

Physiological Comparison of Three Southern Highbush Blueberry Cultivars Under High Tunnel and Open Field Conditions

by

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Abstract

Recent studies have shown that high tunnel production of blueberries generates higher biomass accumulation due to the microclimates created compared to open field production. This study aimed to evaluate the physiological responses and the effect on the growth and development of three southern highbush cultivars under high tunnel conditions and compare it to an open field production system. A total of 180 southern highbush blueberry plants of 'Farthing', 'Indigo Crisp', and 'Legacy' cultivars were planted in March 2022, under high tunnel and open field conditions. Ambient temperature, soil temperature, relative humidity, and daily light integrals were monitored in both conditions. Biomass accumulation, leaf area, and height were measured throughout the season, as well as physiological parameters through gas exchange analysis. The results showed that the microclimate created under the high tunnel affected plants differently across cultivars. Biomass accumulation for 'Legacy' was 16.4% higher in the open field compared to the high tunnel but biomass was not affected by condition for either 'Farthing' or 'Indigo Crisp'. However, all cultivars under the high tunnel showed expedited phenological progression during blooming. Diurnal measurements showed that plants under the high tunnel had a higher photosynthetic rate compared to outside conditions. Biomass accumulation coincides with the physiological measurements since 'Farthing' and 'Indigo Crisp' showed no differences in photosynthetic rate between conditions, and 'Legacy' had higher carbon assimilation outside of the high tunnel compared to plants under high tunnel conditions. This study provided insight into the importance of cultivar selection in high tunnel production. It also allowed a further understanding of the physiological responses of healthy plant growth and development under an alternative system of production.

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List of Abbreviations

HT	High Tunnel
SHB	Southern Highbush
In	Plants located under high tunnel conditions
Out	Plants under open field conditions
GDD	Growing Degree Days
DLI	Daily Light integrals
PPFD	Photosynthetic Photon Flux Density
A	Photosynthetic Rate
gsw	Stomatal conductance
E	Transpiration Rate
Ci	Intercellular Carbon
PhiPS2	Quantum efficiency

Chapter 1

Literature review

1.1 Introduction

The blueberry (*Vaccinium sp.*) production industry has had an extensive history, from traditional use by indigenous populations in the United States to the expansion towards global production (Bowling, 2000). This widespread cultivation has been made possible by breeding programs that have produced new varieties with improved traits that allow cultivation in a wider range of conditions and fruit quality variations (Retamales & Hancock, 2018). These breeding programs have also enabled the development of southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrid), which are now the predominant cultivated variety in many regions (Smrke et al., 2021) In addition, advances in post-harvest technology have allowed for better handling and preservation of blueberries, extending their shelf life (Perkins-Veazie 2016).

Blueberries are a valuable commodity today due to their high nutritional value and health benefits, leading to increased demand and market opportunities for blueberry growers (Retamales & Hancock, 2018). However, unpredictable weather patterns, disease pressure, and limited availability of pollinators are some of the challenges faced by blueberry producers. One potential solution to address these challenges is high tunnel production systems. High tunnels offer a controlled environment that can protect from these challenges, improve fruit quality, and increase development rates (Rodrigues-Saona et al., 2019; Fang et al., 2020).

The literature review will begin by providing a description of blueberry botany, morphology, growth, and development habits. In addition to discussing traditional cultivation practices, this literature review will explore alternative production systems for blueberries, and their potential advantages and limitations. The review will also examine the limits of

photosynthesis in blueberry production and how factors such as light intensity, and temperature affect plant growth and yield. Furthermore, the review will provide a global perspective on blueberry production, discussing how different regions have adapted to the challenges and opportunities of growing this crop. Overall, this literature review seeks to provide a comprehensive and up-to-date understanding of blueberry production, highlighting its potential and limitations, and identifying key research needs and future directions for this important industry.

1.2 Botany

Blueberries belong to the Ericaceae family and the *Vaccinium* genus, subgenus Cyanococcus. (Eck, 1966). Of the 400 species that belong to this genus, about 10% are native to the American continent (Darnell, 2006). About two-thirds are native to Malaysia, 70 are from Southeast Asia, 19 are from Japan, about 5 are from the Pacific region, 5 are from Africa, 6 are from Europe, 25 are native to South America, and 26 are native to North America (Vander, 1988). The species of commercial interest in North America are highbush (*V. corymbosum* L.), southern highbush (SHB) (*V. corymbosum* interspecific hybrid), lowbush (*V. angustifolium* Ait), and rabbiteye (*V. virgatum*) (Perkins, 2016). The *Vaccinium* genus has a basic genome of 12 chromosomes where many polyploids have arisen naturally due to the lack of fundamental sterility barriers between homoploid *Vaccinium* species, meaning that there are no genetic barriers that prevent different *Vaccinium* species from interbreeding and producing viable offspring (Eck 1966). These polyploids, particularly tetraploids ($2n = 48$), have contributed to the wide range of adaptation of the genus by selecting species better adapted to the region and with better fruit characteristics (Eck et al., 1990). The hybridization between these tetraploid subjects created the SHB varieties. This condition facilitates breeding opportunities to create varieties that produce high-quality and quantity berries for different growing conditions (Retamales & Hancock, 2018).

1.3 Morphology and development

1.3.1 Leaves and Stems

Blueberries are small to medium-sized shrubs, with a woody stem and deciduous or evergreen foliage (Darnell, 2006). The categorization of species is grouped by height parameters such as lowbush, half-high, and highbush. Lowbush blueberries won't reach a meter in height, highbush blueberries can range from 1.5 to 5m tall (Eck, 1966) while rabbiteye can reach up to 6m in height. Lowbush blueberries form a rhizomatous colony while rabbiteye and highbush varieties form more of a crown structure. For lowbush blueberries, new shoots are formed on the tip of the rhizomes (Eck et al., 1990), while in highbush and rabbiteye shoots emerge from newly formed or previously dormant buds on the crown. These shoots are called canes and they become hardened or "woody" during the second season of growth (Fig 1.1). The leaves on these half-high and highbush varieties are oval-shaped and arranged alternately on the stems. Overall leaf size and morphology will vary from species to species which can serve as an indicator for identification. Most highbush and rabbiteye species are deciduous but some low-chill varieties can be considered evergreens as long as they don't experience freezing temperatures (Darnell, 2006; Retamal & Hancock, 2018).

1.3.2 Roots

Blueberries develop a shallow root system that is described as dense, fibrous, and lacking root hairs. Root hairs in other woody plants increase the surface area of the root system to increase water and nutrient uptake. In blueberries, the lack of root hairs makes them more susceptible to fluctuating water content levels in the soil. When the plant is fully matured the root structure is described as an inverted cone (Eck, 1966; Bowling, 2000). The root system is divided into two parts by its functionality. The first is the pencil-thick storage and anchor roots which provide

support for the aerial structure, these thick roots can be 11 mm in diameter. The second kind is the feeder roots which are like small threads, from 50 μm to 2 mm in diameter, these are responsible for water and nutrient absorption (Gough, 1994).

About 50% of the root density is around 30 cm from the crown and 80-85% was found within 60 cm. For the total root dry mass, over 80% is found 36 cm from the surface (Spiers, 1986). High mulched soils will result in 83% of the roots located in the upper 15 cm from the surface (Retamales & Hancock, 2018). The root systems are often found inhabited by an endotrophic mycorrhiza which helps the plant transform nitrogen and phosphorous into an available source (Eck, 1966). The symbiotic fungi appear only to infect only the unsubsized tissue which is the growing part of the roots, or root tips (Vander, 1988).

1.3.3 Flowers

The flower structure is typically urn-shaped and is inverted during bloom (Fig 1.2). It possesses a five-layer calyx/sepal to protect the ovary. The corolla/petal is usually white and on some occasions has a pink shade throughout the ribs. Each flower is connected to a pedicle which is connected to a central peduncle called a stalk. A whole peduncle along with the flowers is called a cluster/raceme (Gough, 1994). The pistil can be longer or shorter than the corolla. Dormant one-year-old shoots typically have inflorescence buds at the top with vegetative buds below (Retamales & Hancock, 2018). Depending on species and cultivar, a cluster will hold 8 to 16 flowers (Darnell, 2006). This is negatively correlated with the distance from the tip of the stem. The closer the flowering bud is to the tip, the more flowers in a stalk are found. The shoot thickness is also related to the number of flowers contained in each bud (Eck et al., 1990).

1.3.4 Fruit

The fruit is a small, round berry, typically blue to purple in color, with white or light green flesh depending on the chlorophyll content. The epidermis does not possess stomata and has a cuticle about 5µm thick and produces a waxy bloom on the surface when mature. Blueberry fruit is a true berry, meaning it is formed from one flower, and one mature ovary containing several seeds. It is a five-loculated internal structure that can be visible in larger fruits. The larger fruit can be 2.5 cm in diameter (Gough, 1994). In the northern hemisphere, fruit is typically ripe in mid to late summer, two to three months after flowering, depending on the species and cultivar (Eck, 1966). Fruit size and chemical composition will be determined by the genetic makeup of the variety and the climatic conditions undergone during the blooming stages (Bowling, 2000). Fruit size is correlated with shoot vigor. Early ripening varieties and cultivars will produce larger berries than those with a later ripening characteristic. The number of seeds in a berry is directly related to berry size (Eck et al., 1990).

1.4 Phenology

Temperature plays a critical role in blueberry development. The main driver for phenological progression is chilling hour requirements. The chilling requirements vary between species and define the regions for optimal production (Retamales & Hancock, 2018). Low temperatures can begin the onset of dormancy in blueberries which are followed by the accumulation of chilling hours. These chilling hours start to be accumulated between 7.2- 0°C. Highbush cultivars require a chilling accumulation of 650 to 800, northern highbush requires 800-1200 hours, and most rabbiteye only require 400 to 600 hours with some cultivars only needing 250 (Eck, 1988). Most southern highbush cultivars only require 200 to 400 chilling hours (Santos & Salame-Donoso, 2012).

The *Vaccinium* species' growth behavior is characterized as a perennial where shoots emerge from newly formed buds from the crown. These shoots become canes, and they become woody and hardened in the second year of growth. Dormant one-year-old canes develop floral and vegetative buds. Inflorescence buds are found on the top of the cane while vegetative buds are found below (Retamales & Hancock, 2018). The number of flowers that are developed from each bud is negatively correlated to the distance from the tip of the shoot. Proximal buds may form up to nine to ten flowers while tertiary buds will form only up to seven flowers (Gough, 1994).

Blueberries can either be self-pollinating or cross-pollinated. Fruit that is a result of cross-pollinated through different cultivars tends to result in a larger size (Ehlenfeldt 2001). The blueberry blossom is characterized as an entomophilous flower, meaning that it is adapted for insect pollination. Flowers have a well-developed corolla that is aromatic, and glands at the base that secrete nectar and produce pollen heavy enough to stick to the pollinator's body, all these are characteristics desired by many species of bees that are the main agents of blueberry pollination (Shutak & Marucci, 1966).

1.5 Environmental conditions

1.5.1 Temperature and Photoperiod

The blueberry plant's optimal weather conditions for growth and development will depend on the phenological stage it is currently going through (Lyrene, 2006). Longer photoperiods during the season increase vegetative growth while shorter photoperiods are needed for flower bud initiation. As to temperatures, reports have shown cellular degeneration during temperatures above 48° C. Blueberry root systems do not produce root hairs, limiting water uptake to compensate for water loss during transpiration (Kender & Brightwall, 1966).

1.5.2 Chill hour accumulation

Winter chilling is described as experiencing temperatures equal to or below 7.2° C. Like many other deciduous woody species, blueberries have a chilling requirement to keep them dormant during the winter months and avoid cold damage by keeping plants in dormancy during the winter months. Blueberries break dormancy once temperatures rise (Abreu et al., 2021). In the case of blueberry varieties found in Alabama, northern highbush requires 900 to 1,000 hours, southern highbush 150 to 500 hours, and rabbiteye 400 to 700 hours (Powell et al., 2019). When the chilling hour requirements are not met it will be reflected in physiological symptoms such as reduced and delayed foliation, a reduction in fruit set and increased buttoning, and reduced fruit quality (Fraisie & Whidden, 2010).

Rabbiteye and some northern highbush cultivars are cultivated in the northern region of the state where their chilling requirements can be met. More to the central and southern regions of the state approaching the Gulf of Mexico there is a gradual increase of low chill varieties of rabbiteye, and southern highbush plants cultivated (Powell et al., 1995). Cultivar selection is crucial for successful cultivation. Once dormancy is broken and temperatures are still low, critical temperatures can cause severe loss of yield and decrease fruit quality (Williamson et al., 2004).

1.5.3 Freeze damage

Even though the state of Alabama is characterized for having mild winters, there is still a sequence of freezes reported where temperatures drop 0°C during short periods. These conditions are most common in early fall to late spring and threaten to damage buds, leaves, and fruits of winter crops (Ebel et al., 2008). Critical temperatures will depend on the phenological bloom stage and blueberry cultivar. The tolerance is decreased as the bloom stages progress, meaning they become more sensitive to freezes from one stage to the next (Hicklenton et al., 2002). In the case

of southern highbush, bud swell and bud break can start to suffer damage at -12 to -9° C (10-15° F) and full bloom and petal fall have tolerance as little as to -2.2 to 0° C (28-32° F) (Smith, 2019). For many fruit crops that bloom during or after colder months, there must be protective methods put in place to reduce loss of yield and fruit quality (Williamson et al., 2004).

One of the biggest challenges for blueberry production in the Alabama region is the late winter and early spring frost damage, historically occurring during February and March. (Williamson et al., 2004). Low chill rabbiteye and southern highbush varieties are the most susceptible to these occurrences (Krewer et al., 2008). The most important aspects of protection are cold weather monitoring and understanding of the regional microclimates. Certain topographical features can increase the severity of freezes. Slope direction can influence temperatures, slopes facing north are preferred over south-facing slopes. Also, the lowest part of a slope will present lower temperatures than the most elevated zone, therefore site selection plays a considerable role in freeze protection. Being near bodies of water from sizes of 1 to 10 acres can reduce frost damage (Powell & Himelrick 2000).

In field conditions, the most common method to prevent freeze damage is by implementing overhead irrigation via sprinklers. When overhead sprinkling is applied and the ambient temperature is lower than the water being applied, as the water cools it gives up a fixed degree of heat for each degree of temperature that is lost (Wolf, 1969). The mechanism of protection is the heat that is given off as water freezes, and the continued freezing of the applied water. The energy given out is called “latent heat fusion”. Heat is given out until the water reaches 0°C, the constant application prevents vegetative tissue from going under -0.3 understanding the theory helps determine the frequency at which water must be applied to prevent frost (Smith, 2019). Water application must be constant and uninterrupted through the duration of freezing temperatures.

