ASSESSING SESQUITERPENE LACTONE AND SUGAR CONCENTRATIONS AS INDICATORS OF HEAT TOLERANCE IN FIELD PRODUCED LETTUCE IN OKLAHOMA

By

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IN OKLAHOMA

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Abstract: Lettuce (*Lactuca sativa* L.) is a cool season vegetable typically grown in the spring and fall in Oklahoma by small farmers. Lettuce is not typically grown in the heat of the summer because excessive heat stress causes premature bolting, tip burn and bitter off-flavor. Premature bolting is known to increase the concentration of sesquiterpene lactones (SLs) in lettuce, which cause lettuce to be perceived as bitter when tasted by the consumer. However, sugar production in lettuce can counteract the bitterness imparted by greater amounts of SLs and mask their bitter taste. Eighteen cultivars of lettuce, representing five market types (Loose Leaf, Romaine, Summer Crisp/Batavian, Butterhead, and Salanova®) were grown in field plots during spring, summer and fall harvest seasons. Canopy width and plant height were observed in the field along with yield immediately following harvest. Twelve cultivars were selected for SL and sugar analysis. The concentrations of the SLs lactucin, 8-deoxytactucin, and lactucopicrin were quantified by high performance liquid chromatography (HPLC) along with the concentrations of glucose, sucrose, and fructose, quantified by gas chromatography (GC). Micro sprinkler irrigation technology was also investigated as a mechanism for cooling lettuce in the summer season via evaporative cooling. Spring was the best season to grow lettuce in Oklahoma based on superior Sugar:SL ratios and yields. Romaine and Batavian cultivars are the recommended types of lettuce to grow based on their high yields and consistently high Sugar:SL ratios, in both the spring and fall offseason of production. Salanova® cultivars in general yielded less and had lower Sugar:SL ratios in every season when compared to other types tested. Genetically similar green and red lettuces were investigated and there was no evidence that red lettuce cultivars accumulate less sugar and more SLs compared to green lettuce cultivars. Micro sprinkler irrigation did not increase sugars or decrease SLs, nor did it increase yield, and is not recommended for use to extend the growing season of lettuce.

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CHAPTER I

INTRODUCTION

Lettuce (*Lactuca sativa* L.) can become too bitter for consumers. Because of this, people may not consume as many vegetables as they should. In the United States, less than a quarter of adults and only 7 percent of children are eating the recommended number of daily vegetable servings (Bakke et al., 2018). While lettuce can be thought of as a health food for a variety of reasons, it's obvious that most consumers will not buy a product that they do not enjoy eating. Bitterness is a common complaint in lettuce and most consumers are unlikely to purchase bitter lettuce a second time, though it would likely benefit their health if they consumed more of it.

Consumer complaints of bitterness in purchased lettuce can be traced back to the conditions of lettuce in cultivation, transport, and postharvest handling. The main factor investigated in this study is heat stress during cultivation, and this factor has been linked to the accumulation of bitter perceived molecules within lettuce tissues in the literature (Lee et al., 2015). The ideal field production temperature for lettuce is 18.5 °C, so it is not surprising that this crop is typically grown in the spring and fall to avoid the summer's heat (Zhao and Carey, 2009). This also explains why most lettuce is grown in Mediterranean climates, where temperature extremes are less pronounced. Oklahoma summers are much hotter than lettuces' preferred growing temperature, including periods hotter than 37 °C during the day.

Lettuce is sold to the consumer in a variety of shapes and sizes. Since the late 1980s, there has been more interest in salad mixes and atypical, non-iceberg, lettuce both from growers and consumers (Kuepper and Bachmann, 2002). The shapes of oak leaf and taste profiles of older, loose leaf lettuce types are making a comeback over more conventional head lettuce. Though Kuepper and Bachmann (2002) state that the increasing consumption and demand for the non-conventional lettuces has decreased their price, the premium in price remains over conventional head lettuce. Whether producers grow using an organic or conventional strategy, these products still command a higher price than iceberg and average green head lettuce. Organic producers often offset the increase in production costs, because many of these colorful cultivars are not in the ground very long and are generally harvested early then turned into salad mixes for consumers. This means there is less of a threat from insects that often cause problems in organic production (Kuepper and Bachmann, 2002).

Lettuce in the United States belongs to one of five major groups of cultivars: Crisp Head, Romaine, Loose Leaf, Butterhead, and Summer Crisp types (Mikel, 2007). Salanova® are a newer group of cultivars known for having more leaves. Lettuce is typically a cool season crop. The Romaine, Butterhead, and Summer Crisp lettuce groups are known to be more tolerant to hotter conditions than Loose Leaf lettuce. Batavian cultivars are a subset of Summer Crisp lettuce. The Butterhead types of lettuces are a cross between Romaine and Batavian lineages. Since the middle of the 20th century, breeding programs in the United States have focused on increasing the diversity of the

Romaine and Crisp Head type lettuces. These breeding efforts have given lettuce resistance from many diseases and issues with production, such as downy mildew and leaf drop (Mikel, 2007). These modifications have also addressed the issue of tip burn, which causes unsightly growth in heat stressed lettuce. These breeding efforts have led many to consider some Summer Crisp and Romaine lettuces, along with their hybrids line of Butterheads, to have some degree of heat tolerance. Batavian lettuce, for example, is known to be highly resistant to tip burn when compared with other varieties (Holmes et al., 2019). The other major concern with heat stressed lettuce is that it may bolt during production.

Bolting, broadly, is the physiological event marking the transition from vegetative to reproductive growth. In lettuce, this stage is often defined as beginning at floral initiation, when the shoot apical meristem begins swelling up (Lee and Sugiyama 2006). At the initiation of bolting the growing tip becomes less prominent and loses its conical shape (Hao et al., 2018). Bolting is a very complicated developmental process and as such it involves the influence of many diverse factors, both endogenous and environmental. These factors include temperature, light signals, day length, developmental stage, and endogenous plant hormone activity (Hao et al., 2018). Lettuce is a facultative long day plant, further increasing the risk of bolting in the summer along with elevated temperatures. Hao et al. (2018) illustrated that the concentrations of important plant hormones related to bolting within heat treated lettuce (33 °C day/ 25 °C night) were elevated when compared with lettuce grown at 20 °C day/ 13 °C night.

Exogenous IAA, one of the auxins, promoted and accelerated bolting in lettuce (Hao et al., 2018). This result may not be surprising to many plant scientists, because it fits neatly within current dogma regarding the role of auxin in plant tissues. According to the classical “acid growth theory”, when growing cells are treated with exogenous auxin, the pH of the cell wall is reduced, the cell wall relaxes, and cell elongation occurs. This finding indicates that the genes involved in the synthesis of auxin may be a part of overall regulation of bolting in lettuce. Information such as this is critical to the improvement of lettuce as a food crop, because gene editing and breeding programs may be able to improve lettuce by targeting genes associated with auxin synthesis to delay bolting.

Another explanation for why bolting causes bitterness in lettuce lies in the way that sugars are translocated within the plant from vegetative tissues once bolting is initiated. Lee and Sugiyama (2006) showed that the upper nodes of the plant typically concentrate non-structural carbohydrates once bolting was initiated, and even more dramatically when seeds were set. In particular, Lee and Sugiyama quantified glucose, sucrose, and fructose. Lee and Sugiyama (2006) hypothesized that sucrose and fructans were reserve carbohydrates in lettuce stems, and sucrose and fructans were moved from that source to the sink of the newly developing seeds once flowers began developing (Lee and Sugiyama, 2006). Every other leaf of bolting lettuce was defoliated to test whether loss of leaves would influence the concentration of sugars in the flowers and no significant differences were found between a control group and the defoliated plants. However, Lee and Sugiyama (2006) were not convinced that sugars could not have been moved from leaves before the defoliation, because senescence in the experimental lettuce might have already begun prior to defoliation.

The transition to bolting is not desired by growers, because the flowering stalk and vertical growth in general is known to contain far more bitter molecules (Assefa et al., 2018). These bitter molecules make lettuce less desirable to the consumer, not to mention the unsightly growth habit of bolted lettuce. The concentration of bitter molecules, known as sesquiterpene lactones (SLs), have been demonstrated to increase in the flowering stalks of lettuce when compared with less mature tissue (Sessa et al., 2000). It stands to reason then, that the degree of bolting progression in lettuce would be a very good indicator for how much bitter taste that lettuce will have when it is tasted by consumers.

Lettuce undergoes many other developmental stages prior to bolting in the correct growing environment. These stages, in the order in which they occur, include: the emergence of cotyledon leaves, the rosette stage (circular cluster of leaves), curling towards the growth point of the tips of the inner leaves (cupping stage), overlapping of the cupped leaves and growing point (heading stage), and finally the mature stage where a head of lettuce has reached marketable size (Assefa et al., 2019). Without the right conditions, lettuce may prematurely bolt, skipping the necessary stages of development. When this occurs, the resulting plant is no longer marketable, weighs less, and does not taste the way most consumers expect lettuce to taste.

Another way to visualize lettuce development is to imagine the sigmoidal growth curve that predicts biomass accumulation in lettuce (Spalholz et al., 2020). This curve shows that lettuce starts with slow growth during the seedling stage, then has a very rapid growth stage, and finally remains relatively the same size and weight as it continues toward senescence. If a lettuce plant at any time during the growth curve cycle lacks nutrients, water, or skips developmental stages (due to premature bolting), the resulting plant will not have nearly as much mass as a lettuce plant grown in ideal conditions.

Because there is an association between higher growing temperatures and more bitter molecules, it follows that decreasing the temperature of lettuce closer to its ideal temperature of 18.5 ºC during production is a good practice, wherever it is possible to implement. Temperature reduction would delay bolting and thus prevent developmentally-based bitterness as well. Reducing the temperature whenever possible would also facilitate lettuce to accumulate more biomass and mature through all developmental stages, producing a superior product. Within the literature, many approaches have been examined to extend the growing season of lettuce into summer in temperate climates where bolting is a main concern.

High tunnels have been investigated to reduce bolting in the summer, but less air circulation in a study in Kansas within high tunnels likely kept the temperature more elevated than desired, which caused no reduction of bolting symptoms in this study (Zhao and Carey, 2009). However, Zhao and Carey reported an average reduction of 3.4 °C in soil temperature when comparing shade cloth to open field conditions. For reducing temperature, high tunnels are typically equipped with shade cloth to reduce the solar radiation reaching the lettuce growing within the high tunnel. High tunnels have been successfully utilized to extend the growing season of lettuce farther into the colder months. Shade cloth is not typically used in winter in order to maximize solar heat. High tunnels protect plants from frost and allow plants to be harvested much cleaner than those grown in an open field environment.

Colored shade nets are another approach to the issue of keeping lettuce cool in the summer's heat. These horticultural nets work by reflecting a certain spectra of light, while allowing other portions of the light spectra to reach the crop below. These reflective, colored cloths in many cases reduce temperatures to an even greater degree than their normal black shade cloth counterparts (Laur and University of Georgia Extension Services, 2021). Colored shade cloths also have the advantage of being spectra specific, which could provide more ideal growth habit depending on consumer demand (more compact leaves, more coloration).

The present study utilizes evaporative cooling to reduce growing temperature of field lettuce via a micro sprinkler irrigation system. Evaporative cooling can be difficult to optimize to local conditions and to a specific crop, but it is known to decrease air temperatures. The premise of the technique is simple. When water evaporates over a given surface, for example a lettuce leaf, it absorbs a considerable amount of heat. A group of researchers from Quebec used evaporative cooling to prevent the onset of tip burn on endive (*Cichorium endivia* L.) plants (Jenni et al., 2008). Micro sprinkler mediated evaporative cooling does not work as well when the ambient air prior to sprinkling is humid, because evaporative cooling requires a strong water vapor pressure deficit between the cooled surface (a plant leaf) and the atmosphere. Despite evaporative cooling's idiosyncrasies, micro sprinkler technology has been shown to reduce the air temperature between 3 and 11°C. Additionally, an increase in yield when using micro sprinkler technology has been demonstrated in a study on Chinese lettuce. Micro sprinkler irrigation, when compared with traditional furrow irrigation, increased yield and nutritional content of their chosen lettuce cultivar (Chen et al., 2019).

A group of molecules that impart bitter taste in lettuce are known as sesquiterpene lactones (SLs). These secondary metabolite molecules are thought to have evolved as an antifeedant in lettuce, because the bitterness makes the plants less desirable to herbivores

(Sessa et al., 2000). This claim is further corroborated by the reality that the expression of genes related to creation of these molecules are stress induced (Han et al., 2021). Structurally, sesquiterpene lactones are 15 carbon backbone molecules. Due to the large number of enzymes that act on these molecules, as well as the wide variety of reactions that these enzyme products may undergo, the variety within this class of molecules is astounding (Chadwick et al., 2013).

SLs exist both in a "bound" and a "free" form within lettuce tissue. The bound sesquiterpene lactones are glycosides, bound to a sugar to increase their stability when stored by the plant (Kytidou, 2020). Additionally, glycosides are thought to be more mobile within plant tissues because glycosides pass through the cell membrane more easily than the aglycone form. SLs are concentrated in structures known as lactifers, which are specialized cells that co-occur with vascular tissues in plants within the lettuce family, the Asteraceae (Sessa et al., 2000). It has been hypothesized that when these lactifers break, they release the latex that works to defend plants from tissue damage and deter herbivory, both physically with the latex's viscosity and chemically via the bitter properties of the molecules within the latex.

