Altering light with high tunnel coverings to improve health-promoting phytochemicals of lettuce and tomato

by

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B.S., University of Arkansas, 2014
M.S., Kansas State University, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
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Abstract

High tunnels (HTs) have shown to increase marketability and yield of numerous crops compared to open field production. These structures alter light by utilizing specific polyethylene (poly) films and/or shade cloth, which may negatively affect phytochemical accumulation. This study evaluated the effect of HT coverings on the microclimate, yield, sensory attributes, and phytochemical concentration of tomato (*Solanum lycopersicum* cv. BHN 589) and lettuce (*Lactuca sativa* cv. Two Star, New Red Fire). Field experiments were carried out at the Kansas State University Olathe Horticulture Center in consecutive years from fall 2017 to spring 2019. An adjacent, open field (open) bed was used during the descriptive sensory study. The six HT coverings included standard, UV-stabilized poly (standard); diffuse poly (diffuse); full-spectrum clear poly (clear); UV-A/B blocking poly (block); standard + 55% shade cloth (shade); and removal of standard poly 2 to 3 weeks prior to initial harvest to simulate a movable tunnel (movable). The microclimate measurements of photosynthetic active radiation (PAR) were highest under the movable covering, and exceeded the diffuse and shade covering in the spring and summer seasons. During the fall, a decrease in available PAR, net photosynthesis (Pn), soil and canopy temperatures were observed under all tested coverings, minimizing the effect of the coverings. The coverings with higher soil temperatures in the spring and summer resulted in greater yields of lettuce and tomato. Phytochemical concentration of ascorbic acid (AsA), flavonoids, and phenolic acids were measured in breaker and light red mature fruit at harvest and throughout ripening in tomato and at harvest and after storage in lettuce. For tomato, the fruit under the clear and standard covering increased in AsA compared to the shade and movable coverings. The flavonoids, quercetin and rutin, increased with on-plant ripening, i.e. light red harvested fruit once reaching mature red was higher in concentration compared to breaker harvested fruit once reaching mature red. In the spring red and green lettuce, the flavonoid concentration of isoquercetin and rutin increased under the clear and movable covering. During the fall, a decrease in individual phenolic compounds was observed for both red and green lettuce. Sensory attributes of fall red lettuce were evaluated with a descriptive study at the Center for Sensory Analysis and Consumer Behavior in Manhattan, KS. The lettuce under the open, clear, and movable coverings had redder pigmented leaves relative to the other coverings as determined by both the panelists and instrumental analysis. Results from PCA analysis show a cluster was formed with the movable, clear, and open coverings, and 73% of the perceived variability between the HT coverings was due mostly to color intensity. The results of this work indicate that HTs can significantly alter solar light and temperature, thus altering yield and health-promoting phytochemical concentration of lettuce and tomato and perceived sensory attributes of red lettuce. As growers continue to adopt controlled production systems for increased yields, phytochemical accumulation should be considered in the system design, as it is important for human health.

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Dedication

This research concludes 6 years of M.S. and Ph.D. studies at KSU Olathe. I have been blessed with an unwavering support system both professionally and personally. I would like to dedicate this research to my family, Patrick, Martha, Mary Kate, and Augie Gude, and my boyfriend and best friend of 6 years, Ross Quinn and Hilary Rambeau for keeping me from falling off the edge.

Chapter 1 - Phytochemical Security in Vegetable Crops

Historically, agricultural systems have been yield-driven, focused on meeting the needs of a sharply-rising population. A report in 2013 indicated that vegetable crops have decreased in some phytochemicals from 1950-1999 (Davis et al., 2013). One finding from the study showed that the average red tomato contained 26 mg of ascorbic acid (AsA) /100g fresh weight (FW) in 1950 and only 10 mg AsA/100g FW in 1999. Another found that several crops declined in AsA including cabbage (32% decline), cauliflower (41% decline) collard greens (62% decline), etc., and suggested that soil depletion from over-farming has contributed to the phytochemical loss (Jack, 1998). It is suggested that differences may result from growing produce in different soils, during different weather conditions. However, many of these studies have been dismissed citing too little evidence (Marles, 2017). In tomatoes, is has been shown that there are large tradeoffs between yield and AsA, as well as yield and carotenoid content (Stevens, 1986). Davis et al. (2013) pointed out that a wide range in tomato AsA content existed in 1999 due to cultivar selection, which has been occurring since early farming. They suggested that declines between 1950 to 1999 are best explained by the direction of breeding which selects for yield as opposed to phytochemical accumulation (Davis et al., 2013; Mayer, 1997). Furthermore, sophisticated extraction and detection procedures have increased over time. Selecting cultivars for high yield "may increase the carbohydrate-water fraction in vegetables, without proportionate increases in other nutrients" (Davis et al., 2013, p. 680). Similar trends have been seen with staple crops, such as wheat and rice, comprise over half of the calories of the American diet, and have suffered broader nutrient losses than vegetable crops (Briggs, 1971).

With increasing awareness of the health benefits of fruits and vegetables, there is a growing interest to improve plant phytochemical accumulation, such as the antioxidant capacity (Kalt, 2005). Some studies are looking to enhance carotenoid content through genetic manipulation in tomatoes. Phenolic content has shown to be positively correlated with antioxidant capacity (Kalt et al., 1999). However, most of the leading research is in fruit breeding programs that have selected for increased antioxidant compounds from wild type cultivars (Kalt et al., 2001). Onion breeders have worked to enhance flavonoid antioxidants through cultivar characterization (Marotti & Piccaglia, 2002). Because many genetic and environmental factors can impact secondary metabolites, managing production systems for improved antioxidant capacity brings challenges (Kimura et al., 2003; Oh et al., 2009). This is because various pre-harvest environmental factors can activate genes involved in biosynthetic pathways of secondary metabolites, which leads to the accumulation of numerous health-promoting antioxidants (Dumas et al., 2003). During times of high resource availability (e.g. soil nutrients, irrigation, high light, etc.), plants allocate photosynthate to growth; while during times of intermediate stress (i.e. UV-radiation, high or low temperature, salinity exposure, etc.), plants allocate photosynthate to synthesis of antioxidants (such as secondary metabolites) instead of biomass growth (Loomis, 1932; Loomis, 1953).

Controlled environmental shocks have been used to enhance health-promoting phytochemicals without adversely affecting yield and productivity. The pre-harvest environmental factors that have shown to improve phytochemical concentration include salinity stress (Lorenzo et al., 2006; Parvin et al., 2019; Shalata et al., 2001), heat shock (Oh et al., 2009), chilling stress (Oh & Rajashekar, 2009), water-stress (Boo & Jung, 1999; Yaginuma et al., 2002), and light and UV intensity (Lee et al., 2014; Li et al., 2017; Liu et al., 2018; Tattini et al., 2014). Amongst the environmental factors that affect antioxidant capacity, light characteristics experienced by the crops (both solar intensity and spectral quality) are very important. A study by Oh et al. (2009) evaluated moderate environmental stresses on lettuce to increase antioxidant capacity without negatively impacting yield. They found that introducing stress immediately before harvest through supplemental lighting was more effective at increasing certain phytochemicals than heat shock or chilling stress.

In order to increase yields for rising urban populations, researchers are looking to make larger-scaled semi-controlled and controlled environments more efficient (De Clercq et al., 2018). With two-thirds of the world’s population predicted to live in urban cities, semi-controlled and controlled environmental agriculture can address the needs of urban supply-chains (Shamshiri et al., 2018). Among these systems are high tunnels (HTs), greenhouses, and indoor vertical farms that may improve crop quality and yield by extending the growing seasons, or allowing year-round growing. In the Netherlands, greenhouses now produce 35% of the country’s vegetables, and occupy <1% of the available farmland (De Clercq et al., 2018). However, the use of these growing systems manipulates both light intensity and spectral quality. Light manipulation can result in altered plant biomass, time to flower, phytochrome equilibration and plays an important role in the synthesis of many antioxidants (Aherne & O'Brien, 2002; Stewart et al., 2000).

Secondary metabolite phytochemicals include carotenoids, and phenolic compounds such as flavonoids and phenolic acids. In humans, these secondary metabolites have shown anti-inflammatory, and antitumor activity in the prevention of coronary heart disease and cancer (Arai et al., 2000; Bondonno et al., 2019; Fleshman et al., 2011; Hertog et al., 1993; Olthof et al., 2001), and carotenoids are important to delay or cure age-related eye disorders (Meyers et al., 2013). The secondary metabolite carotenoids, lutein and β-carotene, are vital pigments in plants that minimize damage of photosynthetic components from the chlorophyll molecule (Kopsell et al., 2009). Phenolic compounds provide pigmentation, reduce electrolyte leakage, attract animals to consume, and defend against pest attack with bitter flavor and astringent taste (Ahmad et al., 2010; Brouillard & Dangles, 2017; Samuoliene et al., 2012; Schmelzer et al., 1988; Verstraeten et al., 2003). Consumption of raw vegetables has shown to increase bioavailability of phytochemicals compared to individual dietary supplements (Liu, 2004). Therefore, enhancing the carotenoid and phenolic profile in vegetables is essential for promotion of human health.

Light regulates several developmental processes in plants. Photosynthetic active radiation (PAR) includes the wavelengths that plants use for photosynthesis (400 to 700 nm), which is visible light. In contrast, both visible and ultra-violet (UV) wavelengths alter secondary metabolite production (Krizek et al., 1998). HTs and greenhouses manipulate solar light through overhead coverings with the option of supplemental lighting and shading. Standard polyethylene (poly) film that is used for HT coverings has shown to reduce PAR by 12 to 25% and block UV-B (García-Macías et al., 2007; Gude, 2020 Ch. 2). The loss of secondary metabolite accumulation in HT systems versus open field systems due to solar light alteration has been shown in leafy vegetables (Oh et al., 2011; Woolley et al., 2019; Zhao, Iwamoto, et al., 2007). Standard greenhouse glass has shown to reduce PAR by 15 to 20% and block UV-B (Sytar et al., 2018). Indoor vertical systems are usually lit with artificial light, such as light-emitting diodes (LEDs) (Bula et al., 1991; Wheeler, 2008).

Both the UV and visible spectrum should be considered when implementing a semi-controlled and controlled environment. UV-B receptors, phytochrome, and blue-light receptors are photosystems all involved in the synthesis of phenolic compounds. UV-B radiation has shown to increase UV-B absorbing compounds, such as phenolic and carotenoid compounds, but has shown to be species and cultivar dependent (Becatti et al., 2009; Goto et al., 2016; Pérez et al., 2009; Tsormpatsidis et al., 2008). UV-A radiation has shown to work in conjunction with UV-B and increase phytochemical accumulation (Fuglevand et al., 1996). In addition to UV light, researchers have seen that certain regions of the visible spectrum play a large part in plant growth and phenolic accumulation. Blue light has shown to work synergistically with red light to enhance plant growth (Chen et al., 2011; Stutte et al., 2009), and with UV-A and B to enhance phytochemicals. (Fuglevand et al., 1996) A combination of far-red and red light are absorbed by phytochromes, making them useful for photosynthesis (Cui et al., 2009; Smith & Whitelam, 1990). The addition of far-red light tends to result in flowering and greater leaf elongation than red alone (Cerny et al., 2003; Rajapakse et al., 1999; Stutte et al., 2009). Green light can participate in the photosynthetic process through pigment photoreceptor proteins in cryptochrome-dependent and –independent means, opposing those directed by red and blue wavebands (Folta & Maruhnich, 2007).

The challenges of an efficient controlled environment system include energy management, environmental impact, and the use of natural resources (Shamshiri et al., 2018). A study that highlights the challenge of energy management sought to compare conventionally grown lettuce in Yuma, AZ, to hydroponic greenhouse lettuce using data from crop budgets and governmental agricultural statistics (Barbosa et al., 2015). They found that the hydroponic system offered 11x higher yields but required 82x more energy, due largely to temperature management and lighting. The temperature management contributed to >75% of the cost; however, greenhouses located in more-moderate climates may adopt passive ventilation systems to reduce energy demand.

Light photoperiod is an important system consideration, not only because of its effect on plant growth and phytochemical accumulation, but also because of the energy demand. To speed up growth and yield, photoperiods may be maximized with supplemental lighting from 12 to 24-h photoperiods (Ali et al., 2009; Barbosa et al., 2015; Guo et al., 2016; Shen et al., 2014). Light input will vary by species but many supplemental light systems are designed to add 200 to 300 μmol m^{-2} s^{-1} of light and automatically turn off once solar light exceeds the chosen amount (Bian et al., 2015). Shen et al. (2014) found that net photosynthesis (Pn), total chlorophyll, leaf number, and biomass of lettuce (*Lactuca sativa* L. var. Dasusheng) increased under a 24-h photoperiod at 600 μmol m^{-2} s^{-1} with both white and a 90% red:10% blue light. However, Ali et al. (2009) evaluated photoperiods on the total chlorophyll, total antioxidants, total polyphenol, leaf area, leaf number, and fresh and dry weight of red and green amaranth, swiss chard, red beet, and red spinach. They found that all studied parameters increased under a 12-h photoperiod and decreased under a 24-h photoperiod with white light bulbs at 540 µmol m^{-2} s^{-1}. Light quality combination strategies should be carefully evaluated because the effect of light varies by species and cultivar even under similar cultivation conditions as seen here.

Research has shown species and cultivars of the same plant species respond differently to light (Bian et al., 2015; Krizek et al., 1993). Krizek et al. (1993) found that one cucumber (*Cucumis sativus* L.) cultivar was relatively insensitive to UV-B while another was highly sensitive, but both had a similar biochemical response to UV-B when grown with supplemental light. A separate solar light study by Krizek et al. (1997) used the same cucumber cultivars, and found that leaf area, leaf number, and height increased under UV-A/B solar light exclusion. However, only one of the four cucumber cultivars altered in flavonoid content due to solar UV-A/B presence. They hypothesized that the threshold of UV-B in solar light to affect the content of UV-B absorbing phenolic compounds was not reached.

Researchers have recently begun to study the effect of short-term pre-harvest light alteration to enhance phytochemical accumulation (Becker et al., 2013b; Hipol & Dionisio-Sese, 2014; Oh et al., 2009; Zhao, Iwamoto, et al., 2007). In a greenhouse study, Hipol & Dionisio-Sese (2014) found that 80% shaded red lettuce with 2 weeks of 0% shade prior to harvest increased antioxidant content compared to the lettuce that was moved to 56% shade or the lettuce that remained in 80% shade. In a growth chamber with solar light, Becker et al. (2013) found that lettuce grown in shade for 2 weeks followed by no-shade for 2 weeks accumulated the same antioxidant content as lettuce grown under no-shade conditions for 4 weeks. Both studies suggest that including shade throughout the early part of the growing process could help reduce energy costs needed to cool controlled environments. Additionally, these studies suggest that short-term high intensity illumination pre-harvest may be an effective strategy to enhance phytochemical accumulation without an adverse reaction on yield.

Previous studies have been conducted to understand the effect of temperature, photoperiod, and light conditions on the sensory quality of vegetables (Batziakas et al., 2019; Mølmann et al., 2015; Rosenfeld et al., 1997; Talavera-Bianchi, Chambers, Carey, et al., 2010; Zhang et al., 2019). Mølmann et al. (2015) and Rosenfeld et al. (1997) agreed that temperature and photoperiod were equally determinant of sensory attributes in broccoli and carrot. Recently, researchers have begun to study short-term pre-harvest light alteration to enhance sensory attributes (Wanlai et al., 2013; Zhang et al., 2019). Zhang et al. (2019) found that red lettuce with 7 days pre-harvest red and blue LED illumination was 27% darker, 79% redder, and 47% less yellow. However, consumers tended to prefer lettuce grown without supplemental lighting and judged it to be sweeter with a more likable aftertaste and less bitter.

High tunnel production systems could be a solution to the energy demand challenge introduced with controlled environments. Furthermore, they are more accessible and more easily adopted by growers due to their cost requirements and energy inputs. High tunnel coverings can be used as a tool to manipulate solar light and alter phytochemical content (García-Macías et al., 2007; Li et al., 2017; Oh et al., 2009). However, more strategic research is needed to identify coverings that can enhance phytochemical content with light alteration, in specific species and cultivars. Furthermore, it is inherent to understand consumer perception of altered sensory attributes due to vegetable production in semi-controlled environments. Some of the effects of light quality and quantity on phytochemical changes in vegetables are known, but additional research is needed to better understand the physiological, biochemical, and molecular mechanisms. Furthermore, maturity, temperature, and photoperiod have shown synergistic effect with light and investigation of these parameters in addition to light quality can help us to reveal underlying effects of light quality on plant growth, development and nutritional quality. Therefore, the overall objective of the following chapters was to study the effect of high tunnel coverings on microclimate, crop productivity, phytochemical accumulation, and sensory attributes of lettuce and tomato (*Solanum lycopersicum*).

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Chapter 2 - High Tunnel Coverings Alter Microclimate, Photosynthesis, and Yield of Tomato and Lettuce

Abstract

The implementation of high tunnels (HTs) have shown to increase marketability and/or yield of tomato (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*) crops compared to open field systems. These structures provide the opportunity to alter light intensity and spectral quality by utilizing specific polyethylene (poly) films and/or shade cloth, which may affect microclimate and subsequent crop productivity. However, little is known about how specific HT coverings affect these parameters. The first objective of this study was to evaluate the impact of various HT coverings on the microclimate during spring, summer, and fall. The second objective was to understand the effect of covering on net photosynthesis (Pn) and yield of tomato and lettuce. The coverings included: standard, UV-stabilized poly film (standard); diffuse poly (diffuse); full-spectrum clear poly (clear); UV-A/B blocking poly (block); standard + 55% shade cloth (shade); and removal of standard poly 2 weeks prior to initial harvest to simulate a movable tunnel (movable). Microclimate parameters that were observed included maximum and minimum canopy and soil temperatures, growing degree-days (GDD), and photosynthetic active radiation (PAR). Hybrid red 'BHN 589' tomatoes were grown during the summer; red 'New Red Fire' and green 'Two Star' leaf lettuce were grown in both spring and fall. Trials were conducted from fall 2017 to spring 2019 resulting in two years of data from each season. Differences between seasons were observed and increased temperature, GDD, and PAR was seen during the spring and summer compared to the fall. PAR was greatest under the movable covering in the spring and summer, which was significantly greater than diffuse and shade coverings. The coverings with increased soil temperatures tended to have increased yields in the spring and summer. For tomato, all coverings other than shade produced similar total fruit yield ($P < .001$), with clear producing the greatest amount at 7.39 kg/plant. For red leaf lettuce grown in the spring, the plants under the clear, standard, and diffuse coverings had significantly greater yield than the movable and shade coverings ($P < .001$). Similar trends were observed in the spring green lettuce. The fall lettuce trials had lower PAR, Pn, temperature, and fresh weight compared to the spring trials under all coverings. The findings of this study suggest that HT coverings affect both microclimate and yield of lettuce and tomato.

Keywords: Polyethylene (poly), hoophouses, season extension

2.1 Introduction

HTs are often utilized for environmental protection, increased marketability and an extended production season (Lamont, 2009). In contrast to greenhouses, HTs are less expensive to construct or maintain, as they rely on passive ventilation with a single (or double) layer of polyethylene (poly) stretched over the top of the tunnel (Janke et al., 2017). The two most common warm and cool-season crops grown in HTs in the central U.S. are tomatoes (*Solanum lycopersicum*) and lettuce (*Lactuca sativa*) (Carey et al., 2009). Multiple studies in the central U.S. have found that the yield of tomatoes (Altamimi, 2016; Kumari et al., 2014) and lettuce (Borrelli et al., 2013; Bumgarner et al., 2011; Wallace et al., 2012) grown in a HT were greater than in the open field. Bumgarner et al. (2011) found that the biomass of fall and spring red romaine was positively influenced by increased soil temperatures in the HT environment. One of the advantages of the HT system is that the grower is able to select particular shade cloth and/or poly film coverings in order to maximize yield or other characteristics that may be important for their market.

HT coverings have been found to alter spectral quality, light intensity, plant growth, and phenolic compounds (Flaishman et al., 2015; Oh et al., 2011; Tsormpatsidis et al., 2008; Zhao, Iwamoto, et al., 2007). Additional shade has shown to reduce maximum soil temperature, leaf evapotranspiration and leaf temperature during production periods with temperatures >30 °C which are common to the Central USA (Zhao & Carey, 2009). These extreme temperatures have shown to be detrimental to tomato growth (Saeed et al., 2007). The utilization of a 50% shade screen in a greenhouse study improved marketability by reducing the incidence of tomato blossom end rot (Lorenzo et al., 2006). However, shade has been found to result in a reduction of irradiance, photosynthetic active radiation (PAR), and net photosynthesis (Agati & Tattini, 2010; Li et al., 2017). This was also observed in a HT study in Serbia where black shade with standard poly reduced tomato fruit size compared to a standard poly alone (Ilić et al., 2015).

A movable tunnel is a mobile HT on wheels that can roll down a rail once plants have established themselves, in order to continue planting new crops under the protection of the tunnel (Jett, 2017). The movable tunnel can give growers the ability to alter the quantity of light throughout crop growth, while providing protection to multiple crops per season. However, little is known about the practical application of increasing solar light pre-harvest through removal of HT coverings or with the use of a movable tunnel. A few studies have included pre-harvest full spectrum light exposure coverings 2 to 4 weeks prior to harvest in growth chambers (Becker et al., 2013b), greenhouses (Hipol & Dionisio-Sese, 2014), and HTs (Zhao, Iwamoto, et al., 2007) to study the effects of antioxidant accumulation. Hipol & Dionisio-Sese (2014) sowed 'Red Rapids' and 'Lollo Rosa' red lettuce cultivars under 80% shade and transplanted to various shaded conditions two-weeks prior to harvest. They found that the leaf area and leaf length increased under the 0 or 30% shade compared to the 56 or 80% shade.

PAR is electromagnetic radiation in the waveband between the visible light spectrum of 400 to 700 nm, which is necessary for photosynthesis (Espí et al., 2006). Standard poly used on HT systems has shown to decrease PAR by 23% (Bumgarner et al., 2012). Additionally, standard poly has shown to reduce UV-A light by more than 60% and UV-B by 90% relative to open field plots (Tsormpatsidis et al., 2008). However, sensitivity to UV-B can vary by crop species and cultivar (Krizek et al., 1997; Tevini & Teramura, 1989). Crop growth and elongation is regulated by the indoleacetic acid hormone, which is both absorbed and readily destroyed by UV-B (Mark & Tevini, 1996). A red leaf lettuce study grown with UV-B exclusion had 63% greater fresh weight (FW) compared to the full-spectrum control, while those grown under UV-A/B exclusion had an additional 43% greater FW compared to UV-B exclusion alone (Krizek et al., 1998). Similar findings were found with UV-sensitive cultivars of cucumber (*Cucumis sativus L.*) (Krizek et al., 1997). A HT study with UV-B excluding poly film revealed an increase in leaf enlargement and biomass accumulation with cucumber, soybean (*Glycine max* (L.) Merr.), and New Zealand spinach (*Tetragonia tetragonoides* (Pallas) Kuntze) (Adamse et al., 1997).

To our knowledge, very few studies have examined the effect of altering solar light through the implementation of specific HT coverings. Replicated studies require large, customized tunnel structures in order to impose the coverings in question. Therefore, the first objective of this report was to evaluate the impact of the HT coverings on soil and canopy temperature, growing degree-day (GDD) accumulation, and photosynthetic active radiation (PAR) during spring, summer, and fall. The second objective was to understand the effect of HT covering on net photosynthesis (Pn) and crop productivity of tomato and lettuce.

2.2 Materials and Methods

Trials were conducted at the Kansas State University Olathe Horticulture Center, located in Olathe, in Johnson County, KS (lat. 38.885357 °W). The soil is Chase silt loam (pH 6.5). The trial was conducted in four, "caterpillar" HTs, which are homemade HTs whereby hoops are bent out of galvanized metal fencing material using a specific pipe bender (Quick Hoops Bender; Johnny's Seed, Winslow, Maine, USA). The construction of a homemade HT allowed for customization to suit the needs of the experiment in regards to plot size and tunnel length. The overall design of the caterpillar tunnel is a long and narrow with low ceilings, which provides an ideal structure for an experiment that specifically examines the impact of solar light. The four HTs were 39.6 m long x 3.7 m wide x 2.1 m high. Two beds ran lengthwise in each HT (39.6 m long x 0.61 m wide). The tomato trials were arranged in a randomized complete block design (RCBD) and the lettuce trials utilized a split-plot RCBD, blocked by HT. Each HT had a total of six covering plots (6.1 m long) and an additional 2.1 m "buffer" area at the ends of each HT.

The six coverings included commercially-available greenhouse and HT poly films as well as 55% shade cloth covering and one where the poly was removed. The standard poly (standard) was rated for 92% PAR transmission and blocked <350 nm [single-layer 6-mil (K-50 poly; Klerk's Plastic Product Manufacturing, Inc., Richburg, SC, USA)]. Standard poly + removal (movable) allowed for plant establishment in a protected environment before full solar exposure 2 weeks prior to harvest. The movable covering simulated the potential use of a movable tunnel. Diffuse poly (diffuse) is reported to remove direct radiation of infra-red (IR) light and blocks <380 nm (Luminance; Visqueen Building Products, London, UK). Clear poly (clear) did not contain a UV-inhibitor (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA). UV-A/B block poly (block) blocked <400 nm (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA). A 55% shade cloth + standard poly underneath (shade) reduced plant temperature (Sunblocker Knitted Shade; FarmTek, Dyersville, Iowa, USA).