Depending on the source and costs of water at the time, this method is still more affordable than installing propane heaters (Ballard & Proebsting, 1972).

Since temperature drops aren't as severe in the southeastern United States the implementation of wind machines has shown to be a viable alternative to overhead irrigation (Ribeiro et al., 2006). These wind machines or fans work by mixing the inversion layer, not allowing warmer air to escape, and mixing it with the cold air resulting in elevating temperatures (Evans, 2000). A disadvantage of wind machines is their ineffectiveness during advective freezes. They will not fulfill their purpose during windy conditions. Even though they are more energy and fuel-efficient than electric or propane heaters they are often seen being used in conjunction with these methods (Perry, 1998).

1.6 Soil conditions

Optimal soil composition and chemical characteristics will depend on the species, but the generalization is that most *Vaccinium* blueberries thrive in acidic, sandy, peaty, or organic soils (Vander, 1988) As rabbiteye blueberries are indigenous to the southeastern region, soil amendments may not be required except in cases where the cultivation site experiences nutritional depletion or imbalanced pH levels, which are uncommon in Alabama (Potter & Coneva, 2018). Southern highbush cultivars on the other hand require acidic (pH 4.5-5.5) and well-drained soils (Prodorutti et al., 2007). The use of pine bark to decrease soil pH levels is a common practice, with optimal levels typically ranging between 4-5. It is typically added to the soil prior to planting and has been widely adopted across various production systems, including container cultivation. (Krewer & Ruter, 2009). The incorporation of pine bark in the soil and its application as mulch has become a common practice for the southern highbush variety. A study showed that survival

rate and vigor were significantly higher for plants with pine bark mulch compared to plants without mulch (Nesmith, 2003).

1.7 Cultural Practices

1.7.1 Pruning

Another important aspect of blueberry cultivation is proper pruning. During the growing stage, it is recommended to only prune diseased tissue and dead branches. A fully developed bush should be pruned to avoid overbearing, maintain vigor, and produce quality fruit. Thinning can be used to produce bigger fruit (Powell et al., 2002). Winter pruning, during dormancy combined with pruning in the summer after harvest, resulted in more vigorous growth (Lee et al., 2015). More studies must be done to prove two pruning sessions a year would result in higher yields.

1.7.2 Plastic mulch

The use of plastic mulch or weed fabric has also been a widely adopted practice for blueberry production. The implementation of row covers has shown positive results in weed and pest management by reducing incidences where the plant integrity is exposed (Zang et al., 2021). The use of synthetic material has also been shown to be beneficial compared to wood chips or pine bark mulches since organic mulches tend to influence soil chemistry mainly by lowering pH. Although blueberries prefer acidic soils, pH levels under the recommended range could affect blueberry overall health. This practice is combined with drip irrigation, which is considered the standard for blueberry production, resulting in more efficient use of resources including fertigation practices (Cox, 2009).

1.7.3 Irrigation

In the past, irrigation was not typically used in regions that received 2.5 to 5 cm of precipitation. However, as agriculture has expanded to more arid regions, irrigation has become

essential to ensure high-quality fruit production. While excessive water in the soil can promote shallow root growth, the optimal water content can encourage deeper and more extensive root systems (Kender & Brightwall, 1966). Some of the common methods of irrigation found in blueberries are overhead sprinklers and low-pressure micro-sprayers and drippers. This will depend on water availability since overhead sprinklers consume much more water. Low-pressure systems are becoming more valuable for their sustainability aspect (Retamal-Salgado et al., 2015).

1.8 Propagation

1.8.1 Purposes in propagation methods

Blueberries can be propagated through sexual and asexual methods. Sexual propagation is done by extracting and germinating fertilized seeds. This is mostly exclusive for breeding purposes (Eck, 1966). Asexual propagation is composed of a variety of methods where the most commonly used for commercial purposes are cuttings, rooting of hardwood or softwood cuttings, and tissue culture. There are also grafting techniques such as budding and mound layering that are used for special purposes (Mainland, 1966).

1.8.2 Cuttings

The success of rooting hardwood and softwood cuttings is influenced by the species in question. Regardless of the cutting type, it is crucial to use a well-draining and moisture-retentive medium. The most common materials found in rooting beds are vermiculite, perlite, coarse sand, and (Gough, 1994). Pine bark has also been shown to be an excellent material to include in the substrate for blueberry production, especially since it is so abundant in the southeast (Krewer & Ruter 2009). Acid peat is often seen added to the mix to promote vigorous rooting (Eck, 1988). Bed sizes are preferred to be 15-20 cm high with a galvanized screening on the bottom to prevent rodents from digging through (Gough, 1994). Moisture levels are a very important factor for

successful rooting methods. Misting machines and misting sprinklers have become the conventional method of keeping beds with an adequate water level. Shade and windbreaks have become a necessity for regions with hot and windy environments (Mainland, 2006).

Softwood cutting is typical for highbush and rabbiteye, while propagation through hardwood cuttings is seen more for highbush since rabbiteye varieties have shown poor results through this method. (Eck et al., 1990). Continuous misting is a common practice in softwood cutting propagation to prevent wilting. Recommendations vary depending on the weather conditions of the region but the daily and nightly intervals are usually programmed in short intervals from 5 to 10 seconds every 2 to 10 minutes (Krewer & Cline, 2003). The propagation through hardwood cuttings consists of collecting material from 25-75cm long shoots/whips that contain leaf buds. This material is stored until conditions are optimal for the whips to be cut and placed in beds. The optimal size of the cuttings is 10-15 cm long, hardened, about pencil-thick, and from the midsection of the whip, avoiding flower buds. The best material is collected during late fall and early winter. Cuttings are placed in beds at a 5 by 5 cm density (Eck et al., 1990; Gough, 1994). The same characteristics that are found in hardwood cuttings are used in softwood cuttings. The difference between both methods is the material collection period is after harvest during a growth flush around June to April and is placed in beds shortly after. The condition for softwood cuttings is that the material must be rigid enough to not bend, break, or tear when placed in the medium. An important consideration for both methods is that the source material must be healthy and virus-free (Mainland, 2006).

1.8.3 Tissue culture

Tissue culture propagation is the collection of vegetative buds and portions of the shoot that are placed in a nutrient agar containing culturing solutions to promote cellular growth until it

reaches a desirable size to be placed in a rooting bed (Eck, 1990). Also known as micropropagation, or *in vitro* propagation, this thorough process allows the production of large numbers of similar structures in a short amount of time and from a small amount of source material. This method also allows a disease-free system that ensures healthy plant growth and is also possible to produce new plants year-round (Smagula, 2006).

1.9 Alternative Systems of Production

1.9.1 High Tunnel Production

High tunnels are an infrastructure used in protected cultivation that has gained popularity in the Southeastern United States. The typical design for a high tunnel involves laying a transparent plastic cover, typically made of polyethylene, over high-level hoops with open ends. Variations in design are often seen in the materials used, such as wood and steel (Carey et al., 2009). The increased air temperature within high tunnels resulted in early flower, enhanced yield, and accelerated maturity in blueberry orchards (Ogden & van Iersel, 2009). Southern highbush blueberries have been reported to ripen nearly a month earlier under HT when compared to open field production, creating a market advantage (Retamal-Salgado et al., 2015). There are several highbush cultivars mentioned to increase yields and fruit quality under high tunnels including ‘Duke’, ‘Aurora’, ‘Brigitta’, ‘Snowchaser’, ‘Emerald’, ‘Jewel’, and ‘O’Neal’ (Smrke et al., 2021; Fang et al., 2020). Another southern highbush cultivar that can potentially be incorporated under high tunnels is ‘Farthing’, a University of Florida cultivar, which is characterized as a vigorous growing and compact plant with a 300 chilling hour requirement. It generates a heavy fruit load with medium-sized berries and an extended harvest season. ‘Indigo Crisp’, also a University of Florida cultivar, is known for its great fruit quality and precocity. It is able to tolerate pests and diseases better than most cultivars and has a 300 hour chilling requirement (Williamson et al., 2019). ‘Legacy’ is a USDA cultivar that is very popular in Chilean regions. This cultivar generates

good yields and high-quality fruit while ripening during the mid-season, with chilling requirement of 500 hours (USDA, 1993).

1.9.2 Container production

For southern highbush cultivation, agricultural land is often a limiting factor requiring extensive amendments usually to acidify the growing medium. The use of containers to produce in a soilless substrate or custom mixed substrates is a viable alternative to conventional cultivation (Li & Bi, 2019). This method of production significantly reduces the incidences of soil-borne pests and diseases, also allowing the efficient use of resources such as water and fertilization. Proper selection of container size is critical for a successful production. Factors that influence this decision are years of cultivation and the chilling temperatures of the region. Most selected pots range between 15-to-5-gallon pots (Fang et al., 2020). In the northern region of Spain, container cultivation is widely used and is practically the standard method of production. Influencing pH and resource management are optimal under this method creating cost-efficient agricultural practices (Ciordia et al., 2002). Water management and irrigation scheduling are crucial factors for pot cultivation, if not regulated properly, plants can become water-stressed consequently resulting in reduced yields (Lebaja et al., 2019).

1.10 Photosynthesis in fruit production

1.10.1 Plant Interaction with Light

The interaction between the environment and photosynthesis is the basis of plant growth. It is through these natural exchanges and processes of chemical work that plants generate the necessary fuel to accumulate biomass, and most importantly translocate sugars to fruiting organs. Photosynthesis plays the biggest physiological role in fruit quality and yield (Evans, 2013). It is through photosynthetic modeling that the limitations of photosynthesizing organisms can be

measured. Measuring assimilations and other physiological factors at saturation or depletion has provided the insight necessary to understand and identify ranges of energy source material such as light, H₂O, and CO₂ to maximize photosynthesis as well (Farquhar et al., 2001). Even exploring the physiological responses under conditions out of the comfortable ranges to study the extent of the detrimental conditions provides information for improving photosynthetic processes (Eberhard et al. 2008). In the case of blueberry production under high tunnels, the diffusion factor provided by the polyethylene film results in an increased light distribution throughout the canopy which is reflected in overall higher plant photosynthesis (Retamal-Salgado, 2015).

1.10.2 Photosynthesis to light intensity

Photosynthesis research has increased productivity and is known as an environmental approach as yields increase pounds of food per acre produced, instead of the ancient methods of just expanding agricultural land. Photosynthesis is not only studied at the leaf level but as a whole canopy, where higher leaf area might compensate for the low photosynthetic capacities of certain fruiting crops and light use efficiency increases photosynthetic capacities. The influencing canopy structure, improving the photosynthetic efficiency of Rubisco, also increasing the efficiency of solar radiation interception, are some of the many research topics mentioned in improving photosynthesis to improve biomass production and increase yields (Murchie et al., 2009). The interest of this study is the use of alternative systems of production to reduce stressful conditions to photosynthesis. There is a growing interest in studying the effects of microclimatic conditions created in shading systems, greenhouses, and high tunnels on stomatal conductance and photosynthesis. Polyethylene covers have been shown to dissipate light, reducing the energy intensity reception of leaves, and reducing stress (Demchak, 2009).

Photoinhibition is a limitation in photosynthetic processes caused by excess light interception. The increase in photosynthetic photon flux densities can overwhelm the chlorophyll reaction centers so the excess energy needs to be dissipated through fluorescence and heat. Photosynthesis becomes affected and limited if energy levels are excessive. (Bertamini et al., 2006; Eberhard et al., 2008).

1.10.3 Photosynthesis to Temperature

Additionally, heat stress can also restrict photosynthesis by increasing respiration rates and consuming energy and carbon fixed during photosynthesis. This reduction in net carbon gain can occur due to temperatures outside of the optimal range. Moreover, high temperatures can damage the photosynthetic apparatus and membrane structures, causing oxidative stress and even cell death in extreme conditions (Salvucci & Crafts-Brandner, 2004). Studying alternative systems of production is of great interest in addressing these limitations. Plants and their species have varying photosynthetic capacities, which can be analyzed to determine optimal production conditions. Modifying environmental conditions is often easier than altering a plant's physiological photosynthetic capacity. Improvements in this interaction can promote the growth and development of plants, leading to increased yields (Krizek et al., 2007).

1.11 Historic and global production

It is recorded that commercial cultivation of blueberries started around the 1800s by the North American indigenous population through commodity trades, but the real considerable commercial exploitation did not begin until the 1920s when it started to attract the attention of European settlers (Bowling, 2000). The North American Indians used to dry the berries to prepare for winter and implement them in a variety of dishes. *Vaccinium* leaves and flowers were also used to make tea infusions which were believed to cleanse the blood (Vander, 1988). Since then, a whole industry

has evolved around the fruit production of this perennial shrub. Historically, the United States has led in the production and export of blueberries (Eck & Childers, 1966).

Blueberries have always been a popular food, each year seeing more expansion of production. As a product of globalization, blueberry production has expanded globally for commercial purposes to exports. Blueberry breeding programs have allowed production in a wide variety of conditions, mainly focusing on lowering chilling requirements (Bustamante, 2022). The tendency of blueberry production during the last century has been shifting to regions of warmer and dryer environments. Around 30 countries have been reported to grow blueberries on a commercial scale. The highest growth of blueberry production has been seen in Peru, Mexico, New Zealand/Australia, and China (Bañados, 2006). These countries are creating a stronger impact each season and are noticeable competitors in the demographic. The increase in production is strategically thought through with proper cultivar selection, where the focus is implementing more recent varieties of highbush which are more adapted to their region (Lobos & Hancock, 2015).

Commercial blueberry varieties were introduced to South America in the 1980s. Since then, Peru, Chile, and Mexico are recognized as some of the largest blueberry producers in the world and keep increasing their cultivated area each season (Perez-Ruiz, 2022). The harvest season in South America takes place from September to April, taking advantage of the North American off-season (Bañados, 2006). More recent reports show that Peru has been the country with the highest expansion for blueberry production in the last two years. Last season Peru alone exported 243.4 thousand metric tons of fresh blueberries from August to December 2021 (Ilyas, 2022). At the end of the 2021 season, China leads the total production of blueberries with 477 thousand metric tons, followed by USA, Peru, Chile, and Mexico. (Protzman, 2021, Perez-Ruiz 2022).