Prior research has shown that "bound" SLs are dihydro glycosides (Ferioli and D'Antuono, 2012). The "bound" metabolites cannot typically be tasted until the glycosides are removed and thus form a quickly utilizable surplus in the plant. After cellulase removal of glycoside attachments, the amount of "bound" metabolites could be quantified in each sample by subtracting the concentrations of SLs present in the "free" samples from the "total" sample concentrations incubated with cellulase.

The perception of bitterness within each lettuce can be masked by sweet flavors. Chadwick et al. (2016) established that there was a negative correlation between Sugar:SL ratio and the log of perceived bitterness. Put more simply, for every increase in sugar content or decrease in SL content, consumers were more likely to like a lettuce product more compared to other lettuce samples. This finding means that even lettuce that produces greater amounts of SLs can be perceived as less bitter than another lettuce with fewer SLs but lower sugar content, due to a masking of bitterness by sweetness.

To distinguish between whether certain kinds of lettuce are chemically predisposed to be perceived as bitter, it is important to understand how humans taste bitterness in food. On the surface of the tongue, humans have specialized cells called taste buds that modulate the perception of taste. A subset of receptors is responsible for each general subset of a human's perception of taste (sweet, bitter, savory); the T2R receptors appear to be responsible for the perception of bitterness (Chandrashekar et al., 2000). There are 25 different T2R receptors and some respond to all "bitter molecules" while others only respond to certain classes of molecules (Chadwick et al., 2016). The SLs present in lettuce activate T2R46. Sugars are sensed by only two receptors, T1R2 and T1R3.

While much research is still needed to fully understand bitter taste perception, data supports that there exists a wide variation in human perception of bitterness (Feeney et al., 2011). Observations of the ability of people to sense the bitter chemical propylthiouracil led to the development of several terms, including supertasters (those that taste bitterness at very low thresholds), non-tasters (those people that are "bitter blind"), and a middle phenotype of people that are middle tasters. Supertasters have 16 times more taste buds in general when compared to non-tasters, which also likely impacts their perception of sweetness (Spence, 2015). The proportion of supertasters, middle tasters, and non-tasters was estimated to be 20, 50, and 30 percent respectively (Feeney et al., 2011).

There is not a lot of convincing research showing that “taster status” has any effect on consumer choice (Feeney et al., 2011). This may be especially true for lettuce, because the bitter blindness receptor (T2R38) is a specialist receptor that only activates in the presence of thiouracil like groups, which are not present in lettuce, but are present in other brassica type vegetables (Chadwick et al., 2016). There are also the confounding effects of prior diet, gender, and age which are known to affect bitterness perception outside of the genetics of the people in any given population.

There is a lack of consensus between researchers regarding which of the SL molecules are detected at the lowest thresholds. One study holds that lactucin would be tasted by people at the lowest thresholds (Price et al., 1990). This claim is also supported by another study, including both chicory and lettuce samples, which concluded that lactucin and its associated compounds were most associated with bitter taste perception (Peters et al., 1996). Conversely, another study concluded that 8-deoxylactucin was detected at the lowest thresholds and greater concentrations of 8-deoxylactucin were most correlated with the perception of bitter taste (Chadwick et al., 2016).

As for sugars, glucose was the major sugar present and most highly correlated with sweet perception in lettuce (Chadwick et al., 2016.) The concentration of sugar within lettuce tissue was a function of light intensity and nutrient availability (Blom-zandstra and Lampe, 1984). Blom-zandstra and Lampe documented that nitrate and sugar concentrations were inversely related in a hydroponic Bibb lettuce and that with greater light intensity, more sugar molecules were produced. Because of the discovered inverse correlation between nitrate and sugar in lettuce plants, Blom-zandstra and Lampe concluded that nitrate likely serves as a substitute osmoticum in the cases of lower light intensity or photosynthetic output. Nitrate is taken up by the vacuoles instead of synthesized sugars or organic acids in a low-light scenario, because less ATP is required for the lettuce plant to take up nitrate. Because nitrate helps lettuce plants maintain osmotic pressure, the availability of sufficient nitrogen when growing lettuce is crucial.

Relative sugar concentrations in lettuce also change in accordance with developmental stage. While mono and disaccharides such as glucose, fructose and sucrose predominated in younger plants, as a lettuce plant aged it accumulated more fructans (Lee and Sugiyama, 2006). These researchers hypothesized that fructans acted as a storage carbohydrate and was translocated to the developing seeds when flowers were set. Active translocation from leaves to floral structures provides more evidence that bolting, even in its early stages, could affect the sweetness of a given lettuce plant.

The phenomenon of sweetness and overall flavor sensation is a product of multisensory perception. The way a food looks, including its color, may play a role in how sweet it tastes. Lettuce is not always green. Red and purple lettuces have become a more popular consumer choice. Degree of redness plays a role both on the lettuce's perceived quality at market, but also in the physiology and chemical makeup of the lettuce itself.

Humans perceive red cultivars of lettuce as red because of their relatively higher concentration of anthocyanin pigments. In a study measuring the effective concentrations of pure polyphenol compounds present in different types of natural products, malvidin 3-glucoside (a purple anthocyanin) was shown to have a dramatically lower effective concentration for eliciting a bitter taste response than other polyphenols (Soares et al., 2013). This data lends credibility to the idea that the anthocyanins in red lettuce, though present in very low amounts by percentage of weight, could impart a perceivable threshold of bitter flavor when compared with similarly grown green cultivars.

The human experience of “bitterness” is not the same across different products. The glycosides from anthocyanins and greater amount of phenolics taste more bitter at lower concentrations to supertasters because these compounds impact taste receptors in a similar way to propylthiouracil. In this way, the bitterness caused by the SLs in lettuce is similar, yet distinctly different from the alkaloid influenced bitterness imparted by coffee.

On top of the presence of anthocyanins that may impart noticeable bitterness, red cultivars tend to accumulate more phenolics in general. In a study of French lettuce cultivars, a red cultivar was shown to contain significantly more total phenolic content when compared to similar green cultivars (Nicolle et al., 2004). Total phenolic content includes greater amounts of chlorogenic and hydroxycinnamic acids, which are associated with bitter taste in other fruits and vegetables like carrots and sweet potatoes (Shahidi and Ambigaipalan, 2015). The genes responsible for anthocyanin production also upregulate the genes responsible for polyphenol production, increasing the likelihood that red lettuce could be perceived as more bitter (Zhang et al., 2017).

In any discussion of bitter molecules in natural products, the confounding effects of growing environment, irrigation, pesticide use, temperature, and other associated factors must be considered. To state that all red cultivars contain more of the polyphenol molecules regardless of the way that the plants are grown would be inaccurate. A red cultivar grown at high temperature that is under more stress could logically have more bitter tasting compounds than a red cultivar grown in a less stressful environment, due to the influence of environmentally modulated gene expression. According to Nicolle et al. (2004), elevated growing temperatures in fruits induce phenylalanine ammonia lyase activity, which increases the concentration of bitter polyphenols. Farming practices like irrigation, pesticide and herbicide use, and fertilization are known to impact polyphenol oxidase and peroxidase activity (Nicolle et al., 2004). Although much evidence supports the idea that red cultivars are more likely to be perceived bitter when compared with green cultivar, growing environment can interact to influence the degree of accumulation of bitter molecules.

Because of the presence of anthocyanins, red lettuce contains more natural antioxidants. These antioxidants are more readily utilized by the body from digesting whole foods, like lettuce (Liu, 2004). Natural antioxidant therapy is far more effective in preventing disease than therapy that revolves around only one synthetic antioxidant, such as vitamin C (Shahidi et al., 2015). Additionally, red lettuce cultivars tend to accumulate more polyphenols, which also have antioxidant activity. Therefore, it is possible that red lettuce could be marketed as healthier and thus could generate more revenue for farmers that choose to grow more unusual cultivars.

The objectives of this study were as follows. Heat tolerance in 12 lettuce cultivars was investigated to see how much, if any, effect this genetic “tolerance” and color had on the concentration of SLs and the concentration of sugars across spring, summer, and fall growing seasons. Closely related green and red cultivars were investigated to determine whether lettuce color will have an effect on Sugar or SL accumulation. Micro sprinkler technology was investigated to determine whether the evaporative cooling applied by this type of irrigation benefitted lettuce quality during the summer season of production and would allow farmers to extend the growing season. Cultivars that have more sugars and fewer SLs when compared with other cultivars in each season will be recommended. These molecular recommendations were supported by the growth metrics of yield, plant height, and canopy width for each cultivar tested in each season.

Hypotheses

1. We expect that lettuce grown and cooled under micro sprinkler technology will accumulate fewer SLs than the same cultivars not treated with micro sprinklers in the summer season.
2. We expect that heat resistant cultivars, including the Romaine, Batavian, and Butterhead varieties will have greater Sugar:SL ratios on average in all growing seasons.
3. We expect that red varieties, when compared with their closely related green varieties, will have lower Sugar:SL ratios, regardless of season.

CHAPTER II

ASSESSING SESQUITERPENE LACTONE AND SUGAR CONCENTRATIONS AS INDICATORS OF HEAT TOLERANCE IN FIELD PRODUCED LETTUCE IN OKLAHOMA

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Abstract

Lettuce (*Lactuca sativa* L.) is a cool season vegetable typically grown in the spring and fall in Oklahoma by small farmers. Lettuce is not typically grown in the heat of the summer because excessive heat stress causes premature bolting, tip burn, and bitter off-flavor. Premature bolting is known to increase the concentration of sesquiterpene lactones (SLs) in lettuce, which cause lettuce to be perceived as bitter when tasted by the consumer. However, sugar production in lettuce can counteract the bitterness imparted by greater amounts of SL's and mask their bitter taste. Eighteen cultivars of lettuce, representing five market types (Loose Leaf, Romaine, Summer Crisp/Batavian, Butterhead and Salanova®) were grown in field plots during spring, summer, and fall harvest seasons. Canopy width and plant height were observed in the field along with yield immediately following harvest. Twelve cultivars were selected for SL and sugar

analysis. The concentrations of the SLs lactucin, 8-deoxylactucin, and lactucopicrin were quantified by high performance liquid chromatography (HPLC) along with the concentrations of glucose, sucrose, and fructose quantified by gas chromatography (GC). Micro sprinkler irrigation technology was investigated as a mechanism for cooling lettuce in the summer season via evaporative cooling. Spring was the best season to grow lettuce in Oklahoma based on superior Sugar:SL ratios and yields. Romaine and Batavian cultivars are the recommended types of lettuce to grow based on their high yields and consistently high Sugar:SL ratios, in both the spring and fall offseason of production. Salanova® cultivars in general yielded less and had lower Sugar:SL ratios in every season when compared to other types tested. Genetically similar green and red lettuces were investigated and there was no evidence that red lettuce cultivars accumulate less sugar and more SLs compared to green lettuce cultivars. Micro sprinkler irrigation did not increase sugars or decrease SLs, nor did it increase yield, and is not recommended for use to extend the growing season of lettuce.

Introduction

Lettuce (*Lactuca sativa* L.) ranks second only to potatoes in per capita consumption in the United States (USDA Vegetables Summary, 2021). The ideal field production temperature for lettuce is 18.5 °C, which explains why lettuce is typically grown in the spring and fall in temperate climates to avoid the summer's heat (Zhao and Carey, 2009). This is especially true in Oklahoma, where in the summer temperatures reach above 37 °C. Lettuce in the United States belongs to one of five types of lettuce: Crisp Head, Romaine, Butterhead, Loose Leaf, and Summer Crisp (Mikel, 2007). Many of these different lettuces have been developed to solve the issue of heat stress during cultivation. Heat tolerance in lettuce usually refers to resistance to premature bolting and tip burn caused by heat stress. Batavian lettuce, a subset of the Summer Crisp type, was known to be highly resistant to tip burn when compared with other varieties (Holmes et al., 2019). Summer Crisp and Romaine lettuces, along with their hybrid line of Butterheads also have some degree of heat tolerance.

Heat stress during cultivation was associated with an earlier onset of bolting in lettuce (Hao et al., 2018). Lettuce is also a facultative long day plant, further increasing the risk of bolting during the longer days of summer. Lettuce bolting was defined as beginning at floral initiation, when the shoot apical meristem began to expand (Lee and Sugiyama, 2006). Premature bolting caused lettuce to skip developmental stages, which would likely have an effect on yield, canopy width and plant height (Spalholz et al., 2020). Bolting, aside from unsightly stem elongation, caused an increase in the concentration of bitter molecules, known as sesquiterpene lactones (SLs), especially in the flowering stalks compared to the leaf tissue (Sessa et al., 2000).

SLs exist both in a glycoside (bound) and an aglycone (free) form within lettuce tissue. The bound SLs appeared to increase their stability over the aglycone form when stored by the plant (Kytidou, 2020). Additionally, glycosides are thought to be more mobile within plant tissues because glycosides pass through the cell membrane more easily. SLs are concentrated in structures known as lactifers, which are specialized cells that co-occur with vascular tissues in plants within the Asteraceae family (Sessa et al., 2000). The "bound" metabolites cannot typically be tasted until the glycosides are removed and thus form a quickly utilizable surplus in the plant. Bound SLs could theoretically be converted to free forms during the postharvest stress of lettuce transport and thus influence lettuce taste.

Taste and overall flavor sensation is a product of multisensory perception and the perception of bitterness within each lettuce can be masked by sweet flavors. Chadwick et al. (2016) established that there was a negative correlation between Sugar:SL ratio and the log of perceived bitterness. Lettuce that produced greater amounts of SLs but also relatively greater amounts of sugar could be perceived as less bitter than another lettuce with lower SL content but much lower sugar content, due to the masking of bitterness by sweetness.

