5 ab	82±13 abc
	NAN	0±0 b	31±5 ab	77±14 bc
BV	NEV	3±1 b	46±8 a	88±12 abc
	CHR	12±2 a	28±5 ab	94±16 ab
	SIE	2±1 b	44±9 a	87±17 abc
S	BR	1±1 b	17±4 b	50±6 c
	BG[W]	0±0 b	34±4 ab	52±11 c
	SCR	0±0 b	35±7 ab	66±15 bc
	SCG	0±0 b	28±4 ab	89±14 abc

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.

[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.

[X] Means (n=12) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.05$).

[W] 'Butter Green' missing Spring, 2020 data.

Table 8. Effects for nutrient solution temperature (non-chilled, chilled) on sesquiterpene lactone and sugar content, and plant fresh weight, of lettuce grown for the nutrient-solution chilling study in NFT hydroponics systems during Summer, 2021.

		Cultivar[Z]	**Treatment**[Y]	**Cultivar x Treatment**
Free SL	Lactucin	**[X]	NS	**
	8-Deoxylactucin	***	**	***
	Lactucopicrin	***	***	NS
Bound SL	Lactucin	NS	**	NS
	8-Deoxylactucin	***	**	**
	Lactucopicrin	***	***	NS
Total SL	Total SLs	***	***	**
Soluble Sugars	Fructose	**	**	*
	Glucose	NS	NS	NS
	Sucrose	***	*	**
Total Sugar	Total sugar	**	NS	NS
	Sugar:SL ratio	**	**	*
	Plant fresh wt.	***	**	**

[Z] Nine cultivars in this study included 'Jericho', 'Coastal Star', 'Parris Island', 'Nancy', 'Nevada', 'Cherokee', 'Butter Red', 'Butter Green', 'Sweet Crisp Green'.

[Y] Nutrient-solution temperature treatments (non-chilled, chilled).

[X] Indicates non-significant (NS) at $p > 0.1$, or significant at * $p \leq 0.1$, ** $p \leq 0.05$, or *** $p \leq 0.0001$.

Table 9. Means, standard error, and mean separations for significant effects between two nutrient solution temperatures (non-chilled and chilled) for the nutrient-solution chilling study in NFT hydroponics systems during Summer, 2021

			SL concentration (µg·g^{-1})				**Sugar concentration (mg·g^{-1})**			
Type	**Cultivar**	**Solution-Temperature**	**Free Lactucin**	**Free 8-deoxy-lactucin**	**Bound 8-deoxy-lactucin**	**Total SLs**	**Fructose**	**Sucrose**	**Sugar:SL ratio**	**Plant fresh wt. (g)**
R^{Z}	PI^{Y}	Non-chilled	11±0	0±0	0±0	**118±11 a**	**30±5 b**	16±2	**1091±278 b**	85±13
		Chilled	17±5	0±0	0±0	**77±14 b**	**80±3 a**	25±3	**2733±547 a**	79±8
	JER	Non-chilled	36±4	1±1	0±0	**144±11 a**	54±4	23±3	**1052±142 b**	82±8
		Chilled	27±6	0±0	0±0	**87±18 b**	65±12	26±2	**2097±592 a**	103±12
	CS	Non-chilled	**13±1 b^{X}**	2±1	1±0	**108±7 a**	**39±11 b**	23±4	**1439±381 b**	76±5
		Chilled	**27±8 a**	4±1	3±1	**68±7 b**	**72±18 a**	19±3	**2770±807 a**	60±6
BH	NAN	Non-chilled	**17±6 b**	1±1	0±0	167±10	42±11	**53±8 a**	1068±107	**42±15 b**
		Chilled	**29±8 a**	1±1	1±1	137±14	51±6	**37±10 b**	1159±289	**80±4 a**
BV	NEV	Non-chilled	13±3	7±1	3±0	115±8	74±3	**40±8 a**	2004±339	80±21
		Chilled	17±5	12±2	4±0	88±11	68±6	**21±4 b**	1993±68	70±5
	CHR	Non-chilled	7±4	**50±8 b**	**14±2 b**	**141±20 b**	33±4	22±0	889±79	63±5
		Chilled	26±11	**99±12 a**	**23±5 a**	**195±14 a**	50±6	16±3	701±140	63±14
S	BR	Non-chilled	**44±7 a**	0±0	0±0	**254±31 a**	25±10	16±2	504±120	39±3
		Chilled	**12±0 b**	0±0	0±0	**124±8 b**	26±4	24±1	1063±124	50±5
	BG	Non-chilled	**27±2 a**	1±1	1±0	**224±44 a**	60±15	31±5	799±229	35±3
		Chilled	**14±1 b**	1±1	0±0	**146±20 b**	57±8	30±5	1142±196	49±5
	SCG	Non-chilled	20±2	0±0	0±0	**124±13 a**	35±6	28±2	**1193±60 b**	**72±16 b**
		Chilled	13±1	1±1	0±0	**75±7 b**	51±4	23±3	**1986±201 a**	**118±2 a**

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.