Blueberry production in the southeastern United States has increased rapidly since before the 2000s. The biggest challenge for production is selecting the appropriate varieties and cultivars for each subregion of the Southeast (Ogden & van Iersel, 2009). During the 2017 census, Alabama was reported to have 329 ha of area planted with blueberries for consumption (USDA, NASS, 2017). Rabbiteye blueberries are the predominant variety found in the state, they are native to the southeast, they have a high tolerance for drought and heat, and they can grow on a wide variety of soils (Potter & Coneva, 2018). The challenge of producing rabbiteye is its poor fruit set which is believed to be an issue with cultivar selection and pollination difficulties. A big factor for rabbiteye blueberry production is inadequate chilling accumulation in some regions. This leads to the formation of flowers with a decreased viability to set fruit, indicating the importance of proper cultivar selection (Darnell & Davies, 1990). Southern highbush varieties are very popular in the southeastern United States, especially in Florida and Georgia (Williamson et al., 2004). These low-chilling varieties will have been introduced in Alabama and are becoming an attractive novelty to bring fruit to market before the regular season begins. These are more challenging to produce than rabbiteye cultivars but the benefits of expediting harvest cannot be overlooked (Smith, 2019).

From an agricultural perspective, Alabama is considered a cotton state with a production area of 376,000 acres distributed between 925 farms. A USDA census showed most farms in the state are dedicated to row crops, with corn being grown on 2,112 farms and soybeans on 1,502 farms (Price, 2017). The comparison between the USDA 2012 and 2017 censuses showed that there has been an increase of 63% of farms dedicated to berry production making a total of 885 farms. During this period only blueberry production has seen an increase in acreage, making it 834 acres with a 3% increase. Blackberries sum up 143 acres with a 9% decrease, raspberries barely 5

acres with an 82% decrease and strawberries with 111 acres of production saw a 30% decrease (USDA-NASS, 2012, 2017).

1.12 Objectives

General objective

- The purpose of this study was to compare the physiological responses of three southern highbush blueberry cultivars under high tunnel production and open field conditions.

Specific objectives

- Evaluate southern highbush blueberry growth and development under high tunnel conditions compared to the open field.
- Evaluate and compare the photosynthetic profiles of the studied cultivars between high tunnel and open field conditions.

1.13 Hypotheses

- High tunnel conditions will influence growth by increasing biomass accumulation compared to outside production.
- Plants under the high tunnel were expected to experience accelerated development and early blooming.
- High tunnel production would result in a higher photosynthetic profile across cultivars.
- Open field production was expected to experience higher photosynthetic limitations compared to plants under the high tunnel.

1.14 References

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1.15 Figures

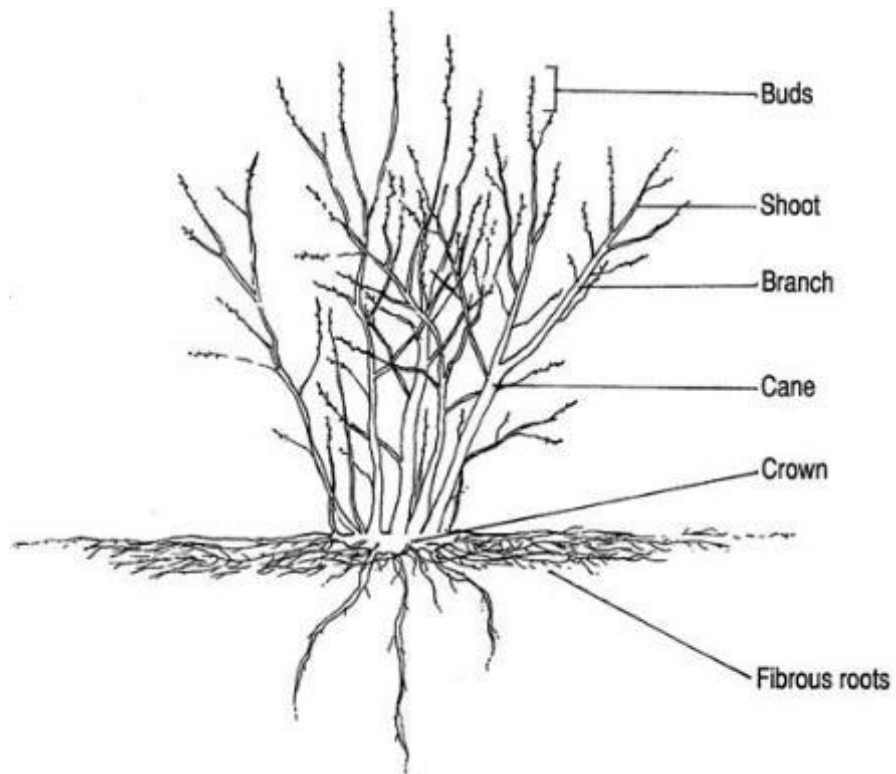


Figure 1.1 Highbush blueberry shrub with indicated parts (NRAES 1992)



Figure 1.2 Southern highbush full bloom (February 2023, EV Smith Research Center, Alabama)

Chapter 2

Influence of High Tunnels on Southern Highbush Blueberry Growth and Development

2.1 Introduction

The blueberry species southern highbush (SHB, *Vaccinium corymbosum* interspecific hybrid) has increased its popularity and implementation in the southern United States by its low chill characteristics. Early blooming blueberries mean reaching the market faster, resulting in an economic advantage (Smith, 2019). Compared to Georgia and Florida which have an affinity for SHB varieties, Alabama blueberry production is predominantly rabbiteye (*V. virgatum*). This makes sense since rabbiteye is native to the region and naturally has a higher chill requirement to avoid the late spring frosts (Potter et al., 2013). There is a growing interest in implementing more southern highbush varieties for economic benefit even considering the production challenges this implies (Ogden & van Iersel, 2009).

Blueberry consumption has increased considerably in the past decade. New varieties and methods of production are researched and implemented at a growing rate to ensure higher yielding, stable, and quality production (Fang et al., 2020). Alabama has immense potential for blueberry production, some of the challenges are cultivar selection by region and method of production. One of the biggest challenges for the early blooming varieties like southern highbush is protecting flowering events from mid-March spring freezes. Due to the typically small sizes of farms in Alabama, not many producers have the economic capacity or the knowledge about implementing freeze protection systems (Thompson, 2022).

The implementation of high tunnels has been a novelty in the past decades, giving producers the ability to influence microclimatic conditions, mostly focusing on ambient and soil temperature, and light interception (Wien, 2009). The use of high tunnels, also known as hoop

houses, is well known for raspberry, blackberry, strawberry, and blueberry production, expediting fruit ripening, and shortening phenological blooming stages compared to conventional systems (Fang et al., 2020). Light and temperature are the main drivers for growth and development, and both characteristics can be managed under high tunnel production systems. Optimal growing conditions will help develop healthier plants which end up benefiting production throughout the season (Spann et al., 2004).

There is still little information regarding high tunnel microclimatic effects on a cultivar-to-cultivar basis, especially in the central Alabama region. Therefore, the objective of this study was to evaluate the growth and development parameters of three southern highbush blueberries under a high tunnel system and an open field production system.

2.2 Material and Methods

2.2.1 Site preparation.

The experiment was carried out at the E.V. Smith Research Station, Plant Breeding Unit (Talladega, Alabama, 32.49679151185915, -85.89036510954459) during the 2022 growing season and the beginning of 2023. A high tunnel was constructed for the use of the study, measuring 267.6 m², and was covered by a polyethylene film. The soil amendment and preparation were finalized before the high tunnel was established. Site preparation began in January 2022 when 15 cm of pine bark was incorporated into the soil to promote soil acidification. Soil tests showed a 4.8 to 5.2 pH level after the soil amendments. Then, 2 cm of pine bark was spread on the raised bed and then covered with weed fabric; the beds were 2.4 m apart measured from the center of the bed. Four beds were raised under the high tunnel and two raised beds were in open field conditions. Irrigation was installed above the weed fabric. Each plant had one micro emitter, and each dripper delivered 1.9 L hour⁻¹.

Plants were spaced 0.91 m apart within rows. The planting holes on the weed fabric were done using a fire torch and a stainless-steel ring cast to mold the circular shape, each hole being 30.5 cm in diameter. The study did not employ a formal experimental design but rather utilized a sampling approach. Also, the study was observational in nature and not randomized in order to evaluate physiological parameters during the first year of establishment. There were 180 plants of southern highbush (*Vaccinium corymbosum* L. interspecific hybrids) used for this study, distributed evenly between these cultivars: ‘Farthing’, ‘Indigo Crisp’, and ‘Legacy’, where 30 plants per cultivar were planted inside of the high tunnel and the remaining 30 plants of each cultivar were planted in outside conditions (Fig 2.1). The destructive sampling consisted of two replicates of each cultivar and location per sampling event. All plant material was sourced from one-year hardwood cuttings from a Florida nursery.

2.2.2 Crop management

Irrigation was scheduled to run three times a day at 45-minute periods during the study. Immediately after planting, all the reproductive organs were eliminated by hand to promote growth. Ammonium sulfate was injected into the system at bi-weekly intervals until September to provide fertilization. Each plant received 8.9 g of fertilizer per application. Plants were pruned twice during the growing season, once during August, to eliminate tissue infected with stem blight (*Botrytis* sp.) and again in October for structural purposes where a third of the height of each plant was cut back to promote vigorous growth for the next season. The utensils used were disinfected from plant to plant during both events.

2.2.3 Environmental Monitoring and Analysis

A ZL6 logger (Meter, Pullman, WA) was placed in the center of the high tunnel and it was connected to an Atmos 14 (Meter, Pullman, WA) was placed under the high tunnel, and it was

connected to an Atmos 14 (Meter, Pullman, WA) weather sensor which recorded ambient temperature (°C) and relative humidity (RH, %). It was also connected to an SQ-521 (Apogee Instruments Inc. Logan, UT) sensor which recorded the photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$). Two A-125 sensors (Watchdog, Spectrum Technologies Inc. Charlotte, NC) were placed under each condition to record soil temperature (°C), and the soil probe was buried 6 inches under the surface line. A Watchdog A-150 (Watchdog, Spectrum Technologies Inc. Charlotte, NC) was used to record ambient temperature and relative humidity, also a LightScout sensor (Spectrum Technologies Inc. Charlotte, NC) to record PPFD in outside conditions. All sensors were programmed to record data every 15 minutes.

PPFD data were converted to daily light interval (DLI, $\text{mol m}^{-2} \text{day}^{-1}$) using the following formula by averaging the 24-hour period and multiplying the value by 3,600 to convert the seconds to days and then dividing by 1,000,000 to convert from μmol to mol . The data was converted to daily values by identifying the daily maximum and minimum values. Growing degree days (GGD) were calculated using the Spec 9 Basic program (Spectrum Technologies Inc. Charlotte, NC) with data retrieved from the four A-125 (Watchdog, Spectrum Technologies Inc. Charlotte, NC). The base temperature used for southern highbush was 7°C (Li & Bi, 2019). The monthly average was calculated for the soil temperature and relative humidity.

2.2.4 Growth Measurements and Analysis

Growth analyses were done through destructive measurements that took place from March to November 2022. A total of eight measurements took place in the last week of each month from March to November. Destructive samplings were performed on two independent replicates that were selected randomly at the beginning of the trial. Each experimental unit was comprised

of *cultivar x condition*. Each measurement took two plants per experimental unit. At the end of the season, a total of 16 plants per experimental unit were used for growth measurements.

2.2.5 Biomass and partitioning analysis

Height was measured before proceeding with every destructive sample. Then the stems were cut from the base of the crown and the leaves were separated from the stems and both elements were weighed. The weight values provided fresh weight (g). The leaves were placed through a leaf area analyzer (LICOR, LI-3100C, Lincoln, NE) that provided leaf area (cm²). The samples were placed into separate marked bags and placed into a dryer for five days. Dry weight was measured separately, and the sum of the dry weights provided aerial biomass (g). For the final biomass measurements in November, leaves and stems were calculated into ratios by cultivar and condition.

2.2.6 Root Scans and Analysis

During the destructive measurements of April, May, and June, the root systems were dug up using a shovel within the 30.5 cm diameter of the planting hole made on the weed fabric. The root systems were washed on a metal mesh table to get rid of debris and soil and placed in marked and sealed plastic bags. The root systems were taken apart and placed on plastic trays with water, which were later put in a root scanner (Epson, Perfection V800 scanner, Los Alamitos, CA). The scanning software (WinRHIZO, Regent, Quebec, Canada) provided root length (cm), surface area (cm²), average root diameter (mm), and root volume (cm³).

2.2.7 Phenology

Phenological bloom progression was monitored every two days during the weekdays, beginning January 2023. Three stems of three plants were marked using orange tape in each cultivar under each condition. The stages recorded were dormant bud, bud swell, early green tip,

bud break, late green tip, tight cluster, early pink bud, late pink bud, early bloom full bloom, and petal fall according to Longstroth (2022).

2.2.8 Statistical analysis

A Generalized Linear Mixed Model (GLIMMIX) was used to evaluate the three-way interaction among conditions (high tunnel and open field), cultivar, and time of sampling on growth data. The fixed effects of condition, cultivar, and time were individually evaluated, as well as their two and three-way interactions using a Tukey grouping to separate the LSMeans. Repetition was randomized with two repetitions per experimental unit to account for within-unit variation. The significance level was set at $p < 0.05$ for the GLIMMIX procedure and mean separation. The end-of-season measurement of dry weight was used to calculate the final partitioning of plant organs. Leaves and stems were calculated into ratios by cultivar and condition and evaluated through an LSMeans and Tukey separation as well as the interaction between cultivar and condition.

An 'NLIN' procedure was used to fit a nonlinear regression model to biomass accumulation and leaf area throughout time. The model was specified using the equation:

$$d = \frac{a}{1 + \exp(-b * (DAT - c))}$$

The 'a' parameter represents the maximum growth values the parameter reached, 'b' represents the rate of growth, and 'c' represents the inflection point at which the growth rate reaches half of its maximum value. The initial parameter values were set in ranges using the 'parameters' statement, to calculate each value by cultivar and condition. The results allowed the estimation of growth parameters between observations.

Growth rates (g/day) were calculated by applying a sigmoidal function to the observed data points using the parameters obtained from the NLIN procedure. The sigmoidal function used to calculate the growth rates is described by the formula:

$$\text{Rate} = \left(\frac{b}{a}\right) * (a * d - d * d)$$

Where 'b' and 'a' are the rate and maximum growth parameters, respectively, obtained from the nonlinear regression model, and 'd' represents the observed growth parameter at a given time point. This formula allows us to estimate the rate of change in growth at any point in time, and to compare the relative rates of growth across cultivars and conditions.