2.2.1 Temperature and growing degree-day (GDD)

HT soil and canopy temperatures (°C) were recorded throughout the six growing seasons (3 seasons/year x 2 years) in the 6 coverings (2 reps per covering). In each rep, one temperature probe (EL-USB-1; Lascar electronics, Erie, PA, USA) was located 10 cm below the soil surface and the second was in the plant canopy (0.15 m above the soil surface for tomato, and at the soil surface for lettuce) similar to Bumgarner et al. (2011). The probes were placed in the north row of the HTs in the center of each plot. Radiation shields were used to protect the canopy probes from direct sunlight and soil temperature probes were placed in sealed metal containers that were designed for the probes (Lascar electronics, Erie, PA, USA). Temperatures were recorded every 30 minutes. For tomato, sensors collected temperature data from July 7 to Oct. 4, 2017 and on the same dates in 2018 (89 days). For fall lettuce, sensors collected temperature from Oct. 27 to Nov. 19 in 2017 (24 days) and Oct. 25 to Dec. 13 in 2018 (50 days). For spring lettuce, sensors collected temperature from Apr. 1 to May 9 in 2018 and on the same dates in 2019 (39 days).

The accumulated values for GDD were determined using the standard equation from McMaster & Wilhelm (1997). The equation is $GDD= \Sigma (T_{MAX} + T_{MIN})/2- T_{BASE}$, where T_{MAX} was the highest canopy temperature recorded during a 24-h day, T_{MIN} was the lowest canopy temperature recorded during a 24-h day, and T_{BASE} was set at 10 °C for summer tomato (O'Connell et al., 2012) and 5 °C for spring and fall lettuce (Gieske et al., 2016). The symbol Σ denotes the sum over a set of terms.

2.2.2 Photosynthetic active radiation (PAR), and net photosynthesis collection

Photosynthetically active radiation (PAR) ($\mu mol/m^2/s$) and net photosynthesis (Pn) ($\mu mol/m^2/s$) rates were measured between 10:00 and 13:00 h on cloudless days prior to harvest using a handheld open gas exchange system, CID-340 (CID Bio Science, Inc., Camas, WA, USA). Three measurements of Pn and PAR were taken in succession per leaf per plot to represent the plot as a whole. Measurements were taken on a plant in the center of the plot in the north bed using a healthy leaf from the lower-most whirl for lettuce, and the 3rd to 5th leaf from the main axis terminal for tomato (one that was large enough to cover the entire inner area of the 11 cm^2 cuvette) (Reddy & Matcha, 2010). Only flat, open leaves in the direct sunlight with no shade from other leaves or structures were chosen. The PAR measurements taken under the movable covering, were considered to be 100% solar transmitting, and were used to quantify the other covering's decrease in radiation (%).

PAR and Pn measurements were taken on tomato plants on Aug. 1, 3, and 5, 2018. Measurements were taken for the fall lettuce on Nov. 16, and Dec. 5, and 9, 2018. The measurements were taken the second year of each trial because the device was not available for use during the first year of the trial. During the spring trials, measurements were taken on lettuce Apr. 30, and May 5, 2019. For PAR, 4 reps were measured during summer, fall, and spring. For Pn, 3 reps were measured in the summer tomato, and 3 reps per lettuce cultivar were measured in the fall, and 2 reps per lettuce cultivar were measured in the spring due to device malfunction in the spring.

2.2.3 Tomato trials

Tomato trials occurred during summer 2017 and 2018 utilizing the hybrid cultivar ‘BHN 589’ (Johnny’s Selected Seeds, Winslow, Maine). The plants were grown in a single row down each bed with 45 cm in-row spacing and 1.5 m spacing between the beds from center-to-center. Tomato seeds were planted Apr. 17, 2017 and Apr. 20, 2018 into 50-cell propagation tray (5 cm cell diameter) (50 Cell Plug Flats; Johnny’s Selected Seeds, Winslow, Maine, USA) with commercial potting mix (Fafard 3B; Conrad Fafard, Agawam, Massachusetts, USA) and grown in the greenhouse. Tomatoes were transplanted into the HTs May 30, 2017 and May 18, 2018. Shade cloth was added to the shade covering on July 11 in 2017 and July 9 in 2018. The removal of the poly in the movable covering treatment occurred Aug. 11 in 2017 and July 22 in 2018. Common cultural methods that were consistent with commercial HT production were implemented (Buller et al., 2016). A custom-blended granular fertilizer mix (31-16-16) was incorporated into the beds prior to planting at a rate of 112 kg/ha nitrogen. Water was applied through drip irrigation. Weeds were suppressed via with plastic mulch in the beds and fabric mulch between the beds. The plants were grown using a stake and weave trellis system.

Fruit with any visible color was harvested weekly from each plot. They were graded as marketable or nonmarketable and the fruit number and weight were recorded. Nonmarketable fruit were determined based on presence of decay, mold, cracking that extended beyond the shoulder, small size (smaller than 4 cm diameter), pest damage, and other defects. Plant counts were taken 3 times in 2017 (July 10, Aug. 31, and Sept. 24) and 3 times in 2018 (July 24, Aug. 15, and Sept. 12) to determine normalized fruit yield (kg/plant). At the end of each growing season, the plants were stripped of all fruit larger than 4 cm (including green) and weighed to determine overall crop productivity. In 2017, tomato fruit were harvested from Aug. 10 to Oct. 4. In 2018, tomato fruit were harvested from July 30 to Oct. 4. Above-ground biomass (g) data was taken annually following the last harvest on Oct. 4, 2017 and Oct. 4, 2018 using the method described by Loewen et al. (2018). One centrally-located plant per plot was cut at the stem 1 cm above the soil (4 plants per covering treatment). The collected sample of the vegetative portion was dried for at least 72 h at 160 °F (71.1 °C) using an industrial oven (SC-400; The Grieve Corporation, Round Lake, Illinois, USA). The yield parameters that were observed included total and marketable yield, total and marketable fruit size (kg/sample), marketability by weight (%), and above-ground biomass.

2.2.4 Lettuce trials

Red ‘New Red Fire’ and green ‘Two Star’ lettuce (Johnny’s Selected Seeds, Winslow, Maine, USA) were grown in trials that occurred during fall 2017 and 2018 and spring 2018 and 2019. The main plots included the coverings as described above (section 2.2.3) and the sub-plots consisted of the two different leaf cultivars that were tested. Lettuce was seeded in the greenhouse into 72-cell propagation trays (3.8 cm diameter) (Pro-Tray 72 Cell Flats; Johnny’s Selected Seeds, Winslow, Maine, USA) with potting mix. The lettuce was transplanted in a staggered double row within each bed (26.7 cm between plant, 26.7 cm between rows).

In the fall, lettuce was seeded in the greenhouse Sept. 7, 2017 and Sept. 19, 2018 and transplanted into the HTs Oct. 6, 2017 and 24, 2018. During fall and in contrast to summer, shade cloth was utilized throughout the entire growing season for the shade cloth covering treatment. Removal of the poly in the movable covering treatment occurred two weeks prior to harvest in 2017 and 2018 (Oct. 27 and Nov. 26, respectively). The fall lettuce season was approximately 4 weeks longer in 2018 due to field flooding that delayed planting, followed by cold winter temperatures that delayed plant growth.

In the spring, lettuce was seeded in the greenhouse Feb. 14, 2018 and Feb. 19, 2019 and transplanted into the HTs March 19, 2018 and Apr. 1, 2019. Shade cloth was added to the shade cloth covering on Apr. 9, 2018 and Apr. 15, 2019. Removal of standard poly over the movable covering treatment happened on the same dates.

If temperatures fell below 0 °C, the covering on the movable covering was replaced during the nighttime until temperatures rose. Additionally, floating row covers (26 g/m^2) were added to the beds at night when temperatures fell below -6 °C. Lettuce was harvested once it reached commercial size. Harvesting was performed in the morning by cutting the above ground part of the plant at the soil level using a harvesting knife (Harris Seeds, Rochester, NY, USA). In the fall, harvest took place 4 weeks after transplanting in 2017 (Nov. 10, 14, and 17). In the 2018 fall trial, much lower temperatures were experienced and the plants were harvested 8 weeks after transplanting (Dec. 10, 12. and 13). In the spring, harvest took place six weeks post-transplant in 2018 (May 3, 7, and 9) and four weeks post-transplant in 2019 (May 6, 8, and 10). Six plants were randomly selected from each plot at each harvest to measure marketable plant fresh weight (g/plant FW).

2.2.5 Statistical analysis

Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC, USA) PROC MIXED with option DDFM=KR in MODEL statement. The summary statistics were presented for soil and canopy maximum and minimum temperatures as well as GDD because only two replications were included in the trial design. The deviation from the mean of the coverings is presented for each covering in those parameters (DFM). PAR and Pn were analyzed using the linear mixed model. Covering was the fixed effect for the PAR model. For tomato Pn, covering was the fixed effect for the model. For lettuce Pn, covering, leaf color, and covering x leaf color were the fixed effects for the model. When significant differences were not observed between leaf colors (in the case of lettuce Pn), the leaf colors were combined within that parameter. The random effects for PAR and Pn included rep and rep x covering interaction. The yield response parameters were analyzed under the linear mixed model. For tomato yield, fixed effects of the model were covering and year. The random effects included the rep x year, and the rep x year x covering interactions. For lettuce, fixed effects of the model were covering, season, year x season, and season x covering. The random effects included the interactions between rep x year x season and rep x covering x year x season. Least squares means (LSMeans) and their standard errors were reported for fixed effects. Pairwise comparisons between 2 coverings were performed based on the 2-sided test for non-zero difference in means. The adjustment for multiplicity was carried out using Tukey’s method. All tests were conducted at the 0.05 significance level.

2.3 Results

2.3.1 Temperature

During the spring trials, the clear covering warmed the soil temperatures compared to the other coverings; the clear covering soil mean was 0.9 °C greater than the shade covering (Table 2.1). The clear covering warmed the summer soil max temperatures compared to the others, with 1.7 °C difference between clear and diffuse coverings. The diffuse covering in the summer had a wide soil temperature range compared to the others and there was no daily difference under the shade covering. During the fall, the mean soil temperatures under the standard, diffuse, and clear coverings were above the season mean.

Table 2.1 Soil maximum and minimum mean temperatures (°C)[a] under high tunnel coverings in Olathe, KS.

Trts[b]	Spring (Lettuce)		Summer (Tomato)		Fall (Lettuce)	
	Max (DFM)[c]	Min(DFM)	Max (DFM)	Min(DFM)	Max (DFM)	Min (DFM)
Standard	16.9 (0.0)	13.9 (0.2)	25.3 (-0.4)	23.8(-0.1)	12.3 (0.2)	10.4 (0)
Movable	16.8 (-0.1)	13.3 (-0.4)	25.5 (-0.3)	23.8(-0.1)	11.7 (-0.4)	10.0 (-0.3)
Diffuse	16.8 (-0.1)	14.2 (0.5)	24.8 (-1.0)	23.7(-0.2)	12.5 (0.4)	10.8 (0.4)
Clear	17.3 (0.4)	14.0 (0.3)	26.4 (0.7)	24.3(0.4)	12.5 (0.4)	10.7 (0.3)
Block	17.4 (0.5)	13.7 (0)	26.2 (0.4)	23.8(-0.1)	12.0 (-0.1)	10.2 (-0.1)
Shade	16.2 (-0.7)	13.3 (-0.5)	25.8 (0)	23.9(0)	11.6 (-0.5)	10.1 (-0.3)

[a] Values are the means of temperatures recorded during two consecutive summers (2017-2018), falls (2017-2018), and springs (2018-2019). Soil temperature probes added 10 cm below the soil surface, recording temperature in 30 min increments throughout each growing season (2 probes per covering).
[b] Trial was arranged in a RCBD, blocked by high tunnel and year, with the following 6 different polyethylene (poly) films randomly assigned within each tunnel: standard poly (standard), poly removal 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B block poly (block), 55% shade cloth on standard poly (shade).
[c] DFM = values in parentheses denote the deviation from the season mean.

During the spring, the highest max canopy temperatures recorded were under the movable and standard coverings, while the lowest recorded were under the clear and shade (Table 2.2). The min canopy temperatures were high under the movable covering, and 3.1 °C greater than the shade covering. The diffuse and block covering displayed a narrow canopy temperature range compared to the other coverings in the spring. The mean summer canopy temperatures under the movable, shade, and block coverings were above the season mean, while the others fell below. In the fall, the canopy mean temperatures were highest under the diffuse, block and clear covering. The fall canopy min temperatures were all below 7 °C.

Table 2.2 Canopy maximum and minimum mean temperatures (°C)[a] under high tunnel covering in Olathe, KS.

Trts[b]	Spring (Lettuce)		Summer (Tomato)		Fall (Lettuce)	
	Max (DFM)[c]	Min (DFM)	Max (DFM)	Min (DFM)	Max (DFM)	Min (DFM)
Standard	26.9 (1.4)	9.8 (-0.7)	29.8 (-0.4)	19.5 (-0.4)	18.2 (0.5)	5.6 (-0.5)
Movable	27.2 (1.6)	12.7 (2.2)	29.2 (-0.9)	21.2 (1.2)	18.1 (0.4)	6.3 (0.1)
Diffuse	25.5 (-0.1)	9.9 (-0.6)	28.8 (-1.3)	19.8 (-0.2)	18.2 (0.5)	6.5 (0.4)
Clear	23.6 (-2.0)	10.8 (0.3)	30.3 (0.1)	19.5 (-0.5)	18.3 (0.6)	6.3 (0)
Block	25.6 (0)	10.2 (-0.3)	30.9 (0.8)	19.4 (-0.6)	18.0 (0.3)	6.5 (0.4)
Shade	24.8 (-0.8)	9.6 (-0.9)	30.3 (0.2)	20.3 (0.4)	15.7 (-2.1)	5.6 (-0.5)

[a] Values are the means of temperatures recorded during two consecutive summers (2017-2018), falls (2017-2018), and springs (2018-2019). Canopy probes, added 0.15 m above the soil surface for summer and at the soil surface for fall and spring, recorded temperature in 30 min increments throughout the growing season (2 probes per covering).
[b] Trial was arranged in a RCBD, blocked by high tunnel, with the following 6 different polyethylene (poly) films randomly assigned within each tunnel: standard poly (standard), poly removal 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B block poly (block), 55% shade cloth on standard poly (shade).
[c] DFM = values in parentheses denote the deviation from the season mean.

The °GDD difference due to covering was noted for all three seasons (Table 2.3). The movable and standard covering increased °GDD during the spring trials. The movable covering deviated from the shade covering by 101 °GDD, the largest deviation observed in the present study. The movable and shade coverings were 68 and 47 °GDD more than the diffuse covering in the summer trials. During the fall, the diffuse, clear, and movable coverings had a high °GDD compared to the shade covering. The plants that were grown under the shade covering decreased the spring and fall soil temperatures, spring and fall canopy temperatures, and spring and fall °GDD more than the other coverings.

Table 2.3 Canopy growing degree days[a] (°GDD; 10 °C base for summer and 5 °C base for fall and spring) in high tunnel trials in Olathe, KS.

Trts[b]	Spring (lettuce)	Summer (tomato)	Fall (lettuce)
	°GDD (DFM)[c]	°GDD (DFM)	°GDD (DFM)
Standard	553 (72)	1246 (-19)	262 (4)
Movable	578 (97)	1304 (39)	275 (17)
Diffuse	498 (17)	1236 (-29)	277 (19)
Clear	479 (-2)	1241 (-24)	276 (18)
Block	505 (24)	1254 (-12)	241 (-17)
Shade	477 (-4)	1283 (18)	218 (-40)

[a] Temperatures were recorded in the summer from July 7 to Oct. 4, 2017 & 2018 (89 d), in the fall from Oct. 27 to Nov. 19, 2017 (24 d) & Oct. 25 to Dec. 13, 2018 (50 d), and in the spring from Apr. 1 to May 9, 2018 and 2019 (39 d). Canopy probes, added 0.15 m above the soil surface for summer and on top of the soil surface for fall and spring, recorded temperature in 30 min increments throughout the growing season (2 probes per covering).

[b] Trial was arranged in a RCBD, blocked by high tunnel, with the following 6 different polyethylene (poly) films randomly assigned within each tunnel: standard poly (standard), poly removal 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B block poly (block), 55% shade cloth on standard poly (shade).

[c] DFM = values in parentheses denote the deviation from the season mean.

2.3.2 Photosynthetic active radiation (PAR)

Overall, higher PAR values were observed during the spring and summer trials as compare to the fall trials (Fig. 2.1). PAR values were significantly higher under the movable covering than under the diffuse and shade coverings in the spring trials. During the summer, the movable covering was statistically higher than all other coverings. During the fall, there was no significant effect amongst the coverings. The PAR observed in the fall was significantly lower than all coverings in the spring and summer covering other than the shade covering, which was the same for all three seasons.

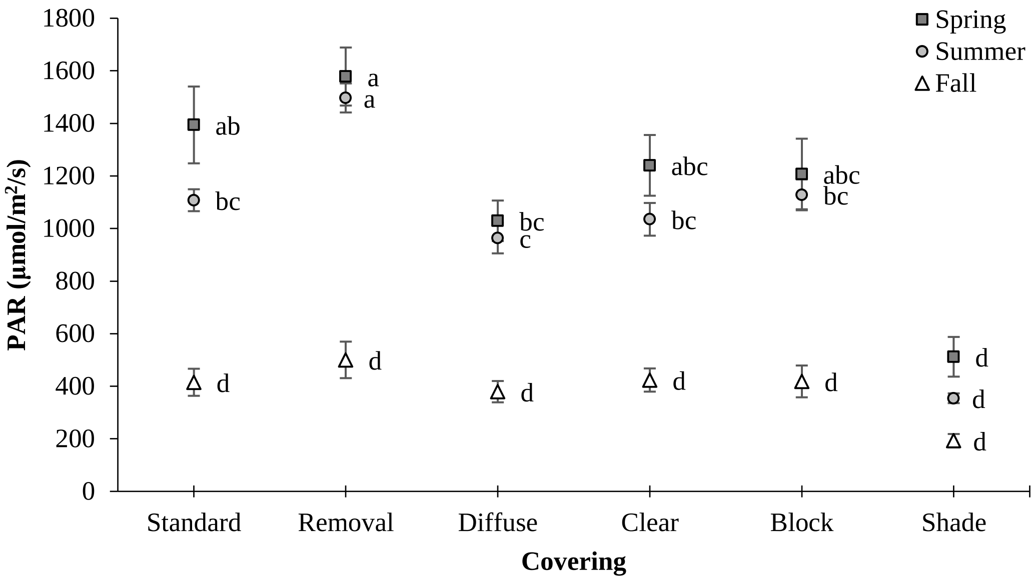

Figure 2.1 Photosynthetic active radiation (PAR (µmol/m^2/s)) between coverings was measured between 10:00 and 13:00 h on cloudless days in summer (Aug. 1, 3, and 5, 2018), fall (Nov. 16 and Dec. 5, 2018), and spring (Apr. 30 and May 5, 2019). Values are three measurements to represent the rep as a whole (four reps per covering). Means (SE) with same letter do not differ significantly at *P* ≤0.05, Tukey's HSD.

During the spring, the standard PAR transmission was 88% and the shade was 32%, compared to the movable covering, where PAR was measured in unobstructed sunlight (Table 2.4). During the summer, the block had 75% and the shade had just 24% PAR transmission compared to the movable covering. During the fall, there was a narrower range in PAR transmission between the movable covering and the other tested coverings, which may be due to the general decrease in PAR transmission as seen in Fig. 2.1.

Table 2.4 The effect of season and high tunnel covering on percent photosynthetic active radiation (PAR)[a] transmission compared to unobstructed light[b] in Olathe, KS.

Trt	Spring (%)	Summer (%)	Fall (%)
Movable	100‡	100	100
Standard	88.3	74.0	83
Block	76.5	75.4	83.6
Clear	78.6	69.1	84.7
Diffuse	65.2	64.4	75.8
Shade	32.4	23.7	38.6

[a] PAR was measured 3 times prior to harvest on cloudless days in summer (Aug. 1, 3, and 5, 2018), fall (Nov. 16 and Dec. 5, 2018), and spring (Apr. 30 and May 5, 2019). Values are three measurements to represent the rep as a whole (four reps per covering).

[b] The movable covering was used to calculate the PAR transmission (%), as light was completely unobstructed during measurements.

2.3.3 Photosynthesis (Pn)

The results from Pn showed no significant difference between the effects of lettuce leaf color, or leaf color x covering interaction within the spring ($P \leq 0.1$, and ≤ 0.7, respectively) or fall ($P \leq 0.2$, and ≤ 0.9, respectively) season, so the leaf colors were combined within seasons. The Pn varied significantly as seen with the covering x season interaction ($P < 0.05$; Fig. 2.2). In the spring, the coverings were statistically similar. In the summer, the shade covering was significantly lower than the other coverings tested. The tested coverings had significantly higher Pn in the spring and summer than in the fall, with the exception of the diffuse and shade covering with lower Pn each season. The shade covering Pn was low regardless of season.

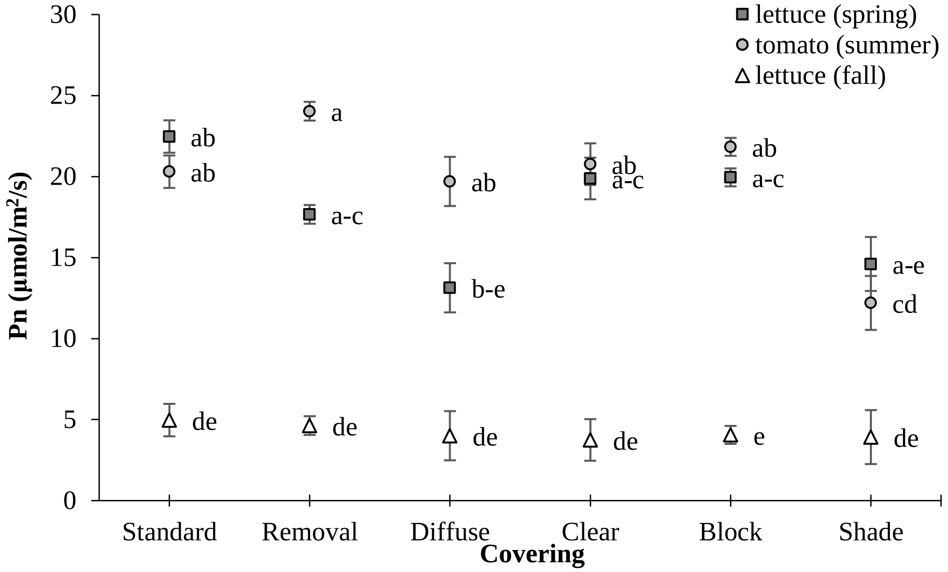

Figure 2.2 The effect of season and poly film covering on net photosynthesis (Pn) in high tunnels located in Olathe, KS. Pn was measured 3 times prior to harvest on cloudless days in summer (Aug. 1, 3, and 5, 2018), fall (Nov. 16 and Dec. 5, 2018), and spring (Apr. 30 and May 5, 2019). Values are three measurements to represent the rep as a whole ($\geq$ 3 reps per covering). Means (SE) with same letter do not differ significantly at $P \leq 0.05$, Tukey's HSD.

2.3.4 Tomato fruit yield

The HT covering had an effect on all crop productivity parameters other than biomass (Table 2.5). A slight effect of year was seen for total yield with 6.97 kg/plant average in 2017 and 5.97 kg/plant average in 2018; however, the effect of year was not seen for the other parameters.

Table 2.5 Probability values[a] of the main effects covering and year on the yield of BHN 589 tomato grown in high tunnels in Olathe, KS in summer 2017 and 2018.

Parameter	Trt[b]	Year
Total Yield (kg/plant)	<.001	<0.05
Total fruit size (kg/sample)	<.001	ns

Marketable Yield (kg/plant)	<.001	ns
Marketable fruit size (kg/sample)	<0.05	ns
Marketability (% by weight)	<0.05	ns
Biomass (g)	ns[c]	ns

[a] A linear mixed model was used to test which factors and interactions had significant effect on the examined quality parameter ($P \leq 0.05$).

[b] The following 6 different polyethylene (poly) films randomly assigned as covering: standard poly (standard), poly removal 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B block poly (block), 55% shade cloth on standard poly (shade).

[c] ns: not significant

For total yield (kg/plant) and total fruit size (kg/sample), it was observed that all coverings performed similarly with the exception of the shade covering (P <.001, and <.001, respectively; Table 2.6). Total and marketable yield increased most under the clear and diffuse coverings, although it did not differ significantly from the standard, movable, and block coverings. However, the movable and shade covering were statistically similar for marketable yield. The marketable fruit size under the shade covering was less compared to the clear and block coverings (P <0.05). However, marketability was high under the shade covering and lower under the movable covering. There was no significant differences observed amongst the coverings for biomass accumulation.

Table 2.6 Yield[a] of ‘BHN 589’ tomato grown in high tunnels in Olathe, KS in 2017 and 2018.

Trt[b]	Total yield (kg/plant)	Total fruit size (kg/sample)	Marketable yield (kg/plant)	Marketable fruit size (kg/sample)	Marketability (% by weight)	Biomass (g)
Standard	6.53 a	0.18 a	5.21 a	0.19 ab	76.5 ab	310
Movable	6.17 a	0.18 a	4.53 ab	0.18 ab	69.5 b	248
Diffuse	7.17 a	0.18 a	5.67 a	0.18 ab	73.3 ab	246
Clear	7.39 a	0.19 a	5.62 a	0.19 a	70.6 ab	278
Block	6.62 a	0.18 a	5.13 a	0.19 a	73.9 ab	311
Shade	4.40 b	0.15 b	3.72 b	0.15 b	77.7 a	209
P-value[c]	<.001	<.001	<.001	<0.05	<0.05	ns

[a] Parameters marked with different letters are significantly different ($P \leq 0.05$). Tukey’s HSD.

[b] Fruit with any visible color was harvested weekly from each plot, graded, marked as marketable or unmarketable and the fruit number and weight were recorded. Tomato fruit were harvested from Aug. 10 to Oct. 4, 2017 and from July 30 to Oct. 4, 2018. Above-ground biomass data taken once each year (four plants per covering measured both years).

[c] The linear mixed model was used to test which coverings had significant effect on the yield parameters ($P \leq 0.05$).

2.3.5 Crop productivity of lettuce

The fresh weight of the green lettuce differed from the red lettuce; therefore, the leaf colors were analyzed separately. Lettuce showed a strong response to the various coverings in the spring growing season. During the spring, the covering had an effect on the fresh weight of red (*P* <.001) and green leaf lettuce (*P* <0.05; Table 2.7).