Evaporative cooling can be difficult to optimize to local conditions and to a specific crop, but it is known to decrease air temperatures. The premise of the technique is simple. When water evaporates over a given surface, for example a lettuce leaf, it absorbs a considerable amount of heat. A group of researchers from Quebec used evaporative cooling to prevent the onset of tip burn for endive (*Cichorium endivia* L.) plants (Jenni et al., 2008). Evaporative cooling is not as effective when the ambient air prior to sprinkling is humid, because evaporative cooling requires a large water vapor pressure deficit between the cooled surface (a plant leaf) and the atmosphere. Despite evaporative cooling's idiosyncrasies, the technology has been shown to reduce the air temperature between 3 and 11 °C (Jenni et al., 2008). An increase in yield when using micro sprinkler technology has been demonstrated in a study on Chinese lettuce. Micro sprinkler irrigation, when compared with traditional furrow irrigation, increased yield and nutritional content of their chosen lettuce cultivar (Chen et al., 2019).

Heat tolerance in 18 lettuce cultivars was investigated in the present study to see how much, if any, effect this genetic “tolerance” had on the concentration of SLs and sugars, as well as yield, across spring, summer, and fall growing seasons in 2 years. Closely related green and red cultivars were investigated to determine whether lettuce color will influence sugar or SL accumulation. Micro sprinkler technology was investigated to determine whether the evaporative cooling applied by this type of irrigation benefitted lettuce quality during the summer season of production and would allow farmers to extend the growing season farther into the summer months.

Materials and Methods

Plant Materials

This study utilized 18 cultivars of lettuce, from five different types of cultivars (Table 1). Lettuce transplants were grown at the Oklahoma State University Greenhouse Learning Center, at Oklahoma State University Campus, Stillwater, OK 74075 (lat. 36.126°N, long. 97.074°W, elevation 300 m) on dates specified in Table 2. Seeds were sown into a propagation media (Fafard® Germinating Mix, Sun Gro Horticulture, Agawam, MA) placed into 200 cell seedling trays and stratified in a walk-in cooler (4ºC) for 3 d to encourage uniform germination before moving to the greenhouse. After the first true leaves emerged (about 1 week in greenhouse), plants were fertigated by hand every other day with 100 ppm N sourced from Jacks 20N-4.8P-13K (Jacks; Allentown, PA). Plants remained in the greenhouse for about 4 weeks prior to transplanting at the

Cimarron Valley Research Station, where plants grew for another 4 weeks prior to harvest.

Field plots were established in a randomized complete block design with four replications at Oklahoma State University Cimarron Valley Research Station, at Perkins, OK 74059 (lat. 35.997°N, long. 97.043°W, elevation 273 m). Plots were made in single rows on raised beds placed 1.83 m apart with a water wheel transplanter, using in row spacing of 30 cm and 7 plants per rep. The soils were sandy loam on top of clay loam in composition (Oklahoma Mesonet, National Weather Center, Norman OK). Irrigation was supplied through drip tape placed below ground during the bedding operation. Drip irrigation emitters had 30 cm spacing and a 2 liter per hour flow rate. Plot temperature, humidity and dew point temperature was monitored using HoboConnect™ probes (Onset Computer Company, Bourne, MA) to record readings every minute throughout the growing season. Leaf surface temperature was determined periodically with a Fluke 62 Mini Max (Fluke Corporation, Everett, WA) infrared sensor to complement the air temperature data.

All plots were irrigated twice a day at 5:30 AM and 5:30 PM for 30 min, except during wet, rainy periods. Plots were fertilized with 10N–13.1P–16.6K (Jacks; Allentown, PA) pre-plant to reach commercially recommended levels of P and K, and fertigated (once per week post-transplant) with 46N–0P–0K to reach our targeted N level, as determined by soil tests prior to planting. Fertigation was achieved by using an injector (R171016, Pentair Corporation, Minneapolis, MN) attached to the drip irrigation. Target N level was 135 kg ha^{-1}, P was 168 kg ha^{-1}, and K was 168 kg ha^{-1}. Weed control was achieved through hand hoeing mostly, although herbicide (Poast; BASF, Florham Park, NJ) was applied in the Summer 2021 growing season at a rate of 1.5 pint per acre along with ammonium sulfate at 2.24 kg ha^{-1} to control grass-type weeds. Pest control was necessary in Summer and Fall 2020. In Summer 2020, the crop was sprayed with 3 oz/acre rate of Mustang Maxx (FMC Corporation, Philadelphia, PA) on 31 July, and in Fall 2020 lettuce was sprayed with Permethrin (JT Eaton Co., Macedonia, OH) on 8 October to control army worms.

Plantings were established in 2020 and 2021 during the spring, summer, and fall on dates indicated in Table 2, with a field growing season of approximately 4 weeks. At time of harvest all plants, except for exceptionally small outliers, in each replicate were measured for height and canopy width, cut at the ground level, and placed into a labeled plastic bag and transported to lab facilities in the Nobel Research Center on the campus of Oklahoma State University in Stillwater. Lettuce was placed into a cold room (4 °C) and processed within 5 h of harvest. Plant weight was determined for each lettuce plant and then lettuce was washed, cored, and placed into frozen storage at -18 °C to await freeze drying and chemical analysis.

During the summer season a subset of cultivars (Salanova® ‘Summer Crisp Green’, Salanova® ‘Summer Crisp Red’, Batavian ‘Nevada’, Butterhead ‘Buttercrunch’, Loose Leaf ‘Black Seeded Simpson’, and Romaine ‘Jericho’) were planted 15 m from the main study but on the same day as the main study. Micro sprinklers (Micro Sprinkler VI Classic SAM610 Model, The Toro Company, Bloomington, MN) affixed to 38 cm stakes were installed at a final micro sprinkler height of 20 cm. In 2020, the duration of micro sprinkler treatment was 30 min per hour between 10 AM and 3 PM for a total of 2.5 h of water application per day. In 2021, the watering duration was reduced to an interval of 5 min per hour between 10:00 AM and 3:00 PM for a total of 25 min of water application per day.

Hobo Connect probes (Onset Computer Company, Bourne, MA) recorded the temperature every minute throughout the growing season in every season except Spring 2020. Spring 2020 temperature data was obtained from the Mesonet station (Oklahoma Mesonet, National Weather Center, Norman OK) near the plots in Perkins, OK. Accumulated growing degree days were calculated for every season, using 10 °C as the base temperature (Lafta et al., 2017; Fig. 1). These probe readings were converted to seasonal averages by adding the temperature on the hour every day within a season and dividing by 24 on a per day basis before taking an overall seasonal average daily temperature (Fig. 2). Average maximum and minimum values were also obtained, by taking the mean of the whole set of maximum and minimum values for each 24 h period of the growing season (Fig. 2).

Sample Preparation for Lab Analysis

Twelve cultivars were analyzed for selected SLs (lactucin, 8-deoxylactucin, and lactucopicrin) and sugars (glucose, fructose, and sucrose). These cultivars were: Romaine ‘Coastal Star’, Romaine ‘Parris Island’, Romaine ‘Jericho’, Butterhead ‘Butter Crisp’, Butterhead ‘Nancy’, Batavian ‘Cherokee’, Batavian ‘Sierra’, Batavian ‘Nevada’, Salanova® ‘Summer Crisp Green’, Salanova® ‘Summer Crisp Red’, Salanova® ‘Green Butter’, and Salanova® ‘Red Butter’. Six lettuce plants (two plants from each of three replications) of each cultivar were processed, frozen, and freeze dried to < 5% moisture content using Harvest Right™ freeze dryers (HRFD-PLrg-SS, Harvest Right, North Salt Lake, UT) with a shelf temperature of 21 °C, vacuum level of <200 Mtorr and a drying duration of approximately 100 h. Samples were then weighed and ground to a fine powder using a UDY cyclone mill (3010-030, UDY Corporation, Fort Collins, CO), fitted with a 1mm screen. A portion of the ground tissue was used for moisture content analysis and the remainder was stored in sealed 120 mL brown glass bottles in a freezer at -20 °C to await analyses.

Moisture Content

Moisture content was determined from triplicate 100 mg samples after drying samples for 48 h at 80 °C. Bins were equilibrated to room temperature in a desiccator prior to weighing. Percent moisture was determined using the following equation:

$$\text{Moisture Content} = 1 - \left(\frac{dryweight - binweight}{wetweight - binweight}\right)$$

Dry weight refers to the weight of the aluminum bin with sample after 48 h in the oven. Bin weight refers to the weight of the aluminum bin taken prior to adding about 100 mg of sample. Wet weight refers to the weight of the aluminum bin plus the weight of the sample before being dried in the oven. Moisture content determinations were utilized to correct analytical results to a dry weight basis.

Reagents Used in Lab Analysis

Ethyl alcohol (190-proof ACS/USP Grade) was purchased from Pharmco (Brookfield, CT). Sugars used for standard runs included D-(+)-glucose ($\geq$ 99.5%), D-

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

Sesquiterpene Lactone Quantification

Free and bound lactucin, 8-deoxylactucin, and lactucopicrin were determined from lettuce samples essentially as described in Feroli and D'Antouno (2012). Sample pairs were weighed in duplicate (200 mg) into 2-dram vials, 20 μg of santonin (Sigma Aldrich, St. Louis, MO) was added as internal standard and 3 ml of 80% methanol plus 2% formic acid was added. Vials were mixed and then incubated at 60 °C for 30 min, mixing every 10 min. After incubation vials were centrifuged at 3,000 g_n for 30 min and the supernatant was decanted into a fresh 2-dram vial. The extraction was repeated once more, and the combined supernatants were re-centrifuged at 3,000 g_n for 30 min to remove remaining sample material and decanted into clean vials. Samples were then dried overnight using a SpeedVac™ vapor evaporator (SPD-121P, Thermo-Savant, Waltham, MA).

For analysis of bound SLs, one of the sample pairs was treated with 25 mg of cellulase enzyme (sourced from *Asperiligus niger* 1.1units/mg, Sigma-Aldrich). After enzyme addition 3 mL of deionized water was added and samples were incubated in a 40 °C heating block for 2 h. The free SL pair of samples were co-incubated in 3 ml of water without enzyme addition. After incubation, 2 mL of ethyl acetate was added, vortexed for 10 sec and vials were then centrifuged for 15 min before decanting the ethyl acetate layer into a new vial. The ethyl acetate addition step was repeated two more times for a total of 6 mL of ethyl acetate layer per sample. The combined ethyl acetate layers were dried using a SpeedVac vapor evaporator for approximately 3 h.

Dried samples from both sample pairs were dissolved into 1 mL of methanol and then overlaid with 5 mL of dichloromethane before being run through 2.8 mL reservoir/500 mg sorbent mass Silica cartridges (Extract-Clean Silica Col., Alltech Associates Inc., Deerfield, IL). Prior to use, each silica cartridge was preconditioned with 6 mL of dichloromethane:isopropanol (1:1 v/v), followed by 6 mL of dichloromethane. Each 6 mL sample was then passed though the column. Flow through column eluates were then dried down using a SpeedVac vapor evaporator and processed for SL determinations. Following sample flow through, each cartridge was reconditioned with 6 mL of dichloromethane:ethyl Acetate (3:2, v/v) and the column eluate was discarded.

SL samples were recovered into HPLC methanol:water (1:1, v/v) before being filtered through Millipore™ filters (Millipore Corportation, Billerica, MA) that were fitted with a 0.45-micron nylon 66 filter (Supelco, Bellefonte, PA) and Whatman 41 (Whatman International, Maidstone, England) quantitative filter paper as a prefilter. The filtered samples were placed into 2 mL glass screw top autosampler vials, fitted with 8 mm septa.

HPLC analyses were performed using a Thermo-Dionex Ultimate-3000 (ThermoFisher Scientific, Waltman, MA) gradient pump and PDA-1 diode array detector. Samples were injected (10 µL) using a Thermo-Dionex Ultimate 3000 autosampler (ThermoFisher Scientific, Waltman, MA). A Kinetex (5 µm) XB C18 [250 x 4.6 mm] column from Phenomenex (Torrance, CA), equipped with a C18 [4 x 3.0 mm] pre-column with cartridges placed in a Security Guard apparatus, was employed. The flow rate was set to 1.0 mL/min and elution solvents were 10% acetonitrile (Solvent A) and 55% acetonitrile (Solvent B). The schedule was set for 48 min. An eluent gradient program was established: 100% solvent A for 5 min, increased to 85% solvent B at 35 min, and 100% solvent B at 36 min. Solvent B was held at 100% until 44 min, and then returned to 100% solvent A over 1 min. Initial conditions of 100% solvent A were held for 3 min prior to a subsequent injection. Chromatograms were detected at 264 nm and analyzed using the chromatography data system Chromeleon 7 (Thermo-Dionex, Waltman, MA). SL quantifications were done relative to the internal standard, santonin. SL concentrations were converted to a dry weight basis using our moisture content analyses on a sample-by-sample basis.

Sugar Content Quantification

Duplicate samples were weighed (200 mg) into 7.4ml vials and extracted with boiling 95% ethanol at 85°C for 20 min. Vials were centrifuged at 3,000 g_n for 15 min and the supernatant was decanted through Whatman 41 filter paper into a 10 ml volumetric flask. Extractions were repeated three more times and the combined supernatants were brought to volume. Duplicate aliquots of 300 µl were added to fresh 7.4ml vials containing 100 µg inositol added as internal standard and dried using a SpeedVac sample concentrator. Samples were deionized to remove organic acids which would interfere with sugar analysis (Davies, 1988) using 250 mg of UCW 3600 mixed bed ion exchange resin (Purolite, King of Prussia, PA) with stirring in 1 ml of deionized water for 2 h at room temperature. Samples were then centrifuged at 3,000 g_n in a SpeedVac centrifuge, and the supernatant was transferred to a clean vial, dried in a SpeedVac sample concentrator, and stored loosely capped over desiccant overnight.