A linear regression model was used to model the height by DAT (time), which allowed the relationship between height and time for each cultivar and condition combination to be analyzed and identify the intercept. After fitting the regression model, the predicted height for each DAT value was calculated using the equation:

$$H = a + bt$$

Where 'a' is the intercept, representing the predicted height at time 0 (t=0), and 'b' is the slope, representing the rate of change in height over time 't' (i.e., the increase in height per unit of time, days). SAS 9.4 was used to generate statistical analyses.

2.3 Results

2.3.1 Environmental Factors

The comparison of ambient temperature between both conditions (Table 2.1) showed that during the trial period, average maximum temperatures were 1.7°C higher under high tunnel conditions compared to the open field. High tunnel values were higher than the open field during the whole trial. Average minimum temperatures showed that high tunnel conditions were 0.2°C higher than open field conditions. The average temperature data during the trial showed that the high tunnel was 0.6°C higher than the open field. The highest difference in the averages between both conditions was during August, September, and October where the high tunnel was 0.8°C, 1°C, and 0.9°C higher temperature, respectively, compared to conventional conditions. The GDD data (Table 2.2) showed that the highest monthly values were seen in July where high tunnel conditions and open field conditions showed an average of 22.5 and 21.6 GDD respectively. The GDD averages during the 2022 season showed that the high tunnel had a 0.99 GDD higher value compared to the outside field. Cumulative data showed that by the end of the 2022 season, the high tunnel accumulated 293.1 GDD more than the open field. During the 2023 phenological monitoring, January accumulated 18 and February 25 more GDDs under high tunnel conditions compared to the open field.

The average soil temperatures during the trial (Table 2.3) showed the temperature under the high tunnel was 23.4°C and 22°C in open field conditions. High tunnel values were higher than the open field during the whole trial. The average difference showed that soil under the high tunnel was 0.9°C higher than the open field. Monthly average differences oscillated during the growing season where the highest values were seen in March, September 2022, and January 2023. Soil temperatures were 1.3°C, 1.2°C, and 1.5°C respectively higher under the high tunnel compared to the open field.

Light data (Table 2.4) showed that by the end of the trial, plants in the open field were receiving 22.5% more direct light compared to plants inside the high tunnel. June showed the highest monthly values for DLI. April showed the highest difference in DLI, where the open field received 30% more direct light compared to the high tunnel.

Relative humidity (Table 2.5) showed that monthly percentage averages oscillated throughout the season where higher values were seen in both conditions. The average values showed that outside conditions were 3.5% more humid compared to high tunnel conditions.

2.3.2 Seasonal growth

Biomass accumulation, leaf area, and height were not affected by the interaction between the fixed effects cultivar, condition, and DAT ($p < 0.05$) (Table 2.6). Biomass was significantly affected ($p = 0.0118$) by the interaction between cultivar and conditions, where ‘Legacy’ was the only cultivar to show a significant difference between conditions. ‘Legacy’-Out showed 22.5 g of more biomass throughout the season compared to ‘Legacy’ under the high tunnel (Table 2.7). For the differences in biomass between cultivars, ‘Legacy’ had higher biomass throughout the season compared to ‘Indigo Crisp’ (Table 2.8). Height was also significantly different between conditions ($p < 0.0001$) where the ‘Legacy’ cultivar was taller compared to ‘Farthing’ and ‘Indigo Crisp’. The maximum growth rate of the growing season was seen between DAT 100 and 150 (Fig. 2.2) which was from June to August, where the three highest monthly average temperatures were recorded (Table 2.1) and some of the highest DLI values of the growing season as well (Table. 2.4).

2.3.2 End-of-season growth.

The interaction between cultivar and condition had a significant effect on end-of-the-season biomass. ‘Legacy’ outside of the high tunnel accumulated 37% more biomass than its high tunnel

counterpart. ‘Farthing’ and ‘Indigo Crisp’ showed no significant differences between conditions (Table 2.9). Even though the leaf area showed a significant effect on the interaction between cultivar and condition (Table 2.9). Final biomass partitioning showed a significant difference between cultivars and conditions. In terms of Biomass partitioning toward leaves, ‘Farthing’-In showed a significantly higher value compared to ‘Farthing’-Out, the difference in portions being 0.1072. ‘Farthing’-In was also the cultivar with a leaf-stem ratio closest to 1:1 in biomass partitioning (Fig. 2.3).

2.3.4 Roots

The test of fixed effects showed no significant interaction between cultivar, condition, and DAT on the root parameters surface area, length, diameter, volume, and biomass (Table 2.10). The greatest number of differences was observed between cultivars where ‘Farthing’ showed a higher surface area and root length compared to ‘Indigo Crisp’ (Table 2.11). ‘Farthing’ also showed a significantly higher root volume compared to ‘Indigo Crisp’ and ‘Legacy’ (Table 2.11). For the biomass estimates, ‘Legacy’ was significantly higher compared to ‘Indigo Crisp’ (Table 2.11). The differences between conditions showed that plants in the open field showed a higher average diameter compared to the high tunnel (Table 2.12). Root volume between conditions showed the contrary, where plants under the high tunnel had a higher overall root volume compared to plants in open field conditions (Table 2.12)

2.3.5 Phenology

Blooming stage progression monitoring during January and February 2023 showed all cultivars under the high tunnel reached further phenological stages compared to the cultivars in outside conditions (Fig. 2.4). By DOY 56, ‘Farthing’-In and ‘Indigo Crisp’-In were at 67%, and ‘Legacy’ was at 33% in petal hall. Also, only 11% of ‘Farthing’-Out and 22% of ‘Indigo Crisp’-

Out reached full bloom on day 56. Legacy-Out showed 11% of the stems under observation in early bloom. The earliest petal fall observation was seen in DOY 46 in 'Farthing'-In and ins DOY 56 for 'Indigo Crisp'-In and 'Legacy'-In. High tunnel conditions showed a higher GDD where the comparison between conditions showed that the high tunnel accumulated 18 more GDD in January and 25 more in February compared to high tunnel conditions (Table 2.2).

2.4 Discussion

Results found by Throop & Hanson (1977) showed *V. corymbosum* blueberry exhibited rapid biomass accumulation between July and early August and minimal changes through August and October. Similar results were observed in this study for all cultivars under both conditions, where maximum growth rates were observed from July to mid-August. Bañados et al. (2006) suggest that the differences in the growth characteristics of each cultivar could be attributed to plant vigor. The DLI monitoring showed that all cultivars in the open field received more direct light compared to plants under the high tunnel. Ogden & van Iersel (2009) agree that higher light interception by the plant will increase overall growth, but also that the high tunnel will provide a diffusion of light that increases light distribution through the plant canopy. In this study, we found that the overall above-ground biomass of highbush blueberries during the first year of establishment was not significantly affected by using a high tunnel system. However, our research did reveal that high tunnels have a considerable impact on the volume of blueberry roots. This is particularly crucial during the establishment phase of the plant's growth cycle considering the perennial nature of *Vaccinium* blueberries, it is essential to develop a robust root system to acquire adequate nutrients and water from the soil. Proper root structure development is also crucial in blueberries considering they have a restricted water uptake capacity due to the lack of root hairs in their structure (Holzapfel et al., 2004).

Ogden & Iersel (2009) reported that high tunnel conditions advanced the dates of petal fall compared to outside conditions. This study observed the same results for the three cultivars under the high tunnel. Santos & Salame-Donoso (2012) also reported similar results, where high tunnel conditions showed evidence of expediting bloom stages. They also attributed this to the higher temperatures observed under the high tunnel. In this study, the GDDs accumulated under the high tunnel were higher during bloom compared to those seen in open field conditions. During January and February the high tunnel accumulated 18 and 25 more GDDs compared to the open field. The results of this study show evidence of accelerated phenological development for all cultivars under the high tunnel. GDD requirements show to vary by cultivar due to the differences in stage development comparing plants in similar conditions. Li & Bi (2019) also observed higher GDDs under the high tunnel compared to open-field conditions. By the end of the phenological progression monitoring ‘Legacy’ under the high tunnel had the least number of inflorescences observed in petal fall compared to ‘Farthing’ and ‘Indigo Crisp’. While the last stage observed in ‘Legacy’ outside of the high tunnel was in early bloom while ‘Farthing’ and ‘Indigo Crisp’ already had inflorescence in full bloom. This could be attributed to the chilling hour requirements since ‘Farthing and ‘Indigo Crisp’ have a lower chilling hour requirement.

Even though there was no significant interaction between the growing conditions and the root characteristics for each cultivar, results showed that all cultivars had a higher length, surface area, root volume, and biomass compared to outside conditions. Spiers (1995) studied the effect of root growth in temperatures of 16, 28, and 38°C, showing that the lowest vigor rating was observed at 38°. The conclusion of that study stated that extremely high temperatures can be detrimental to root growth. During April, May, and June in this study, soil temperatures under the high tunnel were 0.4, 0.1, and 0.7°C higher respectively compared to soil temperatures in outside

conditions and the highest soil temperature observed was 30.2°C under the high tunnel in May. A possibility for the differences in root characteristics is soil water content. The bed in outside conditions received no cover from the rain. It is possible that higher soil water content could have decreased root growth due to sufficient water uptake, as the plants under the high tunnel only received water through irrigation. Wien (2009) also reported a higher soil temperature under high tunnel conditions. He observed a 2°C difference between the high tunnel and the open field.

2.5 Conclusions

The high tunnel showed evidence of influencing microclimatic conditions. These conditions seem to affect SHB blueberry growth differently across cultivars. The results of the study show that rapid biomass accumulation occurs between July and mid-August, with decreased changes through August and November for all cultivars under both conditions. The maximum growth rates were observed from July to mid-August, and cultivars showed their maximum growth rate. The differences in growth characteristics of each cultivar could be attributed to plant vigor and high tunnel conditions. The study also showed evidence of positively affected development by expediting blooming stages throughout all cultivars under the high tunnel, where high tunnel conditions showed higher GDDs compared to the open field. Differences in root parameters were seen mostly between cultivars. The main driver of root systems growth, soil temperature, and soil water content, appeared to not be at a level of difference between conditions to promote any significant differences of the blueberry root systems between conditions.

2.6 References

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2.7 Figures

Cultivar 1		Cultivar 2		Cultivar 3	
Legacy	Indigo	Farthing			

High Tunnels				Open field rows	
Row 1	Row 2	Row 3	Row 4	Row 1	Row 2
C	3	7	10	F	7
1X	4X	B	11X	C	8X
C	A	8	D	1	G
2	5	9	D	2X	F
G	6	E	12X	C	9
H	E	G	A	3	10
F	H	B	F	4	G
H	3	C	9	A	E
A	4	D	A	B	A
F	5X	E	H	E	11X
E	B	C	B	G	12
1	G	7X	10X	5X	D
D	6X	8	11	6	13
2	F	G	12	D	14
G	H	A	F	G	B
1	C	6X	10	1	H
C	4X	7	E	2	A
2X	D	B	11	B	H
A	B	8X	H	3X	C
3	D	E	12	4	B
G	5	9	F	D	E
				C	9
				G	F
				5	10
				G	E
				6	A
				7X	11X
				8	12
				D	13X
				F	14
				1	H
				2X	4
				F	D
				E	5
				C	B
				3	6
				D	7X
				G	8
				B	9X
				H	10
				C	G
				A	11
				F	12X
				E	13
				A	14

Figure 2.1 Distribution map of the three cultivars in the high tunnel and in the open field. The distribution of the plants under the high tunnel starts from the South end of the structure up to the half of the high tunnel.

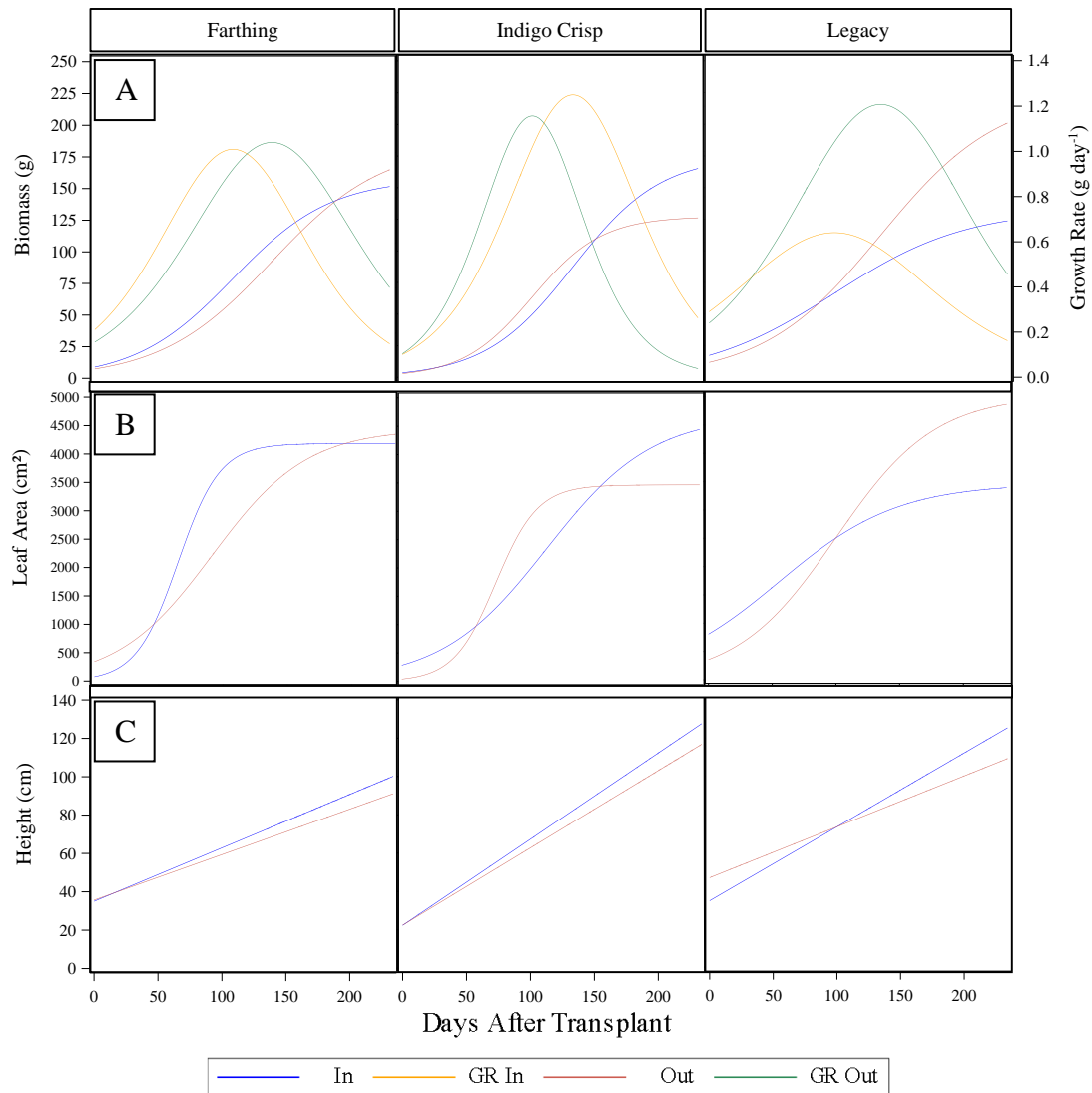


Figure 2.2. Growth parameters as A. biomass accumulation and growth rate, B. Leaf area, and C. Height throughout the season by cultivar and condition. GR = Growth Rate