[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.

[X] Means (N=6) within a column followed by letter indicate significant difference between nutrient-solution temperature treatments at ($p \leq 0.1$) for cultivar using F-Test (SAS sliceby function).

Table 10. Means, standard error, and mean separations for significant main effects within cultivar (four market types) for the nutrient-solution chilling study in NFT hydroponic systems during Summer, 2021.

		SL concentration (µg·g^{-1})		Sugar concentration (mg·g^{-1})
Type	**Cultivar**	**Free Lactuco-picrin**	**Bound Latuco-picrin**	**Total Sugar**
R[Z]	PI[Y]	74±11 bc	8±3 bc	150±19 ab
	JER	74±13 bc	7±1 bc	153±12 ab
	CS	75±9 bc	8±3 bc	152±18 ab
BH	NAN	102±4 ab	20±6 ab	164±15 ab
BV	NEV	60±8 c	10±1 abc	194±14 a
	CHR	50±5 c	4±2 c	130±8 b
S	BR	135±20 a	23±6 a	122±10 b
	BG	141±20 a	20±5 ab	164±15 ab
	SCG	70±9 bc	9±2 bc	142±4 ab

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.
[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.
[X]Means (n=12) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.1$).

Table 11. Means, standard error, and mean separations for significant main effects within nutrient-solution temperature treatment (non-chilled, chilled) in NFT hydroponic systems during Summer, 2021.

	SL concentration (µg·g^{-1})		
Nutrient-Solution Temperature	**Free Lactuco-picrin**	**Bound Lactucin**	**Bound Lactuco-picrin**
Non-chilled	103±8 a	4±1 a	17±2 a
Chilled	65±6 b	2±0 b	7±1 b

[Z]Means (n=27) within a column followed by a lowercase letter are significantly different by pairwise comparison in the model ($p \leq 0.1$).

Table 12. Effects for nutrient solution temperature (non-chilled, chilled) on sesquiterpene lactone and sugar content, and plant fresh weight, of lettuce grown for the nutrient-solution chilling study in NFT hydroponics systems during Winter, 2021.

		Cultivar[Z]	**Treatment**[Y]	**Cultivar x Treatment**
Free SL	Lactucin	**[X]	NS	NS
	8-Deoxylactucin	***	***	**
	Lactucopicrin	***	***	**
Bound SL	Lactucin	**	**	**
	8-Deoxylactucin	**	NS	NS
	Lactucopicrin	**	NS	NS
Total SL	Total SLs	***	***	**
Soluble Sugars	Fructose	***	NS	NS
	Glucose	***	NS	NS
	Sucrose	NS	**	NS
Total Sugar	Total sugar	**	*	NS
	Sugar:SL ratio	NS	NS	NS
	Plant fresh wt.	***	NS	NS

[Z] Nine cultivars in this study included 'Jericho', 'Coastal Star', 'Parris Island', 'Nancy', 'Nevada', 'Cherokee', 'Butter Red', 'Butter Green', 'Sweet Crisp Green'.

[Y] Nutrient-solution temperature (non-chilled, chilled).

[X] Indicates non-significant (NS) at $p > 0.1$, or significant at * $p \leq 0.1$, ** $p \leq 0.05$, or *** $p \leq 0.0001$.

Table 13. Means, standard error, and pairwise comparisons for significant interactions between cultivar (four market types) and nutrient-solution temperature treatments (non-chilled, chilled) for the nutrient-solution chilling study during Winter, 2021.