Samples were derivatized prior to gas chromatography analysis with 50 µl BSTFA plus 1% TMS (Tokyo Chemical Company, Tokyo, Japan), vortexed for 30 s and allowed to incubate for 1 h. Dimethylformamide (100 µl; DMF, Sigma-Aldrich, St. Louis, MO) was added, vials were vortexed for 30 s and incubated for an additional hour. Glucose, fructose and sucrose in samples was then analyzed by injecting 0.5 µl onto the gas chromatography (GC) column.

Samples were injected quickly onto a DB-5 column equipped with a split less injector utilizing a Varian 3400 GC (Varian Medical Systems, Palo Alto, CA). Runs were set for a duration of 20 min and the injector temperature was set and held at 260 °C. The column temperature was adjusted in the following manner: it started and was held at 140 °C for 2 min, then it was increased by 20 °C/min until the column achieved 280 °C, and that temperature was maintained for 9 min. Chromatographic data was collected using Dionex Peaknet software (Dionex Corporation, Sunnyvale, CA). After determining the concentration of sugars in each sample using inositol as internal standard, these concentrations were converted to a dry weight basis using our moisture content analyses on a sample-by-sample basis.

Data Analysis

Subsample measurements on the same experimental unit were averaged prior to the analysis. Growth and laboratory data were analyzed using linear mixed model methods where season, cultivar, and their interaction were the fixed effects, and replication group was the random effect. For significant fixed effects in field data, the treatment means were separated using Tukey's Honestly Significant Difference (HSD) post hoc method. For significant fixed effects in laboratory data, the treatment means were separated using Fisher's Least Significant Difference (LSD) post hoc method. All tests were conducted at the nominal 0.05 level of significance. The data analysis for this experiment was performed using statistical software (SAS ver. 9.4; SAS Inc., Cary, NC), with the Proc Glimmix procedure.

Results

There was a significant effect of cultivar, season, and their interaction on all but six of our parameters of interest (free lactucin, bound lactucin, free 8-deoxylactucin, glucose, sucrose and Sugar:SL ratio) when using a combined analysis of variance

(ANOVA) model at alpha < 0.05 (Table 3). Therefore, the data were analyzed on a per growing season basis to better capture the interaction effect.

The effect of cultivar was significant on mean canopy width in all five measured seasons (Table 4). In Spring 2021, both Romaine type cultivars tested had the greatest canopy widths. Salanova® cultivars, except for 'Summer Crisp Green' were in the lowest statistical group for canopy width in every season. Plant height varied among cultivars and seasons (Table 5). The effect of cultivar on plant height was significant in each of the five measured seasons. One Loose Leaf cultivar 'Black Seeded Simpson' had the greatest plant height in all five measured seasons. All Salanova® and Batavian type cultivars belonged to the lowest statistical grouping for plant height, regardless of season.

The effect of cultivar on yield was significant in all growing seasons except for Fall 2020 (Table 6). In general, yields were greater in the spring growing seasons, regardless of cultivar and year (Data not shown). Butterhead 'Optima' had a dramatic increase in yield between when comparing the Summer 2020 and Summer 2021 growing seasons. This large difference is due to large amounts of transplant death in the Summer 2020 season for this cultivar. In both spring and summer seasons, Romaine 'Jericho' was in the highest mean yield group. In both fall seasons, Batavian cultivars yields were not significantly different from Romaine cultivars. Salanova® type cultivars generally had lower yields than the other cultivar groups, although Salanova® 'Summer Crisp Green' and Salanova® 'Butter Green' were sometimes included within greater yield groupings.

The effect of cultivar on canopy width was significant in both summers of micro sprinkler treatment (Table 7). Romaine 'Jericho' had a larger canopy width than the other cultivars treated with micro sprinklers. The effect of cultivar on plant height was significant in both summers of micro sprinkler treatment (Table 8). Loose Leaf 'Black Seeded Simpson' had the highest plant heights both with and without micro sprinkler treatment (Tables 5 and 8).

While no direct comparison in the form of a statistical test could be conducted, there seemed to be little difference numerically in the yield when the same cultivars were compared between micro sprinkler treatment and drip irrigated plots in Summer 2021 (Tables 6 and 9). Though no impact on yield was observed, lettuce leaf surface temperatures were typically reduced by 3 ^{o}C immediately following micro sprinkler treatment, regardless of cultivar (Data not shown). Batavian 'Nevada' had the greatest yield in the Summer 2020 micro sprinkler plots, while Romaine 'Jericho' had the greatest yield in the Summer 2021 micro sprinkler plots.

The concentrations of Total SL were generally lowest in spring, highest in summer, and intermediate in the fall regardless of cultivar (Data not shown). Total Sugar values were much higher in Spring 2021 compared with summer and fall of that year, but the same trend was not observed in 2020 (Data not shown). The Sugar:SL ratio of the same cultivar in spring is more than six times greater than in summer for many cultivars.

In Spring 2020, the effect of cultivar was not significant for Total SL content, Total Sugar content, or Sugar:SL ratio (Table 10). However, in the Spring 2021 growing season, the effect of cultivar was significant for Total SL, Total Sugar, and Sugar:SL ratio. Salanova® 'Summer Crisp Red' had the greatest concentration of Total SLs. Additionally, Batavian 'Sierra' Butterhead 'Buttercrunch', and Batavian 'Nevada' had the greatest concentrations of Total Sugars. All Salanova® cultivars had the lowest Sugar:SL ratio when compared to other types of cultivars in Spring 2021.

In Summer 2020, The effect of cultivar was significant for the response variables Total SL content and Sugar:SL ratio (Table 10). Batavian ‘Cherokee’ had the greatest value for Total SL content. Batavian ‘Sierra’, Salanova® ‘Butter Green’, and Romaine Jericho had the greatest values for Sugar:SL ratio. In Summer 2021, The effect of cultivar was significant for the variable Total SL content. Batavian ‘Cherokee’ and Salanova® ‘Summer Crisp Green’ had the greatest values for Total SL content.

In Fall 2020, the effect of cultivar was not significant for the response variables Total SL content, Total Sugar content, and Sugar:SL ratio (Table 10). In Fall 2021, the effect of cultivar was significant for the response variables Total SL content and Total Sugar content. Batavian ‘Cherokee’ had the greatest concentration of Total SL, while Batavian ‘Sierra’ had the greatest concentration of Total Sugars.

There was not a consistent trend indicating of SL and sugar profiles when comparing closely related red versus green lettuce varieties. Batavian ‘Cherokee’ which is a red cultivar had higher values of Total SL than the other Batavian cultivars tested in both Summer and Fall 2021 seasons but not in any of the other growing seasons (Table 10). Batavian ‘Cherokee’ also had lower values of Total Sugar in the Spring and Fall 2021 growing seasons. Additionally, Batavian ‘Cherokee’ had lower Sugar:SL ratios in both fall seasons when compared with the other Batavian cultivars in the study, but this was not observed in the spring seasons. The Sugar:SL ratios of Salanova® ‘Butter Red’ Sugar:SL ratios were not different from Salanova® ‘Butter Green’ Sugar:SL ratios in the fall or spring seasons. Additionally, between the red and green Salanova® 'Summer Crisps', no significant differences in Sugar:SL ratio were detected in both years of fall and spring growing seasons.

In all individual growing seasons, lactucin and lactucopicrin were generally the SLs present in highest concentration for most lettuce cultivars tested, except for Batavian 'Cherokee' (Data not shown). Additionally, the SLs lactucin and lactucopicrin were typically present in greater concentrations in the free form than in the bound form. The opposite was true for 8-deoxylactucopicrin, with the bound form generally present in greater concentration than the free form, regardless of season.

In Spring 2020, the effect of cultivar was not significant for any of the SLs except for bound 8-deoxylactucin (Table 11). The Batavian cultivars had the greatest concentrations of bound 8-deoxylactucin. In Spring 2021, the effect of cultivar was significant only for the concentration of free 8-deoxylactucin, and Salanova® 'Summer Crisp Red' had the greatest concentration.

In Summer 2020, there were differences among cultivars for the concentrations of free 8-deoxylactucin, free lactucopicrin, bound 8-deoxylactucin, and bound lactucopicrin (Table 11). Batavian 'Cherokee' had the greatest concentration of both free and bound 8-deoxylactucin. Butterhead 'Buttercrunch', Romaine 'Jericho', and Romaine 'Coastal Star' had the greatest concentrations of free lactucopicrin. Romaine 'Coastal Star' had the greatest concentration of bound lactucopicrin.

In Summer 2021, there were differences among cultivars for the concentrations of all three SLs measured, in both free and bound forms. Salanova® 'Summer Crisp Green' and Salanova® 'Summer Crisp Red' had the greatest concentrations of free lactucin when compared with the other cultivars (Table 11). Batavian 'Cherokee' had the greatest concentration of free 8-deoxylactucin. Salanova® 'Summer Crisp Green' had the greatest concentration of free lactucopicrin. Salanova® 'Summer Crisp Green', 'Summer Crisp Red', and 'Butter Green' had the greatest concentrations of bound lactucin. Batavian 'Cherokee' had the greatest concentration of bound 8-deoxylactucin. Salanova® 'Summer Crisp Green', Salanova® 'Summer Crisp Red', Batavian 'Nevada', and Butterhead 'Nancy' had the greatest concentrations of bound lactucopicrin.

In Fall 2020, there were differences in the concentrations of free 8-deoxylactucin, bound lactucin, and bound 8-deoxylactucin (Table 11). Batavian 'Cherokee' had the greatest concentrations of free and bound 8-deoxylactucin. Salanova® 'Summer Crisp Red' had the greatest concentration of bound lactucin. In Fall 2021, there were differences in the concentration of bound 8-deoxylactucin, but no significant differences in the concentration of the other SLs measured. Batavian 'Cherokee' had the greatest concentration of bound 8-deoxylactucin.

The lettuce treated with micro sprinklers in 2020 did not have numerically lower SL concentration values when compared with those grown only with drip irrigation (Tables 11 and 12). However, in 2021, the Salanova® cultivars grown with micro sprinkler irrigation had numerically lower concentrations of SL's (Tables 11 and 12). For example, Salanova® 'Summer Crisp Red' had a concentration of 209 mg/g lactucin when grown with only drip irrigation and 137 mg/g when grown with drip irrigation and the revised micro sprinkler interval. However, due to experimental design, no direct statistical comparison could be made between the plots irrigated with only drip irrigation and plots treated with micro sprinklers. In Summer 2020, there were significant differences in both free and bound 8-deoxylactucin concentration among cultivars in the micro sprinkler plots (Table 12). Butterhead ‘Buttercrunch’ had the greatest concentration of both free and bound 8-deoxylactucin. In Summer 2021, there were significant differences in the concentration of all free SLs and bound 8-deoxylactucin for plants treated with micro sprinklers. Just as in the Summer 2020 micro sprinkler trial, Butterhead ‘Buttercrunch’ had the greatest concentration of free and bound 8-deoxylactucin. Salanova® ‘Summer Crisp Green’, Romaine ‘Parris Island’, Salanova® ‘Summer Crisp Red’, and Salanova® ‘Summer Crisp Green’ had the greatest concentrations of lactucin, Salanova® ‘Summer Crisp Green’ had the greatest concentration of lactucopicrin.

Sucrose was the only sugar to return a non-significant value for the interaction effect of cultivar and season (Table 3). There were no differences among cultivars for the concentrations of sucrose, except in the Fall 2021 growing season (Table 13). In the Spring 2020 growing season, there were no differences in concentration of fructose, glucose, or sucrose among cultivars (Table 13). However, in Spring 2021, there were differences among cultivars in the concentration of both fructose and glucose. Batavian ‘Nevada’, ‘Sierra’, and Butterhead ’Buttercrunch’ had the greatest concentrations of fructose. Batavian ‘Nevada’, ‘Sierra’, and Butterhead ’Buttercrunch’ also had the greatest concentrations of glucose.

In the Summer 2020 growing season, there were differences in the mean concentration of fructose (Table 13). Batavian ‘Sierra’ and Butterhead ‘Buttercrunch’ had the greatest mean concentrations of fructose when compared to the other cultivars. There were no differences in sugar concentrations among cultivars in Summer 2021.

In the Fall 2020 growing season, there were no differences in concentration for fructose, glucose, or sucrose among cultivars (Table 13). However, in the Fall 2021 growing season, there were differences in concentration among cultivars of all three sugars. Batavian ‘Sierra’, ‘Nevada’, and Salanova® ‘Summer Crisp Green’ had the greatest concentrations of fructose. Batavian ‘Sierra’, ‘Nevada’, and Romaine ‘Coastal Star’ had the greatest concentrations of glucose. Salanova® ‘Summer Crisp Green’, Batavian ‘Nevada’, and Batavian ‘Sierra’ had the greatest concentrations of sucrose.

For micro sprinkler treated plants in Summer 2020, there were significant differences in the concentration of fructose (Table 14). Batavian ‘Nevada’ had the greatest concentration of fructose. In Summer 2021, there were significant differences in the concentrations of fructose and sucrose in the micro sprinkler trials. As in 2020, Batavian ‘Nevada’ had the greatest concentration of fructose. Batavian ‘Nevada’ and Romaine ‘Jericho’ had the greatest concentrations of sucrose.