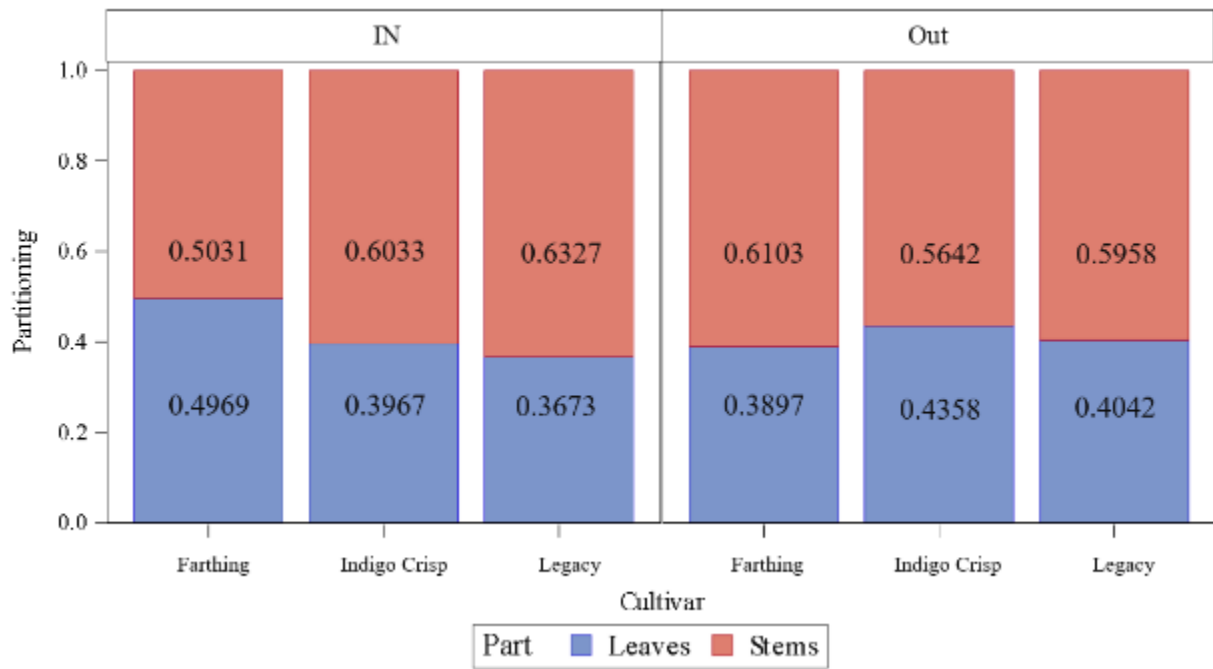


Figure 2.3. Biomass partitioning of final destructive measurement grouped by plant leaves and stems for each cultivar by condition. The level of significance between the interaction between cultivar and condition for partitioning was $p < 0.0001$.

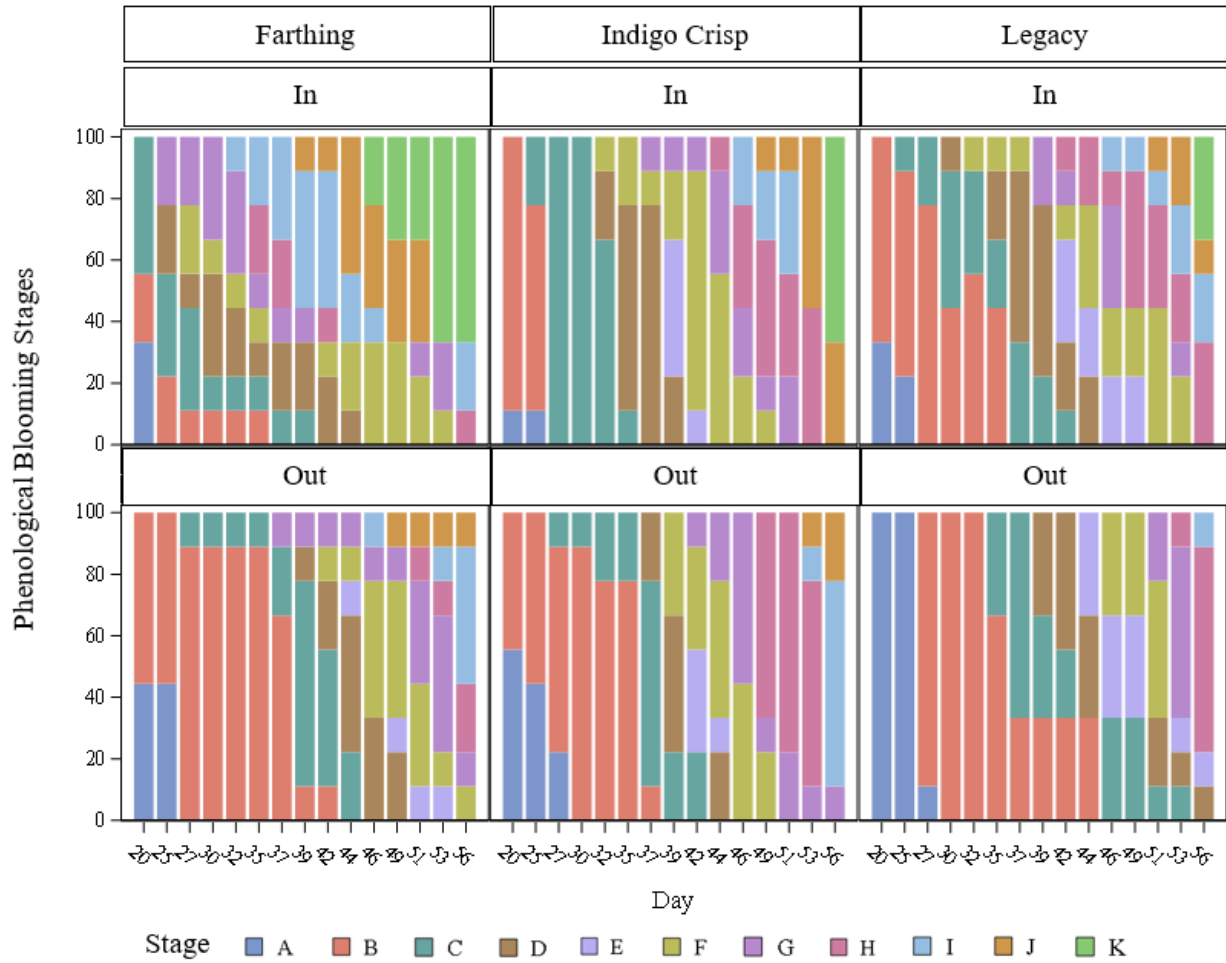


Figure 2.4. Phenological bloom stage progression is shown by frequency, paneled by condition, and cultivar. A=Dormant or tight bud, B=Bud swell, C=Early green tip, D=Bud break/Burst, E = Late green tip F = Tight Cluster G= Early pink bud, H= Late pink bud, I = Early bloom, J = Full bloom, K = Petal fall

2.8 Tables

Table 2.1. Minimum, maximum and average temperature recorded at EV Smith during from March 2022 to February 2023

Month	Year	Max temp (°C)		Min temp (°C)		Average temp (°C)	
		High tunnel	Open field	High tunnel	Open field	High tunnel	Open field
March	2022	35.0	29.9	3.8	3.5	16.3	15.9
April	2022	32.7	31.6	1.8	1.5	17.5	17.1
May	2022	37.3	35.8	10.8	10.5	23.5	22.9
June	2022	40.6	38.3	17.9	17.7	27.6	26.9
July	2022	38.5	36.9	20.7	20.4	27.9	27.3
August	2022	39.2	37.9	19.5	19.3	26.8	26.0
September	2022	39.2	36.5	7.1	6.4	24.2	23.2
October	2022	33.6	31.4	-0.8	-1.2	16.5	15.6
November	2022	38.5	32.8	-4.4	-5.1	13.9	13.3
December	2022	28.7	27.0	-10.6	-10.5	10.1	9.8
January	2023	26.0	25.3	-4.4	-4.2	11.1	10.8
February	2023	36.2	29.3	-4.2	-2.1	14.7	14.2
Total Average		28.6	26.9	12.7	12.5	19.5	18.9

Maximum and minimum daily averages observed by month and average monthly temperatures.

Table 2.2. Monthly cumulative Growing Degree Days (GDD) from March 2022 to February 2023

Month	Year	Monthly Cumulative GDDs	
		High tunnel	Open field
March	2022	126	110
April	2022	345	325
May	2022	542	520
June	2022	655	626
July	2022	697	669
August	2022	669	632
September	2022	554	518
October	2022	354	321
November	2022	280	246
December	2022	209	191
January	2023	205	187
February	2023	276	251
Total Average		409.4	382.9

Growing degree days by month shown for both conditions in daily averages and accumulative values

Table 2.3. Soil Temperature registered at EV Smith inside and outside of the high tunnel during the study.

Month	Year	Average soil temperature (C°)	
		High tunnel	Open field
March	2022	18.8	17.5
April	2022	20.1	19.7
May	2022	25.7	25.6
June	2022	30.4	29.7
July	2022	31.2	30.6
August	2022	30.7	29.8
September	2022	29.1	27.9
October	2022	22.7	21.8
November	2022	18.9	17.9
December	2022	15.1	14.0
January	2023	14.8	13.3
February	2023	15.2	13.7
Total Average		23.4	22.5

Monthly average soil temperature (°C) by condition

Table 2.4. Daily Light Integrals calculated for both conditions inside and outside the tunnel from March 2022 to February 2023

Month	Year	Average DLI ($\mu\text{mol m}^{-2} \text{day}^{-1}$)		Sum of DLI ($\mu\text{mol m}^{-2}$)	
		High tunnel	Open field	High tunnel	Open field
March	2022	35.8	46.0	358	460
April	2022	36.0	51.6	1080	1547
May	2022	40.7	53.7	1263	1664
June	2022	43.1	55.2	1292	1655
July	2022	41.6	50.7	1289	1570
August	2022	35.4	43.8	1096	1359
September	2022	33.2	41.6	995	1249
October	2022	27.0	34.9	837	1083
November	2022	17.2	21.7	516	652
December	2022	12.6	16.8	390	520
January	2023	7.8	16.0	241	497
February	2023	13.1	23.9	368	668
Total Average		28.6	38.0		

Daily light integral ($\mu\text{mol m}^{-2} \text{day}^{-1}$) in monthly averages and the monthly sum of daily light integrals ($\mu\text{mol m}^{-2}$) by condition.

Table 2.5. Relative Humidity registered at EV Smith inside and outside of the high tunnel during the study.

Month	Year	Average Relative Humidity (%)	
		High tunnel	Open field
March	2022	61.9	67.6
April	2022	72.1	74.6
May	2022	75.2	75.0
June	2022	74.2	81.1
July	2022	79.8	81.6
August	2022	82.0	79.6
September	2022	75.0	74.6
October	2022	73.2	76.2
November	2022	77.4	82.0
December	2022	78.9	83.8
January	2023	78.6	86.4
February	2023	78.6	86.4
Total Average		75.6	79.1

Monthly average relative humidity (%) by condition.

Table 2.6. Growing season biomass accumulation, leaf area, and height for the cultivars considered in the study

Cultivar	Condition ^z	Biomass (g)							
		DAT ^y							
		0	33	61	89	125	153	195	234
Farthing	In	5.5	11.0	42.0	66.6	76.4	136.1	130.0	156.9
Indigo Crisp	In	2.6	6.3	25.6	35.2	73.2	109.0	151.9	162.9
Legacy	In	18.2	30.5	37.1	69.5	76.6	98.3	119.7	121.0
Farthing	Out	5.5	17.6	21.2	50.2	77.6	97.1	151.1	161.4
Indigo Crisp	Out	2.6	6.8	24.6	48.5	85.1	112.8	121.1	126.6
Legacy	Out	18.2	22.4	35.0	66.8	97.2	126.4	192.0	193.1

Cultivar	Condition ^z	Leaf Area (cm ²)							
		DAT ^y							
		0	33	61	89	125	153	195	234
Farthing	In	51.3	156.0	2083.3	3265.5	3611.7	4243.0	3990.0	4633.5
Indigo Crisp	In	23.9	289.0	1323.3	1703.7	2800.9	3142.8	4013.7	4586.0
Legacy	In	87.2	264.5	2029.2	3036.7	3015.3	2904.3	3280.6	3477.8
Farthing	Out	51.3	673.3	1210.6	2615.3	3238.5	3044.6	4491.2	4427.6
Indigo Crisp	Out	23.9	297.8	1141.5	2364.6	3596.6	3430.6	3034.3	3699.0
Legacy	Out	87.2	695.7	1137.0	2816.3	2917.2	3852.2	4806.0	4862.0

Cultivar	Condition ^z	Height (cm)							
		DAT ^y							
		0	33	61	89	125	153	195	234
Farthing	In	34.3	45.7	49.5	62.2	69.9	76.2	91.4	99.0
Indigo Crisp	In	24.1	28.8	47.0	59.7	83.8	111.8	106.7	116.9
Legacy	In	55.9	42.8	58.4	59.7	57.2	88.9	125.7	133.7
Farthing	Out	34.3	49.3	38.1	62.2	69.9	72.4	80.0	90.3
Indigo Crisp	Out	24.1	23.0	44.5	64.8	76.2	102.9	95.3	107.6
Legacy	Out	55.9	62.7	59.7	62.2	66.7	80.0	106.7	117.7

		Biomass	Leaf Area	Height
<i>ANOVA</i> <i>p-values</i>	Condition (Cond)	0.3261	0.8782	0.1068
	Cultivar (Cult)	0.0274	0.0669	<.0001
	DAT	<.0001	<.0001	<.0001
	Cond*Cult	0.0118	0.1359	0.7588
	Cond*DAT	0.672	0.7132	0.2416
	Cult*DAT	0.9973	0.9775	<.0001
	Cond*Cult*DAT	0.0651	0.1949	0.9634

^zConditions indicate where the cultivar was located, In = high tunnel and Out = open field. ^yThe DAT = days after transplant which refers to the point in the season when the destructive measurements took place. This is the biomass accumulation through different days after transplant between March and November, by cultivar and condition. P-values show the significance between the interaction of the fixed effects and the significance in differences of each individual factor with a $p = <0.05$.