			SL concentration (µg g^{-1})			
Type	**Cultivar**	**Solution-Temperature**	**Free 8-deoxylactucin**	**Free Lactucopicrin**	**Bound Lactucin**	**Total SLs**
R[Z]	PI[Y]	Non-chilled	1±1	**20±4 b**	0±0	**25±5 b**
		Chilled	1±0	**34±8 a**	0±0	**42±6 a**
	JER	Non-chilled	0±0	7±1	2±1	12±1
		Chilled	1±0	9±2	0±0	16±2
	CS	Non-chilled	**0±0 b**	11±1	1±0	22±3
		Chilled	**3±1 a**	11±1	0±0	26±4
BH	NAN	Non-chilled	1±1	29±2	0±0	44±9
		Chilled	1±1	40±4	1±1	57±6
BV	NEV	Non-chilled	1±0	14±3	**6±3 a**	28±8
		Chilled	2±0	21±5	**1±0 b**	33±7
	CHR	Non-chilled	**11±0 b**	9±1	**3±2 a**	44±3
		Chilled	**17±2 a**	14±1	**1±0 b**	44±2
S	BR	Non-chilled	0±0	**24±2 b**	0±0	**45±7 b**
		Chilled	1±0	**64±13 a**	0±0	**92±12 a**
	BG	Non-chilled	**1±1 b**	**30±9 b**	0±0	61±7
		Chilled	**6±1 a**	**42±5 a**	2±1	67±7
	SCG	Non-chilled	**0±0 b**	**21±6 b**	1±0	**29±7 b**
		Chilled	**2±1 a**	**38±3 a**	1±1	**58±9 a**

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.
[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.
[X] Means (n=6) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.1$).

Table 14. Means, standard error, and mean separations for significant main effects within cultivar (four market types) for the nutrient-solution chilling study in NFT hydroponic systems during Winter, 2021.

		SL concentration (µg g^{-1})			Sugar concentration (mg g^{-1})			
Type	**Cultivar**	**Free Lactucin**	**Bound 8-deoxy-lactucin**	**Bound Latuco-picrin**	**Fructose**	**Glucose**	**Total Sugar**	**Plant fresh wt. (g)**
R[Z]	PI[Y]	4±1 abc	0±0 b	1±1 b	23±3 abc	39±1 ab	95±5 ab	40±3 bc
	JER	3±1 bc	0±0 b	2±1 b	24±3 abc	35±2 ab	96±6 ab	65±4 a
	CS	7±2 abc	0±0 b	3±1 ab	31±4 a	44±3 a	105±7 a	53±3 ab
BH	NAN	8±3 abc	1±0 ab	5±2 ab	13±2 cd	25±2 c	74±7 b	40±4 bc
BV	NEV	3±0 c	2±1 ab	3±1 ab	22±2 abcd	38±2 ab	97±6 ab	36±4 c
	CHR	9±2 abc	5±3 a	2±1 b	22±2 abcd	36±2 ab	87±7 ab	32±3 c
S	BR	15±3 ab	0±0 b	9±1 a	10±2 d	30±4 bc	71±8 b	31±5 c
	BG	16±6 a	4±2 a	5±2 ab	14±2 bcd	25±1 c	72±5 b	37±4 bc
	SCG	8±2 abc	0±0 b	4±2 ab	26±3 ab	41±2 a	92±5 ab	35±2 c

[Z] Lettuce types: (R) - Romaine, (BH) - Butterhead, (BV) - Batavian, (S) - Salanova®.

[Y] Lettuce cultivars: (PI) 'Parris Island', (JER) 'Jericho', (CS) 'Coastal Star', (BC) 'Buttercrunch', (NAN) 'Nancy', (BR) 'Butter Red', (BG) 'Butter Green', (SCR) 'Sweet Crisp Red', (SCG) 'Sweet Crisp Green'.

[X] Means (n=12) within a column followed by the same lowercase letter are not significantly different by pairwise comparison in the model ($p \leq 0.1$).

Table 15. Means, standard error, and mean separations for significant main effects within nutrient-solution temperature treatment (non-chilled, chilled) for the nutrient-solution chilling study in NFT hydroponic systems during Winter, 2021.

	Sugar concentration (mg g^{-1})	
Nutrient-Solution Temperature	**Sucrose**	**Total sugar**
Non-chilled	37±2 a[Z]	91±4 a[Y]
Chilled	29±1 b	85±3 a

[Z] Means (n=27) within a column followed by a lowercase letter are significantly different by pairwise comparison in the model ($p \leq 0.1$).

[Y] Significant effect was observed for total sugar ($p = 0.089$), but pairwise comparison in the model did not produce significant differences.