Discussion

The objective of this study was to provide cultivar recommendations to the small farmers of Oklahoma for each season of lettuce production, as well as evaluate whether micro sprinkler irrigation systems can be used to extend the growing season of lettuce farther into the summer months. Based on greater yields and relatively high Sugar:SL ratios in both spring and fall off-season of production, Romaine and Batavian cultivars are recommended (Tables 4-6 and 10). Spring was the ideal season to grow lettuce in Oklahoma due to greater yields and Sugar:SL ratios. Additionally, no cultivar appears to possess sufficient heat tolerance to develop a marketable flavor profile (as measured in SL and sugar content) during the summer months, therefore lettuce should not be grown during the summer months in Oklahoma at all in outdoor production on a commercial scale. Free SL's were typically present in greater concentration than Bound SL's, with the exception of 8-deoxylactucin. Micro sprinkler treatment did not appear to significantly impact either Sugar:SL ratios or growth parameters, indicating that this treatment should not be used to extend the growing season of Oklahoma lettuce farther into the summer. Red cultivars also did not always have lower Sugar:SL ratios when compared to closely related green cultivars.

Romaine cultivars tended to have greater mean yields and canopy widths than the other cultivars, regardless of season (Tables 4 and 6). This result is supported by a similar variety trial conducted in Louisiana, where Romaine cultivars comprised the group with the largest field weight and head width when compared with Butterhead and Batavian lettuces that were also included in this study (Afton, 2018). These results should give confidence to small farmers that growing Romaine lettuces will result in superior yields and thus more investment return on the cost incurred in growing them and transporting them to market. Greater yields are vital because wholesale lettuce is sold by the pound, thus greater yielding types generate more revenue (Afton et al., 2020). Lettuce sold by the head can either be sold on a weight basis or as a set price per head (Afton et al., 2020). Thus, Romaine lettuces make an ideal choice based on our data which shows that they yield more than other cultivars while retaining a relatively high Sugar:SL ratio (better flavor profile). Batavian lettuces have similar yields in the fall off-season of production, while retaining relatively similar Sugar:SL ratios (Tables 4 and 10).

The spring growing seasons had the lowest mean temperatures of the three growing seasons in both years (Fig. 2). Because greater concentrations of SLs are associated with premature bolting and heat stress, low Sugar:SL ratios observed in both summer growing seasons likely resulted from that excessive heat (Sessa et al., 2000). Because the lettuce was exposed to more temperature extremes, greater amounts of SLs were produced in the summer seasons compared to sugars. Due to the dramatically low Sugar:SL ratios in both summer seasons, including the studies' more heat tolerant cultivars, growing any lettuce outdoors during the middle of the summer in Oklahoma is not recommended (Table 10).

The SLs lactucin and lactucopicrin were typically greater in concentration in the free form, while 8-deoxylactucopricrin, when present, typically had greater concentrations in the bound form. According to the literature, our values for the µg/g concentrations of SLs within lettuce tissue in a normal growing season are very reasonable. Scientists using a similar method to extract the three principal bitter SLs on Korean lettuce cultivars found that the total concentrations of SLs varied from 14.6 – 67.7 µg/g dry wt. (Seo et al., 2009). These results corroborate the concentrations we obtained in the spring season, but still appear lower than the summer and fall seasons (Table 10). However, these scientists tested the concentration of SLs from hydroponically grown plants, therefore they do not provide an exact match to our study system as the plants were grown outside and not in controlled conditions. Another study by Korean scientists on the concentration of lactucin and lactucopicrin in the germplasm of 572 different accessions of lettuce from around the world found that free lactucin ranged from trace amounts to 235.3 µg/g dry wt. and lactucopicrin ranged from 66.3 – 3188.5 ug/g dry wt. basis (Sung et al., 2016). Sung et al., (2016) also utilized a similar method of field transplanting (4 weeks in plastic cells before transplant), except plastic house structures were used rather than open field as in the present study. These results indicate that our values are within the established range quantified by other scientists.

Our result showing that 8-deoxylactucin, while typically present at lower concentrations compared to the other two SLs, tends to accumulate more in the bound form when compared to the free form agrees with the results published by Price et al. (1990). Their results also showed that bound lactucin in lettuce, when present, tended to be present in greater concentration than free lactucin. The opposite is true of this studies' dataset. In fact, Price et al. (1990) only identified free lactucin in 10 out of 25 cultivar samples, while our results show consistent presence of free lactucin in all samples (Tables 11). Lettuce plants from the study of Price et al. (1990) were grown in controlled conditions, while the plants in the present study were grown under open field conditions. Price et al. (1990) also utilized a different purification scheme and chromatographic conditions from those used in the present study.

We have not found studies that have investigated SLs and sugars for lettuce grown under the extreme, open field conditions of this study. Growing lettuce under open field conditions provides many more opportunities for plant stress when compared with growth chamber and greenhouse conditions. Under greenhouse and growth chamber conditions, SLs are likely not produced in as high concentration as under open field conditions. The average total concentration of the same three SLs in a study on Korean lettuce cultivars grown in a greenhouse environment was 37.5 µg/g (Seo et al., 2009). This average value is lower than almost all of our values for total SL content, including in the ideal spring season (Table 10). However, another study on SL content in lettuce that also transplanted lettuce into field (covered) conditions had similar, greater values of total SL content, ranging between 120.1 and 2286.0 µg/g (Sung et al., 2016).

These reported differences in total SL content may indicate that growing lettuce in field conditions increases the concentration of SLs. Additionally, this idea is corroborated by the findings of a hydroponic partner study carried out simultaneously with the present study. In this partner study, observed total SL content was generally lower when growing the same cultivars (McLemure 2022, unpublished data). Additionally, a study comparing two different types of growth chamber environments (hydroponic versus soil based) found that lettuce grown in soil-based growth chambers had significantly higher levels of lactucopicrin and 8-deoxylactucin (Tamura et al., 2018). Our observations of relatively high SLs in summer (highest temperature), moderate SLs in fall (moderating temperatures) and lower SLs in spring (temperatures closest to optimum for lettuce) (Figs. 1 and 2; Data not shown) appear to fall in line with Han et al. (2021) who indicated that the genes that upregulate the production of SLs are stress (especially temperature stress) induced.

Because of the multitude of plant stresses ever present in field research, future studies on SL's in field produced lettuce should implement a planned covariance structure into the experimental design to better account for the natural variation present in the field. Soil moisture, for example, could be measured in regular intervals for each cultivar in each replication group in every season. This covariance structure in turn would likely pull away some of the excessive variation in measures of concentration, like that present in our results, that was obtained from using a simple physical block as the only method of accounting for field variation. With greater statistical power, more precise recommendations could be made. Future variety trial studies of this type would also benefit from utilizing qualitative quality ratings of the cultivars evaluated, such as tip burn ratings and ratings of head firmness (Gaudreau et al., 1994). The present study reports yields but these yields do not necessarily measure marketable quality. More quality indices would also improve recommendations to farmers.

Without a taste panel, this study's results rely fully on prior results from another lettuce taste study to provide our recommendation of an ideal lettuce taste profile based on high Sugar:SL ratios (Chadwick et al., 2016). Taste is far more complex and can be influenced by many more molecules than were quantified in this study. For instance, red lettuces contain anthocyanins which could be tasted as bitter at a very low threshold (Soares et al., 2013). Thus, these findings cannot truly answer whether red cultivars have a propensity to taste more bitter than their closely related green counterparts. Additionally, there is no consensus regarding which of the 3 SLs quantified in our study tastes bitter at the lowest thresholds. Some studies say the most bitter of the SL molecules is lactucin (Price et al., 1990), while others say 8-deoxylactucin (Chadwick et al., 2016).

When sugars are considered, our data agree with the established literature that glucose is the sugar typically present in the highest concentration, in all seasons except for the spring season (Chadwick et al., 2016).

Micro sprinkler treatment appeared to negatively influence yield in the Summer 2020 season (Table 9). This reduction in yield was likely caused by too much water delivered to the plots. Too much water was likely applied because standing water was observed many times in the Summer 2020 micro sprinkler plots. This excessive irrigation likely caused abnormal amounts of plant death as well as stunted growth, thus necessitating a change of the sprinkling interval in the Summer 2021 growing season. The amount of drip irrigation reaching the sprinkled plots in the Summer 2021 season was also manually adjusted to be lower and the interval of sprinkling was decreased. However, even with this change in interval and drip irrigation, the micro sprinkler plots did not appear to have higher yields, lower plant heights, or larger canopy widths when compared with the drip irrigation plots (Tables 4-6 and 7-9). This result is supported by a study on iceberg lettuce grown in Poland (Rolbiecki and Rolbiecki, 2000). There was no noticeable difference in yield between lettuce grown with micro sprinklers and drip irrigation in their experiment. As for the differences in the concentrations of the three bitter SL's, micro sprinkler mediated evaporative cooling did not appear to affect their accumulation either (Tables 11 and 12). Without, any demonstrated benefit, micro sprinkler use in lettuce production is not recommended.

Conclusions

These data do not support the use of micro sprinklers to extend the growing season of lettuce farther into the summer months. Micro sprinklers did not increase the yield, increase sugar content, or decrease SL content in the summer months in Oklahoma when compared with plots without micro sprinklers. Red cultivars in this study did not always have lower Sugar:SL ratios when compared with closely related green cultivars. The ideal lettuce cultivars to grow in spring, the best season for lettuce growth in Oklahoma, are Romaine and Batavian cultivars due to their relatively greater yields and superior Sugar:SL ratios. Salanova® cultivars had lower yields and Sugar:SL ratios when compared with the other types of lettuce in the study. If farmers wish to utilize fall offseason production, Romaine and Batavian cultivars are again a superior choice because of greater yields and Sugar:SL ratios.

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APPENDICES

Tables and Figures

Table 1. Lettuce cultivars and seed sources utilized in this study

Type	Cultivar	Vendor	Pelleted?
Loose Leaf	'Black Seeded Simpson'	Johnny's	N[i]
	'Waldman's Dark Green'	Johnny's	N
	'Panisse'	Johnny's	Y
Romaine	'Parris Island'[ii]	Johnny's	N
	'Jericho'[ii]	Johnny's	N
	'Coastal Star'[ii]	Johnny's	Y
Butterhead	'Nancy'[ii]	Johnny's	Y
	'Optima'	High Mowing	N
	'Buttercrunch'[ii]	Johnny's	N
Batavian	'Nevada'[ii]	Johnny's	Y
	'Cherokee'[ii]	Johnny's	Y
	'Sierra'[ii]	Harris	N
Salanova®	'Red Butter'[ii]	Johnny's	Y
	'Green Butter'[ii]	Johnny's	Y
	'Red Sweet Crisp'[ii]	Johnny's	Y
	'Green Sweet Crisp'[ii]	Johnny's	Y
	'Red Oakleaf'	Johnny's	Y
	'Green Oakleaf'	Johnny's	Y

[i]N refers to seeds that were not pelleted, Y refers to seeds that were pelleted

[ii]Cultivars were included in the lab analysis

Table 2. Lettuce sowing, transplanting, and harvesting dates.

Season	Sowing Date	Transplant Date	Harvest Date
Spring 2020	2 Mar. 2020	2 Apr. 2020	6 May 2020
Summer 2020	1 June 2020	16 July 2020	24 Aug. 2020
Fall 2020	27 Aug. 2021	24 Sep. 2020	26 Oct. 2020
Spring 2021	4 Mar. 2021	9 Apr. 2021	19 May 2021
Summer 2021	30 May 2021	9 July 2021	3 Aug. 2021
Fall 2021	20 Aug. 2021	28 Sep. 2021	2 Nov. 2021

Table 3. ANOVA analysis of the main effects of season, cultivar, and their interaction

		Main Effect and *P* value		
Type of variable	Response variable	Season	Cultivar	Season*Cultivar
Growth Metrics[i]	Canopy width (cm)	*P* < 0.001	*P* < 0.001	*P* < 0.001
	Plant height (cm)	0.016	*P* < 0.001	*P* < 0.001
	Yield (g)	*P* < 0.001	*P* < 0.001	*P* < 0.001
Free SL[ii]	Lactucin (ug/g)	*P* < 0.001	0.097	0.027
	8-deoxylactucin (ug/g)	0.110	*P* < 0.001	0.013
	Lactucopicrin (ug/g)	0.002	*P* < 0.001	*P* < 0.001
Bound SL[ii]	Lactucin (ug/g)	0.002	0.007	0.097
	8-deoxylactucin (ug/g)	*P* < 0.001	*P* < 0.001	*P* < 0.001
	Lactucopicrin (ug/g)	0.003	0.001	0.028
Sugars[ii]	Glucose (mg/g)	0.011	0.096	0.026
	Fructose (mg/g)	*P* < 0.001	*P* < 0.001	*P* < 0.001
	Sucrose (mg/g)	0.001	0.008	0.679
Bitterness[ii] measures	Total SL (ug/g)	*P* < 0.001	*P* < 0.001	0.033
	Total Sugar (mg/g)	*P* < 0.001	*P* < 0.001	0.007
	Sugar:SL ratio	0.008	*P* < 0.001	0.058

[i]Reflects all growing seasons except Spring 2020, because growth metrics were not measured in that season.
[ii]Reflects all growing seasons except Summer 2020, because not enough laboratory reps were taken from some cultivars to complete the model.