Table 2.7 Probability values[a] of the main effect covering on the yield parameters of red 'New Red Fire' and green 'Two Star' leaf lettuce grown in high tunnels in Olathe, KS in the fall (2017 and 2018) and spring (2018 and 2019).

Parameter	Leaf Color	Trt	Season	Year (Season)	Season x Trt
Fresh Weight (g/plant)	Red	<.001	<.001	<.001	<0.05
	Green	<0.05	<.001	<.001	ns

[a] A linear mixed model was used to test which factors and interactions had significant effect on the examined quality parameter ($P \leq 0.05$).

The spring red lettuce yield was statistically similar under the standard, clear, diffuse, and block covering and greater than the shade covering (Table 2.8). Similarly, the green lettuce yield was greatest under the same four coverings (standard, clear, diffuse, and block) but there were no significant pairwise comparison differences observed. During the fall, the covering did not have a significant effect on fresh weight yield of either the green or red leaf lettuce (Table 2.8). However, plants under the shade covering had lower mean compared to the other coverings for both the red and green lettuce during the fall.

Table 2.8 Total Yield[a] data (g/plant) of red 'New Red Fire' and green 'Two Star' leaf lettuce grown in high tunnels in Olathe, KS in the fall (2017 and 2018) and spring (2018 and 2019).

Trt[b]	Spring		Fall	
	Red	Green	Red	Green
Standard	204 a	218	41	54
Movable	158 bc	181	36	53
Diffuse	195 a	221	41	53
Clear	201 a	223	38	50
Block	179 ab	221	34	43
Shade	140 c	183	24	32
P-value[c]	<.001	<0.05	ns	ns

[a] Lettuce was harvested in the early morning using a lettuce knife at the soil level to remove the full plant along with outer whirl leaves, minus the root system (6 plants per covering per high tunnel). From transplant to final harvest, fall lettuce was in the tunnel from Oct. 6 to Nov. 19, 2017 and Oct. 24 to Dec. 13, 2018. From transplant to final harvest, the spring dates were Apr. 2, 2018 to May 8, 2018 and Apr. 1 to May 9, 2019. Trial was arranged in a RCBD, blocked by high tunnel with leaf colors alternating between the north and south bed of adjacent tunnels.

[b] The following 6 different polyethylene (poly) films coverings assigned within each tunnel: standard poly (standard), poly removal 2 weeks prior to harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B block poly (block), 55% shade cloth on standard poly (shade). Parameters marked with different letters are significantly different ($P \leq 0.05$). Tukey's HSD.

[c] The linear mixed model was used to test which coverings had significant effect on the yield parameter within season ($P \leq 0.05$).

2.4 Discussion

The goal of this study was to document the impact of various HT coverings on the microclimate and crop productivity of lettuce and tomato during spring, summer, and fall. Both covering and season affected temperature, °GDD and PAR within the HTs. It has been well documented that PAR is highest from May to July and lowest in December but shifts based on location within the

USA (Wilson & Meyers, 2007). Likewise, PAR was highest in the spring and lowest in the fall. The coverings had a large effect on PAR in the spring and summer but little effect in the fall.

The standard poly was rated for 92% PAR transmission and blocked UV-B and some UV-A radiation. In our trials, the microclimate under the standard covering was very similar to each of the three given season means for soil max and min temperatures. The standard poly trapped heat with higher maximum temperatures, which contributed to higher °GDD accumulation in the spring, when compared to other coverings besides movable. °GDD is a heat index used to predict when a crop will reach maturity and may positively influence yield (McMaster & Wilhelm, 1997). Once the movable covering was removed and the plants were exposed to full sun, the highest PAR values were observed under the standard poly in the spring with 88% transmission and 83% transmission in the fall. The results are slightly greater than in a HT study in Ohio, where standard poly had spring and fall PAR transmission of 77% relative to full sunlight (Bumgarner et al., 2012).

The movable covering simulated the use of a movable tunnel, and the PAR values can be compared to an open field control. Greater PAR was measured under the movable covering in all three seasons compared to the other coverings in the given season. To our knowledge, no previous research has been conducted to study the effect of a movable covering on microclimate and productivity within a HT system or with the use of a movable tunnel. The removable covering cooled the maximum and minimum soil temperatures below the season mean in the spring, summer and fall. During the spring and summer, the movable covering increased °GDD compared to the other tested coverings. The low outdoor temperatures in the late part of the fall season often required floating row covers and/or the replacement of poly over the movable covering (section 2.2.4). Careful steps were taken to prevent unintended bias in regards to light. However, this protocol likely altered the movable covering canopy temperatures in the fall. It is important to note that a grower using any of these coverings would have to implement similar methods to ensure the success of the crop and so the microclimate data reported here is still very valuable for growers even though it may be impacted by other factors than HT covering alone.

The poly film used for the clear covering did not contain a UV-inhibitor and degraded quickly because it was not manufactured for greenhouse or HT use. This film was not made for HT production, but it may provide a greater insight into the role of light on crop productivity and quality. During the spring, summer, and fall, the clear covering warmed the soil temperature more than the other tested coverings. In a hydroponics system, Moorby & Graves (1979) hypothesized that high soil temperatures encourage plant growth due to a greater rate of carbon fixation, achieved by more efficient light interception. The clear poly reduced PAR by 21% in the spring, 31% in the summer, and only 15% in the fall compared to the unobstructed PAR under the movable covering. A HT study with a UV-clear poly film [single-lay 6-mil (Hummert International, Topeka, KS, USA)] found that summer PAR was reduced by 17% (Woolley et al., 2019).

The block covering had a slow rate of degradation from UV, due to an added UV inhibitor that blocked <400 nm, and a 75% PAR transmission rate (Krizek et al., 2006), similar to spring and summer measurements in the present study. The average spring and summer soil temperatures under the block covering were above the mean, similar to the clear covering. In the fall, the block covering cooled soil temperatures below the season mean. In Buffalo, NY, a UV exclusion study found that a UV-transmitting covering increased PAR transmission by 10% compared to the UV-blocking covering. Although the trial design differed from the one utilized in this study, we found that the PAR under the block, clear, diffuse and standard coverings were statistically similar. Future research should scale-up the trial design to have one covering assigned to individual HTs. In addition to PAR, UV-A and UV-B measurements should be taken to get a more complete picture of the effect of covering on solar light.

The diffuse poly is marketed to diffuse light, reduce IR, leaf temperature, and transmit UV spectra (Oh et al., 2011). Overall, our study confirmed these claims as the max summer soil temperatures (1.7 °C cooler than the clear covering) and max summer canopy temperatures (2.1 °C cooler than the block covering) were reduced under the diffuse poly. In the fall, the diffuse poly warmed the soil and canopy temperatures, which resulted in higher fall °GDD than all other tested coverings. Diffuse poly reduced PAR transmission more than all coverings other than the shade in each season and has previously been reported to transmit just 40 to 50% PAR (Lang, 2014).

Many HT growers are currently using or are interested in utilizing shade cloth in order to protect their crops from the effects of high temperature or heat stress (Zhao & Carey, 2009). In the spring and fall, the shade covering cooled the soil temperatures more, and the °GDD was reduced relative to the other tested coverings. In the summer, the shade had a high °GDD relative to the other coverings besides movable. During each season, PAR transmitted only 15 to 40%

PAR under the shade covering, which has been previously observed under shade (Agati & Tattini, 2010; Li et al., 2017; Zhao & Carey, 2009).

We investigated the effect of HT coverings on net photosynthesis (Pn) and yield parameters of tomato and lettuce to understand if patterns existed between yield and microclimate parameters. Since our RCBD limited the ability to correlate microclimate to yield results, future research may consider using a completely randomized trial design to incorporate regression analysis.

Several investigators have reported positive correlation between PAR, Pn, and yield (Agati & Tattini, 2010; Lorenzo et al., 2006; Oh et al., 2011). It is likely that a reduction of PAR caused by the use of shade cloth resulted in the reduction of Pn and yield (Agati & Tattini, 2010). The summer PAR and tomato Pn increased under the movable covering, but did not differ significantly from standard, diffuse, clear, and block covering. However, marketable yields decreased under the movable covering, and it may have been due to fruit cracking driven by summer rain events as suggested in O'Connell et al. (2012). °GDD was used to predict when crops will reach maturity, but higher °GDD did not positively influence marketable tomato yields. The accumulated °GDD in the movable and shade coverings were higher and marketable yields were lower than the other coverings. It has been shown that total yield and size of tomato increased with higher soil temperatures in a hydroponics system (Moorby & Graves, 1979). Similarly, the clear plastic increased summer soil temperatures as well as total yields when compared to the shade covering. It should be noted that although the use of shade cloth resulted in lower tomato yield and size compared to the other coverings, the marketability was highest of the tested coverings. This has been found in other tomato studies that evaluated the effect of shade (Lorenzo et al., 2006) and may contribute to current grower-adoption of shade cloth in growing regions like the Central U.S. The coverings with the greatest tomato biomass accumulation in the present study didn't result in the greatest total yields, as past research has found (Loewen et al., 2018). Although it was not significant, it was observed that the above ground biomass increased most under the block and standard coverings, but the total and marketable yield was significantly increased under the clear and diffuse coverings compared to the others.

The implementation of HT systems has been particularly important for cool-season crops like leafy greens where the growing season can be dramatically extended (Carey et al., 2009). It has been observed that location, season, leaf color, and environment alter yield and quality of cool-season crops grown in a HT system (Borrelli et al., 2013; Bumgarner et al., 2011; Wallace et al., 2012; Zhao & Carey, 2009). The standard, diffuse, clear and block coverings increased soil temperatures and marketable fresh weight of the red and green spring-grown lettuce compared to movable and shade coverings. Others have reported that the yield of romaine lettuce grown in a HT was positively influenced by increased soil temperatures in both spring and fall (Bumgarner et al., 2011). Decreased soil temperatures amongst the shade and movable coverings may have contributed to a decrease in yield for both the red and green lettuce tested in this report. A previous study that utilized the same red and green cultivars as tested here, found that 50% (black) shade cloth reduced soil temperature, canopy temperature and yield compared to the control (Li et al., 2017). Similar to our findings with tomato, increased GDD did not determine increased productivity in spring and fall lettuce trials. Wallace et al. (2012) observed that a HT site resulted in less GDD but greater lettuce yield compared to the open field during one year of the two-year study. Blocking UV-A/B in past UV exclusion studies has resulted in greater biomass production of leaf lettuce (Adamse et al., 1997; Krizek et al., 1997, 1998; Shioshita et al., 2006). Presently, the block covering resulted in greater spring yields in the red leaf lettuce compared to shade. However, it was statistically similar in fresh weight to the other coverings that allowed more UV light transmittance.

The fall temperatures, PAR, Pn, and yield values were lower compared to the summer and spring values. The fall lettuce yields may have been impacted by season due to a decrease in UV radiation, PAR, and temperature in the fall relative to the spring (McKenzie et al., 1996; Singer et al., 2015; Wilson & Meyers, 2007). A previous study testing the same red lettuce, found that an average canopy max and min temperature of 22.1 and 13.4 °C, respectively, had a fresh weight average of 298 g/plant in a two year study (Singer et al., 2015). The fall max and min temperatures were approximately 5 °C less in the present study. There effect due to covering in the fall was not significant; however, the fresh weight under the shade covering was consistently lower than the rest of the red and green lettuce.

2.5 Conclusion

This results of this study showed that specific HT coverings could be utilized to further affect the microclimate and productivity of crops. Additionally, it was observed that the soil temperatures and PAR impacts yield parameters more than °GDD. Higher soil temperatures in the three studied seasons, tended to result in greater yields for summer tomato, and spring and fall lettuce. The shade covering reduced PAR in every season and cooled soil temperature in the spring and fall. The decrease in marketable yields under the shade covering during each season may have been due to decreased PAR and soil temperature. The increase in tomato total and marketable yields under the clear covering was likely due to warmed soil temperatures. The decrease in red and green lettuce marketable yields under the movable covering during the spring trials was likely due to decreased soil temperatures. While during the fall, the decrease in Pn and yields of lettuce under all the coverings may have been due to decreased temperatures and PAR. This study showed that HT coverings alter both microclimate and yield during multiple seasons. Further research could include the effect of the various HT coverings on the health-promoting phytochemical content of lettuce and tomato. These findings are especially important for growers in the central USA to incorporate coverings that maximize solar light and soil temperature into their HT system. As HTs are more popularly utilized, it is important to understand the impact that the chosen covering can have on the crop yield and productivity. Future work may consider the effect of coverings on health-promoting phytochemical, as they are vital for human health.

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Chapter 3 - Effect of high tunnel coverings on vitamin C and phenolic antioxidants of breaker and light red tomatoes at harvest and during ripening.

Abstract

The implementation of high tunnels (HTs) has shown to increase marketability and yield of tomato compared to open field systems. These structures utilize specific polyethylene (poly) films and/or shade cloth to alter light intensity and spectral quality, which has shown to affect health-promoting phytochemicals. The effect of altering solar light with the use of HT coverings on the phytochemical content of tomato fruit was evaluated in a two-year study in Olathe, KS. The 6 different coverings included a standard poly (standard), diffuse poly (diffuse), clear poly (clear), UV-A/UV-B blocking poly (block), 55% shade cloth + standard poly (shade), and removal of standard poly 2 weeks prior to the initial harvest that simulated the use of a movable tunnel (movable). AsA, isoquercetin, quercetin, rutin, chlorogenic acid, and ferulic acid compounds were quantified using UPLC-MS. The accumulation of AsA in the fruit under the standard and clear coverings was greater than the movable and shade coverings ($P < .001$). The AsA concentration of the fruit harvested at the light red stage was 10% greater than the fruit harvested at the breaker stage by the time it reached mature red stage (or the point of consumption (POC)). Both the rutin and quercetin flavonoids were greater in fruit harvested at light red stage than at breaker stage by the POC ($P < .001$, and $< .001$, respectively). Chlorogenic acid was the highest accumulating phenolic compound measured. Chlorogenic acid and ferulic acid were affected by the covering x maturity stage interaction ($P < 0.01$, and < 0.05, respectively). At the POC, the fruit harvested at the light red stage had 60% higher chlorogenic acid concentration under the movable covering than those harvested at the breaker stage, 55% under the shade, and 43% under the block covering. Based on the results of this study, both the HT covering and harvest maturity altered primary and secondary metabolite accumulation by the POC. These compounds are known to play an important antioxidant role in human nutrition. As HT production expands for tomato and other fruiting vegetable crops, poly films with specific light transmission properties may be valuable for maintaining health-promoting phytochemicals and furthering global food security.

Keywords: hoophouse, season extension, solanum lycopersicum

3.1 Introduction

Tomato (*Solanum lycopersicum*) is the most common warm-season crop grown in HTs in the USA (Carey et al., 2009). The HT system allows a grower the opportunity to select particular polyethylene (poly) and/or shade cloth coverings to maximize yield and other quality parameters that are important to their market (Cowan et al., 2014; Z. S. Ilić et al., 2015; Wells & Loy, 1993). Shade cloth is often added to reduce high temperatures (>32 °C) that have shown to impair cell division (Flaishman et al., 2015; Gude, 2020 Ch. 2). Several reports have suggested that although HT production may increase tomato biomass, it may decrease phenolic content (Isman & Duffey, 1982; Stamp, 1990, 2003; Wilkens et al., 1996). Tomato fruit are a source of antioxidants like ascorbic acid (AsA) and phenolic compounds (George et al., 2004; Stewart et al., 2000). The poly films and shade cloth that are utilized to cover HTs have shown to alter spectral quality, light intensity, plant growth, and antioxidant compounds (Cowan et al., 2014; Ilić et al., 2015; Oh et al., 2011; Romani et al., 2002; Selahle et al., 2014; Tsormpatsidis et al., 2008; Wilkens et al., 1996). Photosynthetic active radiation (PAR) and ultraviolet (UV) radiation have been shown to decrease under HT poly film (Zhao, Carey, et al., 2007) and shade cloth (Agati & Tattini, 2010), which resulted in decreased antioxidant capacity of lettuce (*Lactuca sativa* L.*)* (Oh et al., 2011; Romani et al., 2002) and tomato (Ilić et al., 2015; Selahle et al., 2014).

Antioxidants can reduce reactive oxygen species (ROS) that are by-products of a disrupted electron transport chain in times of environmental stress (Ahmad et al., 2010). Environmental stresses can include UV, light intensity, and some visible regions of the light spectra that have shown to impact the biosynthesis of health-promoting primary and secondary metabolites, such as AsA and phenolic compounds in HT systems (Antonious et al., 2009; Luthria et al., 2006; Oh & Rajashekar, 2009). AsA, phenolic compounds and other antioxidants counter the harmful effects of ROS (Ahmad et al., 2010; Takahama & Oniki, 1992). AsA displays antioxidant synergism by regenerating vitamin E (a-tocopherol) to protect against photo-oxidation. This ascorbate regeneration system is a primary response to mitigate oxidative stress as seen in both tomatoes (Shalata et al., 2001) and rice (*Oryza sativa*) (Boo & Jung, 1999). Studies show that increased solar radiation can lead to AsA accumulation in the tissues of HT and greenhouse-grown tomato fruits (El-Gizawy et al., 1993; Giuntini et al., 2005; Selahle et al., 2014; Ward, 1963). Similarly, fruit positioning on the plants to expose fruits to high light tends to result in greater AsA content (Ward, 1963), due to direct light.

UV-B radiation activates enzymes such as phenylalanine ammonium-lyase (PAL) (Krizek et al., 1993) and chalcone synthase (CHS) (Kubasek et al., 1992), which are both important enzymes in the phenylpropanoid pathway and are responsible for the synthesis of phenolic compounds. PAL is the key enzyme in phenolic acid biosynthesis (Jones, 1984). Phenolic acids, such as cinnamic acids like chlorogenic acid and others are UV-B absorbing compounds and may serve as UV-B receptors in plants (Yaginuma et al., 2002). Previous studies on cucumber (*Cucumis sativus*) show that seedling exposure to UV-B radiation caused up to 78% increase in PAL (Krizek et al., 1993). CHS is the first step in the flavonoid biosynthesis pathway (Winkel-Shirley, 2001) and previous studies have found that both blue light and UV-A light enhance the response of CHS to UV-B synergistically (Fuglevand et al., 1996; Kubasek et al., 1992). It has been shown that full-spectrum, solar radiation can increase the amounts of certain phenolic compounds, such as flavonoids and phenolic acids due to photo-oxidative stress (Li et al., 2017; Sytar et al., 2018; Tomás-Barberán & Espín, 2001; Woolley et al., 2019; Yaginuma et al., 2002). However, responses are species-specific, and it has been suggested that solar UV-B may be insufficient to promote flavonoid production (Krizek et al., 1997; Tevini & Teramura, 1989), therefore, supplemental radiation may result in increased phenolic compound accumulation.

Very few studies have examined the effect of manipulation of full-spectrum solar radiation with HT coverings on phenolic compounds in tomato. One such study in Maryland, found that tomatoes grown with UV-transmitting poly induced accumulation of both phenolic acid and flavonoid compared to UV-blocking poly (Luthria et al., 2006). Levels of caffeic acid, p-coumaric acid, and ferulic acid in tomatoes, were 20% higher under UV-transmitting poly. In a greenhouse study with tomatoes, leaves had a four-fold increase in rutin and chlorogenic acid content under a 30% shade cloth compared to plants that were grown under a 73% shade cloth (Wilkens et al., 1996). HT coverings have demonstrated the ability to alter microclimate differentially (Gude, 2020 Ch. 2), and tomatoes have shown to increase phenolic content when mean temperatures are between 20 and 22 °C (Dannehl et al., 2012). Preliminary studies with lettuce show that brief increases in light intensity prior to harvest results in greater antioxidant accumulation (Becker et al., 2013b; Hipol & Dionisio-Sese, 2014; Zhao, Iwamoto, et al., 2007). These studies suggest that the use of a movable tunnel could provide protection and season extension during plant establishment and growth, followed by full spectrum exposure to encourage antioxidant accumulation.

Maturity stage is another factor determining the antioxidant capacity of vegetables. During ripening, tomato fruit undergoes a wide range of physiological changes that affect the final fruit composition (Gierson & Kader, 1986). AsA acts as a growth regulator in plant development and as an electron donor (Poiroux-Gonord et al., 2010). Early studies show that tomato fruit harvested at the mature green stage and ripened at 20 °C to mature red contained less AsA than those harvested at mature red stage (Betancourt et al., 1977; Adel A Kader et al., 1977). ROS are known to increase with ripening due to increased respiration, which results in increased antioxidant activity resulting from of higher AsA and phenolic compounds (Vinha et al., 2013). In tomato there is a significant increase in AsA synthesis, recycling, and degradation during ripening (Tyapkina et al., 2019). It has been suggested that phenolic compounds are involved in the stability of AsA, as both AsA and total phenolic content in pink stage fruit stored at 12 °C increased during a 15-d storage period (Vinha et al., 2013). This may be due to a strong reducing capacity of some flavonoids (e.g. quercetin) and phenolic acids (e.g. chlorogenic acid) (Pietta, 2000). In a greenhouse study, it was found that AsA, and phenolic compounds, rutin and caffeic acid, increased with ripening, while chlorogenic acid has been shown to decrease during ripening (Gautier et al., 2008).

Tomato consumption in the USA ranks second to the potato (*Solanum tuberosum*), with 43% from raw consumption (Reimers & Keast, 2016). Because of this, the average U.S. citizen receives a large amount of dietary AsA and phenolic compounds through tomato consumption (Institute of Medicine, 2000). Since these compounds are known to play an important role as antioxidants in human nutrition, the UV transmission properties of poly coverings are important for phytochemical accumulation. Therefore, the objectives of this study were to evaluate the effect of HT covering, and maturity stage at harvest throughout ripening, on AsA, and flavonoid and phenolic acid concentration in tomato fruit.

3.2 Materials and Methods

3.2.1 Plant material and experimental design

Trials were conducted at the Kansas State University Olathe Horticulture Center, located in Olathe, Kansas, during summers 2017 and 2018. The hybrid cultivar ‘BHN 589’ tomatoes

(Johnny's Selected Seeds, Winslow, Maine, USA) were grown in four, "caterpillar" HTs (39.6 m long x 3.7 m wide x 2.1 m high), which are homemade HTs whereby hoops are bent out of galvanized metal fencing material using a specific pipe bender (Quick Hoops Bender; Johnny's Seed, Winslow, Maine, USA). The construction of a homemade HT allowed for customization to suit the needs of the experiment in regards to plot size and tunnel length. The overall design of the caterpillar tunnel is a long and narrow with low ceilings, which provides an ideal structure for an experiment that specifically examines the impact of solar light. Two beds ran lengthwise in each HT (39.6 m long x 0.61 m wide). The trials were arranged in a randomized complete block design (RCBD), using HTs as blocks. Each HT, or rep, had six randomly assigned HT coverings (6.1 m long) and an additional 2.1 m "buffer" area at the ends of each HT. The coverings included 1. standard poly (standard) that was rated for 92% PAR transmission and blocked radiation <350 nm [single-layer 6-mil (K-50 poly; Klerk's Plastic Product Manufacturing, Inc., Richburg, SC, USA)]. 2. Standard poly with poly removal 2 weeks prior to the initial harvest to allow for full spectrum exposure of crops, simulating a movable tunnel (movable). 3. Diffuse poly (diffuse) is reported to remove direct radiation of infra-red (IR) light and blocks <380 nm (Luminance; Visqueen Building Products, London, UK). 4. Clear poly (clear), had full spectrum radiation (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA). 5. UV-A/UV-B blocking poly (block) blocked radiation <400 nm (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA). 6. A 55% black shade cloth + standard poly underneath (shade) reduced light intensity and canopy temperature (Sunblocker Knitted Shade; FarmTek, Dyersville, Iowa, USA).

Cultural practices were consistent with those used in commercial HT production (Gude, 2020 Ch. 2). The tomatoes were transplanted May 30, 2017 and May 18, 2018 in a single row in the center of each bed (45 cm between plants, with 1 m spacing between the beds from center-to-center). Buffer zones (1.5 m) were implemented at either end of each plot to minimize interplot interference. Shade cloth was added July 10, 2017 and July 9, 2018 to the shade covering. The poly was removed Aug. 7, 2017 and July 22, 2018 over the movable covering.

3.2.2 Tomato collection for AsA and phenolic compound analysis

For AsA and phenolic compound analysis, fruit were collected from two harvests each year (Aug. 10 and 30, 2017 and Aug. 6 and 15, 2018). Marketable fruit that was free of decay, mold, cracking that extended beyond the shoulder, small size (<4 cm diameter), pest damage, and other defects, were utilized to determine nutritional quality. The fruit were transported in an air-conditioned vehicle for further evaluation at the postharvest physiology lab at KSU-Olathe. Fruit were sorted in two maturity stages based on skin color (Table 1) with two measurements taken at opposite 45° angles from the blossom end of the fruit using an A5 Chroma-Meter (Minolta CR-400; Minolta Co. Ltd., Osaka, Japan) (Andrés & Perla, 2004). The two harvest maturity stages studied were breaker (a noticeable break in color with lesser than 10% of other than green color), and light red (between 60 and 90% red) (U.S. Department of Agriculture, 1997).

Table 3.1 Color determines maturity stage based on L* (-black to +white), a* (-greenness to +redness), b* (-blue to +yellow), Chroma $(a^{*2} + b^{*2})^{0.5}$, and hue angle $[\tan^{-1} (b^*/a^*)]$.