CHAPTER II FIGURES

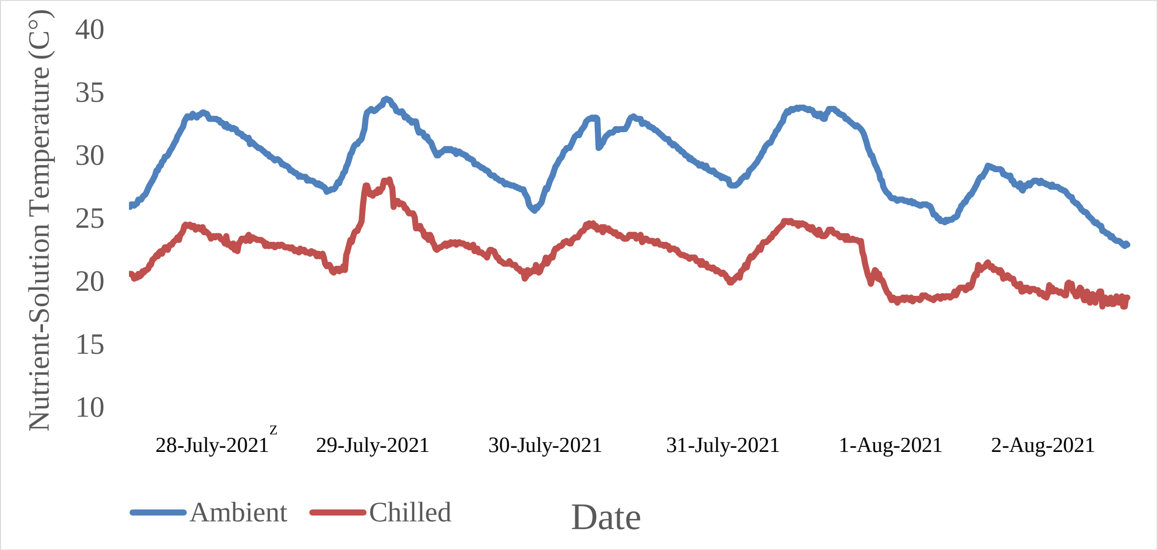

Figure 2. Hydroponic nutrient-solution temperature for non-chilled and chilled treatments over 10-min time intervals for Summer, 2021

[Z]Thermocouple sensor deleted data except six days prior to harvest (28-July 2021 through 2-Aug-2021)

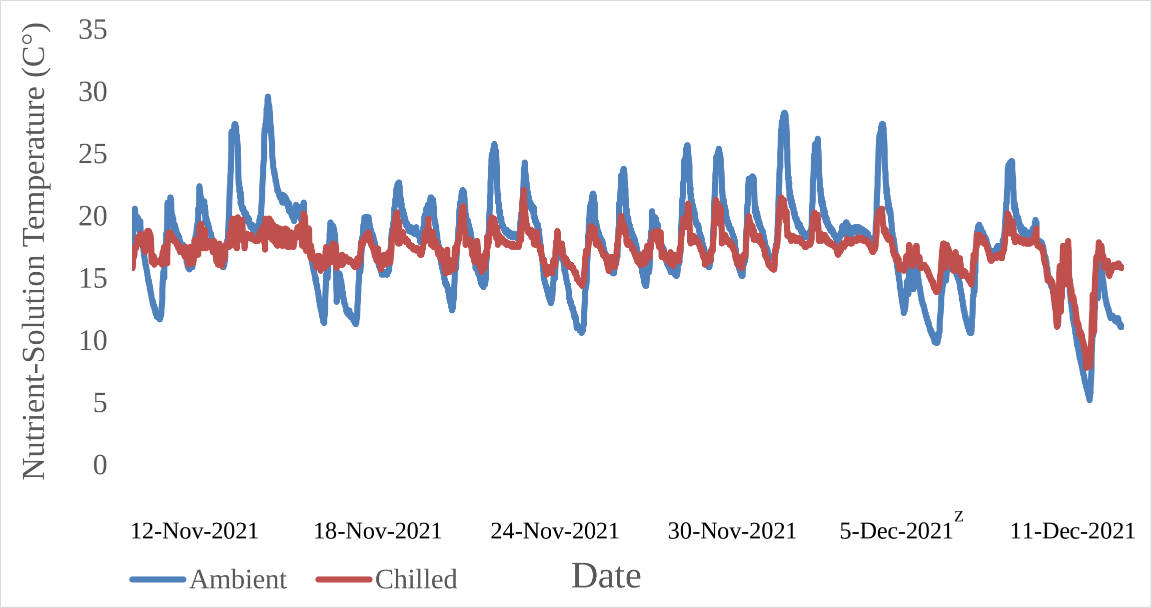

Figure 3. Hydroponic nutrient-solution temperature for non-chilled and chilled treatments over 10-min time intervals for Winter, 2021

^{Z}Thermocouple recorded data for entire duration of harvest excluding five days (11-Dec-2021 through 16-Dec-2021)

VITA

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Master of Science

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