Table 4.
Least square mean canopy widths (cm) of 18 lettuce cultivars

Type	**Cultivar**	**Summer 2020**	**Fall 2020**	**Spring[i] 2021**	**Summer 2021**	**Fall 2021**
Batavian	Cherokee	12.4abc[ii]	16.9abcde	22.5cde	18.7efg	22.4abcd
Batavian	Nevada	10.3bc[iii]	16.8abcde	21.4cdef	21.7bcd	20.1abcd
Batavian	Sierra	16.2ab	17.3abcd	21.9cdef	20.3cde	22.7abcd
Butterhead	Buttercrunch	12.5abc	14.6cdefgh	18.5efg	15.9ghi	18.1bcd
Butterhead	Nancy	7.7c	14.8cdefgh	23.6cd	22.3bcd	18.9abcd
Butterhead	Optima	8.0bc	15.0cdefg	22.3cde	23.7b	24.0abc
Loose Leaf	Black Seeded Simpson	10.1bc	16.2bcdef	28.5b	17.1fghi	18.3bcd
Loose Leaf	Panisse	8.1bc	14.1defgh	20.6cdef	18.3efgh	18.7abcd
Loose Leaf	Waldman's Dark Green	10.3bc	18.2abc	25.3bc	NA[iv]	22.3abcd
Romaine	Coastal Star	17.4a	19.6ab	NA[iv]	23.7b	27.0a
Romaine	Jericho	16.0ab	18.9ab	36.0a	27.5a	25.5ab
Romaine	Parris Island	12.0abc	20.5a	34.2a	23.0bc	26.9a
Salanova®	Butter Green	10.4bc	11.0h	15.2g	12.7i	13.8d
Salanova®	Butter Red	8.5bc	12.0gh	17.2fg	13.9i	14.9d
Salanova®	Oakleaf Green	8.3bc	11.2gh	17.9efg	15.2i	14.7d
Salanova®	Oakleaf Red	11.1abc	14.5cdefgh	20.1defg	15.4hi	16.7cd
Salanova®	Summer Crisp Green	10.3bc	13.1efgh	24.4bcd	19.5def	18.6abcd
Salanova®	Summer Crisp Red	7.5c	12.5fgh	19.6defg	13.5i	16.7cd

[i]Spring 2020 growing season was not measured.
[ii]Canopy width was measured from largest leaf to opposite leaf. Canopy widths were measured within a week of harvest in each season.
[iii]The effect of cultivar on canopy width was significant in all seasons, means sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05
[iv]Cultivars marked with NA indicate crop failure, there were not enough plants to measure.
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Table 5.
Least square mean (cm) plant heights of 18 lettuce cultivars

Type	Cultivar	Summer 2020	Fall 2020	Spring 2021[i]	Summer 2021	Fall 2021
Batavian	Cherokee	3.9bcde[ii]	1.3ef	1.8b	3.2de	1.6b
Batavian	Nevada	2.1e[iii]	1.5ef	1.6b	1.9e	1.5b
Batavian	Sierra	2.2de	1.1f	1.3b	1.8e	1.8b
Butterhead	Buttercrunch	4.9bcde	1.7def	2.1b	3.0de	2.2b
Butterhead	Nancy	2.4cde	1.9cdef	1.9b	2.9de	1.5b
Butterhead	Optima	4.4bcde	2.8cd	2.6b	4.6de	2.2b
Loose Leaf	Black Seeded Simpson	13.8a	5.7a	10.9a	26.8a	39.4a
Loose Leaf	Panisse	2.3cde	2.2cdef	2.9b	2.4e	1.5b
Loose Leaf	Waldman's Dark Green	7.4b	4.4b	6.3ab	NA[iv]	9.6b
Romaine	Coastal Star	6.1bc	3.0c	NA[iv]	9.4bc	4.7b
Romaine	Jericho	4.9bcde	2.2cdef	2.8b	10.5b	3.6b
Romaine	Parris Island	5.8bcd	2.4cde	3.5b	6.7cd	2.4b
Salanova®	Butter Green	3.0cde	1.5ef	2.4b	1.7e	1.0b
Salanova®	Butter Red	2.5cde	1.4ef	2.3b	1.9e	0.9b
Salanova®	Oakleaf Green	2.8cde	1.0f	1.6b	1.6e	0.7b
Salanova®	Oakleaf Red	3.1cde	1.6ef	1.7b	2.1e	1.2b
Salanova®	Summer Crisp Green	1.9e	1.6def	2.0b	1.5e	0.7b
Salanova®	Summer Crisp Red	2.4cde	1.2ef	1.1b	1.7e	0.6b

[i]Spring 2020 growing season was not measured.
[ii]Lettuce plant height was measured from ground level to shoot apical meristem. Heights were measured within a week of harvest in each season.
[iii]The effect of cultivar on plant height was significant in all seasons, groups sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05
[iv]Cultivars marked with NA indicate crop failure, there were not enough plants to measure.

Table 6.
Least square mean yield (kg/ha)[i] of 18 lettuce cultivars

Type	Cultivar	Spring 2020	Spring 2021	Summer 2020	Summer 2021	Fall 2020	Fall 2021
Batavian	Cherokee	1807bcd[ii]	3100cdefg	941ab	1344cde	1122a	1825abc
Batavian	Nevada	2460abcd	2770defg	456b	1812cde	1317a	1555abc
Batavian	Sierra	2174abcd	2750defg	1839a	1613cde	1313a	2434abc
Butterhead	Buttercrunch	2488abc	2482cdefg	1413b	1414bcde	1084a	1428abc
Butterhead	Nancy	2935abcd	3094efg	343ab	2003cde	961a	1798abc
Butterhead	Optima	2801abcd	2898defg	107b	3354ab	1112a	3567a
Loose Leaf	Black Seeded Simpson	2949ab	4347cd	1002ab	1800cde	1137a	2069abc
Loose Leaf	Panisse	2139abcd	3301cdef	258b	1388cde	962a	1768abc
Loose Leaf	Waldman's Dark Green	2721abcd	3849cde	738b	NA[iii]	1702a	1986abc
Romaine	Coastal Star	3487a	NA[iii]	1215ab	2534abcd	1116a	3143ab
Romaine	Jericho	3521a	7663a	1436ab	3696a	1195a	2287abc
Romaine	Parris Island	2932abc	6350ab	824b	2641abc	1208a	2277abc
Salanova®	Butter Green	NA[iii]	2281efg	1474ab	1289cde	1074a	1250bc
Salanova®	Butter Red	1443cd	2092fg	329b	748e	773a	885c
Salanova®	Oakleaf Green	1745bcd	2157fg	505b	1129de	941a	1276bc
Salanova®	Oakleaf Red	1372d	2388efg	859b	896e	846a	999bc
Salanova®	Summer Crisp Green	2792abcd	4689bc	601b	1365cde	916a	1192bc
Salanova®	Summer Crisp Red	1439d	1547g	233b	555e	849a	944c

[i]Average yield per ha was calculated using an in-row spacing of 1 ft, between row spacing of 6 feet.
[ii]The effect of cultivar on yield was significant in every season except for Fall 2020, groups sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05
[iii]Cultivars marked with NA indicate crop failure, there were not enough plants to measure.

Table 7.
Least square mean canopy widths[i] (cm) of lettuce in micro sprinkler plots

Type	**Cultivar**	Summer 2020	Summer 2021
Batavian	Nevada	12.7a[ii]	18.7b
Butterhead	Buttercrunch	10.7ab	16.2b
Loose Leaf	Black Seeded Simpson	8.0bc	17.9b
Romaine	Jericho	13.0a	27.6a
Salanova®	Summer Crisp Green	8.8b	17.9b
Salanova®	Summer Crisp Red	5.0c	13.6b

[i]Lettuce canopy width was measured from largest leaf to opposite leaf. Canopy Widths were measured within a week of harvest in each season.
[ii]The effect of cultivar on canopy width was significant in both seasons, groups sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05

Table 8.

Least square mean plant heights (cm)[i] of micro sprinkler plots

Type	Cultivar	Summer 2020	Summer 2021
Batavian	Nevada	3.1bc[ii]	1.5c
Butterhead	Buttercrunch	4.1bc	2.9c
Loose Leaf	Black Seeded Simpson	16.6a	26.1a
Romaine	Jericho	7.4b	14.9b
Salanova®	Summer Crisp Green	2.3c	2.3c
Salanova®	Summer Crisp Red	3.2bc	2.5c

[i]Lettuce plant height was measured from ground level to shoot apical meristem. Heights were measured within a week of harvest in each season.

[ii]The effect of cultivar on plant height was significant in both seasons, groups sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05

Table 9.
Least square mean yields[i] (kg/ha) in micro sprinkler plots

Type	Cultivar	Summer 2020	Summer 2021
Batavian	Nevada	1087a[ii]	1742b
bbButterhead	Buttercrunch	952b	1578bc
Loose Leaf	Black Seeded Simpson	NA[iii]	1480bc
bcRomaine	Jericho	933[iv]	3511a
Salanova®	Summer Crisp Green	376b	1282bc
Salanova®	Summer Crisp Red	NA[iii]	582 c

[i]Average yield was calculated using an in-row spacing of 1 ft, between row spacing of 6 feet.

[ii]Groups sharing the same letter are not significantly different when tested with Tukey's HSD at alpha=.05

[iii]Cultivars marked with NA indicate crop failure, there were not enough plants to measure.

[iv]Statistical grouping is not provided because of failure of one of 4 replicate plots.

Table 10. Total SL content, Total sugar content, and Sugar:SL ratio of lettuce in six seasons.

		Spring 2020		
Type	Cultivar	Total SL (µg/g)	Total Sugar (mg/g)	Sugar:SL ratio[i]
Romaine	Parris Island	79 ± 6a[ii]	189 ± 59a	2.4 ± 1a
	Jericho	49 ± 13a	122 ± 65a	2.7 ± 2a
	Coastal Star	65 ± 12a	158 ± 67a	2.6 ± 1.5a
Butterhead	Buttercrunch	57 ± 4a	116 ± 62a	2 ± 1.1a
	Nancy	72 ± 56a	148 ± 75a	3.5 ± 2.9a
Batavian	Nevada	110 ± 56a	139 ± 59a	1.5 ± 0.9a
	Cherokee	87 ± 32a	146 ± 38a	1.8 ± 0.3a
	Sierra	122 ± 58a	144 ± 29a	1.5 ± 1.1a
Salanova	Butter Red	77 ± 38a	83 ± 17a	1.4 ± 1.1a
	Butter Green Summer	NA[iii]	NA[iii]	NA[iii]
	Crisp Red Summer	72 ± 54a	85 ± 39a	1.2 ± 0.8a
	Crisp Green	96 ± 8a	100 ± 44a	1.4 ± 0.8a
	P value[v]	0.283	0.173	0.264

		Spring 2021		
Type	Cultivar	Total SL (µg/g)	Total Sugar (mg/g)	Sugar:SL ratio
Romaine	Parris Island	71 ± 18c	172 ± 25bcd	2.5 ± 0.4abc
	Jericho	65 ± 20c	172 ± 38bcd	3 ± 1.6abc
	Coastal Star	NA[iii]	NA[iii]	NA[iii]
Butterhead	Buttercrunch	68 ± 29c	221 ± 36abc	3.9 ± 2.2ab
	Nancy	36 ± 12c	146 ± 70de	4.5 ± 2.9a
Batavian	Nevada	78 ± 4c	234 ± 24ab	3 ± 0.5abc
	Cherokee	112 ± 49bc	163 ± 72cd	1.9 ± 1.6bc
	Sierra	71 ± 40c	248 ± 35a	4.6 ± 2.9a
Salanova	Butter Red	68 ± 27c	94 ± 15e	1.5 ± 0.4c
	Butter Green Summer	117 ± 89abc	108 ± 21de	1.4 ± 1.2c
	Crisp Red Summer	210 ± 130a	129 ± 15de	0.9 ± 0.7c
	Crisp Green	183 ± 117ab	125 ± 29de	0.9 ± 0.5c
	P value[v]	0.023	$P < 0.001$	0.022

Table 10 Continued

		Summer 2020								
Type	Cultivar	Total SL (µg/g)			Total Sugar (mg/g)			Sugar:SL ratio		
Romaine	Parris Island	497[iv]			202			0.41		
	Jericho	378	±	77cd	161	±	18a	0.43	±	0.08abc
	Coastal Star	539	±	146b	153	±	31a	0.31	±	0.16cd
Butterhead	Buttercrunch	490	±	38c	160	±	11a	0.33	±	0.04bcd
	Nancy	349			187			0.54		
Batavian	Nevada	209			176			0.85		
	Cherokee	753	±	102a	143	±	16a	0.19	±	0.03d
	Sierra	351	±	10cd	183	±	30a	0.52	±	0.08a
Salanova	Butter Red	216			104			0.48	±	
	Butter Green	315	±	84d	148	±	28a	0.48	±	0.1ab
	Summer Crisp Red	536			188			0.35		
	Summer Crisp Green	665			156			0.23		
	P value	0.001			0.417			0.008		

		Summer 2021								
Type	Cultivar	Total SL (µg/g)			Total Sugar (mg/g)			Sugar:SL ratio		
Romaine	Parris Island	278	±	81cd	65	±	6a	0.2	±	0.07a
	Jericho	246	±	127d	79	±	8a	0.4	±	0.2a
	Coastal Star	269	±	31cd	67	±	2a	0.3	±	0.04a
Butterhead	Buttercrunch	413	±	67bc	65	±	4a	0.2	±	0.01a
	Nancy	313	±	79bcd	64	±	7a	0.2	±	0.03a
Batavian	Nevada	334	±	24bcd	75	±	16a	0.2	±	0.05a
	Cherokee	718	±	71a	80	±	41a	0.1	±	0.06a
	Sierra	431	±	55b	106	±	49a	0.2	±	0.09a
Salanova	Butter Red	345	±	148bcd	94	±	44a	0.3	±	0.25a
	Butter Green	412	±	63bc	77	±	33a	0.2	±	0.12a
	Summer Crisp Red	442	±	123b	62	±	19a	0.1	±	0.04a
	Summer Crisp Green	700	±	130a	47	±	28a	0.1	±	0.04a
	P value	P < 0.001			0.218			0.066		