Table 2.7. Seasonal growth parameters by cultivar and condition

Cultivar	Condition^z	Biomass (g)	Leaf Area (cm²)	Height (cm)
Farthing	In	78 ab	2754.3	66
Indigo Crisp	In	70.8 b	2235.4	72.4
Legacy	In	71.4 b	2261.9	77.8
Farthing	Out	72.7 ab	2469.1	62.1
Indigo Crisp	Out	66 b	2198.5	67.3
Legacy	Out	93.9 a	2646.7	76.5
<i>p-value</i>	Cultivar*Condition	0.0118	0.1359	0.7588

^zConditions indicate where the cultivar is located, In = high tunnel and Out = open field. Growth parameters biomass, leaf area, and height are shown as the LSMeans in response to the effect of the interaction between cultivar and condition. P-values show the significance of the fixed effects mentioned, with a confidence level of $p < 0.05$.

Table 2.8. Seasonal growth parameters by cultivar

Cultivar	Biomass (g)	Leaf Area (cm²)	Height (cm)
Farthing	75.4 ab	2611.7	64.0 b
Indigo Crisp	68.4 b	2217.0	69.8 b
Legacy	82.6 a	2454.3	77.1 a
<i>p-value</i> Cultivar	0.0274	0.0669	<.0001

Growth parameters biomass, leaf area, and height are shown as the LSMeans in response to the cultivar effect. P-values show the significance of the fixed effects mentioned, with a confidence level of $p < 0.05$.

Table 2.9. End-of-season growth parameters

Cultivar	Condition^z	Biomass (g)	Leaf Area (cm²)	Height (cm)
Farthing	In	156.9 ab	4633.53 a	99.0
Indigo Crisp	In	162.9 ab	4585.95 a	116.9
Legacy	In	121.0 b	3477.8 a	133.7
Farthing	Out	161.4 ab	4427.62 a	90.3
Indigo Crisp	Out	126.6 ab	3699 a	107.6
Legacy	Out	193.1 a	4862.03 a	117.7
<i>p-value</i>	Condition	0.1608	0.6641	0.2791
	Cultivar	0.3803	0.3271	0.1097
	Cultivar x Condition	0.0054	0.0172	0.9470

^zIndication of the condition the cultivar was located where In = inside the high tunnel, and Out = open field. Growth parameters biomass, leaf area, and height values at the end of the growing season. The destructive measurement was taken in November. P-values show the significance of the interaction between the cultivar and the condition factors on the effects of the growth parameters. Tukey grouping was placed in parameters that show a significant effect ($p < 0.05$) on the interaction between the factors: cultivar and condition.

Table 2.10. Root parameters during destructive measurements in April, May, and June

		Surface Area (cm ²)					
		Farthing		Indigo Crisp		Legacy	
DAT		High Tunnel	Open Field	High Tunnel	Open Field	High Tunnel	Open Field
	33	1708.1	2440.1	1665.1	1494.2	2764.4	1912.6
	61	3359.5	2789.6	2731.9	2891.9	3254.2	2199.6
	89	6963.4	6246.6	4455.1	2817.0	4734.6	3755.8
		Root Length (cm)					
		Farthing		Indigo Crisp		Legacy	
DAT		High Tunnel	Open Field	High Tunnel	Open Field	High Tunnel	Open Field
	33	31245.0	35355.0	33063.0	25895.0	50469.0	30395.0
	61	44341.0	36401.0	36417.0	37986.0	45356.0	29395.0
	89	91248.0	84334.0	55088.0	36477.0	57688.0	50888.0
		Average Diameter (mm)					
		Farthing		Indigo Crisp		Legacy	
DAT		High Tunnel	Open Field	High Tunnel	Open Field	High Tunnel	Open Field
	33	0.174	0.219	0.161	0.184	0.176	0.202
	61	0.241	0.246	0.240	0.242	0.231	0.241
	89	0.246	0.239	0.257	0.246	0.265	0.244
		Root Volume (cm ³)					
		Farthing		Indigo Crisp		Legacy	
DAT		High Tunnel	Open Field	High Tunnel	Open Field	High Tunnel	Open Field
	33	7.5	13.5	6.7	6.9	12.1	9.8
	61	20.5	17.1	16.4	17.6	18.8	13.2
	89	42.6	37.2	28.9	17.4	31.2	22.4
		Biomass (g)					
		Farthing		Indigo Crisp		Legacy	
DAT		High Tunnel	Open Field	High Tunnel	Open Field	High Tunnel	Open Field
	33	3.3	4.8	2.0	1.9	6.3	8.0
	61	10.6	6.4	5.2	6.7	10.1	9.0
	89	18.1	16.9	13.1	8.7	19.7	19.0
		Surface Area	Root Length	Average Diameter	Root Volume	Biomass	
	Condition (Cond)		0.0656	0.066	0.0475	0.0482	0.531
	Cultivar (Cult)		0.0083	0.0244	0.4377	0.0034	0.0041
	DAT		<.0001	0.0001	<.0001	<.0001	<.0001
<i>p-value</i>	Cond*Cult		0.5548	0.6169	0.492	0.4872	0.9027
	Cond*DAT		0.3694	0.9429	0.0007	0.055	0.5662
	Cult*DAT		0.0201	0.0235	0.063	0.0215	0.6087
	Cond*Cult*DAT		0.7201	0.7186	0.7667	0.7118	0.7741

Root parameters were analyzed from the destructive measurements made in DATs 33, 61, and 89 which are equivalent to April, May, and June. P-Values indicate the level of significance of the interaction between the three main factors and individual factors as well with a level of significance if $p < 0.05$.

Table 2.11. Root parameters by cultivar

Cultivar	Surface Area (cm²)	Root Length (cm)	Average Diameter (mm)	Root Volume (cm³)	Biomass (g)
Farthing	3917.9 a	53821 a	0.2274	23.1 a	9.98 ab
Indigo Crisp	2675.9 b	37488 b	0.2218	15.6 b	6.25 b
Legacy	3103.5 ab	44032 ab	0.2265	17.6 b	11.98 a
<i>p-value</i>	0.0083	0.0244	0.4377	0.0034	0.0041

Root parameters were compared between the cultivar factors. Values in the table are the estimates retrieved from the ANOVA results in the statistical analysis. P-values indicate the level of significance of the differences in each parameter estimated between cultivars. level of significance is $p < 0.05$.

Table 2.12. Root parameters by condition

Condition^z	Surface Area (cm²)	Root Length (cm)	Average Diameter (mm)	Root Volume (cm³)	Biomass (g)
In	3515.15	49435	0.2212 b	20.5211 a	9.7889
Out	2949.7	40792	0.2293 a	17.2239 b	9.0167
<i>p-values</i>	0.0656	0.066	0.0475	0.0482	0.531

^zCondition indicates where the cultivar is located, In = high tunnel, and Out = open field. The table shows the differences between the condition effect and the LSMeans generated for the three days after the transplant where root characteristics were analyzed. Only the root characteristics with a significant difference ($p < 0.05$) show Tukey grouping.

Chapter 3

Physiological Response of Southern Highbush Blueberries to High Tunnel Conditions

3.1 Introduction

Solar radiation is the primary driver of photosynthesis, a process in which energy and sugars are created for the generation of biomass and other biochemical reactions. Water and CO₂ are two other elements needed for this process to take place (Giuliani et al., 1997). The leaf stomata which regulate water loss through transpiration and carbon uptake also play an important role in photosynthesis. For researchers and producers, the abundance of CO₂ in the atmosphere is not a concern for this process, while water availability will depend on management practices (Franck & Vaast, 2009). To increase food crop production, it is essential to not only provide nutrients, water, and cultural management but also to optimize environmental conditions that promote photosynthetic responses. Achieving this goal requires a better understanding of the complex interactions between plants, soil, and climate (Wang et al., 2007).

One of the most concerning limitations of photosynthetic activity is photoinhibition. This process is caused by excessive light absorption from the leaves creating an excess of light energy in the chlorophyll reaction centers of the chloroplasts which results in their impairment and inactivation (Bertamini et al., 2006). Heat stress is another concern in photosynthetic limitations. Temperatures above the optimal ranges limit photosynthesis by increased photorespiration rates, which consume the energy and carbon fixed during photosynthesis, leading to decreased net carbon gain. High temperatures can also cause damage to the photosynthetic apparatus and membrane structures, leading to oxidative stress and cell death, of course under extreme conditions (Salvucci & Crafts-Brandner, 2004). It should not be forgotten that cultivar selection is a critical factor in maximizing crop productivity and quality. When choosing a cultivar, it is essential to

consider not only its fruit quality and yield potential but also its adaptability to the local environment (Lin & Binns, 1991).

Alternative systems of production create these optimal conditions that improve the productive qualities of fruiting crops if properly managed of course. (Fang et al., 2020). High tunnels have been widely popularized for short-cycle vegetables and fruit crops. It is more recently that perennial fruiting crops are being produced under high tunnels. The microclimatic environment generated inside hoop houses can optimize conditions for increasing production if properly managed (Wien, 2009). Blueberries have shown to be an ideal crop for high tunnel production. This fruit-producing woody shrub has increased in popularity in the past decades and is considered a novelty crop not only because of its flavor but also because of its health benefits. Researchers and producers continuously work towards improving production in all directions (Santos & Salame-Donoso, 2012).

Physiological responses can help understand internal responses to environmental conditions, but there is still little information regarding blueberry cultivars under alternative systems of production. The objective of this study is to compare the photosynthetic responses of the southern highbush blueberries, 'Farthing', 'Indigo Crisp', and 'Legacy', grown under high tunnels and open field conditions. The study will provide insights into the adaptive mechanisms of the cultivars to different environmental conditions and the potential benefits of using high tunnels for blueberry production. The results of this study will contribute to the development of evidence-based recommendations for selecting cultivars and optimizing management practices for blueberry production in high tunnels and open fields.

3.2 Material and Methods

3.2.1 Site Preparation

During the 2022 growing season and the beginning of 2023, an experiment was conducted at the E.V. Smith Research Station, Plant Breeding Unit located in Tallahassee, Alabama at coordinates 32.49679151185915, -85.89036510954459. The study utilized a high tunnel, measuring 267.6 m², which was covered with polyethylene film. Prior to the establishment of the high tunnel, soil preparation, and amendment were completed, including the incorporation of 15 cm of pine bark into the soil to promote soil acidification. Soil tests revealed a pH level between 4.8 to 5.2 after the amendments. Raised beds, each 2.4 meters apart when measured from the center of the bed, were created using 5 cm of pine bark covered with weed fabric. There were four raised beds located within the high tunnel and two in open field conditions.

Plants were spaced 0.91 m apart within rows. The planting holes on the weed fabric were done using a fire torch and a stainless-steel ring cast to mold the circular shape, each hole being 30.5 cm in diameter. The study did not employ a formal experimental design but rather utilized a sampling approach. Also, the study was observational in nature and not randomized in order to evaluate physiological parameters during the first year of establishment. There were 180 plants of southern highbush (*Vaccinium corymbosum* L. interspecific hybrids) used for this study, distributed evenly between these cultivars: ‘Farthing’, ‘Indigo Crisp’, and ‘Legacy’, where 30 plants per cultivar were planted inside of the high tunnel and the remaining 30 plants of each cultivar were planted in outside conditions (Fig 2.1) All physiological measurements consisted of three replicates of each cultivar and location during each measurement event. All plant material was sourced from one-year hardwood cuttings from a Florida nursery.

3.2.2 Crop management

To promote growth, all reproductive organs were manually removed immediately after planting. During the vegetative growth period from March to November of 2022, irrigation was applied in three 45-minute irrigation events per day using micro emitters with a 1.9 liter per hour discharge. Ammonium sulfate was injected into the system every two weeks until September, with each fertilization supplying 8.9 grams per plant. Plants were pruned twice during the growing season, once in August to eliminate infected tissue affected by stem blight (*Botrytis* sp.) and again in October for structural purposes. A third of the plant's height was trimmed to encourage robust growth in the following season. The equipment used during both events was disinfected between each plant.

3.2.3 Environmental Monitoring and Analysis

A ZL6 logger (Meter) was placed in the center of the high tunnel and linked to an Atmos 14 (Meter) weather sensor to record ambient temperature ($^{\circ}\text{C}$) and relative humidity (RH, %). Additionally, it was connected to an SQ-521 (Apogee Instruments Inc.) sensor to record photosynthetic photon flux density (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$). Two A-125 sensors (Watchdog, Spectrum Technologies Inc.) were placed beneath each condition to document soil temperature ($^{\circ}\text{C}$), with the soil probe being buried ten centimeters below the surface. To measure ambient temperature and relative humidity in outside conditions, a Watchdog A-150 (Watchdog, Spectrum Technologies Inc.) was used, along with a LightScout sensor (Spectrum Technologies Inc.) to record PPFD. Data was recorded every 15 minutes by all sensors.

To obtain the daily light interval (DLI, $\text{mol m}^{-2} \text{day}^{-1}$), the PPFD data were averaged over a 24-hour period and multiplied by 3,600 to convert seconds to days, then divided by 1,000,000 to convert from μmol to mol . The resulting DLI was averaged monthly. The ambient temperature

data was used to determine the daily maximum and minimum values. Growing degree days were calculated for southern highbush using the Spec 9 Basic program (Spectrum Technologies Inc.), utilizing data from the four A-125 sensors (Watchdog, Spectrum Technologies Inc.). The base temperature for southern highbush was 7°C (Li & Bi, 2019). The soil temperature and relative humidity were averaged monthly.

3.2.4 Physiological Measurements and Analysis

A portable gas exchange analyzer (LICOR, LI-6800) was used to measure photosynthetic rate (A , $\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration, (E , $\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_{sw} , $\text{mol m}^{-2} \text{s}^{-1}$), intercellular CO_2 concentration (C_i , $\mu\text{mol mol}^{-1}$), and quantum efficiency (PhiPS2).