Maturity	L*	a*	b*	Chroma	Hue°
Breaker	61.10 (± 1.5)	-4.79 (± 1.5)	27.00 (± 1.5)	27.92 (± 1.5)	100.11 (± 1.5)
Light Red	47.78 (± 1.5)	22.34 (± 1.5)	32.68 (± 1.5)	39.75 (± 1.5)	55.87 (± 1.5)
Mature Red	44.84 (± 1.5)	27.94 (± 1.5)	31.17 (± 1.5)	40.79 (± 1.5)	48.98 (± 1.5)

Analyses were conducted at breaker stage on day 0 (BR), light red stage (BR_LR), and mature red stage (BR_MR) or the POC (Table 3.2). Mature red stage is fruit with more than 90% red skin color (U.S. Department of Agriculture, 1997).To mimic commercial retail, BR_LR fruit were stored at optimum conditions of 12.5 °C at 90% RH (Kader, 1996) in environmental chambers (Forma Environmental Chambers; ThermoFisher Scientific Inc., Asheville, NC) until day 10 or the corresponding a* value. BR_MR fruit were stored at 12.5 °C and 90% RH until day 10 or the corresponding a* value + market shelf conditions of 21 °C and 65% RH for 5 days or the corresponding a* value. This process mimics commercial retail distribution where unripe fruit is stored in cooler temperatures to slow ripening and then moved to the retail shelves in ambient temperatures to increase the rate of ripening (Kader, 1996).

Light red mature fruit was first analyzed on day 0 (LR) and then once reaching mature red stage (LR_MR) or the POC (Table 3.2). LR_MR fruit were held at market shelf conditions of 21 °C and 65% RH for 5 days or the corresponding a* value. The goal was to mimic the commercial distribution chain where the fruit waits in ambient conditions until reaching mature red stage at the POC.

Table 3.2 Maturity stage at analysis based on redness a* (-greenness to +redness) with corresponding codes.

Maturity stage at harvest	Maturity stage at analysis	Redness at analysis (*a)	Code
Breaker	Breaker	-4.79 (± 1.5)	BR
	Light Red	22.34 (± 1.5)	BR_LR
	Mature Red	27.94 (± 1.5)	BR_MR
Light Red	Light Red	22.34 (± 1.5)	LR

Mature Red	27.94 (± 1.5)	LR_MR

On the analysis days, the four replicates of each covering treatment were comprised of three fruit. Approximately 2 g of fresh sample was homogenized with 20 mL of 6% meta-phosphoric acid with 2N acetic acid solution frozen at -20 °C until AsA analysis. The remaining fresh sample was lyophilized in the freeze dryer (Harvest Right, Salt Lake City, Utah, USA), and ground (Waring WSG30; Conair Corporation, Torrington, Connecticut, USA) for phenolic analysis.

3.2.3 Standards, reagents, and equipment

For AsA analysis, AsA, potassium phosphate monobasic, and meta-phosphoric acid were purchased from Fisher (Fisher Scientific, Hampton, New Hampshire, USA). A calibration curve was created (2.5-50 μg/mL) in 10% meta-phosphoric acid solution and stored for 1 month at -20 °C. For phenolic compound analysis, commercial standards were all of analytical grade and included chlorogenic acid, quercetin, isoquercetin, rutin, and ferulic acid, as well as formic acid (purity > 99%) purchased from Acros Organics (Geel, Belgium). The stock standard solutions of individual compounds were prepared as described by Alarcón-Flores et al. (2013). Ammonium acetate, methanol, ethanol, glacial acetic acid (all HPLC grade) were purchased from VWR (VWR, Radnor, Pennsylvania, USA). Equipment used includes an analytical balance (Mettler Toledo, Columbus, Ohio, USA), sonicator (Ultrasonic Bath; Fisher Scientific, Hampton, New Hampshire, USA), and centrifuge (Avanti J-E; Beckman Coulter, Indianapolis, Indiana, USA).

3.2.4 Extraction and analysis of AsA

AsA was determined using the method developed by Klimczak & Gliszczyńska-Świgło (2015) with some modifications. Prior to analysis, samples were vortexed, centrifuged (7393 rpm, 10 min, 4 °C), and supernatant was poured into test tube. Sample extract (200 μL) was combined with 800 μL of 6% meta-phosphoric acid with 2N acetic acid and filtered through a 1 mL 96-well plate using a 0.22 μm filter (Supor; Pall Co., Port Washington, NY, USA). Samples of 5 uL were injected in to the Waters Acquity UPLC System (Waters Co., Milford, MA, USA), equipped with a binary solvent manager (part number: 186015001), a column manager (part number: 186015009), a sample manager (part number: 186015005), a photodiode array UV/vis detector (PDA part number: 186015026), and a QDa mass detector (part number: 186006511) for further confirmation, using Empower 3 chromatography data software. The chromatographic separation was achieved on a High Strength Silica (HSS) T3 column (100 mm x 2.1 mm, 1.8 μm particle size) with methanol + 1% formic acid (eluent A) and 5 mM potassium phosphate monobasic (KH_2PO_4) at pH 2.65 (eluent B) as the mobile phases. The linear gradient started with 5% A, increased to 15% A over 1 min, then to 35% A over 1 min, and returned to initial conditions within the next 4 min with a flow rate of 0.2 mL/min. The eluate was detected using the PDA and the chromatogram was recorded at 245 nm. Quantification of AsA was performed with an external standard. AsA was quantified, related to the corresponding standard, and results were expressed as mg AsA/100g fresh weight (FW). For each extract, three subsamples were made.

3.2.5 Extraction and analysis of phenolic compounds

Each replicate of the covering was extracted and analyzed in a darkened room with a red safety light to avoid oxidation of the analytes, following the procedure of Vallverdú-Queralt et al. (2013), with some modifications. Lyophilized tomato fruit (0.2 g) were homogenized with 4 mL of extraction solution (ethanol/water, 80/20, v/v) and vortexed (20 s), sonicated (5 min), and centrifuged (4000 rpm, 15 min, 4 °C). The supernatant was transferred into a test tube and the extraction was repeated. Both supernatants were combined and evaporated to dryness under nitrogen flow (2-6 ppm). The residue was reconstituted with 3 mL of 30 mM ammonium acetate in de-ionized (D.I.) water with 5 pH adjusted with formic acid (eluent B) and filtered through a 25 mm 0.22 μm filter (Supor; VWR, Radnor, PA, USA) into several 1.5 mL Eppendorf tubes for reserve and the sample extract was stored in darkness at -70 °C until analysis. Prior to analysis, a portion of each extracted sample was thawed, vortexed and 100 μL sample extract was added to 900 μL 50:50 eluent A (methanol + 0.1% formic acid):eluent B (10% dilution) for injection.

The analysis was carried out using a method adapted from Alarcón-Flores et al. (2013). Samples of 3 uL were injected in to the Waters system (section 3.2.4), and the separation was achieved on Waters Acquity Ethylene Bridged Hybrid (BEH) C18 column (100 mm x 2.1 mm, 1.7 μm particle size) at 30 °C. Eluting peaks were monitored at 254 nm and 325 nm. The elution was performed with 5% A for 1.5 min, and a linear gradient was then installed to reach 30% A at 4 min, 85% A at 8 min, and 100% A at 10 min. These conditions were maintained for 1.5 min, before being returned to the initial conditions in 30 s. The flow rate was set at 0.2 mL/min. Quantification of phenolics was performed with external standards. The phenolic compounds of rutin, quercetin, isoquercetin, ferulic acid and chlorogenic acid were quantified, related to their corresponding standard based on retention time and confirmed by their absorption spectrum in

UV. Results were expressed as mg/kg dry weight (DW). For each extract, three subsamples were made.

3.2.6 Statistical analysis

The data was subjected to natural log (ln) transformation before subjected to linear mixed model analysis. With phytochemical compound analysis, assuming log transformation is common to normalize the data. The fixed effects of the model were year, HT covering, maturity stage and maturity stage x covering interaction. Random effects of the model were the HT x year, and the HT x year x covering interactions. Fixed effects were evaluated via type III tests. Each value is presented in the results as the back-transformed least square means (LSMeans) ± back-transformed standard error. The pairwise comparison was carried out using Tukey's method at the 0.05 significance level. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=KR in MODEL statement.

3.3 Results

Table 3.3 shows the probability values of the effect of HT covering, maturity stage of the tomato at harvest, and their interactions on the AsA and phenolic compound concentration.

3.3.1 AsA concentration

The AsA concentration was affected by HT covering and maturity stage (*P* <.001, and <.001, respectively).

Table 3.3 Probability values[a] of high tunnel covering, maturity stage, and maturity stage x covering on the antioxidant parameters of tomato fruit grown in high tunnels in Olathe, KS in 2017 and 2018.

Individual antioxidant	Covering[b]	Maturity stage[c]	Maturity stage x Covering
Ascorbic Acid (mg AA/100g FW)	<.001	<.001	ns
Isoquercetin (mg/kg DW)	ns	ns	ns
Rutin (mg/kg DW)	ns	<.001	ns
Quercetin (mg/kg DW)	ns	<.001	ns
Chlorogenic acid(mg/kg DW)	<0.01	<.001	<0.01
Ferulic acid (mg/kg DW)	<0.01	<0.01	<0.05

[a] linear mixed model was used to test if covering, maturity stage, or maturity stage x covering had significant effect on the examined parameter ($P \leq 0.05$).

[b] Covering includes the following 6 different high tunnel coverings: standard poly, standard poly with removal two weeks prior to the first harvest, diffuse poly, clear poly, UV-A/B blocking poly, and 55% shade cloth over standard poly.

[c] Maturity stage includes 5 analysis time points: breaker harvested fruit (BR), breaker harvested fruit at 'light red' maturity (BR_LR), breaker harvested fruit at 'mature red' maturity (BR_MR), light red harvested fruit (LR), light red harvested fruit at 'mature red' maturity (LR_MR).

The standard and clear coverings were statistically similar and greater than the movable and shade coverings (Fig. 3.1). AsA concentration decreased most under the shade covering compared to the other coverings.

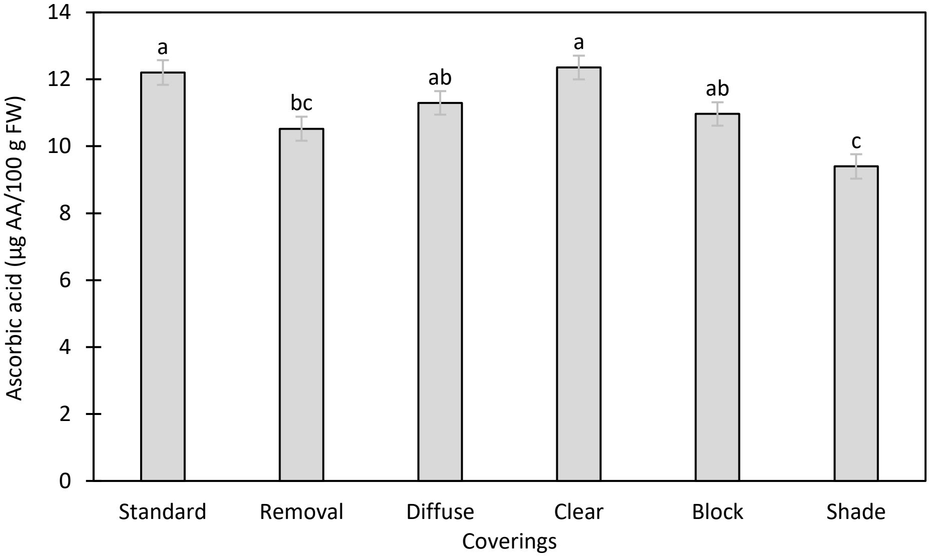

Figure 3.1 Ascorbic acid concentration (AsA) of tomato fruit grown under the following 6 different high tunnel coverings: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade). Lsmeans (±SE) with same letter do not differ significantly at $P < 0.05$, Tukey's HSD.

AsA concentration was statistically similar between the breaker harvested fruit at the light red stage (BR_LR), light red fruit at harvest (LR), and the light red harvested fruit at the mature red stage (LR_MR), and greater than the breaker fruit at harvest (BR) (Fig. 3.2). At the POC, the AsA concentration in LR_MR fruit was statistically similar to the breaker harvested fruit at the mature red stage (BR_MR).

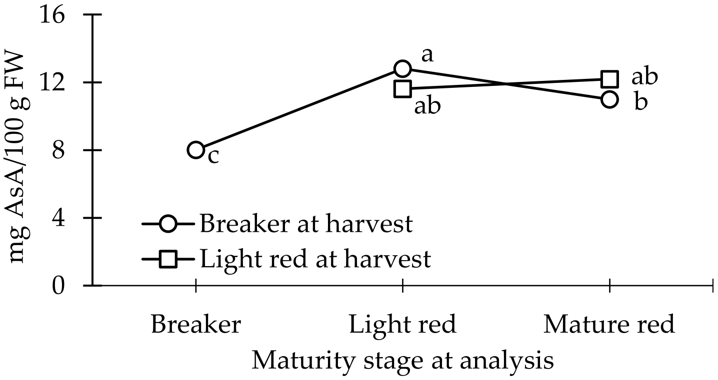

Figure 3.2 The effect of maturity stage on ascorbic acid concentration (mg AsA/100g DW) of tomato grown in high tunnels in Olathe, KS in 2017 and 2018. Fruit were harvested at breaker stage and analyzed at d0, at light red stage (stored at 12.5 °C at 90% RH until d 10 (or an a* value of 22)), and mature red stage (stored at 12.5 °C and 90% RH until d 10 (or an a* value of 22) + market shelf conditions of 21 °C and 65% RH 5 d (or an a* value of 28)). Fruit were also harvested at light red stage and analyzed at d0, and at mature red stage (market shelf conditions of 21 °C and 65% RH for 5 d (or an a* value of 28)). Lsmeans (±SE) with same letter do not differ significantly at *P* <0.05, Tukey's HSD.

3.3.2 Phenolic compound accumulation

Three flavonoids and two phenolic acids and were identified from their absorption spectra in the tomato samples. The flavonoids included three flavonols, quercetin, rutin, and isoquercetin. No significant differences were observed under the various HT coverings for the flavonol accumulation (Table 3.3). The maturity stage affected the amount of quercetin and rutin in the tomato fruit (*P* <.001, and <.001, respectively; Fig. 3.3). The quercetin concentration in the BR_LR, LR, and LR_MR fruit increased compared to BR fruit. The rutin concentration in BR_LR, LR, and LR_MR fruit increased compared to BR_MR fruit. Both rutin and quercetin accumulation increased in LR_MR fruit relative to BR_MR fruit by 19% and 7.5%, respectively. Similar to AsA, the LR fruit maintained the rutin and quercetin concentration at the POC, or LR_MR.

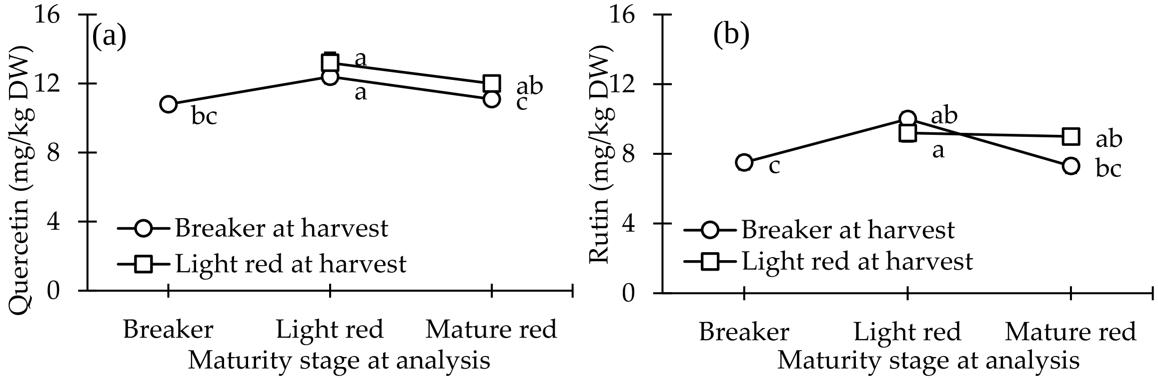

Figure 3.3 The effect of maturity stage on quercetin (a), and rutin (b) concentration (mg/kg DW) of tomato grown in high tunnels in Olathe, KS in 2017 and 2018. Fruit were harvested at breaker stage and analyzed at d0, at light red stage (stored at 12.5 °C at 90% RH until d 10 (or an a* value of 22)), and mature red stage (stored at 12.5 °C and 90% RH until d 10 (or an a* value of 22) + market shelf conditions of 21 °C and 65% RH 5 d (or an a* value of 28)). Fruit were also harvested at light red stage and analyzed at d0, and at mature red stage (market shelf conditions of 21 °C and 65% RH for 5 d (or an a* value of 28)). Lsmeans (±SE) with same letter do not differ significantly at *P* <0.05, Tukey's HSD.

The phenolic acids, chlorogenic acid and ferulic acid, were impacted by the maturity stage x covering interactions (*P* <0.01, and <0.05, respectively; Table 3.3). Chlorogenic acid was the highest accumulating phenolic compound in the tomato fruit. The accumulation of chlorogenic acid differed between covering in BR and BR_MR fruit (*P* <0.05, and <0.01, respectively; Table 3.4), with increased amounts observed in the fruit grown under the clear covering. In the LR_MR fruit, chlorogenic acid differed between coverings (*P* <0.01), with the block resulting in increased amounts. The amount of chlorogenic acid was highest in BR and LR fruit at harvest, and lowest in BR_MR and LR_MR fruit. At the POC, chlorogenic acid concentration in the LR_MR fruit was significantly greater than BR_MR fruit under the movable, block, and shade coverings (*P* <0.01, <0.05, and <0.05, respectively).

Table 3.4 The effect of high tunnel covering and maturity stage on chlorogenic acid concentration (mg/kg DW) of tomato at analysis and comparing the point of consumption (POC) after grown in high tunnels in Olathe, KS in 2017 and 2018.

Covering[a]	BR[b]	BR_LR	BR_MR	LR	LR_MR	POC (BR_MR and LR_MR)
Standard	52 (5.4)	37 (5.8)	14 (2.8)	42 (6.8)	20 (2.3)	ns[c]
Movable	45 (4.9)	29 (4.2)	7 (1.4)	52 (7.7)	18 (2)	**
Diffuse	41 (4.2)	23 (3.3)	17 (3.2)	64 (9.5)	23 (2.5)	ns
Clear	67 (6.9)	32 (4.7)	21 (4.1)	58 (7.7)	25 (2.8)	ns
Block	56 (5.8)	29 (4.7)	18 (3.4)	62 (8.2)	32 (3.5)	*
Shade	60 (6.8)	41 (6.3)	15 (3.2)	46 (6)	27 (3.2)	*
Mean	54	32	15	54	24	
P-value	<0.05	ns	<0.01	ns	<0.01	

[a] Coverings include: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade).

[b] Maturity stage includes 5 analysis time points: breaker at harvest (BR), breaker harvested fruit at 'light red' maturity (BR_LR), breaker harvested fruit at the POC or 'mature red' stage (BR_MR), light red at harvest (LR), light red harvested fruit at the POC or 'mature red' stage (LR_MR). To mimic commercial retail, BR_LR fruit were stored at optimum conditions of 12.5 °C at 90% RH until d 10 (or an a* value of 22). BR_MR fruit were stored at 12.5 °C and 90% RH until d 10 (or an a* value of 22) + market shelf conditions of 21 °C and 65% RH 5 d (or an a* value of 28). LR_MR fruit were held at market shelf conditions of 21 °C and 65% RH for 5 d (or an a* value of 28).

[c] Significance between BR_MR and LR_MR for individual coverings, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq .001$

The ferulic acid concentration differed between coverings in the BR fruit (*P* <0.05; Table 3.5), with increased amount observed under the standard and movable coverings. In the BR_MR fruit, a covering difference was observed (*P* <0.05), and the ferulic acid concentration was highest under the shade covering. It was observed that the values increased for the breaker mature fruit during off-plant ripening. In the LR fruit, ferulic acid concentration differed among coverings (*P* <0.05), and increased under the block covering. In LR_MR fruit, ferulic acid concentration differed among coverings (*P* <0.05) and increased under the clear covering. Inconsistent observations between coverings were observed at various maturity stages for ferulic acid concentration. Unlike the other compounds, the ferulic acid concentration at the POC between the BR_MR fruit exceeded that of the LR_MR under the standard and shade coverings (*P* <0.05, and <0.01, respectively).

Table 3.5 The effect of high tunnel covering and maturity stage on ferulic acid concentration (mg/kg DW) of tomato at analysis and comparing the point of consumption (POC) after grown in high tunnels in Olathe, KS in 2017 and 2018.

Covering[a]	BR[b]	BR_LR	BR_MR	LR	LR_MR	POC (BR_MR and LR_MR)
Standard	3.2 (0.7)	2.3 (0.7)	3.6 (2.8)	2.7 (0.7)	1.4 (0.4)	* [c]
Movable	2.2 (0.5)	2.7 (0.8)	1.7 (1.4)	1 (0.3)	1.1 (0.3)	ns
Diffuse	1.9 (0.4)	2.4 (0.7)	2.1 (3.2)	1.8 (0.4)	1.4 (0.4)	ns
Clear	1.1 (0.2)	2.6 (0.8)	3.5 (4.1)	2.6 (0.6)	3.3 (0.9)	ns
Block	1.4 (0.3)	2.1 (0.7)	3.6 (0.9)	2.9 (0.6)	2.9 (0.8)	ns
Shade	1.6 (0.4)	5 (1.5)	6.3 (1.8)	2.2 (0.5)	1.6 (0.5)	**
Mean	1.9	2.9	3.5	2.2	2.0.	
P-value	<0.05	ns	<0.05	<0.05	<0.05	

[a] Coverings include: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade).

[b] Maturity stage includes 5 analysis time points: breaker at harvest (BR), breaker harvested fruit at 'light red' maturity (BR_LR), breaker harvested fruit at the POC or 'mature red' stage (BR_MR), light red at harvest (LR), light red harvested fruit at the POC or 'mature red' stage (LR_MR). To mimic commercial retail, BR_LR fruit were stored at optimum conditions of 12.5 °C at 90% RH until d 10 (or an a* value of 22). BR_MR fruit were stored at 12.5 °C and 90% RH until d 10 (or an a* value of 22) + market shelf conditions of 21 °C and 65% RH 5 d (or an a* value of 28). LR_MR fruit were held at market shelf conditions of 21 °C and 65% RH for 5 d (or an a* value of 28).

[c] Significance between BR_MR and LR_MR for individual coverings, *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq .001$

3.4 Discussion

In this study we examined the changes in antioxidants (AsA and phenolic compounds) as the result of manipulating light with the use of different HT coverings. We found that AsA was affected by maturity stage and light, which is in agreement with previous studies on the effects of harvest maturity and growing environment (Boo & Jung, 1999; Gautier et al., 2008; Islam et al., 1996; S. K. Lee & Kader, 2000; Shalata et al., 2001; Ward, 1963). The shade covering had only 24% PAR transmission (Gude, 2020 Ch. 2) and the least AsA accumulation of the coverings tested. Environmental stressors like high light has shown to increase AsA concentration due to generated ROS (Selahle et al., 2014; Sørensen et al., 1995; Toivonen et al., 1994). Previous studies have shown that light intensity has the greatest influence on AsA accumulation (Hamner et al., 1945; Selahle et al., 2014; Ward, 1963). By limiting light with shade, photosynthetic activity and synthesis of ascorbate and α-tocopherol reduces (Yamamoto & Bassi, 1996). As described in Gude (2020 Ch. 2), the shade covering lowered net photosynthesis in the tomato plant compared to the other tested coverings. Hamner et al. (1945) found a 66% increase in AsA when plants were transferred from shade to full solar spectrum when the fruit were mature green. Interestingly, the movable covering that received the most unobstructed light, accumulated less in AsA compared to the standard and clear covering. As described in Gude (2020 Ch. 2), the block poly transmitted 75% PAR, the standard poly with 74% PAR, the clear poly with 69% PAR, and the diffuse with 64% PAR. However, according to the manufacturer, the standard poly blocks UV-B but transmits most UV-A, while the clear poly was UV-transmitting. In our trials, AsA accumulated in fruit under coverings with greater UV/Vis radiation compared to the shade covering. However, no difference in AsA was observed from fruit under the block covering compared to the standard, diffuse, and clear coverings. A study with solar UV-B exclusion found no significant difference in AsA when comparing a UV-B excluding film to a standard poly film (Papaioannou et al., 2012). Future work should consider measuring UV-A/B transmittance into the measured parameters, to enhance the study.

No differences between coverings were detected for the three flavonol compounds that we studied; however, all three were found to be in the ranges as determined by previous tomato studies (Alarcón-Flores et al., 2013, 2016; Hallmann, 2012; Minoggio et al., 2003; Slimestad & Verheul, 2009; Vallverdu-Queralt et al., 2011). The aforementioned tomato studies did not specify the production system and it is possible that their samples came from the open-field. Light-induced production of flavonoids in the outer tissues of cornflower (*Centaurea cyanus L.*) and sorghum (*Sorghum bicolor* Moench, cv. Acme Broomcorn) provide a well-known protective mechanism against intense solar radiation (Takahashi et al., 1991a; Yatsuhashi & Hashimoto, 1985). However, UV-A/B sensitivity has been demonstrated to be crop and cultivar specific (Krizek et al., 1993) so it is not clear if this mechanism is active in tomato. Quercetin was the highest accumulating flavonoid in the tomato samples, as found previously (Crozier et al., 1997). The molecular structure of the studied flavonols are similar in that a glucose molecule in isoquercetin and a rutinose disaccharide in rutin have replaced the 3-O hydroxyl group on the C ring of quercetin (Pietta, 2000). The presence of the catechol group on the B ring indicate that the flavonols in question are highly active against ROS.

Previous studies have found that the combined amount of the identified phenolic acids was 36% higher than the flavonoid concentration in conventionally grown, open-field tomatoes (Hallmann, 2012). The antioxidant potential of field grown tomatoes in Italy was highly correlated with phenolic acid concentration versus flavonoid concentration (Minoggio et al., 2003). Chlorogenic acid was the highest accumulating phenolic compound in our trials, which was consistent with previous studies, although concentrations range widely from <10 to 805 mg/kg DW (Alarcón-Flores et al., 2013, 2016; Minoggio et al., 2003; Vallverdu-Queralt et al., 2011). The large range could be explained by variable conditions such as cultivar, environmental conditions, location, maturity, as well as storage conditions and duration. In the breaker fruit, chlorogenic acid concentration increased under the clear covering in both BR and BR_MR fruit. Likewise, Luthria et al. (2006), found that total phenolic and individual phenolic acid concentrations of tomato fruit at harvest increased by 20% when cultivated under UV-transparent (or clear) film compared with UV-blocking film. In this study, the chlorogenic acid concentration increased by 16% and 14% under the clear covering compared with the block covering in BR and BR_MR fruit, respectively. Unlike the breaker fruit, the chlorogenic acid concentration in light red fruit increased by 44% under the block covering in LR_MR fruit compared to the movable covering, with no UV or light obstruction. It has been suggested that intermediate stress is beneficial for secondary metabolite accumulation (Loomis, 1932; Loomis, 1953).