Table 10 Continued

		Fall 2020		
Type	Cultivar	Total SL (µg/g)	Total Sugar (mg/g)	Sugar:SL ratio
Romaine	Parris Island	85 ± 5a	203 ± 73a	2.4 ± 0.76a
	Jericho	125 ± 150a	170 ± 47ab	3.4 ± 2.58a
	Coastal Star	161 ± 189a	173 ± 22ab	2.5 ± 1.96a
Butterhead	Buttercrunch	205 ± 221a	119 ± 21bc	1.2 ± 0.98a
	Nancy	71[iv]	159	2.2
Batavian	Nevada	65 ± 17a	155 ± 13abc	2.5 ± 0.47a
	Cherokee	300 ± 215a	98 ± 14c	0.4 ± 0.23a
	Sierra	117 ± 20a	127 ± 27bc	1.6 ± 0.09a
Salanova	Butter Red	119 ± 3a	89 ± 28c	1.1 ± 0.32a
	Butter Green	179 ± 15a	141 ± 48abc	0.8 ± 0.21a
	Summer Crisp Red	399 ± 292a	118 ± 23bc	0.4 ± 0.23a
	Summer Crisp Green	185 ± 113a	163 ± 56ab	1 ± 0.41a
	P value	0.207	0.053	0.065

		Fall 2021		
Type	Cultivar	Total SL (µg/g)	Total sugar (mg/g)	Sugar:SL ratio
Romaine	Parris Island	95 ± 42b	60 ± 7c	0.8 ± 0.47a
	Jericho	73 ± 52b	63 ± 14bc	1.2 ± 0.76a
	Coastal Star	92 ± 13b	66 ± 10bc	0.7 ± 0.13a
Butterhead	Buttercrunch	68 ± 11b	59 ± 7c	0.9 ± 0.25a
	Nancy	78 ± 25b	58 ± 6c	0.8 ± 0.32a
Batavian	Nevada	70 ± 26b	79 ± 20ab	1.3 ± 0.84a
	Cherokee	433 ± 312a	51 ± 11cd	0.2 ± 0.24a
	Sierra	104 ± 110b	90 ± 9a	1.7 ± 1.42a
Salanova	Butter Red	140 ± 69b	32 ± 5e	0.3 ± 0.13a
	Butter Green	56 ± 19b	49 ± 11cde	1 ± 0.4a
	Summer Crisp Red	125 ± 5b	39 ± 7de	0.3 ± 0.06a
	Summer Crisp Green	77 ± 35b	80 ± 12ab	1.2 ± 0.54a
	P value	0.01	P < 0.001	0.111

[i]Sugar:SL ratio expressed in mg/g per ug/g, to keep values smaller
[ii]Lsmeans followed by different letters indicate a significant difference using Fishers LSD at α=0.05
[iii]NA indicates complete crop failure
[iv]Samples without standard deviation did not have 3 replications, therefore no value is displayed due to an unfair comparison
[v] Each parameter was modeled separately to detect whether there were statistically significant differences, with replication group as a random effect.

Table 11. Least square mean concentrations (μg/g) of SLs of lettuce in six seasons

Spring 2020

		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxylactucin	Lactucopicrin
Romaine	Parris Island	42±5a[i]	2± 2c	10± 7a	15±11a	5± 3cd	9±7a
	Jericho	28±2a	1± 0c	7±13a	9± 5a	3± 3d	2±2a
	Coastal Star	28±3a	5± 4bc	11± 9a	12±14a	7± 2cd	2±1a
Butterhead	Buttercrunch	17±6a	11± 8abc	10± 9a	3± 3a	13± 3bcd	2±1a
	Nancy	54±6a	2± 2c	11±54a	3± 2a	3± 1d	2±2a
Batavian	Nevada	29±2a	6± 4bc	7±16a	32±26a	34±18abc	3±3a
	Cherokee	20±5a	18±14a	5±12a	3± 1a	38±18ab	2±2a
	Sierra	44±4a	2± 2c	12±28a	3± 1a	54±43a	7±4a
Salanova®	Butter Red	44±4a	15±11ab	8±27a	4± 3a	2± 2d	4±3a
	Butter Green[i]	NA	NA	NA	NA	NA	NA
	Summer Crisp Red	41±4a	12±14abc	6±12a	15± 6a	22±30bcd	2±2a
	Summer Crisp Green	30±9a	10±8abc	10± 2a	16±15a	1± 1d	5±2a
	P value[y]	0.386	0.084	0.539	0.075	0.012	0.126

Spring 2021

		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxy	Lactucopicrin
Romaine	Parris Island	29±15a	7±12b	24± 23a	10± 6a	51± 0a	2± 2a
	Jericho	45±16a	1± 2b	15± 3a	0± 0a	1± 0a	1± 1a
	Coastal Star[i]	NA	NA	NA	NA	NA	NA
Butterhead	Buttercrunch	35±27a	4± 2b	16± 4a	0± 0a	13± 4a	0± 0a
	Nancy	5± 2a	3± 3b	16± 4a	6± 8a	5± 3a	2± 1a
Batavian	Nevada	32±18a	1± 1b	30± 19a	7± 9a	6± 6a	2± 1a
	Cherokee	19± 7a	15±13b	21± 9a	4± 3a	0±45a	1± 1a
	Sierra	19±15a	3± 2b	18± 4a	11±15a	18±15a	2± 1a
Salanova®	Butter Red	15±15a	0± 0b	35± 6a	11±15a	1± 1a	6± 3a
	Butter Green[z]	24±18a	2± 3b	63± 60a	13± 6a	9±14a	6± 8a
	Summer Crisp Red	44±29a	62±54a	29± 9a	13± 9a	58±95a	4± 1a
	Summer Crisp Green	29±18a	9± 8b	98±114a	2± 2a	25±40a	19±32a
	P value[ii]	0.258	0.010	0.214	0.441	0.469	0.493

Table 11 Continued

Type	Cultivar	Summer 2020 Free Lactucin	Free 8-Deoxylactucin	Free Lactucopicrin	Bound Lactucin	Bound 8-Deoxylactucin	Bound Lactucopicrin
Romaine	Parris Island	91[iii]	1	383	2	4	16
	Jericho	104± 8a	0± 0b	215±51ab	39± 56a	9±10b	9±13b
	Coastal Star	154±48a	10± 6b	204±52ab	90±114a	40±25b	66±36a
Butterhead	Buttercrunch	111±70a	12± 3b	255±41a	48± 76a	53± 9b	11± 8b
	Nancy	240	0	439	16	3	0
Batavian	Nevada	43	2	138	2	25	25
	Cherokee	73±47a	116±19a	181±15b	5± 7a	382±73a	3± 3b
	Sierra	99±26a	8± 2b	150± 4b	39± 41a	48± 2b	7± 6b
Salanova®	Butter Red	98	0	91	12	4	12
	Butter Green	93±18a	9± 3b	159±74b	3± 3a	31±10b	15± 2b
	Summer Crisp Red	178	0	317	20	9	13
	Summer Crisp Green	48	14	252	306	7	37
	P value[ii]	0.347	P < 0.001	0.048	0.293	P < 0.001	0.005

Type	Cultivar	Summer 2021 Free Lactucin	Free 8-Deoxylactucin	Free Lactucopicrin	Bound Lactucin	Bound 8-Deoxylactucin	Bound Lactucopicrin
Romaine	Parris Island	71± 20d	1± 1c	171±45b	12± 8cde	2± 1c	21±11cde
	Jericho	67± 24d	16±14bc	112±38cd	13±11cde	26±44c	12±16de
	Coastal Star	104± 44cd	3± 3c	130±21bcd	6±11de	7± 5c	20±13cde
Butterhead	Buttercrunch	126± 32bcd	20±15bc	159±62bc	15± 9cde	67±59b	26±21bcd
	Nancy	106± 28cd	0± 0c	155±35bcd	18± 7bcde	4± 2c	28± 7abcd
Batavian	Nevada	76± 20d	12± 1bc	123±22bcd	12±11cde	72± 4b	36±13abc
	Cherokee	80± 54d	113±10a	105±38d	13±14cde	405±29a	2± 4e
	Sierra	118± 44bcd	13± 3bc	172±41b	22±12bcd	81± 9b	25±11bcde
Salanova®	Butter Red	197±102abc	9±11bc	106±24d	3± 3e	2± 2c	18± 3cde
	Butter Green[z]	134± 42bcd	20± 2bc	123±18bcd	34±15ab	66± 5b	25± 3bcde
	Summer Crisp Red	209± 90ab	1± 1c	162±45bc	30±4abc	9± 3c	46±16ab
	Summer Crisp Green	235± 94a	46±76b	317±64a	46±22a	5± 4c	51±33a
	P value[ii]	0.012	P < 0.001	P < 0.001	0.003	P < 0.001	0.020

Table 11 Continued

		Fall 2020					
		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxylactucin	Lactucopicrin
Romaine	Parris Island	14± 5a	0± 0b	63± 1a	3± 0b	1± 0b	3± 1a
	Jericho	30± 40a	1± 0b	90±112a	1± 1b	1± 1b	2± 3a
	Coastal Star	23± 22a	2± 1b	120±161a	6± 3b	9± 6b	5± 4a
Butterhead	Buttercrunch	55± 73a	6± 2b	106±116a	4± 5b	27± 14b	8±11a
	Nancy	7[iv]	12	39	1	4	8
Batavian	Nevada	9± 7a	1± 0b	38± 11a	1± 1b	13± 4b	2± 1a
	Cherokee	18± 19a	45±23a	62± 45a	2± 1b	174±138a	5± 4a
	Sierra	17± 8a	3± 3b	64± 1a	5± 3b	14± 6b	5± 0a
Salanova®	Butter Red	18± 0a	1± 0b	76± 0a	1± 0b	1± 0b	11± 3a
	Butter Green	28± 12a	10± 2b	91± 12a	4± 4b	41± 21b	7± 5a
	Summer Crisp Red	107±130a	4± 4b	223±118a	25±21a	6± 3b	35±32a
	Summer Crisp Green	20± 10a	2± 1b	133± 80a	4± 5b	3± 3b	23±28a
	P value[iii]	0.469	P < 0.001	0.281	0.030	0.005	0.115

		Fall 2021					
		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxylactucin	Lactucopicrin
Romaine	Parris Island	16± 9a	3± 4a	63±41a	8± 8a	5± 4b	5± 6a
	Jericho	8± 1a	0± 0a	29± 9a	5± 2a	12± 13b	12±18a
	Coastal Star	24±12a	1± 1a	58±27a	3± 2a	7± 4b	0± 0a
Butterhead	Buttercrunch	14± 9a	2± 3a	26± 4a	1± 1a	17± 12b	10± 5a
	Nancy	12± 4a	0± 0a	47±21a	2± 1a	15± 18b	0± 1a
Batavian	Nevada	20±20a	2± 1a	34± 9a	3± 5a	5± 2b	5± 3a
	Cherokee	34± 6a	69±104a	41±36a	8± 5a	264±206a	7± 7a
	Sierra	12± 8a	18± 29a	41±41a	4± 4a	17± 23b	17±17a
Salanova®	Butter Red	22±10a	17± 25a	72±34a	11±12a	6± 4b	12±10a
	Butter Green	17±16a	3± 2a	28± 3a	2± 2a	4± 2b	1± 1a
	Summer Crisp Red	26±16a	2± 2a	68± 4a	9± 2a	20± 23b	4± 2a
	Summer Crisp Green	17± 8a	11± 19a	36± 5a	5± 3a	4± 4b	5± 3a
	P value[iii]	0.186	0.423	0.174	0.266	0.001	0.321

[i]Lsmeans followed by different letters indicate a significant difference using Fishers LSD at α=0.05.
[ii]No values shown because this cultivar was not planted in the Spring 2020 growing season.
[iii]Each molecule and configuration were modeled separately to detect whether there were statistically significant differences, with replication group as a random effect.
[iv]Cultivar means reported without standard deviation did not have 3 extractions reps, thus measures of spread and post hoc test lettering were not reported.

Table 12. Least square mean concentrations of SLs (ug/g) of lettuce in micro sprinkler plots

		Summer 2020 micro sprinkler trial					
		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxylactucin	Lactucopicrin
Romaine	Parris Island	141±12ab[i]	2±1b	285± 36a	12±2a	2±0c	0± 0a
Butterhead	Buttercrunch	278±163a	17±9a	421±108a	8±8a	32±9a	14±12a
Batavian	Nevada	49± 13b	5±1b	218± 21a	6±4a	18±5b	19±14a
Salanova®	Summer Crisp Red	NA[ii]	NA[ii]	NA[ii]	NA[ii]	NA[ii]	NA[ii]
	Summer Crisp Green	139±102b	2±1b	260±120a	6±4a	1±1c	20± 3a
	P value	0.108[x]	0.021	0.104	0.579	P < 0.001	0.209
		Summer 2021 micro sprinkler trial					
		Free			Bound		
Type	Cultivar	Lactucin	8-Deoxylactucin	Lactucopicrin	Lactucin	8-Deoxylactucin	Lactucopicrin
Romaine	Parris Island	136±29ab	1±2b	140±48bc	5± 1a	9±13b	12± 5a
Butterhead	Buttercrunch	66±17c	23±9a	111±38bc	12± 3a	80±38a	21± 8a
Batavian	Nevada	86±43bc	7±5b	75± 19c	7±11a	35±15b	19± 7a
Salanova®	Summer Crisp Red	137±46ab	2±2b	153± 29b	80±84a	5± 3b	21±11a
	Summer Crisp Green	186± 6a	1±1b	285± 39a	15±13a	4± 2b	9±13a
	P value	0.007[x]	0.002	P < 0.001	0.162	0.004	0.418

[i]Lsmeans followed by different letters indicate a significant difference using Fishers LSD at α=0.05

[ii]Excessive plant death made this cultivar unavailable in this season.