Diurnal photosynthesis measurements

To monitor diurnal changes, measurements were taken every two hours between 6:00 am and 6:00 pm, with a frequency of every 30 to 35 days using a portable gas exchange analyzer (LI-6800, LICOR INC., Lincoln, Nebraska, USA). The measurements were conducted from April to September 2022. Measurements were done on three plants of each cultivar in each condition. The leaves selected for each plant for each measurement were newly matured leaves at the top-middle of the canopy. The environmental factors temperature, and relative humidity used during each measurement were retrieved from the environmental sensors at each hour of measurements and the light intensity selected for each measurement was determined by the external quantum sensor of the LI-6800. The CO_2 levels were set to 410 ppm. Environmental factors were set at the beginning of each hour of measurements for diurnal and midday measurements. The integral of the total photosynthetic rate and stomatal conductance of each individual diurnal calculated using the methods reported by Sanz-Saez (2017).

Midday photosynthesis measurements

During this study, midday measurements were conducted every 15 to 20 days between 10:00 and 11:00 am, coinciding with the daily peak of photosynthesis as identified during diurnal measurements. The measurements were carried out on three plants of each cultivar in each condition, from April to September of 2022. Newly matured leaves at the top-middle of the canopy were selected for each measurement, and environmental factors such as temperature and relative humidity were recorded at the start of each measurement event using environmental sensors. Light intensity for each condition was determined using the external quantum sensor of the LI-6800, while CO₂ levels were maintained at a constant 410 ppm throughout the study. These standardized measurement procedures ensure that the data collected on photosynthesis rates are reliable and comparable across the different plants and conditions evaluated in this study.

Photosynthesis to C_i curves

The Maximum photosynthesis at saturation was adapted from the AC_i measurements according to Soba et al. (2020). These measurements took place during July and October between 8:00 am and 12:00 pm on three independent samples in each condition for each cultivar. The carbon dioxide concentration of the reference chamber was set to 410, 300, 200, 100, 50, 0, 410, 410, 600, 800, 1,000, and 1,200 $\mu\text{mol mol}^{-1}$. The light saturation point was determined at 2,000 $\mu\text{mol mol}^{-1}$ by a light response measurement where the assimilation was measured at 2,500, 2,200, 2,000, 1,800, 1,500, 1,200, 900, 600, 300, 150, 50, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light on three independent samples of each cultivar under the high tunnel.

3.2.5 Statistical analyses

This study analyzed the integrated diurnal and midday physiological parameters and maximum photosynthetic rate observations with their corresponding stomatal conductance values

at saturated CO₂. A Generalized Linear Mixed Model was used to evaluate the three-way interaction among condition, cultivar, and time. Additionally, fixed effects of condition, cultivar, and time were individually evaluated, as well as their two-way interactions using a Tukey grouping to separate the LSMeans. Repetition was randomized with three repetitions per experimental unit to account for within-unit variation. The significance level was set at $p < 0.05$. The relationship between Diurnal and midday photosynthesis and the air temperature was measured using a regression model. Statistical analyses were done using SAS 9.4.

3.3 Results

3.3.1 Environmental Data

Under high tunnel conditions, the average maximum temperatures were 1.7°C higher than those in the open field. Throughout the trial, the high tunnel values remained higher than those in the open field. In terms of average minimum temperatures, high tunnel conditions were 0.2°C higher than those in the open field (Fig. 3.1) According to the light data, plants in the open field received 22.5% more direct light than those inside the high tunnel at the end of the trial. The month of June had the highest monthly values for DLI. In April, the open field received 30% more direct light than the high tunnel, which was the highest difference in DLI (Fig. 3.1).

3.3.2 Diurnal measurements

The analysis of the diurnal integrals showed no significant interaction effects between cultivar, condition, and DAT on photosynthetic rate and stomatal conductance (Fig. 3.2.) Both parameters showed significant changes in DAT which indicate that there were different responses throughout the season (Fig 3.2). The test of fixed effects showed a significant interaction between cultivar and condition on the photosynthetic rate. The only significant differences between cultivars and conditions showed that ‘Farthing’ and ‘Indigo Crisp’ in both conditions had a higher

diurnal photosynthetic rate compared to ‘Legacy inside and outside of the high tunnel (Table 3.1). This is supported by the significant differences between cultivars, where ‘Farthing’ and ‘Indigo Crisp’ showed a significantly higher photosynthetic rate compared to the ‘Legacy’ cultivar (Table 3.1). The condition also showed a significant effect on the photosynthetic rate, where plants under the high tunnel showed higher photosynthetic values compared to the open field (Table 3.1). The differences between conditions of the same cultivar for each individual DAT, using a mean separation analysis showed that ‘Farthing’ and ‘Indigo Crisp’ under high tunnel conditions had a higher photosynthetic rate compared to the outside counterpart during the diurnal measured of DAT 96. During this same analysis, ‘Legacy’ showed a higher stomatal conductance in the open field compared to the high tunnel during DAT 165 (Fig 3.2).

3.3.3 Midday measurements

The test of fixed effects showed a significant interaction between cultivar, condition, and DAT on photosynthetic rate, stomatal conductance, and transpiration (Table 3.2). The ‘Farthing’ and ‘Indigo Crisp’ showed more instances of significantly higher photosynthetic rates in high tunnel conditions compared to the open field throughout the midday measurements (Fig. 3.3). All cultivars under open field conditions showed significantly higher values of stomatal conductance, transpiration, and intercellular carbon compared to plants under the high tunnel (Table 3.2). Plants outside showed $0.019 \text{ mol m}^{-2} \text{ s}^{-1}$ more midday stomatal conductance compared to high tunnel conditions. ‘Legacy’ showed the highest numeric difference for stomatal conductance between conditions, $0.0280 \text{ mmol m}^{-2} \text{ s}^{-1}$, followed by ‘Farthing’ with $0.0102 \text{ mmol m}^{-2} \text{ s}^{-1}$, and lastly by ‘Indigo Crisp’ with $0.0102 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Table 3.2). Quantum efficiency was significantly different between the condition and cultivar as ‘Farthing’ and ‘Indigo Crisp’ under the high tunnel showed a higher quantum efficiency under the high tunnel compared to plants outside and the

‘Legacy’ cultivar (Table 3.2). All plants under the high tunnel showed a 0.046 significantly higher ratio compared to open field conditions (Table 3.2).

3.3.4 Photosynthesis at Saturation

Photosynthesis at saturation for all cultivars, conditions, and DATs ranged between 13.3 and 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with stomatal conductance ranging between 0.0580 and 0.1678 $\text{mol m}^{-2} \text{s}^{-1}$. The photosynthetic rate at saturation and its respective stomatal conductance showed no significant effect from the interaction between cultivar, condition, and DAT (Table 3.3). However, the photosynthetic rate showed significant differences at a cultivar level, where ‘Farthing’ and ‘Indigo Crisp’ were significantly higher compared to ‘Legacy’ (Table 3.3). Also, stomatal conductance was significantly different between conditions, as all cultivars showed higher values outside compared to the high tunnel (Table 3.3).

3.5 Discussion

The monitored weather parameters revealed notable differences between the high tunnel and open field conditions. The high tunnel provided higher average and maximum temperatures and an average of 22% less direct light interception. Wien (2009) reported higher temperatures under the high tunnel during the day throughout the growing season compared to outside conditions. He also observed rapid decreases in temperature to those outside once the day came to an end. Retamal-Salgado et al. (2015) reported similar results as well as an average 25% difference in light interception between conditions. historical studies observed a range between 20 to 30% difference in light interception between conditions (Retamales-Salgado et al., 2015; Smrke et al., 2021). Hao et al., (2019) evaluated different northern and southern highbush blueberry (*V. corymbosum*) cultivars under different temperature ranges of 5°C. They determined that some cultivars reached

peak photosynthesis between 25 to 30°C while other cultivars reached peak values between 30 and 35°C. What all cultivars had in common was decreased photosynthesis beyond 35°C.

The photosynthetic responses at elevated CO₂ were similar to those observed by Petridis et al. (2018) where the maximum photosynthetic rates at saturation ranged between 15 and 25 μmol m⁻² s⁻¹ across their evaluated northern highbush (*V. corymbosum*) cultivars. They state that the differences in photosynthetic rates at saturation are attributed to the maximum carboxylation rates of 1,5-bisphosphate carboxylase oxygenase (Rubisco), which can indicate a photosynthetic limitation. The significantly higher stomatal conductance observed in plants in outside conditions could be attributed to the higher light acclimation that was generated throughout the season.

Between species, shade-acclimated plants are light-saturated at a lower PPFD compared to plants acclimated at full sun. Light saturation can easily be reached causing plants to consume stored energy in sugars to dissipate light energy. Plants under full sun show to have a higher photosynthetic capacity (Tateno & Taneda 2007). This was the case for the ‘Legacy’ midday measurements since it showed an overall higher photosynthetic rate in open field conditions. Dai et al. (2009) showed that *Tetrastigma hemsleyanum* plants under full sun showed some levels of photoinhibition and a lower photosynthetic rate compared to plants under 50% shade. During our midday measurements, ‘Farthing’ and ‘Indigo Crisp’ showed higher photosynthetic rates in more instances through different DATs under the high tunnel compared to the open field suggesting that these cultivars may be more sensitive to photoinhibition in the outside conditions. Plants appear to benefit from light diffusion at a photosynthetic level. Retamales & Hancock (2018) state that a big part of commercial blueberry cultivars originated from genetic material that thrives and is found naturally in understories of forests so optimal growing conditions would be intermediate light intensities as well as diffused light sources. This can be seen reflected in this study since the overall

diurnal photosynthetic rate was higher under high tunnel conditions. The differences showed between cultivars indicate that the 'Legacy' cultivar was the least adapted to the region regardless of the condition considering it showed a lower photosynthetic profile in both the midday and diurnal measurements compared to 'Farthing' and 'Indigo Crisp'

The higher stomatal conductance observed in plants outside of the high tunnel could be attributed to a higher PPFD exposure. Acclimation plays a big role in physiological responses. Long-term light exposure levels have a lasting effect on the stomatal activity as well as size and density. (Halland Nicholas, 2009). Similar studies also observed higher stomatal conductance in plants under higher PPFD compared to those under reduced direct light (Kim et al., 2011). They reported changes in leaf morphology such as increased leaf area and stomatal characteristics such as size and density and attribute this to be the reason for the differences in stomatal conductance between conditions. Higher PPFD exposure increased stomatal conductance which increased carbon uptake as well as water loss through transpiration.

3.6 Conclusions

Photosynthetic parameters are observed to be influenced differently by high tunnel conditions across cultivars. Overall photosynthesis was higher in plants under the high tunnel during the diurnal measurements. The comparison between cultivars showed that 'Legacy' is the least adapted between cultivars. Stomatal conductance seems to not be affected by conditions or cultivars during the diurnal measurements. During the midday measurements, the 'Legacy' cultivar showed a higher photosynthetic profile in open field conditions considering the midday measurements showed higher photosynthetic values compared to the high tunnel. 'Farthing' and 'Indigo Crisp' midday photosynthesis performed better under the high tunnel compared to their outside counterparts and the 'Legacy' cultivar when observing the individual measurements in

each day after transplant. The stomatal conductance was higher under more direct light interception, consequently increasing overall transpiration during the midday measurements and the photosynthesis at saturation measurements.

Overall, the findings suggest that high tunnel conditions have a cultivar-specific effect on photosynthetic parameters in southern highbush blueberry production. The variability observed in the responses of different cultivars indicates the importance of careful and appropriate cultivar selection that is adapted to the specific environmental conditions of high tunnels in southern highbush blueberry growth. It is interesting to note that the ‘Legacy’ cultivar showed a higher photosynthetic profile in open field conditions, indicating that this cultivar may not be well-suited for high tunnel production in this region. However, the ‘Farthing’ and ‘Indigo Crisp’ cultivars performed better under high tunnel conditions compared to their outside counterparts and the ‘Legacy’ cultivar, indicating that these cultivars may be better adapted to the conditions found in southern highbush blueberry high tunnels.

The higher stomatal conductance observed under more direct light interception in high tunnel conditions suggests the benefits of the reduction of light intensity in southern highbush blueberry high tunnel systems to optimize plant growth and development. It is important to note that physiological responses to high tunnel conditions during the first year of the establishment were significant, and further studies are needed to determine whether these trends persist over multiple growing seasons.

As a concluding remark, these results demonstrate the potential of high tunnel production to improve southern highbush blueberry physiological parameters. Careful cultivar selection and management practices are key to optimizing plant growth and production in southern highbush blueberry high tunnels. Further research in this area is needed to fully understand the complex

interactions between cultivar selection, environmental conditions, and plant physiology in southern highbush blueberry high tunnel production.