Ferulic acid and chlorogenic acid have been found to produce strong antioxidant activities by inhibiting lipid oxidation and scavenging ROS (Cheng et al., 2007). Ferulic acid concentration was lower than the other measured phenolic compounds in this study, but the amounts were 10-fold greater than previous findings from HT tomatoes (Luthria et al., 2006). Within both the breaker and light red fruit, no consistent differences were observed between coverings. Few studies aimed to identify phenolic compound differentiation based on HT covering, but Luthria et al. (2006) also found no consistent differences in ferulic acid accumulation between HT coverings that altered UV light. Furthermore, the BR_MR fruit grown under the shade covering resulted in the greatest accumulation of ferulic acid, which is in contrast with previous tomato studies (Liu et al., 2018). At the POC, the ferulic acid concentration of the BR_MR fruit increased by 61% under the standard covering and 75% under the shade covering compared to the LR_MR fruit.

We investigated the effect of the maturity stage of the tomato fruit on antioxidant concentration (AsA and phenolic compounds) at harvest and during ripening. AsA increased with harvest maturity, which several studies have found to be true (Betancourt et al., 1977; Gautier et al., 2008; Islam et al., 1996; Adel A Kader et al., 1977; Ward, 1963). Islam et al.

(1996) found that AsA increased by 26% in light red harvested fruit compared to breaker harvested fruit in a greenhouse study. Similarly, Gautier et al. (2008) found that fruit accumulated AsA during ripening on or off the plant, but the increase was greater for those with a later harvest maturity. The AsA for the fruit from the light red harvest maturity did not change significantly during ripening, while the fruit from the breaker harvest maturity did. The AsA for the breaker harvest maturity increased throughout maturity stages, and peaked in BR_LR fruit. AsA is known to decrease with prolonged periods of storage (Lee & Kader, 2000). This may be the reason for such a large decrease in AsA from BR_LR to BR_MR fruit. Similar reports from Selahle et al. (2014) show that 21-d storage at 10 °C followed by storage for 2 d at 25 °C resulted in significant AsA loss. The authors suggested that ripening encourages ROS production, and that ascorbate-oxidase and peroxidase is released from the cell walls due to water loss and tissue damage from storage. In comparing the two harvest maturities at the POC, the AsA concentration was 10% higher in the LR_MR fruit, but did not differ statistically from the BR_MR fruit.

We found the flavonoids, quercetin and rutin, to vary by maturity stage at harvest and during ripening. At harvest, LR fruit contained significantly greater quercetin and rutin concentration compared to BR fruit. In addition, rutin and quercetin increased from BR to BR_LR stage and then decreased by the POC (BR_MR) to levels that were similar in BR fruit. In a previous greenhouse study, rutin in cherry tomatoes increased both with harvest maturity and storage temperature (Gautier et al., 2008). The BR_MR fruit was stored at 12.5 °C for 10 d + 5 d shelf life at 21 °C. Although those storage and shelf life conditions are optimal for quality assurance (Kader, 1996), the change in temperature may have initiated the decrease in flavonoid concentration in the BR_MR stage fruit. The rutin concentration at LR_MR stage increased significantly by 19% compared to BR_MR fruit. The quercetin concentration at LR_MR stage increased by 7.5% compared to the BR_MR stage, although the difference was insignificant.

During ripening, the chlorogenic acid of the LR fruit did not differ from the BR fruit, as other studies have found (Gautier et al., 2008; Wardale, 1973). Chlorogenic acid was the only measured phenolic compound to decrease throughout off-plant ripening, decreasing 2-fold from one maturity stage to the next in both breaker and light red harvest mature fruit. The results are similar to findings from a greenhouse study, where chlorogenic acid decreased to 60% of the concentration of mature green fruit by mature red stage (Gautier et al., 2008). They found that

PAL is the key enzyme in phenolic acid biosynthesis and has been reported to decrease during fruit ripening, without being a limiting factor for synthesis of phenolic compounds (Fleuriet & Macheix, 1981; Gautier et al., 2008; Hunt & Baker, 1980; Winter & Herrmann, 1986). Previous studies have suggested that AsA may be spared at the expense of chlorogenic acid due to its lower redox potential (Slimestad & Verheul, 2005). However, the redox potential for chlorogenic acid was later found to be higher at 0.57 V compared to AsA at 0.28 V (Bors et al., 1995; Namazian & Zare, 2005). Another theory is that chlorogenic acid is associated with auxin metabolism and decreases during ripening (Buta & Spaulding, 1997). The study found that chlorogenic acid paralleled the decline in indole-3-acetic acid (IAA) levels in the pericarp of two tomato varieties during maturation. Although chlorogenic acid decreased during ripening, we observed 37% greater chlorogenic acid concentration in LR_MR stage fruit than in BR_MR stage fruit.

It has also been suggested that chlorogenic acid may be further catabolized during ripening to produce caffeic acid derivatives, like ferulic acid (Gautier et al., 2008; Luthria et al., 2006). In this study, ferulic acid did increase throughout ripening for the breaker mature fruit. Ferulic acid accumulation was different from the other measured metabolites, in that BR_MR exceeded that of LR_MR under the standard and shade coverings. Ferulic acid was found to be within the normal range for tomato (Alarcón-Flores et al., 2016; Minoggio et al., 2003; Vallverdu-Queralt et al., 2011).

3.5 Conclusion

The results of this study show that HT covering and maturity stage significantly affect AsA and phenolic compounds in tomato fruit. Three flavonols and two phenolic acids (quercetin, rutin, isoquercetin, chlorogenic acid, and ferulic acid) were quantified from tomatoes at breaker, light red and mature red stages. Quercetin was the predominant flavonoid measured, but chlorogenic acid was the predominant phenolic compound measured throughout the two-year trial. The most consistent effect of HT covering was observed under the clear covering with increased amounts of AsA in light red and breaker mature fruit, as well as chlorogenic acid concentration in breaker mature fruit. Throughout ripening, AsA, rutin, quercetin, and ferulic acid all increased. These results indicate that the accumulation of these antioxidants in tomato fruit is significantly affected by the spectral quality of ambient solar radiation and harvest maturity. Further research with tomatoes could include the effect of HT covering on tomato pigment compounds. Since

AsA and phenolic compounds are important antioxidants for human nutrition, subtle differences in nutrient composition between the HT coverings as a result of differences in the light transmission properties of these different coverings is important for tomato production.

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Chapter 4 - Effects of various high tunnel coverings on color and phenolic compounds of red and green leaf lettuce (*Lactuca sativa*)

Abstract

High tunnels (HTs) have been shown to improve crop productivity of and these systems have been rapidly adopted across the USA. However, HT coverings have can reduce light intensity and spectral quality, thus negatively affecting pigmentation and accumulation of several important phytochemicals in lettuce. Therefore, the objectives of this study were to evaluate the effect of growing season (spring vs fall) and HT covering on red and green lettuce at harvest and after 5 days of storage with respect to leaf color and phenolic acid and flavonoid accumulation. The coverings included: standard poly (standard), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), 55% shade cloth over standard poly (shade), and standard poly removed 2 to 3 weeks prior to the initial harvest to simulate a movable tunnel (movable). Red 'New Red Fire' and green 'Two Star' leaf lettuce trials were conducted from fall 2017 to spring 2019 in Olathe, KS. Trials were arranged in a split-plot, randomized complete block design and four individual tunnels served as replications. Phenolic acid compounds (chicoric acid, chlorogenic acid, caffeic acid, and ferulic acid) and flavonoid compounds (rutin and isoquercetin) were measured with the use of the UPLC-MS. The clear and movable coverings resulted in darker red leaves (lower chroma and hue angle values) within the red lettuce during both seasons (P <.001). In contrast, the shade covering resulted in the least pigmented leaves. Chlorogenic acid and chicoric acid were the most prevalent phenolic compounds in both green and red lettuce. The phenolic compound accumulation in the red lettuce was significantly greater in the spring than the fall for all measured compounds other than caffeic acid. In the spring, the flavonoids increased under the movable covering for both red and green lettuce. During the spring trials, the isoquercetin concentration of the red lettuce was 72% higher under the movable coverings compared to the shade covering (P <0.01). The chlorogenic acid of the fall green lettuce was similar for all coverings and higher than the shade covering (P <.001). The effect of covering and season on phenolic compound concentrations after 5 days of storage was inconsistent. The results of this study indicate that health-promoting phytochemicals can be altered due to solar light manipulation with the use of various HT coverings and growers could implement specific poly films to improve their overall production system.

Keywords: spectral quality, light intensity, UPLC-MS

4.1 Introduction

Phenolic compounds are available to humans through plant consumption, and play a diverse role in both plants and humans. As antioxidants in humans, the phenolic compounds have shown anti-inflammatory and antitumor activity in the prevention of coronary heart disease and cancer (Arai et al., 2000; Hertog et al., 1993; Olthof et al., 2001). In plants, phenolic compounds also act as antioxidants (Ahmad et al., 2010; Tomás-Barberán & Espín, 2001), and provide pigmentation (Brouillard & Dangles, 2017; Giovannoni, 2001; Shioshita et al., 2006; Tattini et al., 2004). In vitro, flavonoids have shown to reduce electrolyte leakage in response to membrane oxidation (Verstraeten et al., 2003).

Lettuce, especially red leaf lettuce, is a good source of phenolic acids and flavonoids, including quercetin glucosides, anthocyanin conjugates, and caffeic acid derivatives (DuPont et al., 2000; García-Macías et al., 2007; Llorach et al., 2008; Ribas-Agustí et al., 2011; Sytar et al., 2018; Tomás-Barberán et al., 1997). More specifically, the phenolic acids, chicoric acid (dicaffeoyltartaric acid), chlorogenic acid (5-caffeoylquinic acid), caffeic acid, and ferulic acid have shown anti-diabetic effects, prevented formation of mutagenic compounds, and can inhibit lipid peroxidation (Cheng et al., 2007; Kono et al., 1995; Poiroux-Gonord et al., 2010). Quercetin derivatives in lettuce include quercetin 3-O-(6”-O-malonyl)-glucoside, isoquercetin (quercetin-3-O-glucoside), rutin (quercetin-3-O-rutinoside), and quercetin 3-O-glucoronide, which have shown anti-inflammatory effects (Middleton et al., 1981). The flavonols, isoquercetin and rutin, contain a catechol group on the B ring which makes them highly active antioxidants (Pietta, 2000).

Plants exposed to various environmental stresses during production will generate reactive oxygen species (ROS) (Ahmad et al., 2010). Non-enzymatic antioxidants, such as phenolic compounds, are upregulated with increased stress to scavenge ROS and avoid oxidative stress. Thus, it has been shown that phenylalanine ammonia-lyase (PAL), a key gateway enzyme of phenylpropanoid pathway, is typically upregulated in response to environmental and biotic stresses (light, temperature, pest damage) (Dixon & Paiva, 1995; Krizek et al., 1993, 1998; Tomás-Barberán & Espín, 2001). Ultraviolet light (UV-B) is known to increase PAL activity in lettuce and cucumber (*Cucumis sativus* L.) (Becker et al., 2015; Krizek et al., 1993, 1998). Krizek et al (1998) found that ambient UV-B increased the PAL content by 27 to 83% in lettuce, which resulted in increased accumulations of anthocyanins and other flavonoids. Induction of chalcone synthase (CHS), a key enzyme in flavonoid biosynthesis has also been shown to result from a synergistic relationship between UV-B and blue light (Fuglevand et al., 1996; Kubasek et al., 1992).

HTs and other forms of protected environment growing systems have shown to increase the crop productivity of lettuce by minimizing environmental stresses and providing extension of the growing season (Borrelli et al., 2013; Bumgarner et al., 2011; Singer et al., 2015; Wallace et al., 2012). The HT system has shown to alter spectral quality, light intensity, plant growth, and antioxidant compounds through polyethyelene (poly) films and shade cloth (Cowan et al., 2014; Ilić et al., 2015; Oh et al., 2011; Romani et al., 2002; Tsormpatsidis et al., 2008; Wilkens et al., 1996). Photosynthetic active radiation (PAR) and UV radiation has been shown to decrease under HT poly film (Zhao, Carey, et al., 2007) and shade cloth (Elad et al., 2007; Ilić et al., 2015; Li et al., 2017; Tattini et al., 2004). Several lettuce studies have shown secondary metabolites accumulate at higher levels with increasing moderate stress, which coincides with decreased biomass accumulation (García-Macías et al., 2007; Krizek et al., 1998; Tevini & Teramura, 1989).

When grown in open field conditions with higher light intensity, both green and red lettuce can accumulate higher phenolic compounds compared to those grown in HTs with standard poly (Oh et al., 2011), and clear poly (Woolley et al., 2019). Similarly, Li et al. (2017) found that the use of 50% black shade cloth increased fresh weight of red and green leaf lettuce, but reduced phenolic compounds compared to the open field. Studies also show that the phenolic accumulation in lettuce is especially affected 2 to 4 weeks prior to harvest (Becker et al., 2013a; Hipol & Dionisio-Sese, 2014). The concept of subjecting crops to full spectrum light prior to harvest is feasible with a movable tunnel, where the plant is established before the tunnel is moved (Jett, 2017). Currently, published studies related to movable HTs and the impact that their use has on crop physiology and yield are lacking.

Phenolic compound accumulation may be impacted by season due to reduced UV radiation, PAR, and temperature in the fall compared to the spring (McKenzie et al., 1996; Wilson & Meyers, 2007). During a study in Spain from February to May, Marin et al. (2015) found that temperature and radiation positively correlated with phenolic acid and flavonoid accumulation in open field red lettuce. However, Marin et al. (2015) found that red lettuce pigment was enhanced with wide temperature ranges, and was darker when the plants experienced temperatures below °7 C.

Storage is another factor that is known to affect phenolic compound accumulation, due to continued phenolic metabolism after harvest. In addition, the physiological maturity at the time of harvest and pre-harvest conditions have shown to influence lettuce phenolic compound concentrations during storage (Becker, Klaering, Schreiner, et al., 2014; DuPont et al., 2000). Higher PAR and surface temperature pre-harvest, has shown to increase respiration rate and moisture loss throughout storage, resulting in decreased phytochemical compounds (Ntsoane et al., 2016).

Lettuce is one of the most common crops grown in HTs across the U.S. (Carey et al., 2009), an important vegetable and a good source of phytochemicals in the American diet (Conrad et al., 2017). HTs have been increasingly utilized in leafy vegetable production across the U.S., but its yield and phytochemical content vary depending on the environmental conditions. Thus, it is critical to investigate how different HT coverings affect leaf color and phenolic compounds in both spring and fall season at harvest and during postharvest storage. Therefore, the objectives of this study were to evaluate the effects of growing season (spring vs fall), various types of HT coverings, and storage day (day 0 and day 5) on red and green lettuce, with respect to leaf color and accumulation of phenolic acids and flavonoids in leaf tissue.

4.2 Materials and Methods

4.2.1 Experimental design

Red leaf lettuce (RL) and green leaf lettuce (GL) trials occurred from fall 2017 to spring 2019 at the Kansas State University Olathe Horticulture Center, located in Olathe, Kansas. ‘New Red Fire’ and ‘Two Star’ were used for the RL and GL trials, respectively. The trials were conducted in four, “caterpillar” HTs (39.6 m long x 3.7 m wide x 2.1 m high) in a split-plot randomized complete block design. There were four individual HTs and each served as a replication as described by Gude (2020 Ch. 2). Two beds ran lengthwise in each HT (39.6 m long x 0.61 m wide).

The six main plot coverings were randomly assigned to 6.1 m long plots within each HT and an additional 2.1 m buffer area was implemented at the end of each HT. The red and green lettuce types were randomly distributed between the north and south beds within each tunnel and served as the sub-plots. The coverings included 1. standard poly (standard) that was rated for 92% PAR transmission and blocked radiation <350 nm [single-layer 6-mil (K-50 poly; Klerk's Plastic Product Manufacturing, Inc., Richburg, SC, USA)]. 2. Standard poly with poly removal (movable) 2 to 3 weeks prior to harvest to allow for full spectrum exposure of crops. The movable covering simulated a movable tunnel and allowed plants to be established in a protected environment and then be exposed to full spectrum light prior to harvest. 3. Diffuse poly (diffuse) blocked direct radiation of infra-red (IR) light and radiation <380 nm (Luminance; Visqueen Building Products, London, UK). 4. Clear poly (clear), had full spectrum radiation (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA). 5. UV-A/B blocking poly (block) blocked radiation <400 nm (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA). 6. 55% shade cloth + standard poly (shade) reduced light intensity and canopy temperature (Sunblocker Knitted Shade; FarmTek, Dyersville, Iowa, USA). During both the spring and fall seasons, the poly over the movable covering was replaced at night when outdoor temperatures fell below 0 °C. Additionally, floating row covers (26 g/m) were added to all the beds at night when outdoor temperatures fell below -6 °C. These steps were put in to place to mitigate freezing damage to the crop, but careful steps were taken to ensure that any bias on light exposure was minimal.

4.2.2 Lettuce trials

Red 'New Red Fire' and green 'Two Star' lettuce (Johnny's Selected Seeds, Winslow, Maine, USA) were grown in trials that occurred during fall 2017 and 2018 and spring 2018 and 2019. Lettuce was seeded in the greenhouse into 72-cell propagation trays (3.8 cm diameter) (Pro-Tray 72 Cell Flats; Johnny's Selected Seeds, Winslow, Maine, USA) with potting mix. The lettuce was transplanted in a staggered double row within each bed (26.7 cm between plant, 26.7 cm between rows).

In the fall, lettuce was seeded in the greenhouse Sept. 7, 2017 and Sept. 19, 2018 and transplanted into the HTs Oct. 6, 2017 and 24, 2018. During fall, shade cloth was utilized throughout the entire growing season for the shade cloth covering. Removal of the poly in the movable covering treatment occurred two weeks prior to harvest in 2017 and 2018 (Oct. 27 and Nov. 26, respectively). The fall lettuce season was approximately 4 weeks longer in 2018 due to field flooding that delayed planting, followed by cold winter temperatures that delayed plant growth.

In the spring, lettuce was seeded in the greenhouse Feb. 14, 2018 and Feb. 19, 2019 and transplanted into the HTs March 19, 2018 and Apr. 1, 2019. Shade cloth was added to the shade cloth covering on Apr. 9, 2018 and Apr. 15, 2019. Removal of standard poly over the movable covering treatment happened on the same dates.

If temperatures fell below 0 °C, the covering on the movable covering was replaced during the nighttime until temperatures rose. Additionally, floating row covers (26 g/m^2) were added to the beds at night when temperatures fell below -6 °C. Lettuce was harvested once it reached commercial size. Harvesting was performed in the morning by cutting the above ground part of the plant at the soil level using a harvesting knife (Harris Seeds, Rochester, New York, USA). In the fall, harvest took place 4 weeks after transplanting in 2017 (Nov. 10). In the 2018 fall trial, much lower temperatures were experienced and the plants were harvested 8 weeks after transplanting (Dec. 10). In the spring, harvest took place six weeks post-transplant in 2018 (May 3) and four weeks post-transplant in 2019 (May 10). Six plants were randomly selected from each plot at each harvest to measure marketable plant fresh weight (g/plant FW).

Lettuce was harvested in early morning by cutting the above ground part of the plant, including any outer whirl leaves, using a lettuce knife at the soil level (Harris Seeds, Rochester, New York, USA). Six plants were chosen randomly from each covering plot, within the four replications, placed in plastic bags, and transported in an air-conditioned vehicle to the postharvest physiology lab at KSU-Olathe. Analysis occurred on day of harvest, day 0, and after 5 days of storage in optimum conditions of 1.5 °C and 90% RH in environmental chambers (Forma Environmental Chambers; ThermoFisher Scientific Inc., Asheville, North Carolina, USA). Following color analysis, samples were combined by replications, lyophilized in the freeze dryer (Harvest Right, Salt Lake City, Utah, USA), and ground (Waring WSG30; Conair Corporation, Torrington, Connecticut, USA) for phenolic analysis.

4.2.3 Color

Color of RL and GL was measured on day 0 and day 5 after harvest. Color was determined based on three plants per replication and four measurements per plant. An undamaged outermost leaf was chosen from each plant and two measurements were taken on left and right side of the midrib, 1 to 3 cm from the tip (Ilić et al., 2017). Color measurements were made using an A5 Chroma-Meter (Minolta CR-400; Minolta Co. Ltd., Osaka, Japan). The instrument was calibrated with the Minolta calibration standard white reflector plate before sampling lettuce leaves. L*, a*, and b* readings were transformed to those of the L, a, b color space and finally to hue angle and chroma according to Setser (1984) and as recommended by McGuire (2019). Hue angle was expressed on a 360° color wheel where 0° and 360° represents red, 90° represents yellow, 180° represents green, and 270° represents blue. Chroma indicates color purity or saturation (high values are more vivid) (McGuire, 2019).

4.2.4 Standards, reagents, and equipment

For phenolic compound analysis, commercial standards were all of analytical grade and included caffeic acid, chicoric acid, chlorogenic acid, isoquercetin, rutin, and ferulic acid, as well as formic acid (purity > 99%) purchased from Acros Organics (Geel, Belgium). The stock standard solutions of individual compounds (with 1000 μg/mL concentrations) were prepared with methanol and stored at -20 °C in dark bottles as described by Alarcón-Flores et al. (2013). Ammonium acetate, methanol, and ethanol (all HPLC grade) were purchased from VWR (VWR, Radnor, PA, USA). Equipment used includes an analytical balance (Mettler Toledo, Columbus, OH, USA), sonicator (Ultrasonic Bath; Fisher Scientific, Hampton, NH, USA), and centrifuge (Avanti J-E; Beckman Coulter, Indianapolis, IN, USA).

4.2.5 Extraction and analysis of phenolic compounds

Each of the four replicates of the covering was comprised of three lettuce plants and was extracted and analyzed in a darkened room with a red safety light to avoid oxidation of the analytes, following the procedure of Vallverdú-Queralt et al. (2013), with some modifications. Lyophilized lettuce (0.2 g) were homogenized with 4 mL of extraction solution (ethanol/water, 80/20, v/v), vortexed (20 s), sonicated (5 min), and centrifuged (4000 rpm, 15 min, 4 °C). The supernatant was transferred into a test tube and the extraction was repeated. Both supernatants were combined and evaporated to dryness under nitrogen flow (2-6 ppm) and recovered with 4 mL of 30 mM ammonium acetate in de-ionized (D.I.) water with 5 pH adjusted with formic acid (eluent B) and filtered through a 25 mm 0.22 μm filter (Supor; VWR, Radnor, PA, USA) into several 1.5 mL Eppendorf tubes for reserve and the sample extract was stored in darkness at -70 °C until analysis. Prior to analysis, a portion of each extracted sample was thawed, vortexed and 100 μL sample extract was added to 900 μL 50:50 eluent A (methanol + 0.1% formic acid):eluent B (10% dilution) for injection.

The analysis was carried out using a method adapted from Alarcón-Flores et al. (2013). Samples of 3 uL were injected in to the Waters Acquity UPLC System (Waters Co., Milford, MA, USA), equipped with a binary solvent manager (part number: 186015001), a column manager (part number: 186015009), a sample manager (part number: 186015005), a photodiode array UV/vis detector (PDA part number: 186015026), and a QDa mass detector (part number: 186006511) for further confirmation, using Empower 3 chromatography data software. The separation was achieved on Waters Acquity Ethylene Bridged Hybrid (BEH) C18 column (100 mm x 2.1 mm, 1.7 µm particle size) at 30 °C. Samples were kept at 10 °C in the sample manager. Eluting peaks were monitored at 254 nm and 325 nm. The elution was performed with 5% A for 1.5 min, and a linear gradient was then installed to reach 30% A at 4 min, 85% A at 8 min, and 100% A at 10 min. These conditions were maintained for 2 min, before being returned to the initial conditions in 30 s. The flow rate was set at 0.2 mL/min. The phenolic compounds isoquercetin, rutin, caffeic acid, chicoric acid, chlorogenic acid and ferulic acid, were quantified, related to their corresponding standard based on retention time, and confirmed by their absorption spectrum in UV. Results were expressed as mg/kg dry weight (DW). For each extract, three subsamples were repeatedly made.

4.2.6 Statistical analysis

Data for the two lettuces were analyzed separately, and subjected to linear mixed model analysis. These models use two types of explanatory variables: fixed effects, which affect the mean of the response variable; and random effects, which affect the variance of the response. The fixed effects of the linear mixed model for color were season, year nested within season, covering, storage day, season x covering, season x storage day, storage day x covering, and season x storage day x covering. Random effects of the model include rep x year x season, and rep x covering x year nested within season. The values are means (±SE).

The phytochemical data was subjected to natural log (ln) transformation before subjected to linear mixed model analysis. With phytochemical compound analysis, assuming log transformation is common to normalize the data. The fixed effects of the model are season, year nested within season, covering, storage day, season x covering, season x storage day, storage day x covering, and season x storage day x covering. Random effects of the model include rep x year nested within season, and the rep x covering x year nested within season interaction. Within a season and storage day, the multiple comparison procedure was carried out using Tukey's method at the 0.05 significance level. Also, within a season, storage day effect for each covering was evaluated. Back-transforming the LSmean differences to the original scale corresponds to the ratio of medians. Statistical analysis was executed via Statistical Analysis Software (SAS version 9.4; Cary, NC) PROC MIXED with option DDFM=KR in MODEL statement.