[iii]Each molecule and configuration were modeled separately to detect whether there were statistically significant differences, with replication group as a random effect.

Table 13. Sugar concentrations (mg/g) in six seasons.

Type	Cultivar	Spring 2020 Fructose	Spring 2020 Glucose	Spring 2020 Sucrose[v]	Spring 2021 Fructose	Spring 2021 Glucose	Spring 2021 Sucrose
Romaine	Parris Island	74±28a[i]	64±24a	51±10a	80± 7bc	68±11cd	15± 2a
	Jericho	38±26a	39±22a	45±24a	78±27bcd	74± 7bc	20±12a
	Coastal Star	63±29a	51±22a	43±18a	NA[y]	NA	NA
Butterhead	Buttercrunch	41±25a	42±20a	33±21a	111±26ab	95±15a	20± 4a
	Nancy	53±20a	42±24a	50±36a	65±45cde	62±17cd	18± 9a
Batavian	Nevada	50±28a	47±15a	42±17a	121±11a	95± 9a	18± 8a
	Cherokee	55±38a	47± 3a	44±10a	79±53bc	70±15cd	13± 7a
	Sierra	42±20a	51±10a	50±22a	135±22a	94±17ab	20± 4a
Salanova®	Butter Red	8± 2b	38± 9a	36±12a	26±11e	62±11cd	17±15a
	Butter Green Summer	NA[ii]	NA	NA	39± 7de	53±12d	16± 2a
	Crisp Red Summer	25±13a	31±16a	30±13a	66±14cd	53± 2d	10± 2a
	Crisp Green	27±11a	32±11a	41±21a	49±17cde	63± 2cd	12± 4a
	P value[iii]	0.113	0.091	0.762	P < 0.001	P < 0.001	0.439

Type	Cultivar	Summer 2020 Fructose	Summer 2020 Glucose	Summer 2020 Sucrose	Summer 2021 Fructose	Summer 2021 Glucose	Summer 2021 Sucrose
Romaine	Parris Island	58	137	13	11± 2a	44± 3a	10± 2a
	Jericho	44± 7bc	103±26a	14± 8a	14± 2a	51± 1a	14± 5a
	Coastal Star	40±15bc	93±13a	20±21a	13± 2a	45± 4a	11± 3a
Butterhead	Buttercrunch	56± 6ab	97±12a	7± 2a	14± 1a	43± 2a	8± 1a
	Nancy	57[iv]	110	20	10± 2a	44± 3a	9± 4a
Batavian	Nevada	68	89	19	14± 7a	46± 7a	16± 8a
	Cherokee	35± 7c	104±13a	4± 2a	14± 7a	58±27a	8± 7a
	Sierra	71±22a	100±20a	13± 4a	23±15a	66±25a	18±10a
Salanova®	Butter Red	14	85	5	11± 9a	71±29a	12± 7a
	Butter Green Summer	36±14bc	95±29a	17±12a	12± 5a	56±23a	9± 5a
	Crisp Red Summer	61	116	10	7± 2a	50±19a	6± 2a
	Crisp Green	51	91	13	12± 6a	61±26a	10± 6a
	P value	0.023	0.917	0.281	0.278	0.205	0.057

Table 13 Continued

Type	Cultivar	Fall 2020 Fructose	Fall 2020 Glucose	Fall 2020 Sucrose	Fall 2021 Fructose	Fall 2021 Glucose	Fall 2021 Sucrose
Romaine	Parris Island	78±27a	69±21a	62±41a	14± 4bc	31± 6bcd	15±0abc
	Jericho	53±42a	69±11a	48±13a	16±10bc	35± 4bc	14±3abc
	Coastal Star	54±21a	72±25a	47±10a	12± 2bc	38± 4ab	16±8abc
Butterhead	Buttercrunch	26± 4a	58±22a	42±11a	14± 5bc	37± 4b	8±5cde
	Nancy	54[iv]	51	54	12± 2bc	36± 5bc	13±7bcd
Batavian	Nevada	47±16a	57±10a	50±21a	22± 9ab	38± 5ab	19±8ab
	Cherokee	24±17a	51±18a	29±6a	14± 3bc	33±10bcd	6±4de
	Sierra	24±21a	50±12a	55±5a	28± 9a	45± 5a	17±2ab
Salanova®	Butter Red	18± 5a	49± 9a	24±23a	5± 0c	22± 4e	6±1de
	Butter Green Summer	47±47a	49±14a	45±9a	12± 6bc	26± 4de	11±2bcde
	Crisp Red Summer	22±13a	70±17a	33±22a	7± 1c	28±10cde	4±2e
	Crisp Green	50±38a	59±26a	53±13a	22±15ab	36± 0bc	22±7a
	P value	0.280	0.362	0.460	0.025	P < 0.001	0.002

[i]Lsmeans followed by different letters indicate a significant difference using Fishers LSD at α=0.05

[ii]NA indicates cultivar was not grown in that season

[iii]Each molecule was modeled separately to detect statistically significant differences, with replication group as a random effect.

[iv]Cultivar means reported without standard deviation did not have 3 extractions reps, thus measures of spread and post hoc test lettering were not reported.

[v]Although the interaction effect of cultivar and season was not significant for this parameter; it was included in this interaction table to complete the dataset.

Table 14. Sugar concentrations (mg/g) of lettuce in micro sprinkler plots

		Summer 2020 Micro Sprinkler Trials			Summer 2021 Micro sprinkler Trials		
Type	Cultivar	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
Romaine	Jericho	73± 3b[i]	125± 3a	9±0a	27±5b	79±8a	27±15ab
Butterhead	Buttercrunch	61±23b	121± 4a	8±2a	21±2bc	76±7ab	19±10bc
Batavian	Nevada	113±16a	128± 9a	10±7a	35±6a	84±8a	40± 7a
Salanova®	Summer Crisp Red	NA[ii]	NA	NA	14±2d	67±7b	5± 1c
	Summer Crisp Green	40±14c	110±13a	8±9a	18±0cd	80±3a	16± 8bc
	P value[iii]	0.006	0.136	0.955	*P* < 0.001	0.077	0.011

[i]Lsmeans followed by different letters indicate a significant difference using Fishers LSD at $\alpha=0.05$

[ii]NA indicates complete crop failure

[iii]Each molecule was modeled separately to detect statistically significant differences, with replication group as a random effect.

Fig. 1. Accumulated heat units in all growing seasons. 10 °C used as the base temperature for calculation.

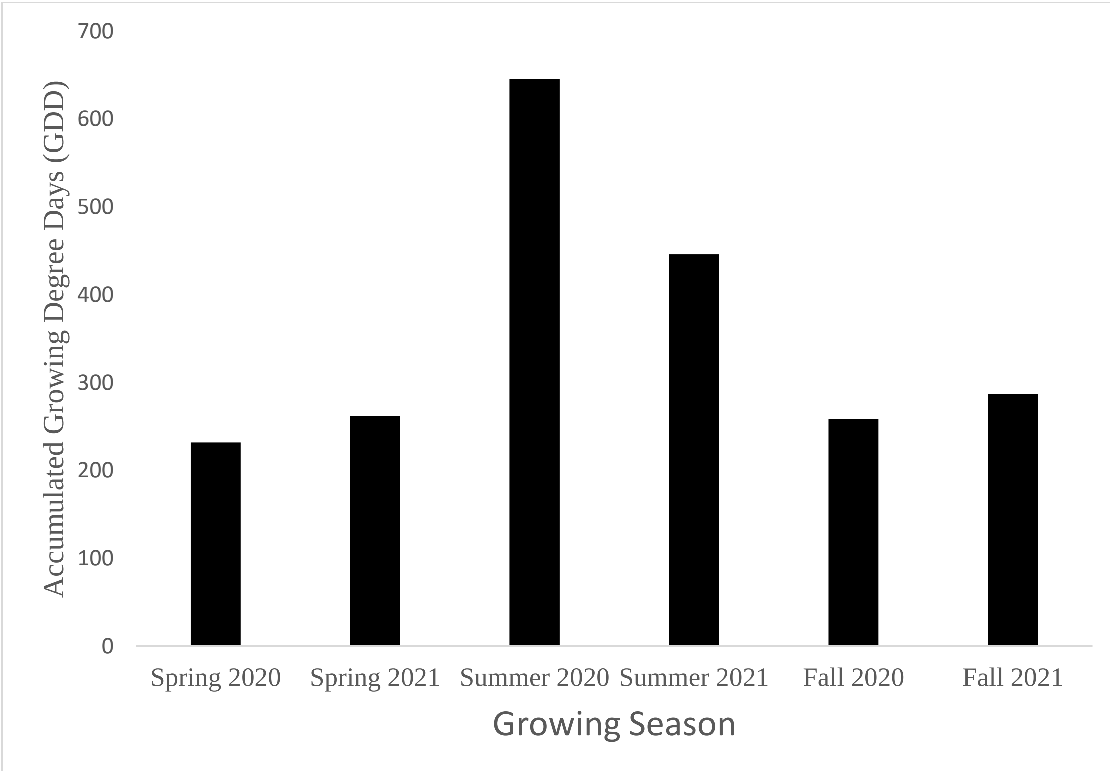
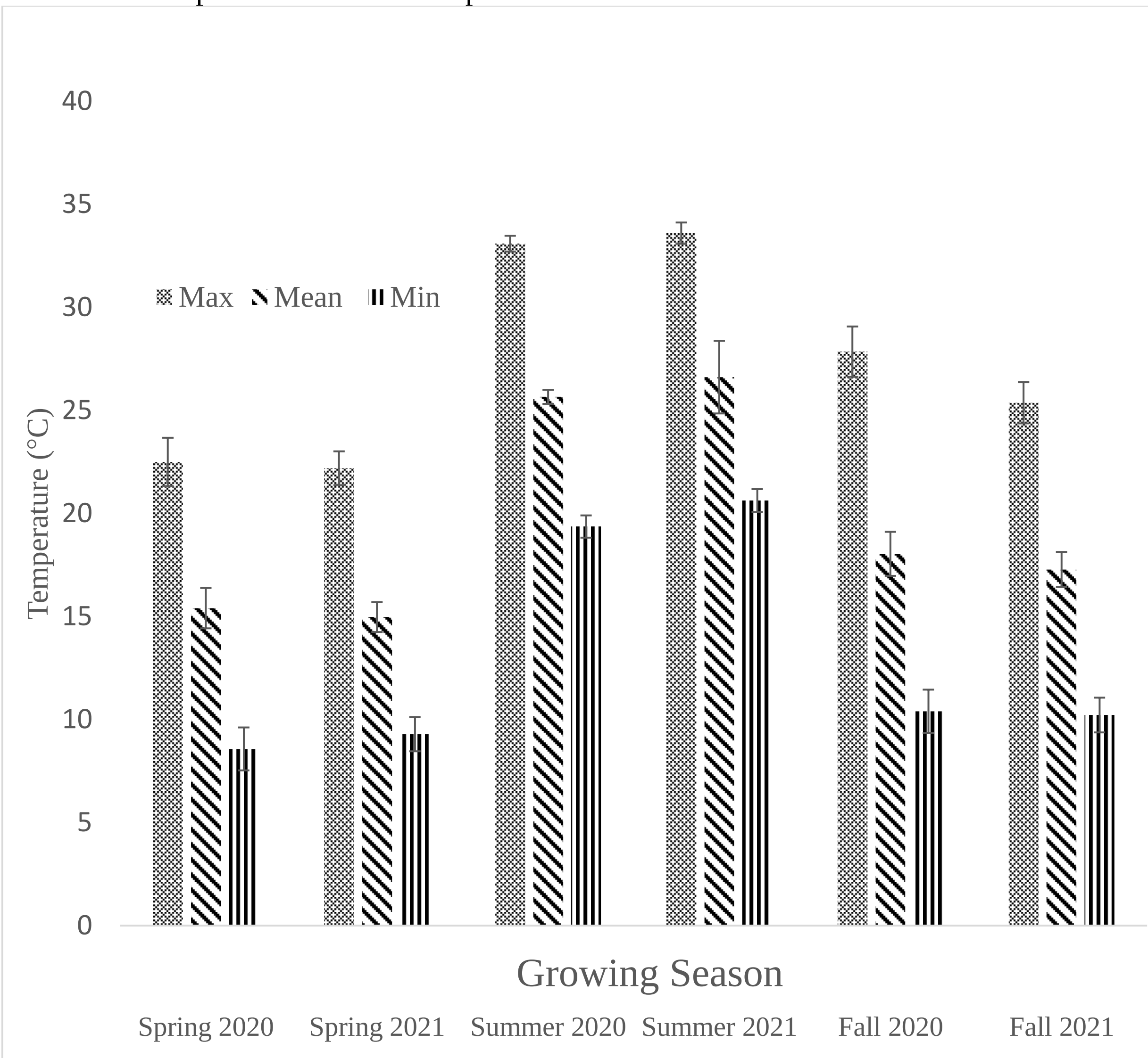


Fig. 2. Maximum, mean, and minimum temperatures in all growing seasons. Error bars represent standard error of the mean. Spring 2020 data was compiled from the Perkins Mesonet station on the research station, while the other seasons' data came from HOBOConnect probes at the research plots.



VITA

John Alexander Unterschuetz

Candidate for the Degree of

Master of Science

Thesis: ASSESSING SESQUITERPENE LACTONE AND SUGAR CONCENTRATIONS AS INDICATORS OF HEAT TOLERANCE IN FIELD PRODUCED LETTUCE IN OKLAHOMA

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