4.3 Results

4.3.1 Color

Color was analyzed to examine the effects and interactions of season, HT covering, and storage days in RL and GL. The hue angle of RL differed significantly by season, covering, and storage day (*P* <.001, <.001, and <0.05, respectively; Table 4.1). Similarly, the chroma values of RL differed significantly by season, covering, storage day, and interaction were observed between season x storage day (*P* <.001, <.001, <.001, and <0.05). The chroma and hue of fall RL was darker (lower chroma and hue values) compared to the spring.

Table 4.1 Probability values[a] of effects and their interactions on chroma and hue angle of red 'New Red Fire' and green 'Two Star' leaf lettuce grown in high tunnels[b] in Olathe, KS in the fall (2017 and 2018) and spring (2018 and 2019) at the day of harvest and on day 5.

Parameter	Lettuce color	Season (S)	Covering (C)	S x C	Storage Day (D)	S x D	C x D	S x C x D
Chroma	Red	<.001	<.001	ns	<.001	<0.05	ns	ns
	Green	ns	<.001	<0.05	<0.01	<.001	ns	<0.05
Hue °	Red	<.001	<.001	ns	<0.05	ns	ns	ns
	Green	<.001	<0.01	<0.05	ns	<0.01	<0.05	<0.05

[a] linear mixed model was used to test if effects and interactions had significant effect on the examined parameter (P ≤0.05).

[b] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly, standard poly with removal two weeks prior to the first harvest, diffuse poly, clear poly, UV-A/B blocking poly, and 55% shade cloth over standard poly.

During the spring RL trials, the hue angle of lettuce grown under the shade covering was higher than the ones under the movable and clear coverings (Fig. 4.1). The fall RL hue angle was highest under the shade covering and it differed significantly from the movable, diffuse, and clear coverings at day 0, and from the clear and block at day 5. The chroma of spring and fall RL under the shade covering was higher than all other tested coverings at harvest. After 5 days storage during the spring- the RL chroma decreased under the diffuse, clear, block, and shade coverings (*P* <0.05, <0.01, <0.05, and <0.01, respectively). During the fall, the block covering decreased significantly after 5 days of storage for hue angle and chroma (*P* <0.01, and <0.05).

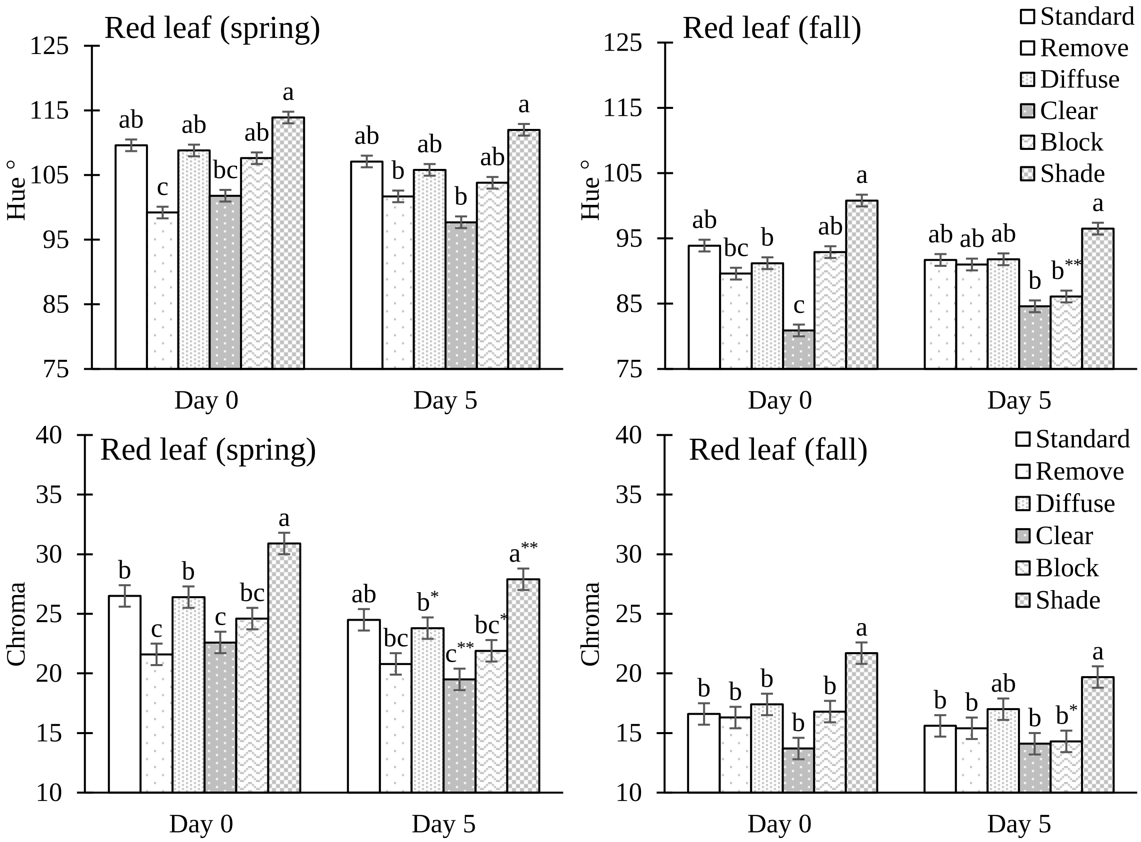

Figure 4.1 Effect of high tunnel covering on red 'New Red Fire' leaf color (hue angle [$\tan^{-1}(b^*/a^*)$] and chroma $(a^{*2} + b^{*2})^{0.5}$). Columns with same letter (per day) do not differ significantly between coverings at $P \leq 0.05$, Tukey's HSD. Within each covering, $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq .001$ denotes significant differences between days. Coverings include: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade).

The GL hue angle differed by season and covering ($P < .001$, and < 0.01; Table 4.1). Hue angles in the spring GL were significantly higher under the standard, block, and shade coverings, than the other tested coverings at harvest (Fig. 4.2). Similarly, the GL chroma was affected by covering and storage day ($P < .001$, and < 0.01). Chroma for the spring GL was statistically similar between the standard, block, and shade coverings, which was higher than the movable and clear coverings. By day 5, hue angle and chroma of spring-grown GL increased for the movable, diffuse, and clear coverings.

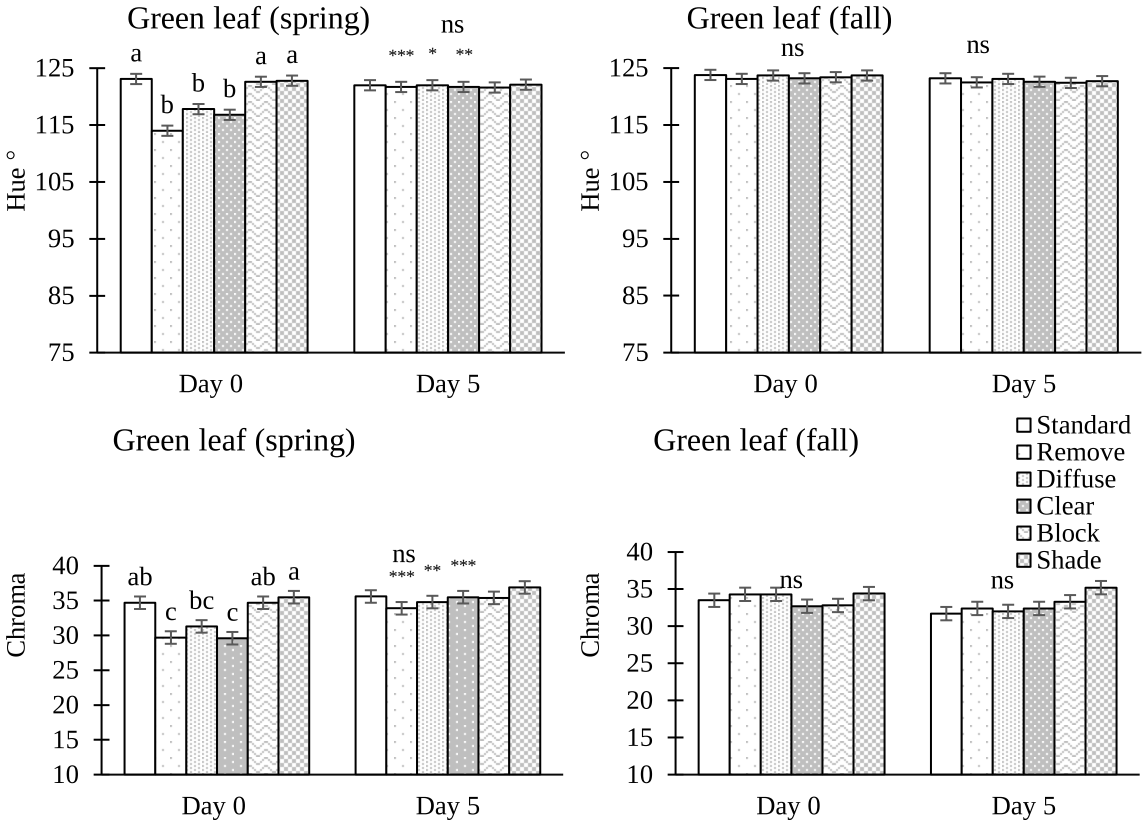

Figure 4.2 Effect of high tunnel covering on green 'Two Star' leaf color (hue angle [tan^{-1} (b*/a*)] and chroma (a*^{2} + b*^{2})$^{0.5}$). For each day, columns with same letter do not differ significantly between coverings at $P \leq 0.05$, Tukey's HSD. Within each covering, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq .001$ denotes significant differences between days. Coverings include: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade).

4.3.2 Phenolic acid compound accumulation of red leaf lettuce

Individual phenolic acids were analyzed to examine the effects and interactions of season, HT covering, and storage day in RL and GL (Table 4.2).

Table 4.2 Probability values[a] of effects and their interactions on phenolic acid concentration of red 'New Red Fire' and green 'Two Star' leaf lettuce grown in high tunnels[b] in Olathe, KS in the fall (2017 and 2018) and spring (2018 and 2019) at the day of harvest and on day 5.

Parameter	Leaf color	Season (S)	Trt (T)	Storage Days (D)	(S x T)	(T x D)	(S x T x D)
Caffeic acid	Red	ns	<0.05	ns	ns	ns	ns
	Green	ns	ns	ns	<0.05	ns	ns
Ferulic acid	Red	<.001	ns	ns	ns	ns	ns
	Green	ns	ns	ns	ns	ns	ns
Chlorogenic acid	Red	<.001	<0.05	<.001	ns	<0.05	ns
	Green	<0.01	ns	<0.05	<0.01	<0.05	<.001
Chicoric acid	Red	<.001	ns	ns	ns	ns	ns

	Green	<0.05	ns	ns	<0.05	<0.01	<.001

[a] linear mixed model was used to test if effects and interactions had significant effect on the examined parameter (P ≤0.05).

[b] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly, standard poly with removal two weeks prior to the first harvest, diffuse poly, clear poly, UV-A/B blocking poly, and 55% shade cloth over standard poly.

There was an effect of HT covering on caffeic accumulation for RL (*P* <0.05); however, this difference did not exist after storage when nested within season (Table 4.3). Chlorogenic acid and chicoric acid were found to be the dominant phenolic compounds. After 5 days of storage the concentration of chlorogenic acid was decreased for all coverings The overall effect of covering x storage day was observed with fall chlorogenic acid, as concentration was higher under the diffuse, clear, and block coverings compared to the movable and shade coverings at day 5 (*P* <0.05).

During the spring, the caffeic acid concentration under the standard, movable, block and shade coverings decreased by day 5 (*P* <0.05, <0.01, <0.05, and <0.01, respectively). The chlorogenic acid concentration in the spring RL that was grown under the clear and shade coverings decreased by day 5 (P <0.05, and <0.01, respectively). During the fall, the caffeic acid concentration under standard, movable, clear, and shade covering increased by day 5 (*P* <0.01, <0.05, <0.05, and <0.05, respectively). The fall RL ferulic acid concentration under the movable and clear covering increased by day 5 (*P* <0.05, and <.001, respectively). Furthermore, a significant decrease in fall RL chlorogenic acid was noted for the standard and movable coverings after 5 day's storage (P <.001, and <.001, respectively). During the fall trials, the diffuse, clear, and block coverings approximately doubled their chicoric acid concentration after 5 days in storage (*P* <0.05, <0.05, and <0.05, respectively).

Table 4.3 Phenolic acid concentration (mg/kg DW) of red 'New Red Fire' leaf lettuce in plants grown in the fall (2017 and 2018) and spring (2018 and 2019) under six high tunnel coverings[a] at harvest and after 5 d in storage at 1.5 °C.

Lettuce (season)	Trt	Caffeic acid		Ferulic acid		Chlorogenic acid		Chicoric acid	
		Day 0	5	0	5	0	5	0	5
Red (spring)	Standard	10.2	5.8^{*b}	9	4.9	555	375.9	195.8	128.6
	Movable	12.7	5.8**	7.1	4	566.8	326.2	234.6	140.1
	Diffuse	6.6	6.6	5.8	3.6	535.4	394.1	164.8	188.4
	Clear	10.3	6.2	9.2	3.3	781.7	414.6*	208.4	172.2
	Block	11.1	6.1*	4.7	3.4	596.8	462.2	184.3	206.6
	Shade	8.2	4.1**	4	2.4	587.2	264.8**	197.6	144.4
	P-value	ns	ns	ns	ns	ns	ns	ns	ns
Red (fall)	Standard	6.1	10.8*	1.3	1.4	280.5	75.2 bc***	143.2	163.1
	Movable	5.7	10.2*	0.8	2.5*	184.1	66.6 c***	105.1	153.8

Diffuse	5	8	1.6	1.8	201.6	189.5 a	91.6	179.4^{*}
Clear	4.4	7.9^{*}	0.6	4.5^{***}	142.2	180 a	78.4	154.2^{*}
Block	4.7	6.7	1.7	2.1	160.2	165.3 ab	97.4	183.2^{*}
Shade	4.6	7.9^{*}	0.8	1.9	132.5	85.4 c	106.4	149.6
P-value	ns	ns	ns	ns	ns	<0.05	ns	ns

[a] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade). Columns with same letter do not differ significantly between coverings at $P \leq 0.05$, Tukey's HSD. DW, dry weight; Trt, covering. ns; not significant.

[b] For each covering within each compound, significant differences throughout storage are noted: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq .001$.

4.3.3 Phenolic acid compound accumulation of green lettuce

Season was found to be a prominent effect in GL with the spring season resulting in greater chlorogenic acid and chicoric acid accumulation (*P* <0.01, and <0.05; Table 4.2). The spring GL chlorogenic acid accumulation was higher under the standard and shade coverings than the diffuse covering at harvest (*P* <0.05; Table 4.4). However different from the spring lettuce, the fall GL chlorogenic acid accumulation was decreased under the shade covering relative to the others at harvest (*P* <.001). During the fall GL trials, the caffeic acid accumulation from the standard and clear coverings increased relative to the shade covering at harvest (*P* <0.01). On day 5 in the fall GL, the chicoric acid concentration was higher under the clear covering compared to the diffuse covering (*P* <0.05).

After 5 days in storage, the spring GL ferulic acid concentration decreased under the standard, movable, and diffuse coverings (*P* <0.05, <0.01, and <0.05, respectively). Similarly, the chlorogenic acid concentration decreased significantly by day 5 in the spring GL under the standard and diffuse coverings (*P* <0.05, and <0.05, respectively). In the spring GL, a large increase in chicoric acid was observed after 5 days under the diffuse covering (*P* <0.01). In the fall GL after 5 days storage, the chlorogenic acid concentration decreased under the standard, movable, and diffuse coverings (*P* <0.05, <0.01, and <0.05) and increased under the shade covering and (*P* <0.01). By day 5, the chicoric acid concentration in the fall GL decreased under the movable and diffuse covering (*P* <0.01, and <0.05), and increased under the block and shade coverings (*P* <0.05, and <.001).

Table 4.4 Phenolic acid concentration[a] (mg/kg DW) of green 'Two Star' leaf lettuce in plants grown in the fall (2017 and 2018) and spring (2018 and 2019) under six high tunnel coverings at harvest and after 5 d in storage at 1.5 °C.

Lettuce	Trt	Caffeic acid		Ferulic acid		Chlorogenic acid		Chicoric acid	
(season)		Day 0	5	0	5	0	5	0	5

Green (spring)	Standard	9.5	8.5	22	7*[b]	160.1 a	58.8*	131.7	89.2
	Movable	10.6	8.4	37.2	6.8**	60 ab	53.5	116	178.3
	Diffuse	9.7	11.3	24.2	6.9*	40.3 b	110.8*	60.1	247.9**
	Clear	9.6	8.8	28.1	11.4	132.4 ab	62.3	155.3	85.5
	Block	8.7	9.2	25.9	8.8	72.7 ab	58.5	83.3	102.5
	Shade	12.8	8.3*	18.6	8	140.3 a	69.7	154.7	127.9
	P-value	**ns**	**ns**	**ns**	**ns**	**<0.05**	**ns**	**ns**	**ns**
Green (fall)	Standard	12.8 a	9.5	4.7	15.9	106.5 a	35.8*	166.6	95.3 ab
	Movable	11.3 ab	9.1	12.9	16.3	93.1 a	24.5**	178	56.5 ab**
	Diffuse	9.9 ab	8.9	9	16.4	81.4 a	28*	118.4	48.1 b*
	Clear	12.1 a	12.3	8.2	20.9	48 a	55.4	82.5	168.5 a
	Block	8.3 ab	11.6	8.8	20.3	35.5 a	53.4	58.3	141.3 ab*
	Shade	6.7 b	8.3	9.9	16.9	9.3 b	40.7**	11.8	116.8 ab***
	P-value	**<0.05**	**ns**	**ns**	**ns**	**<.001**	**ns**	**ns**	**<0.05**

[a] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade). Columns with same letter do not differ significantly between coverings at *P* ≤0.05, Tukey's HSD. DW, dry weight; Trt, covering. ns; not significant.

[b] For each covering within each compound, significant differences throughout storage are noted: **P* ≤0.05, ***P* ≤0.01, ****P* ≤.001.

4.3.4 Flavonoid compound accumulation of red lettuce

The individual flavonoid compounds, isoquercetin and rutin, were analyzed to examine the effects of season, HT covering, and storage day of RL and GL (Table 4.5). Similar to RL phenolic acid accumulation, the spring season resulted in increased isoquercetin and rutin accumulation compared to the fall (*P* <.001, and <.001).

Table 4.5 Probability values[a] of effects and their interactions on flavonoid concentration of red 'New Red Fire' and green 'Two Star' leaf lettuce grown in high tunnels[b] in Olathe, KS in the fall (2017 and 2018) and spring (2018 and 2019) at the day of harvest and on day 5.

Parameter	Lettuce	Season (S)	Trt (T)	Storage Day (D)	(S x T)	(T x D)	(S x T x D)
Isoquercetin	Red	<.001	<.001	ns	ns	ns	ns
	Green	<.001	<0.01	<0.01	<.001	ns	ns
Rutin	Red	<.001	ns	ns	ns	ns	ns
	Green	<.001	ns	ns	ns	ns	ns

[a] linear mixed model was used to test if effects and interactions had significant effect on the examined parameter (P ≤0.05).

[b] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly, standard poly with removal two weeks prior to the first harvest, diffuse poly, clear poly, UV-A/B blocking poly, and 55% shade cloth over standard poly.

During the spring RL trials, the movable covering increased isoquercetin accumulation by 72% compared to the shade covering (*P* <0.01; Table 4.6). In the fall RL, rutin accumulation increased under the clear, block, and shade covering compared to the standard and movable coverings (*P* <0.01). The fall RL isoquercetin concentration increased under the movable covering compared to the shade covering on day 5 (*P* <0.05).

After 5 days of storage in spring RL, rutin decreased significantly for all coverings. The fall RL isoquercetin concentration decreased by day 5 under the shade covering (*P* <0.05). While the fall RL rutin concentration decreased significantly by day 5 under the clear, block and shade coverings.

Table 4.6 Flavonoid concentration[a] (mg/kg DW) of red 'New Red Fire' leaf lettuce in plants grown in the fall (2017 and 2018) and spring (2018 and 2019) under six high tunnel coverings at harvest and after 5 d in storage at 1.5 °C.

Season	Trt	Red leaf			
		Isoquercetin		Rutin	
		Day 0	Day 5	Day 0	Day 5
Spring	Standard	43.3 ab	26.1	15.9	4.3***[b]
	Movable	99.3 a	52	29.8	6.3***
	Diffuse	36.5 ab	50.7	13.1	4**
	Clear	60.4 ab	57.9	21.1	4.2***
	Block	40.8 ab	58.7	17	4.2***
	Shade	27.7 b	21.7	11.3	3.5**
	P-value	**<0.01**	**ns**	**ns**	**ns**
Fall	Standard	14.1	18 ab	2.2 b	3.4
	Movable	16.7	24.5 a	3.2 b	5.7
	Diffuse	12.7	19.8 ab	5.1 ab	6
	Clear	23.2	16.5 ab	12.2 a	3.3***
	Block	19.1	16.4 ab	10.3 a	2.7**
	Shade	15.7	8.1 b*	10.2 a	4.2*
	P-value	**ns**	**<0.05**	**<0.01**	**ns**

[a]Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade). Columns with same letter do not differ significantly between coverings at *P* <u>≤</u>0.05, Tukey's HSD. DW, dry weight; Trt, covering. ns; not significant.

[b]For each covering within each compound, significant differences throughout storage are noted: **P* ≤0.05, ***P* ≤0.01, ****P* ≤.001.

4.3.5 Flavonoid compound accumulation of green lettuce

In the GL, the isoquercetin and rutin accumulation increased in the spring, relative to the fall (P <.001, and <.001, respectively; Table 4.7). The shade covering increased in isoquercetin concentration by day 5 in fall GL (*P* <0.05).

Table 4.7 Flavonoid concentration[a] (mg/kg DW) of green 'Two Star' leaf lettuce in plants grown in the fall (2017 and 2018) and spring (2018 and 2019) under six high tunnel coverings at harvest and after 5 d in storage at 1.5 °C.

Season	Trt[a]	Green leaf			
		Isoquercetin		Rutin	
		Day 0	Day 5	Day 0	Day 5
Spring	Standard	49.0	47.4	22.7	13
	Movable	104.0	60.8	38.8	35.2
	Diffuse	40.2	52.5	22.4	20

	Clear	83.1	47.8	27.4	20.7
	Block	54.7	47.9	25.8	21.4
	Shade	45.4	40.3	17	10.8
	P-value	**ns**	**ns**	**ns**	**ns**
Fall	Standard	4.7	6.3	3.3	8.9
	Movable	6.5	11.1	5	4.3
	Diffuse	5.3	4.9	4.5	1.3
	Clear	7.2	16.5	11.9	11.9
	Block	8.9	21.6	21.1	4.5
	Shade	9.1	11.7*	11.9	4
	P-value	**ns**	**ns**	**ns**	**ns**

[a] Trial was arranged in a randomized complete block design, blocking by high tunnel and year. Covering includes the following 6 different coverings: standard poly (standard), standard poly with removal two weeks prior to the first harvest (movable), diffuse poly (diffuse), clear poly (clear), UV-A/B blocking poly (block), and 55% shade cloth over standard poly (shade). Columns with same letter do not differ significantly between coverings at $P \leq 0.05$, Tukey's HSD. DW, dry weight; Trt, covering. ns; not significant.

[b] For each covering within each compound, significant differences throughout storage are noted: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq .001$.

4.4 Discussion

Deep, red color intensity of the leaf pigmentation is an important characteristic when purchasing red leaf lettuce (Gazula et al., 2007). The chroma and hue angle were lower in the fall RL, resulting in darker leaves. The fall canopy temperatures were 6.3 to 17.7 °C, while the spring were 10.5 to 25.6 °C (Gude, 2020 Ch. 2), which may have contributed to the leaf darkening. Previous studies found that darker RL was observed when cultivated in cooler temperatures compared to warmer ones (Becker, Klaering, Schreiner, et al., 2014; Marin et al., 2015).

Hue angle and chroma in the spring RL was lowest under the movable and clear coverings. According to the hue angles, the shade covering presented the most green leaf coloring, while the clear and movable presented the reddest (Marin et al., 2015). Gude (2020 Ch. 2) showed that spring PAR transmission under the movable covering was 100% with no light obstruction, and lowest under the shade with only 32% PAR transmission. The standard had 88%, the clear had 79%, the block had 77% and the diffuse had 65% PAR transmission. Although the standard covering had higher PAR transmission than the clear, the standard covering absorbed UV-B, while the clear covering transmitted both UV-A and B. This suggests that the full UV radiation under the movable and clear covering may have contributed to the dark red pigmentation during the spring. Our findings of covering differences in lettuce color induction by UV-radiation and light intensity are in agreement with past reports (Marin et al., 2015; Shioshita et al., 2006; Sytar et al., 2018). Shioshita et al. (2006) found that the effect of UV radiation is more determinate of darker RL pigmentation compared to high light intensity

(PAR). Similarly, Dussi et al. (1995) found that exposure to lower wavelengths light resulted in darker, less chromatic and redder pear fruit. To our knowledge, this is the first study to report the effect of a movable covering on lettuce color development at harvest and after 5 days of storage. During the fall, it was the clear covering over the RL that had the lowest hue angle and chroma at day 0 (more red). In the fall, the low temperatures often required replacement of the standard poly over the movable covering (section 4.2.1), which may have reduced the movable covering effect of unobstructed light prior to harvest.

After 5 days in storage, the spring RL chroma reduced under the diffuse, clear, block and shade coverings resulting in less chromatic lettuce. The fall hue angle and chroma reduced under the block covering after 5 days in storage. Previous studies have found that higher PAR and temperature pre-harvest resulted in darkening (decreased hue) of skin color during postharvest storage of red sweet peppers (Selahle et al., 2015). However, this pattern was not observed in the present study, as PAR was most reduced under the diffuse and shade coverings (Gude, 2020 Ch. 2).

For the spring GL at day 0, chroma and hue angle increased under the shade, standard, and block coverings relative to the other tested coverings. Similarly, Ilić et al. (2017) found that a 50% black shade net increased chroma of a green lettuce at harvest compared to the open field control. However, this effect was not detected after 5 days in storage.

The phenolic compounds reported here included chicoric acid, chlorogenic acid and quercetin glycosides, which were previously identified as the prominent phenolic compounds in lettuce (Oh et al., 2011; Romani et al., 2002; Woolley et al., 2019). Our study focused on the effects of season and HT covering influences on these major lettuce phenolics at harvest and after 5 days in storage. Effects of covering on phenolic compound accumulation varied with season. Season was found to be the prominent factor with the spring lettuce accumulating greater phenolic acid accumulation in RL, with the exception of caffeic acid. The phenolic acid accumulation of ferulic acid, chlorogenic acid, and chicoric acid of the RL was significantly less during the fall. Similarly, the phenolic acid accumulation of chlorogenic acid and chicoric acid of the GL was also less during the fall. Typically, the fall seasons have lower levels of UV-radiation (McKenzie et al., 1996), PAR, and temperature (Gude, 2020 Ch. 2), which may have contributed to the decrease in secondary metabolites due to altered phenolic metabolism. Studies have shown RL phenolic compound accumulation to positively correlate with solar radiation and temperature; although, it has also been shown to vary with cultivar (Marin et al., 2015; Sytar et al., 2018).

Previous studies have shown that solar UV-radiation resulted in increased phenolic compound accumulation in HT lettuce (García-Macías et al., 2007; Oh et al., 2011; Turtola et al., 2005; Woolley et al., 2019; Zhao, Carey, et al., 2007; Zhao, Iwamoto, et al., 2007). During the spring, the RL phenolic acid accumulation of caffeic acid, ferulic acid, chlorogenic acid, and chicoric acid was highest under the UV-transparent coverings (movable or clear covering) at day 0. Chlorogenic acid accumulation of the spring RL grown under the clear covering was 32% greater than that under the diffuse covering, although the differences were not significant. It has been suggested that dark red RL color is positively influenced by total phenolic accumulation (Sytar et al., 2018), which was found true in spring RL. In HTs, García-Macías et al. (2007) found that individual flavonoid and phenolic acid accumulation of lettuce under UV-transparent poly were greater than those grown under UV-reduce or UV-block poly coverings. To our knowledge, this is the first report to investigate the utility of a movable covering with phenolic compound accumulation in lettuce. However, a few studies have included pre-harvest light altering coverings into their trial design (Becker et al., 2013a; Hipol & Dionisio-Sese, 2014; Zhao, Iwamoto, et al., 2007). A study with accidental poly removal from HTs due to weather events, found that RL with 4 weeks of full solar exposure prior to harvest, resulted in the same antioxidant accumulation as the open field (Zhao, Iwamoto, et al. 2007).

Although the effect of covering in storage was inconsistent in the spring RL, caffeic acid and chlorogenic acid decreased for some coverings after 5 days. In the fall RL, chlorogenic acid decreased for some coverings by day 5, while caffeic acid, ferulic acid, and chicoric acid increased for some coverings by day 5. The decrease in chlorogenic acid has been well documented in tomato (Gautier et al., 2008; Slimestad & Verheul, 2005), and lettuce (Ferreres et al., 1997; Tomás-Barberán et al., 1997). It is suggested that the rise in levels of caffeic acid derivatives may happen at the expense of chlorogenic acid, due to chlorogenics high redox potential (Gautier et al., 2008). Another theory is that chlorogenic acid is a good polyphenol oxidase substrate (Ferreres et al., 1997). One study found that the total phenolic accumulation increased throughout optimum storage in romaine and red leaf lettuce grown in HTs, indicative of active phenolic metabolism throughout storage (Zhao, Carey, et al., 2007).

Unlike the RL, the spring GL chlorogenic acid accumulation increased under the shade and standard covering relative to the diffuse covering. Similar to the RL, the fall GL chlorogenic acid increased under all coverings except the shade covering. However, the fall standard poly covering resulted in 91% increase in chlorogenic acid compared to the shade covering. The fall GL chicoric acid accumulation under the movable covering was increased by 93% more than the shade covering, although the two were not significantly different. Furthermore, the fall GL caffeic acid accumulation increased under the standard and clear covering relative to the shade. In agreement, green lettuce total phenol accumulation decreased under a 55% black shade relative to a pearl shade cloth (Ilić et al., 2017). At day 5, the fall GL chicoric acid concentration increased under the clear covering compared to the diffuse. Compared to the red lettuce, it has been suggested that the green lettuce allocates less carbon to the biosynthesis of phenolics and more to biomass growth, which may explain the metabolite difference in cultivar (Becker et al., 2015).

It has been suggested that higher PAR and surface temperature pre-harvest, may result in phytochemical loss throughout storage due to increased plant respiration (Ntsoane et al., 2016). However, the effect of covering throughout storage was inconsistent for GL, as seen with RL. Previous studies have suggested that phenolic acid and total phenolic concentration remain stable or increase after storage of green lettuce (Ferreres et al., 1997; Zhao, Carey, et al., 2007), but we did not find this to be true.

Similar to the effect of season on phenolic acid accumulation, the flavonoids isoquercetin and rutin in the RL was significantly less during the fall. Similarly, the GL isoquercetin and rutin accumulation were also less during the fall. The fall solar radiation was decreased compared to the spring (Gude, 2020 Ch. 2), and studies have shown that solar UV-radiation activate genes involved in flavonoid biosynthesis (Fuglevand et al., 1996; Kubasek et al., 1992).

It was found that the flavonoid isoquercetin increased under the movable covering (full PAR and UV-transmission) compared to the shade covering in the spring RL, with rutin following a similar trend. Becker et al. (2015) also observed an increase in flavonoids, in response to the full solar spectrum. Similarly, Li et al. (2017) found that the quercetin derivative accumulation in red and green leaf lettuce decreased under a 50% black shade cloth relative to the open field. A growth chamber study tested the effect of switching RL from shade to no shade and vice versa on quercetin derivative accumulation by harvest (Becker et al., 2013). They found that the lettuce grown under shade and then moved to no shade 2 weeks before harvest had the same accumulation of isoquercetin and other quercetin derivatives as the lettuce grown under no shade throughout the full trial. In the fall RL, the shade covering resulted in a decreased isoquercetin concentration by day 5 in storage.

The UV-A/B block covering in the present study did not affect flavonoid compound accumulation of RL or GL in either season. In greenhouse conditions, UV-radiation increased the concentrations of flavonoids compared to lettuce grown under UV-B exclusion (Krizek et al., 1998). It has been suggested that perhaps endogenous flavonoids were depleted from other antioxidant scavenging functions, or perhaps the flavonoid inducing threshold of UV-B needed to was not reached in the ambient solar light (Krizek et al., 1997). Future work with larger scale-up capacity may consider assigning one covering per HT, to possibly amplify the effect of the covering on available solar light.

Similar to spring RL, the isoquercetin and rutin accumulation of the spring GL increased under the movable covering at harvest by 61% and 56%, respectively, compared to the shade. In agreement, Ilić et al. (2017) found that the black shade cloth resulted in a decreased total flavonoid content of green lettuce compared to a pearl colored shade cloth. In the fall, there was no noticeable trend for GL.

4.5 Conclusion

This research studied the effect of HT coverings on color, and phenolic acid and flavonoid concentration in the spring and fall seasons in red and green leaf lettuce. HT coverings that alter light intensity and UV, can potentially add value to protected salad crops, such as red and green lettuce. Overall effectiveness of covering varied by season. Both temperature and light intensity (PAR and UV-radiation) increased under all coverings in the spring compared to the fall, which may have contributed to the RL and GL phenolic compound decline in the fall. Based on the chroma and hue angle, we showed that pigmentation of RL was heavily influenced by solar light intensity and UV-radiation. In the spring, the movable and clear covering resulted in lettuce with more red/purple leaf pigmentation. In the fall, the RL clear covering hue angle stood apart from the other tested coverings, with the shade resulting in the least red/purple leaf pigmentation. Biosynthesis of phenolic compounds in the spring was enhanced by growing the crop under the clear and movable coverings that allowed a higher exposure to UV radiation. This was most pronounced with the isoquercetin and rutin accumulation of both RL and GL. However, chlorogenic acid concentration in the spring RL was highest under the clear covering with a 32% increase compared to the diffuse covering. Chlorogenic acid concentration decreased throughout storage for both RL and GL during the spring and fall trials. However, the effect of storage was mostly inconsistent and altered by season. A general decrease of isoquercetin was observed throughout storage of spring GL; however, a general increase of isoquercetin was observed throughout storage of fall GL. This study provides scope to consider clear poly or movable coverings in the production of red ‘New Red Fire’ and green ‘Two Star’ lettuce, to enhance phenolic compounds. Since phenolic compounds are known to play an important role as antioxidants in human nutrition, the UV transmission properties of these coverings may be important from a nutritional significance. With increasing consumer regard for antioxidant potential in food crops and increasing utilization of the HT system, growers may strategically choose coverings to increase solar intensity without adversely affecting yield.

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Chapter 5 - Effect of light characteristics on the sensory properties of red lettuce (Lactuca sativa)

Abstract

Production of leafy greens in high tunnels (HTs) results in increased yields, improves visual quality, and extends the production period. HT production systems also provide the opportunity to utilize various coverings such as polythlene (poly) film and/or shade cloth to maximize yield as well as phytochemical concentration. Light spectral quality and intensity influences phytochemical accumulation, which can be responsible for plant pigmentation and taste. The objective of this study was to examine the effect of various HT coverings on the sensory quality, microclimate, and physical quality of red leaf lettuce (*Lactuca sativa* cv. New Red Fire). The coverings included standard poly (standard), standard poly with removal 2 weeks prior to harvest to simulate a movable tunnel (movable), diffuse poly (diffuse), clear poly (clear), UV-A and UV-B blocking poly (block), standard poly with 55% shade cloth (shade), and no poly in the open field (open). Lettuce was produced at the Kansas State University Olathe Horticulture Center during fall 2017 as part of a larger study that examined the effect of HT coverings on yield, quality, and storage life. A highly trained descriptive panel evaluated the samples using a 15-pt scale from 0 (none) to 15 (extremely high). The sensory panel determined a list of 20 attributes to describe the appearance, texture, flavor, and mouthfeel of the lettuce samples. The color intensity attribute had the most differentiation between coverings and the open covering was higher (i.e. darker) than the others at 7.5 ($p<.0001$), followed by clear and movable coverings at approximately 6.8, while the block covering scored a 4.3, and the shade covering scored a 2. The instrumental color measurements showed similar trends of darker red leaves from the open, clear, and movable coverings. The initial crispness was similar for movable, standard, diffuse, and block coverings at approximately 5.3, which was higher than the open covering at 4 ($P < 0.01$). The open covering was less mature at harvest, which has shown to result in softer tissue. Water-like mouthfeel was another differing attribute amongst the studied coverings ($p<0.005$). Based on the results of this study, HT growers can implement specific HT coverings in order to cater to markets that value.

Keywords: Lexicon, visible light, ultra-violet light, hoop-house, season extension

5.1 Introduction

Fruit and vegetable consumption may lead to the reduction of certain diseases, due to their antioxidant properties (Wihelmina Kalt et al., 1999; Martin et al., 2000). The phenolic compounds are the largest category of phytochemicals that act as antioxidants scavenging free radicals, neutralizing them to prevent lipid oxidation (Babbar et al., 2014; Liu, 2004). Phenolic acids (such as caffeic acid) and flavonoids (such as anthocyanin and quercetin) act as antioxidants pre- and post-consumption in both plant tissues and consumers. In humans, they have shown to reduce arteriosclerotic plaques and inflammation (Wang & Goodman, 1999), and habitual consumption is associated with decreased mortality due to cardiovascular and cancer (Bondonno et al., 2019).

In plants, the phenolic compounds provide pigmentation, attract animals to consume, aid in seed dispersal, and also defend against pest attack with bitter flavor and astringent chemesthetic sensation (Brouillard & Dangles, 2017; Samuoliene et al., 2012; Schmelzer et al., 1988). Amongst the environmental factors that affect crop phytochemical quality, light characteristics (both solar intensity and spectral quality) are particularly important (Tsormpatsidis et al., 2008; Zhang et al., 2019). Light-induced production of flavonoids and phenolic acids in the outer tissues of plants provides a well-known protective mechanism against intense solar radiation (Takahashi et al., 1991b; Yatsuhashi & Hashimoto, 1985). Sensory properties are very important for the assessment of vegetable quality by consumers and for their purchase behavior. The red pigment in lettuce leaves is due to phenolic compound accumulation and is an important appearance attribute responsible for commercial value of red leaf lettuce (Gazula et al., 2007; Kader et al., 1973).

With recent growth in local food production, there has been an expansion of HT systems (Carey et al., 2009). HTs are utilized by growers for environmental protection, increased marketability and an extended production season (Lamont, 2009; Wells & Loy, 1993). HTs may be a more accessible and cost effective option for lettuce production as compared to a greenhouse (Janke et al., 2017). The most common cool-season crop grown in HTs is lettuce (Carey et al., 2009). Typically, HTs are covered with 6-mil polyethylene (poly) film that is UV-stabilized for durability. However, the HT system allows for a grower to select particular poly films and/or shade cloth in order to affect the spectral quality of light, microclimate and crop growth (Gude, 2020 Ch. 2), as well as phenolic compounds (Gude, 2020 Ch. 4; Oh et al., 2011; Tsormpatsidis et al., 2008).

Looking specifically at natural light sources, UV light obtained from solar radiation is a large factor affecting certain phenolic compound accumulation, and subsequent plant pigmentation and appearance (García-Macías et al., 2007; Goto et al., 2016; Luthria et al., 2006; Marin et al., 2015; Rajabbeigi et al., 2013; Shioshita et al., 2006; Tsormpatsidis et al., 2008). Shioshita et al. (2006) found that the red leaf lettuce grown under full solar UV-radiation had a deeper red pigmentation compared to the other coverings with partial UV transmission. In agreement, Goto et al. (2016), found in an environmentally controlled study that UV-B stimulated the biosynthesis of antioxidant polyphenols. Marin et al. (2015) reported that both solar radiation and temperature have a positive correlation with color and the phenolic acid and flavonoid content of three red leaf lettuces. They found that plant pigmentation positively correlated with total phenolic content and increased as the season progressed. In partial agreement, Gazula et al. (2007) suggested that low temperature, and not light, exerted the strongest positive influence on anthocyanin and red pigmentation in lettuce.

There have been a few studies that report the effects of HT vegetable production and its effect on sensory quality (Batziakas et al., 2019; Mølmann et al., 2015; Talavera-Bianchi, Chambers, Carey, et al., 2010). Talavera-Bianchi, Chambers, Carey, et al. (2010) examined pac choi grown with varying levels of conventional and organic fertilizer in both HT and open field production systems. A trained panel determined that pac choi from within the HT had higher intensities of most attributes (umami, crispness, sulfur, green overall or woody), regardless of fertilization management. Batziakas et al. (2019) studied the detectable differences between store-bought spinach, and locally grown spinach from both the HT and the open field. They observed that the overall likeness, flavor, and texture for HT grown spinach was preferred to open field grown or store-bought spinach in both a large consumer study and a descriptive panel.

This study examines the individual sensory attributes of the red lettuce grown under different HT coverings and compares them to soil temperature and leaf color. Identification of these attributes and the evaluation of lettuce quality will help future growers gain more information on how the light quality affects each of the examined sensory attributes. This study is a portion of a larger study that researched the effect of HT coverings on microclimate, productivity, phytochemical accumulation, and now- sensory attributes. The first objective of this study was to develop sensory profiles by a highly trained descriptive panel to describe color, flavor, texture and mouthfeel characteristics of red leaf lettuce to determine if any differences exist when grown under different HTs coverings. The second objective was to quantify the effects of the coverings on microclimate and coloration and compare them to the perceived sensory attributes.

5.2 Materials and Methods

Trials were conducted at the Kansas State University Olathe Horticulture Center, located in Olathe, Kansas. The trial was conducted in four “caterpillar” HTs that were 39.6 m long x 3.7 m wide x 2.1 m high. The construction of a homemade HT allowed for customization to suit the needs of the experiment in regards to plot size and tunnel length. The overall design of the caterpillar tunnel is a long and narrow with low ceilings, which provides an ideal structure for an experiment that specifically examines the impact of solar light. Each HT had a total of six covering plots (6.1 m long) and an additional 2.1 m “buffer” area at the ends of each HT. The lettuce trials utilized a split-plot RCBD, blocked by HT. Two beds ran lengthwise in each HT (39.6 m long x 0.61 m wide), and the lettuce variety, ‘New Red Fire’ (Johnny’s Selected Seeds, Winslow, ME), alternated between the north and south bed at every other tunnel. Lettuce was transplanted in a staggered double row within the bed (26.7 cm between plants, 26.7 cm between rows).

Each tunnel, or rep, included six individual plots that were randomly assigned the six coverings. An open field (open) bed was added adjacent to the HTs, but the replicates were not randomized, and is used for comparison purposes. The six HT coverings included commercially-available greenhouse and HT poly films as well as shade cloth. The standard poly (standard) was rated for 92% PAR transmission and blocked <350 nm [single-layer 6-mil (K-50 poly; Klerk’s Plastic Product Manufacturing, Inc., Richburg, SC, USA)]. Standard poly + removal (movable) allowed for plant establishment in a semi-protected environment before full solar exposure 2 weeks prior to harvest. The movable covering simulated the potential use of a movable tunnel (Gude, 2020 Ch. 2). Diffuse poly (diffuse) removed direct radiation of infra-red (IR) light and blocked <380 nm (Luminance; Visqueen Building Products, London, UK). Clear poly (clear) did not contain a UV-inhibitor (6-mil Clear Plastic Sheeting; Lowes, Mooresville, NC, USA). UV-A/B block poly (block) blocked <400 nm (Dura Film Super 4; BWI Companies, Inc., Nash, TX, USA). A 55% shade cloth + standard poly underneath (shade) reduced plant temperature

(Sunblocker Knitted Shade; FarmTek, Dyersville, Iowa, USA). The HT coverings, codes, and expanded descriptions are seen in Table 5.1.

Table 5.1 Polyethylene (poly) coverings in high tunnel system and corresponding codes and descriptions for the studied lettuce samples.

Covering	Code	Description
Standard poly	Standard	Single layer, 0.15 mm rated for 5 year lifetime.
Standard poly (removal 2 weeks prior to harvest)	Movable	Allows full spectral light once removed.
Diffuse poly	Diffuse	Removes direct radiation of IR light, reducing leaf temperature, deepens light penetration.
Clear poly	Clear	Allows full spectral light without much filtration, increased rate of degradation because doesn't contain UV-inhibitor.
UV-A/B Blocking (380-400 nm)	Block	Slows degradation of plastic.
55% Shade Cloth + Standard poly	Shade	Reduces light intensity and temperature.
Open Field	Open	Allows full spectral light without filtration.

5.2.1 Lettuce Trials

Lettuce was seeded Sept. 7, 2017 into 72-cell propagation trays (4 cm diameter) (Pro-Tray 72 Cell Flats; Johnny's Selected Seeds, Winslow, Maine, USA) with potting mix and transplanted four weeks later (Oct. 6, 2017). Common cultural methods for the region were practiced during production (Buller, Rivard, & Oxley, 2016). Lettuce was harvested the morning of Nov. 7 at random within each plot, bagged, and boxed in coolers of ice for transport to the testing facilities in Manhattan, KS for sensory evaluation in an air-conditioned vehicle.

This study used the methods described in the manuscript, "Lexicon to Describe Flavor of Fresh Leafy Vegetables" (Talavera-Bianchi, Chambers, & Chambers, 2010). The study was conducted in six days: two days for orientation and lexicon development, and the remaining four days for evaluation of the samples in triplicate (90 min for each day). Two lettuce plants were used for each replication (a total of 52 lettuce plants were used for the entire study).

5.2.2 Lexicon development and evaluation procedure

A highly trained descriptive panel (n=5) composed of four females and one male between 58 and 77 years of age from the Center for Sensory Analysis and Consumer Behavior at Kansas State University (Manhattan, KS) evaluated the samples. These panelists completed at least 120 hr of training and had a minimum of 2,000 h of sensory testing experience. More specifically, panelists had previous experience in the evaluation of vegetables and other vegetable products

(Batziakas et al., 2019; Talavera-Bianchi, Chambers, & Chambers, 2010). During the development of the lexicon, the panelists were asked to examine terms that had previously been developed to describe the sensory attributes of a wide variety of green vegetables, including lettuce (Talavera-Bianchi, Chambers, & Chambers, 2010), as well as terms used to describe "green" aroma in foods (Hongsoongnern & Chambers, 2008). Starting from this previous work, the existing lexicons were adjusted to only include terms applicable to lettuce. Batziakas et al. (2019) used the same panelists as well as the previously mentioned lexicons with minor adjustments for fresh spinach. This early work with spinach helped the panel in the development of the lexicon used in this study with lettuce; both studies used fresh, leafy vegetables and the lexicons were mostly similar. Development sessions lasted 90 min and up to six samples were evaluated in each session. Several products were reviewed during the development phase to adjust the lexicon and familiarize the panel with the product category. Table 5.2 shows a complete list of the attributes used for evaluation, including the attribute definitions, references, and intensities for attributes not obtained from (Talavera-Bianchi, Chambers, & Chambers, 2010).

Table 5.2 Sensory categories and attributes used to describe color, flavor and texture of red leaf lettuce.

Category	**Attributes**	**Definition**	**Reference**
Appearance	Color Intensity (Redness)	Intensity or strength of the color from light to dark.	Pantone color chips 2042U = 8.0
Texture	Initial Crispness	The intensity of audible noise at first bite with the molars.	Fresh Baby Spinach Leaf =2.5 Snow Pea = 8.0 Fresh Spinach – Place 5 in Ziploc Snack bags; Snow Pea – Serve 3 in Ziploc Snack bags; Fold leaf in half from leafy end to stem end. Take bite at center of fold.
Flavor	Green, Overall	Aromatic characteristics of plant-based materials. A measurement of the total green characteristics and the degree to which they fit together. Green attributes include one or more of the following: green-unripe, green-peapod, green-grassy/leafy, green-viney, and green-fruity. These may be accompanied by musty/earthy, pungent, astringent, bitter, sweet, sour, floral, beany, minty, and piney.	(Talavera-Bianchi, Chambers, & Chambers, 2010)
	Green, Peapod	A green aromatic associated with green peapods and raw green beans; characterized by increased musty/earthy.	

	Green, Grassy/Leafy	A green Aromatic associated with newly cut-grass and leafy plants; characterized by sweet and pungent character.
	Green, Viney	A green aromatic associated with green vegetables and newly cut vines and stems; characterized by increased bitter and musty/earthy character.
	Lettuce	Green, slightly musty and sometimes bitter water-like aromatics associated with lettuce like Bibb and Iceberg.
	Spinach	The brown, green, slightly musty, earthy aromatics associated with fresh spinach.
	Parsley	The clean fresh green, bitter, pungent aromatics associated with fresh parsley.
	Woody	Brown, musty aromatics associated with very fibrous plants and bark.
	Musty/Earthy	Aromatics associated with damp, wet soil
	Sweet, Overall	Aromatics associated with the impression of sweet substances such as fruit or flowers.
	Sour	The fundamental taste sensation of which citric acid is typical.
	Bitter	A basic taste factor of which caffeine is typical.
	Salty	The fundamental taste factor of which sodium chloride in water is typical.
	Umami	Flat, salty flavor enhances naturally occurring in some tomatoes.
Mouthfeel	Water-like	Liquid perception during mastication of some fruits and vegetables such as watermelon, peaches, tomatoes, and lettuce.
	Tooth-etch	A chemical feeling factor perceived as drying/dragging when the tongue is rubbed over the back of the tooth surface.
	Astringent	The drying, puckering sensation on the tongue and other mouth surfaces.
	Metallic	An aromatic and mouthfeel associated with tin cans or aluminum foil.

The evaluation method used was adapted from the flavor profile method (Caul, 1957; Keane, 1992). This method uses a panel consensus in which the panelists must come to an agreement on definitions, attributes, and reference products in the development of the lexicon. Sample evaluation was executed by panelists individually and in triplicate, using a 0-15 intensity scale with 0.5 increments, with 0 meaning "none" and 15 meaning "extremely high", and was compliant with ASTM standards (ASTM E3041-17, 2017)

The red lettuce samples were evaluated after two days in closed plastic bags in refrigerated storage (4 °C). For preparation, each lettuce sample was rinsed with deionized water and dried using a salad spinner. Random, similar leaves with no evidence of deterioration were chosen for evaluation (whole leaves without petiole removal). Each replicate for each lettuce covering was served in sample sizes of four to six leaves, and three replications were utilized in total. The leaves were served on 4-inch foam plates with random 3-digit codes to reduce bias. If needed, leaves were cut to fit onto the plates. To test the lettuce, panelists were instructed to fold the leaf in half through the middle and take one bite from the middle of the fold. Panel room had neutral colors, was well lit, ventilated, temperature controlled, and it was compliant with ASTM standards (ASTM E1871-17, 2017).

5.2.3 Soil temperature

Various aspects of the microclimate were observed in the trials and details are reported in Gude (2020 Ch 2). HT soil temperatures (°C) were continuously recorded with two probes (EL-USB-1; Lascar electronics, Erie, PA, USA) per plot were used for this purpose; the soil probe was buried 10 cm below the soil surface as done by Bumgarner et al. (2011). The probes were placed in the north row of the HTs in the center of each covering plot. All sensors were connected to a programmable data logger to record temperature in 30-minute increments. Sensors collected temperature from Oct. 27 to Nov. 19, 2017 (24 days).

5.2.4 Color

To determine leaf color, mature lettuce plants were harvested early in the morning, using a lettuce knife (Harris Seeds, Rochester, NY, USA) at the soil level to remove the full plant along with any outer whirl leaves, minus the root system. Color measurements were made using an A5 Chroma-Meter (Minolta CR-400; Minolta Co. Ltd., Osaka, Japan). The values include 4 reps with 3 plants per rep and 4 measurements per plant. Two measurements were taken on left and right side of midrib on an undamaged outermost leaf, 1 to 3 cm from the tip. Color results were expressed by the chromatic coordinates CIE L*, a*, b*, hue, and Chroma (Bakker & Timberlake, 1986).

5.2.5 Analysis

Analysis of variance (ANOVA) and Fisher's LSD (Least Significant Difference) were conducted on the dataset to determine significant differences between covering groups. The fixed effects of the 3-way ANOVA model were product, panelist, and replication. Multivariate analysis was also done in the form of a principal component analysis (PCA) and cluster analysis using the Ward method to assess distances and to further compare relationships between covering groups. The analysis was performed using XLSTAT 2018.5.52459 (Addinsoft, New York, NY, USA).

The temperature and color response parameters were analyzed under the linear mixed model. Fixed effects of the model were covering. The random effect of model is HT. Pairwise comparisons between coverings were performed based on the 2-sided test for non-zero difference in means. The adjustment for multiplicity was carried out using Tukey's method at the 0.05 significance level. The analysis was performed using JMP Software (JMP Pro 14.1.0; Cary, NC, USA).

5.3 Results and Discussion

A lexicon of 20 attributes (Table 5.2) was developed to describe appearance, texture, flavor, and mouthfeel characteristics of the red lettuce. Significant differences were found in eight attributes (color intensity, initial crispness, water-like, toothetch, parsley, woody, sweet overall and astringent) (Table 5.3). Full sensory profiles were generated for each covering.

Table 5.3 Descriptive analysis attribute mean[a] scores and *P*-value of red leaf lettuce grown in high tunnel systems under seven different plastic lighting coverings.

Coverings	Color Intensity	Water-Like	Initial Crispness	Toothtech	Green Overall
Open[d]	7.5 (0.1) a[b]	3.8 (0.3) bc	4.0 (0.3) c	2.7 (0.2) a	5.0 (0.2)
Clear	6.8 (0.1) b	3.6 (0.2) c	4.6 (0.3) bc	2.3 (0.1) bc	5.1 (0.2)
Movable	6.8 (0.2) b	4.3 (0.3) b	5.6 (0.3) a	2.7 (0.2) a	5.2 (0.2)
Standard	5.3 (0.3) c	5.1 (0.2) a	5.3 (0.2) ab	2.6 (0.2) ab	5.2 (0.2)
Diffuse	4.7 (0.3) d	4.2 (0.2) bc	5.5 (0.2) a	2.4 (0.2) abc	5.1 (0.2)
Block	4.3 (0.1) d	4.1 (0.3) bc	5.0 (0.2) ab	2.3 (0.1) c	5.1 (0.2)
Shade	2.0 (0.0) e	3.8 (0.3) bc	4.6 (0.3) bc	2.6 (0.1) a	4.8 (0.2)
P-value	< 0.0001	0.0005	0.0004	0.0164	ns[c]
Coverings	Green Peapod	Green, Grassy/ Leafy	Green Viney	Lettuce	Spinach
Open	1.9 (0.3)	3.9 (0.2)	2.5 (0.2)	4.3 (0.1)	1.8 (0.3)
Clear	2.2 (0.4)	4.2 (0.3)	2.4 (0.4)	4.6 (0.2)	2.0 (0.3)
Movable	2.5 (0.3)	4.3 (0.2)	2.2 (0.3)	4.6 (0.1)	1.7 (0.3)
Standard	2.5 (0.4)	4.5 (0.2)	2.7 (0.3)	4.4 (0.2)	1.9 (0.3)
Diffuse	2.5 (0.3)	4.2 (0.3)	2.4 (0.3)	4.5 (0.2)	1.6 (0.3)
Block	2.3 (0.3)	4.0 (0.3)	2.0 (0.3)	4.5 (0.1)	1.9 (0.3)
Shade	2.8 (0.3)	4.1 (0.2)	2.4 (0.3)	4.1 (0.2)	1.7 (0.3)
P-value	ns	ns	ns	ns	ns
Coverings	Parsley	Woody	Musty Earthy	Sweet Overall	Sour
Open	1.9 (0.2) c	2.2 (0.3) cd	2.9 (0.3)	0.3 (0.2) c	0.7 (0.2)
Clear	2.5 (0.2) a	2.2 (0.3) d	2.4 (0.4)	1.2 (0.3) ab	0.8 (0.2)
Movable	2.1 (0.2) abc	2.3 (0.3) bcd	3.0 (0.3)	1.1 (0.2) ab	0.5 (0.2)
Standard	2.5 (0.2) ab	2.7 (0.3) ab	3.0 (0.4)	0.8 (0.2) bc	1.1 (0.2)
Diffuse	2.0 (0.3) bc	2.8 (0.2) a	3.1 (0.2)	1.8 (0.3) a	0.4 (0.2)
Block	2.1 (0.2) bc	2.6 (0.2) abc	2.7 (0.3)	1.3 (0.2) ab	0.5 (0.2)
Shade	1.8 (0.3) c	2.4 (0.3) abcd	2.8 (0.4)	1.1 (0.2) b	0.6 (0.2)

P-value	0.0147	0.0241	ns	0.0038	ns
Coverings	Bitter	Salty	Umami	Astringent	Metallic
Open	5.9 (0.2)	0.2 (0.1)	2.6 (0.2)	1.9 (0.2) a	0.3 (0.2)
Clear	5.5 (0.2)	0.5 (0.2)	3.1 (0.2)	1.5 (0.2) bc	0.7 (0.2)
Movable	5.7 (0.2)	0.3 (0.2)	3.2 (0.2)	1.7 (0.2) abc	0.7 (0.2)
Standard	6.1 (0.1)	0.2 (0.1)	2.6 (0.2)	1.9 (0.1) ab	0.5 (0.2)
Diffuse	5.5 (0.2)	0.2 (0.1)	3.1 (0.2)	1.6 (0.2) abc	0.6 (0.2)
Block	5.6 (0.2)	0.3 (0.2)	3.5 (0.2)	1.4 (0.2) c	0.4 (0.2)
Shade	5.6 (0.3)	0.3 (0.2)	2.8 (0.3)	1.6 (0.2) abc	0.5 (0.2)
P-value	ns	ns	ns	ns	ns

[a] Data are LSmean values (SE) on a 15-point scale.

[b] For each attribute not sharing the same letter within the same column were significantly different at $P < 0.05$ (Fisher's protected LSD).

[c] ns is not significant.

[d] Coverings: open field (open); clear poly (clear); standard poly with removal two weeks prior to harvest (movable); standard poly (standard); diffuse poly (diffuse); UVA + UVB blocking (block); standard poly with shade cloth (shade).

Regarding flavor, the "green" flavors in this study included green-overall, green-peapod, green-grassy/leafy, and green-viney. Of the green flavors listed, the mean intensity scores of each covering were low to moderate and not significant (Table 5.3). In addition to the "green" flavors, three common leafy vegetables were used as reference on the scale for comparison: lettuce, spinach, and parsley. The samples scored low on spinach and parsley, and moderately on lettuce flavor. For parsley flavor, it was noted that clear and standard coverings scored high in comparison to open and shade coverings (p<0.0147). The flavor attributes of woody and musty/earthy scored low for all coverings. However, the woody attribute was high for the diffuse covering in comparison to open, clear, and movable coverings. Other terminologies, such as sweet-overall, sour, bitter, salty and umami, were used to describe taste. All lettuce samples scored very low for all of these attributes, except for the "bitter" category, where it scored moderately. However, no significant differences were noted between coverings, with the exception of overall sweetness. Diffuse scored significantly higher than standard, shade and open coverings.

The texture characteristic of initial crispness scored similarly between movable, standard, block and diffuse coverings and greater than open (p<0.004; Table 5.3). In the present study, the soil temperature of the open covering was cooler compared to all other coverings (p<.0001; Table 5.4). It has been reported that immature lettuce leaves have softer tissue (Martínez-Sánchez et al., 2012), which may have contributed to the perceived lack of crispness of the open covering by the sensory panel. It has been shown that increased soil temperatures in HTs result in increased lettuce yields (Bumgarner et al., 2011; Gude, 2020 Ch. 2; Wallace et al., 2012). A reason being is that environmental temperature plays a role in maturation (Gray & Morris, 1978; Singer et al., 2015). Gray & Morris (1978) found that lettuce sowed with outdoor temperatures below 11 to 12 °C took longer to mature. All tested coverings had statistically higher soil temperatures compared to open at 9.8 to 11 °C (Table 5.4), meaning that the open covering may have had an earlier maturity at harvest. For the mouthfeel characteristic, water-like, the standard covering scored statistically higher than all other coverings (Table 5.3). For toothetch, all samples scored low, and the open, movable, standard, and diffuse coverings were statistically similar and greater than the block covering. Mouthfeel characteristics, water-like and toothetch, were used to describe products that possessed "green" attributes (Hongsoongnern & Chambers, 2008; Talavera-Bianchi, Chambers, & Chambers, 2010), along with astringent and metallic mouthfeel. For both astringent and metallic, all lettuce coverings scored low. However, some differences were noted for astringent where the open covering provided lettuce with the highest astringency and was statistically greater than the clear and block coverings.

Table 5.4 Soil maximum and minimum mean temperatures (°C)[a] under high tunnel coverings[b] in Olathe, KS. Within the same column, means with different letters are different ($P \leq 0.05$), Tukey's HSD.

Coverings	Max		Min	
Open	11.0	c	9.8	c
Clear	14.0	ab	12.8	ab
Movable	12.5	b	11.8	b
Standard	13.5	ab	12.5	ab
Diffuse	13.6	a	13.3	a
Block	14.1	ab	12.4	ab
Shade	13.4	ab	12.3	ab
P-value		<.0001		<.0001

[a] Soil temperature probes added 10 cm below the soil surface, recording temperature in 30 min increments (2 probes per covering), from Oct. 27 to Nov. 19, 2017 (24 days).

[b] Trial was arranged in a split-plot RCBD, blocked by high tunnel, with the following 6 coverings randomly assigned within each tunnel: clear poly (clear); standard poly with removal two weeks prior to harvest (movable); standard poly (standard); diffuse poly (diffuse); UVA + UVB blocking (block); standard poly with shade cloth (shade). An open field bed (open) was adjacent to the high tunnels and replicated plots were not randomized.

Color intensity was the attribute that had the most differentiation between samples as compared to the others (p<.0001; Table 5.3). The shade covering was significantly lower in color intensity compared to the other coverings. The open covering scored significantly higher than the other coverings, but was followed closely by the clear and movable coverings. The open covering had direct sunlight exposure throughout the growth process, similar was the movable covering 2 weeks prior to harvest, and the clear covering transmitted both UV-A and UV-B. Clear and movable coverings maximized light exposure with photosynthetic active radiation

(PAR) at 85 to 100% transmission (Gude, 2020 Ch. 2). Previously, it has been shown that the amount of light exposure a plant receives during the growth process has the greatest impact on pigmentation (Agati & Tattini, 2010; Shioshita et al., 2006; Zhang et al., 2019; Zhao, Carey, et al., 2007). We found that open, movable and clear (100% UV and 100% light intensity) had lower L* values (i.e. more black), higher a* values (i.e. redder), and lower chroma values (i.e. darker in color) compared to the shade covering (Table 5.5). Similarly, Shioshita et al. (2006) found that UV-radiation is a greater determinate on coloration of red lettuce than overall light intensity. Specifically, plants were the darkest red with 100% UV-radiation in the open field, and intermediate in red coloration when grown with 50% UV-radiation under poly film with 50% shade cloth. The diffuse, standard, and block coverings were intermediate in their coloring. The shade covering scored lower for color intensity as it was covered with a shade cloth for the majority of its growth period. Zhao et al. (2007) found that baby spinach and baby pac choi grown within a HT covered with a 39% shade cloth resulted in a significant decrease in antioxidant accumulation in comparison to the open field counterplots. Because phenolic content is a major component to antioxidant accumulation (Howard et al., 2002), and contributes to pigment accumulation (Giovannoni, 2001), plant pigmentation/color may decrease with minimized direct light exposure (Zhang et al., 2019). A greenhouse study by Zhang et al. (2019), observed that red lettuce grown with supplemental light in addition to solar light for 7 d prior to harvest had darker red leaves than its counterpart without supplemental light.

Table 5.5 Effect of high tunnel covering[a] on color, based on L* (-black to +white), a* (-greenness to +redness), b* (-blue to +yellow), Chroma $(a^{*2}+ b^{*2})^{0.5}$, and hue angle $[\tan^{-1}(b^*/a^*)]$ of lettuce grown in Olathe, KS in fall 2017. Within the same column, means[b] with different letters are different ($P \leq 0.05$), Tukey's HSD.

Covering	**L***		**a***		**b***		**Chroma**		**hue (°)**	
Open	34.8	c	2.5	a	12.1	d	12.8	d	75.3	d
Clear	37.7	bc	-0.4	ab	16.3	c	16.6	c	89.6	c
Movable	38.1	bc	-1.4	bc	16.8	c	17.3	c	91.9	bc
Standard	38.5	b	-3.5	bcd	18.7	bc	19.4	bc	98.4	abc
Diffuse	40.7	b	-5.6	de	21.3	ab	22.2	bc	102.9	abc
Block	40.3	b	-4.3	cd	19.7	bc	20.5	bc	100.3	abc
Shade	45.3	a	-8.5	e	25.1	ab	26.7	a	107.7	abc
P-value		<.0001		<.0001		<.0001		<.0001		<.0001

[a] Trial was arranged in a RCBD, blocked by high tunnel, with the following 6 coverings randomly assigned within each tunnel: clear poly (clear); standard poly with removal two weeks prior to harvest (movable); standard poly (standard); diffuse poly (diffuse); UVA + UVB blocking (block); standard poly with shade cloth (shade). An open field bed (open) was adjacent to the high tunnels and replicated plots were not randomized.

[b] Values are means of 36 lettuce plants (4 measurements per plant, 3 plants per rep).

Furthermore, lower environmental temperatures have shown to increase plant pigmentation while slowing down plant maturation (Gazula et al., 2007; Marin et al., 2015; Mølmann et al., 2015). In the present study, the open covering had significantly lower soil temperatures, as previously discussed (Table 5.4), and scored the highest in color intensity (Table 5.3). In a baby red leaf lettuce study comparing climactic variables to plant pigment and phenolic content, Marin et al. (2015) found that lettuce harvested in February showed a longer growing cycle and was darker red than its warmer counterpart harvested in May. They noted a positive relationship between hue angle in relation to cold temperatures below 7.2 °C during the week before harvest. A study by Mølmann et al. (2015) with a highly trained sensory panel, observed many appearance-based differences with broccoli florets that were grown in controlled environmental chambers at two temperatures. Florets grown in lower temperatures resulted in a darker hue compared to those grown at a higher temperature. Furthermore, previous studies have shown pigment responsible anthocyanin content to increase in cool cultivated versus warmer-cultivated red lettuce (Becker, Klaering, Kroh, et al., 2014; Gazula et al., 2007; Marin et al., 2015). They hypothesized that the biosynthesis of phenolic compound responsible for pigmentation was a method to improve cold temperature tolerance; however, both Marin et al. (2015) and Gazula et al. (2007) found it to be species and cultivar specific in lettuce.

The largest differences were related to color intensity ($p<.0001$), initial crispness ($p<0.004$) and water-like attributes ($p<0.005$; Table 5.3). Those three attributes helped to formulate two clusters of coverings that were evident from the principle component analysis (PCA) and confirmed by cluster analysis under the Ward method (Fig. 5.1). The first cluster included lettuce grown within the open, clear, and movable coverings, this cluster is characterized by lettuce with the highest color intensity which explains about 73% of variability among coverings by itself. The second cluster was formed by samples from the diffuse, standard, and block coverings. This cluster was characterized by the samples with highest initial crispness and water-like attributes which explains about 13% of the total variability. These coverings may have a better-quality considering lettuce/green/crispness attributes influence consumer perception of product freshness (Cardello & Schutz, 2003). A single covering, shade, was not grouped into either cluster because it was different from the rest with low color intensity. Flavor attributes did not have a huge effect explaining the differences between coverings.

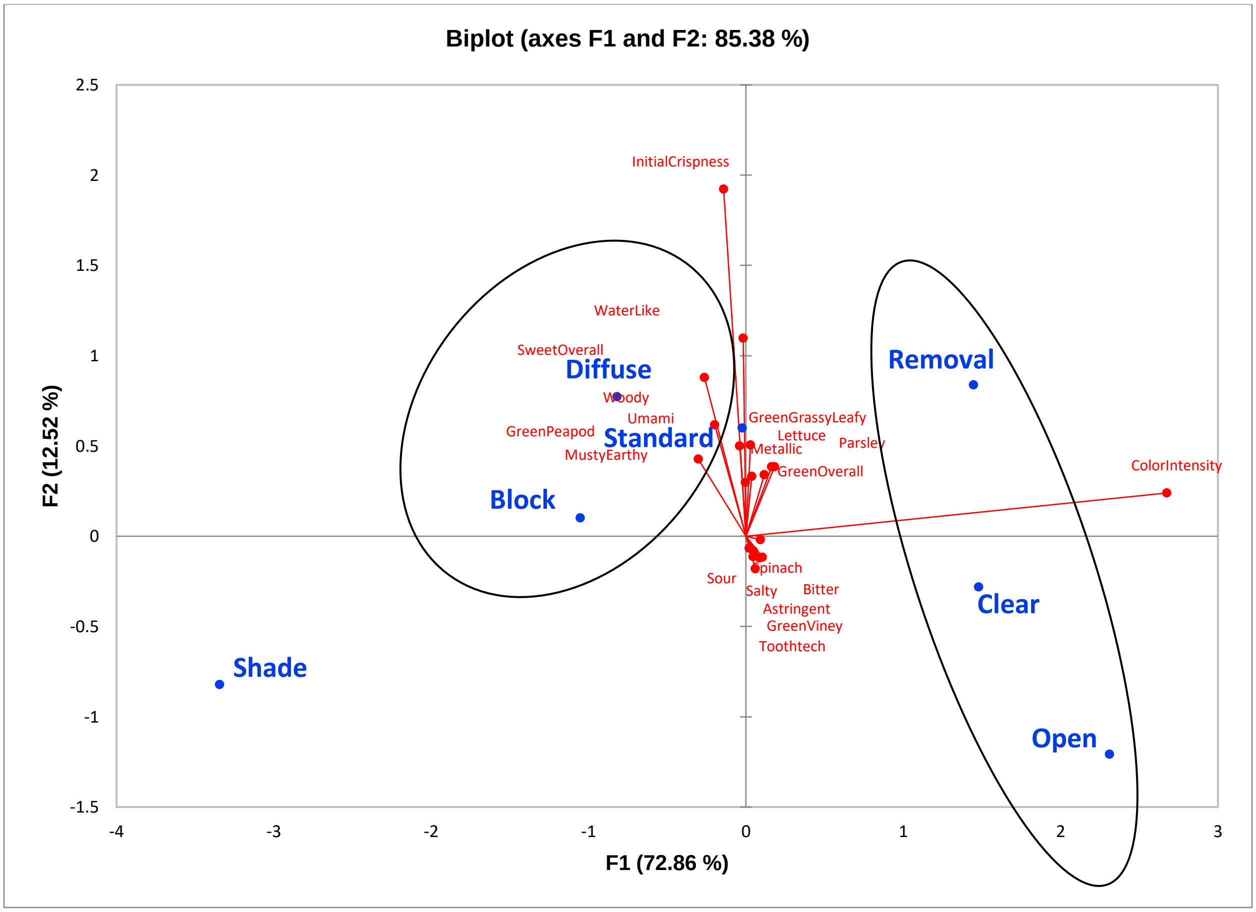

Figure 5.1 Representation of the polyethylene light coverings with principal component analysis (PCA) map of factor 1 (visual; movable, clear, and open) vs. factor 2 (texture; diffuse, standard, block). Covering codes: open field (open); clear poly (clear); standard poly with removal two to three weeks prior to harvest (movable); standard poly (standard); diffuse poly (diffuse); UVA + UVB blocking (block); standard poly with shade cloth (shade).

5.4 Conclusion

This study clearly shows that light and temperature variations during growth have significant effects on color intensity and texture of red lettuce, explaining most of the differentiation between coverings. Because consumers purchase based on appearance, and prefer redder red leaf lettuce- growers will want to design their HT system to promote leaf pigmentation. From an appearance perspective, open, clear and movable coverings provided the consumer a darker red leaf lettuce. However, clear and movable coverings also benefitted from the controlled environment while maintaining more pigmented tissue. The shade covering differed from the others, as it resulted in lettuce with the least red-pigment. From a texture and mouthfeel perspective, the diffuse, standard, and block coverings had high initial crispness and water-like attributes which is another important consumer quality. There was little difference in the flavor attributes between lettuce coverings. These results indicate that the spectral quality of ambient solar UV radiation alters perceived texture, mouthfeel, and visual parameters. Since these parameters are known to play an important role in commercial value of red leaf lettuce, covering materials may be of considerable importance from a sensory standpoint.

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Chapter 6 - Conclusion

This dissertation presented the results of four studies focused on altered light in a high tunnel (HT) system due to the covering and its effect on microclimate, productivity, and phytochemical content of tomato and lettuce, and sensory quality of red lettuce. The overarching purpose of the research was to improve the phytochemical content without adversely affecting yield.

The first study investigated the effects of HT coverings and season on the microclimate, yield, and productivity at harvest of tomato and red and green lettuce. Many previous studies have shown that HT systems are effective for increasing quality and yield of both lettuce and tomato. We wanted to identify any changes due to the tested HT coverings on the microclimate and yield parameters and compare the results between the two. The second study focused on the effect of the HT coverings on antioxidant content (AsA and phenolic compounds) of tomato harvested at breaker and light red maturity stage and ripened to the point of consumption. We wanted to assess the difference between the present day commercial practice, which is to harvest at mature green or breaker stage and artificially ripen the fruit versus the local market practice with a closer consumer base, which is to harvest at light red to red stage, and compare the differences by the point of consumption (POC). The third study examined the effect of HT covering, season, and storage day (day 0 and 5), on color and phenolic compound accumulation of red and green lettuce. We wanted to evaluate the tested coverings over the same crops in two distinct seasons. The fourth study determined any perceived sensory changes due to HT coverings of fall red leaf lettuce. Very few studies have researched the effect of HT production on sensory attributes of leafy greens.

Our results showed that the soil temperatures and PAR impacted yield parameters more than the other microclimate parameters. Higher soil temperatures in the three studied seasons, tended to result in greater yields for summer tomato, and spring and fall lettuce. The clear covering warmed the summer soil more than the other coverings, resulting in increased total yields for tomato. The shade covering cooled soil temperature in the spring and fall, and reduced PAR and yields in every season relative to the other coverings. While during the fall, the decrease in Pn and yields of both the red and green lettuce may have been due to decreased temperatures and PAR.

The most consistent effect due HT covering in phytochemical concentration during the tomato trials was that the clear covering resulted in increased amounts of AsA in both light red and breaker mature fruit, as well as chlorogenic acid concentration in breaker mature fruit. The light red harvested fruit at the POC increased the concentration of AsA by 10%, chlorogenic acid by 38%, quercetin by 8%, and rutin by 19% compared to the breaker harvested fruit at the POC. However, chlorogenic acid content was the only compound to decrease throughout storage and decreased 2-fold from one maturity stage to the next.

The fall red lettuce color was darker than the spring, but the phenolic acid and flavonoid accumulation (with the exception of caffeic acid) was higher in the spring than the fall in red lettuce. Similar results were observed with the spring green lettuce chroma coloration, along with increased chlorogenic acid, chicoric acid, isoquercetin, and rutin accumulation relative to the fall. Both temperature and light intensity (PAR and UV-radiation) increased under all coverings in the spring, which may have contributed to the red lettuce and green lettuce phenolic compound decline in the fall.

Both the movable and clear covering in the spring and the clear covering in the fall resulted in the most red/purple leaf pigmentation of the tested coverings. The shade covering resulted in the least red/purple leaf pigmentation. Biosynthesis of phenolic compounds in the spring was enhanced by growing the crop under the clear and movable coverings that allowed a higher exposure to UV radiation. This was most pronounced with the isoquercetin and rutin accumulation of both red and green lettuce. However, chlorogenic acid concentration in the spring red lettuce was highest under the clear covering with a 32% increase compared to the diffuse covering. As seen with tomato, chlorogenic acid concentration decreased after 5 days in storage for both red and green lettuces during the spring and fall trials. However, the effect of storage was mostly inconsistent and altered by season.

Similar to the instrumental analysis, from a visual appearance perspective, the clear and movable coverings provided the consumer a darker red leaf lettuce. These results indicate that the full spectral quality those two coverings altered the perceived visual attribute of color. The shade covering differed from the others, from a visual perspective, as it was the least red-pigmented covering tested. From a texture and mouthfeel perspective, the diffuse, standard, and block coverings had high initial crispness and water-like attributes that may be important to the consumer. There was little difference in the flavor attributes between lettuce coverings. This study clearly shows that light and temperature variations during growth have significant effects on appearance and texture of red lettuce, explaining most of the differentiation between coverings. Since these parameters are known to play an important role in commercial value of red leaf lettuce, solar light intensity and UV-radiation can be manipulated through HT coverings to enhance the red pigmentation.

Overall, the results indicate that HT coverings can be used as a tool to enhance phytochemicals in lettuce and tomato production in the central U.S. Since this production system is heavily utilized, growers can adopt strategic practices based on the crop and season to enhance antioxidant capacity. Although several compounds in this research increased throughout storage, more research is needed to address the concern of phenolic loss by consumption. This study provides scope to consider clear poly in the production of ‘BHN 589’ tomato and clear or a movable covering in the production of red ‘New Red Fire’ and green ‘Two Star’ lettuce, to enhance phenolic compounds. HT coverings that alter light intensity and UV, can potentially add value to these semi-protected crops. Since phenolic compounds are known to play an important role as antioxidants in human nutrition, the UV transmission properties of these coverings may be important from a nutritional significance. Future research will consider a clear poly covering that has been engineered for HT utilization. Future scale-up designs may include single covering designation per HT, in order to amplify the effect of HT covering on microclimate.