3.7 References

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3.8 Figures

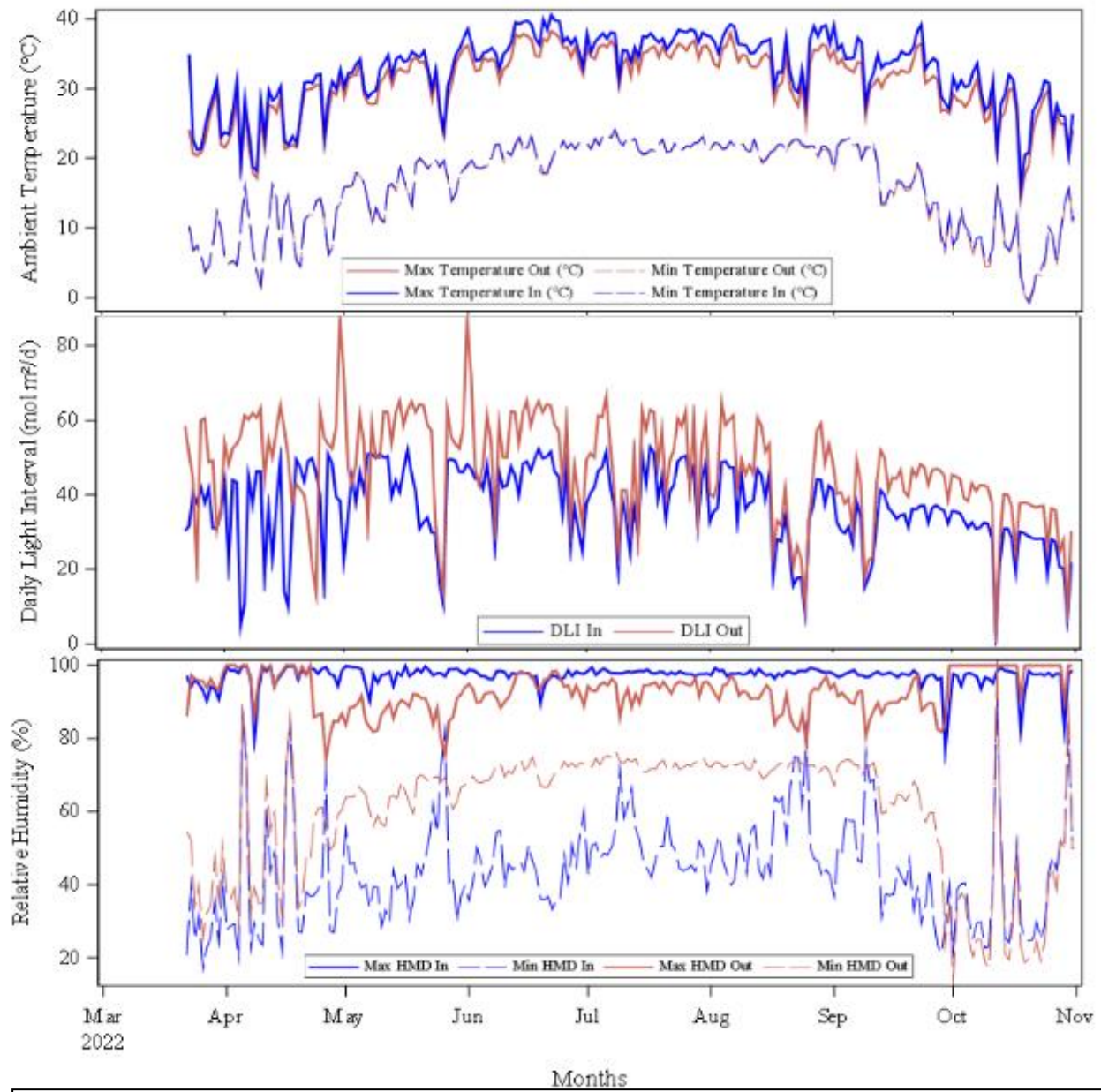


Figure 3.1. Maximum and minimum air temperature, daily light integrals, and maximum and minimum relative humidity under both conditions.

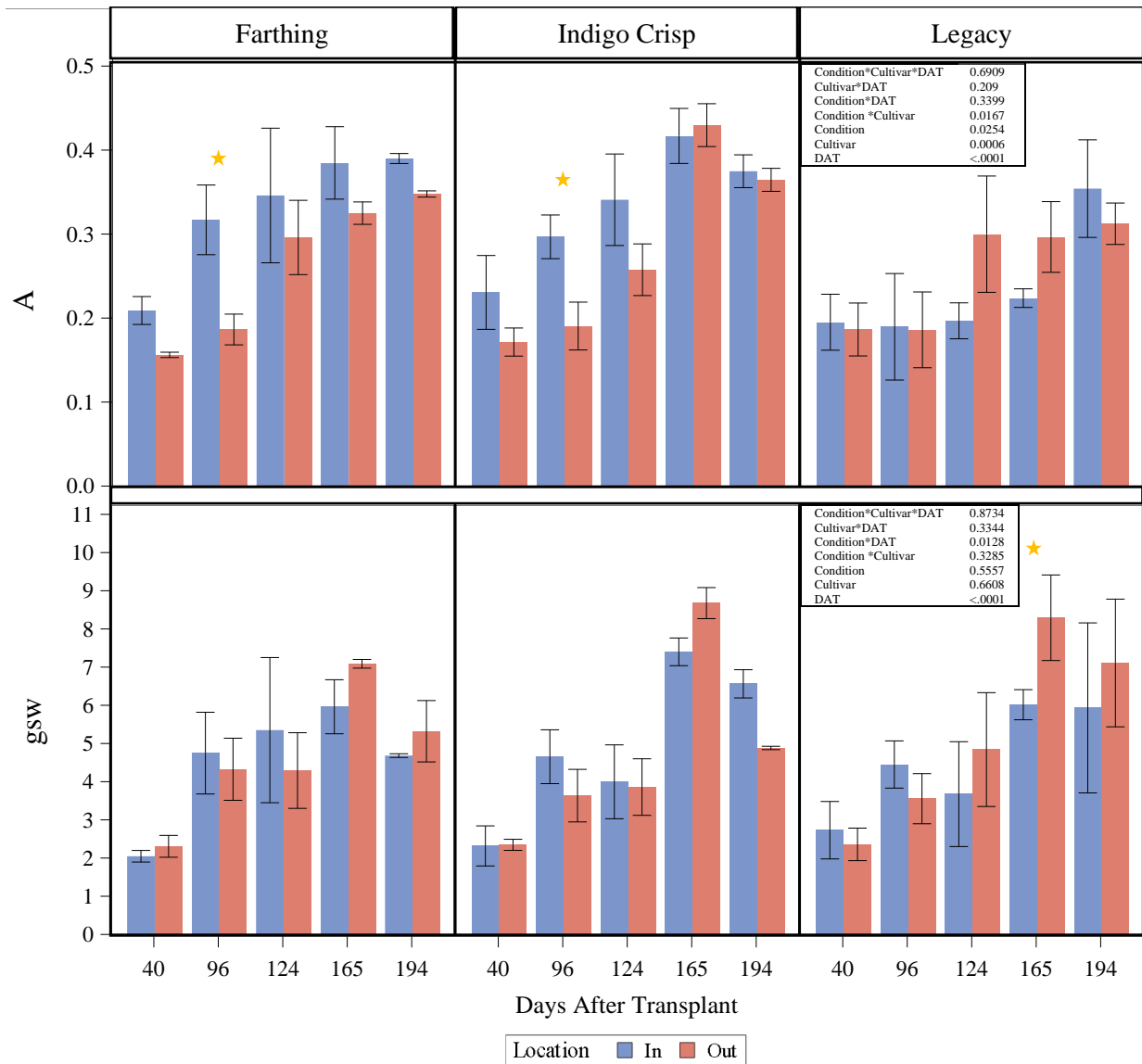


Figure 3.2 Integral diurnals of A photosynthetic rate ($\text{mmol m}^{-2} \text{day}^{-1}$) and gsw stomatal conductance ($\text{mol m}^{-2} \text{day}^{-1}$) throughout the season by cultivar and condition. P-Values showed for each variable with the significance of the effect of each factor and their respective interactions. The significance level was analyzed as $p < 0.05$. The star figure indicates the significance of the differences within each cultivar to their condition counterpart for every individual DAT.

A

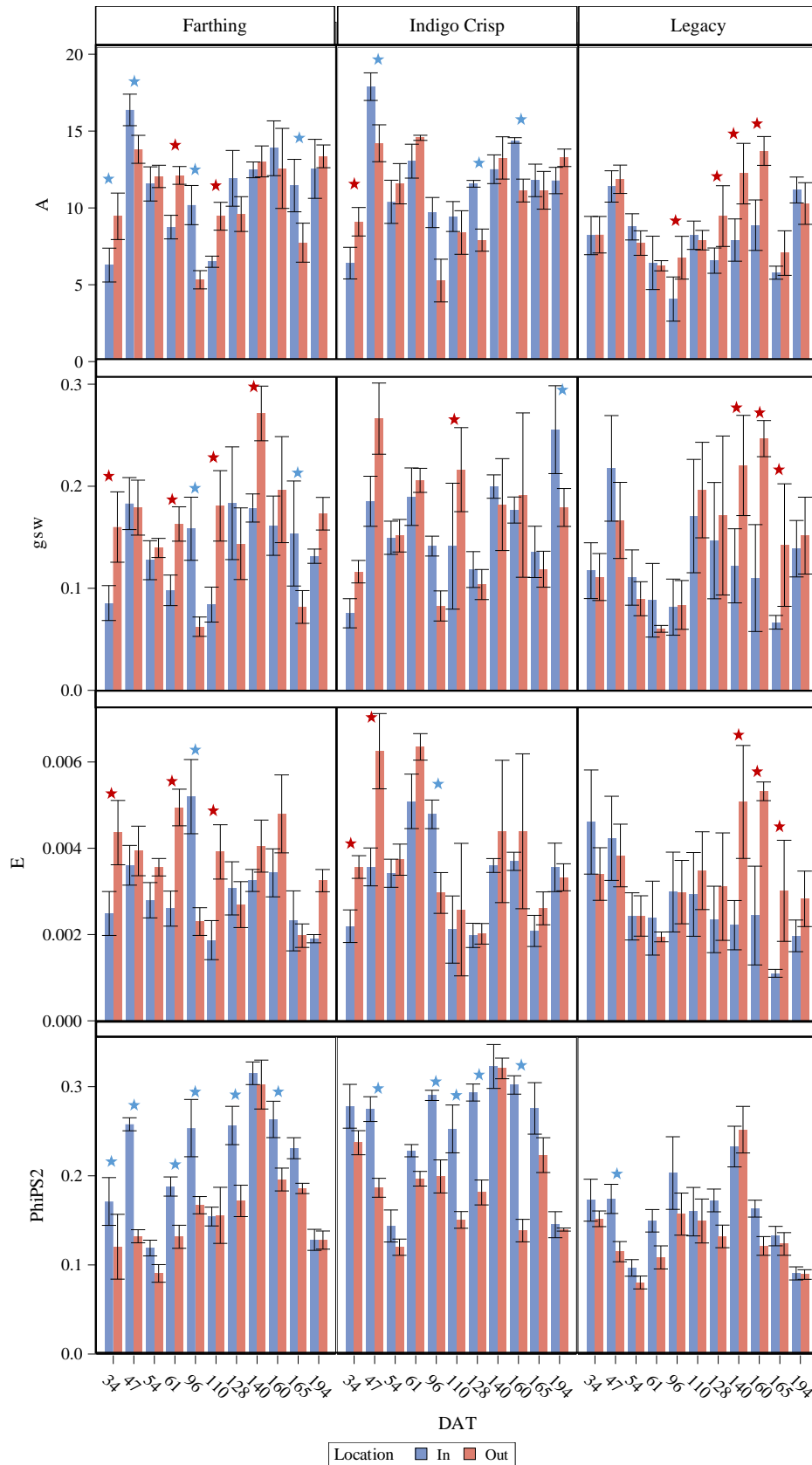


Figure 3.3 Midday A ($\mu\text{mol m}^{-2} \text{s}^{-1}$), gsw ($\text{mol m}^{-2} \text{s}^{-1}$), E ($\text{mol m}^{-2} \text{s}^{-1}$) PhiPS2 showed the cultivars in each condition by DAT. The star icons represent any significant differences between conditions within each cultivar for every individual DAT where red signifies a higher value in outside conditions and blue indicates higher values under the high tunnel.

3.9 Tables

Table 3.1. Diurnal measurements shown by cultivar and condition, cultivar, and condition

Cultivar	Condition^z	A mmol m⁻² s⁻¹	gsw mol m⁻² s⁻¹
Farthing	In	0.3293 ab	4.5576
Indigo			
Crisp	In	0.332 a	4.9855
Legacy	In	0.2318 c	4.5595
Farthing	Out	0.2622 bc	4.6659
Indigo			
Crisp	Out	0.2828 ab	4.6791
Legacy	Out	0.2562 c	5.2303
Cultivar		A mmol m⁻² s⁻¹	gsw mol m⁻² s⁻¹
Farthing		0.2958 a	4.6118
Indigo Crisp		0.3074 a	4.8323
Legacy		0.244 b	4.8949
Condition^z		A mmol m⁻² s⁻¹	gsw mol m⁻² s⁻¹
In		0.2977 a	4.7009
Out		0.2671 b	4.8584
<i>p-values</i>	Effects	A	gsw
	Condition*Cultivar*DAT	0.6909	0.8734
	Cultivar*DAT	0.209	0.3344
	Condition*DAT	0.3399	0.0128
	Condition*Cultivar	0.0167	0.3285
	Condition	0.0254	0.5557
	Cultivar	0.0006	0.6608
	DAT	<.0001	<.0001

^zCondition indicates the location of the cultivar where In = high tunnel and out = open field. Diurnal physiological parameters measurements show the significance of the interaction between cultivar and condition and the significant differences between the condition effect and the cultivar effect. P-values of the midday photosynthesis measurements, the significance level of each effect, and the interaction between effects, including the three-way interaction between the three main effects.

Table 3.2. Midday measurements shown by cultivar and condition, and by condition

Cultivar	Condition^z	A μmol m⁻² s⁻¹	gsw mol m⁻² s⁻¹	E mol m⁻² s⁻¹	PhiPS2
Farthing	In	10.39 A	0.1217	0.00246	0.210 B
Indigo Crisp	In	11.02 A	0.1413	0.00277	0.253 A
Legacy	In	7.20 C	0.1016	0.00209	0.158 D
Farthing	Out	10.08 A	0.1394	0.00309	0.161 D
Indigo Crisp	Out	10.37 A	0.1515	0.00346	0.189 C
Legacy	Out	8.53 B	0.1296	0.00289	0.133 E

	Condition^z	A μmol m⁻² s⁻¹	gsw mol m⁻² s⁻¹	E mol m⁻² s⁻¹	PhiPS2
	In	9.5357	0.1215 B	0.002558 B	0.2069 A
	Out	9.6565	0.1402 A	0.003248 A	0.161 B

	Effects	A	gsw	E	PhiPS2
<i>p-value</i>	Condition*Cultivar*DAT	<.0001	<.0001	<.0001	0.2324
	Condition *DAT	0.0011	0.0028	0.0012	<.0001
	Cultivar*DAT	<.0001	<.0001	<.0001	<.0001
	Condition *Cultivar	0.0014	0.504	0.9201	0.0007
	Condition	0.606	0.0032	<.0001	<.0001
	Cultivar	<.0001	0.0004	0.0076	<.0001
	DAT	<.0001	<.0001	<.0001	<.0001

^zCondition indicates the location of the cultivar where In = high tunnel and out = open field. Midday physiological parameters measurements show the significance of the interaction between cultivar and condition and the significant differences between the condition effect. P-values of the midday photosynthesis measurements, the significance level of each effect, and the interaction between effects, including the three-way interaction between the three main effects.

Table 3.3. Photosynthetic rate and stomatal conductance at saturation

		² DAT			
		129		186	
Cultivar	³ Condition	A $\mu\text{mol m}^{-2}\text{ s}^{-1}$	gsw $\text{mol m}^{-2}\text{ s}^{-1}$	A $\mu\text{mol m}^{-2}\text{ s}^{-1}$	gsw $\text{mol m}^{-2}\text{ s}^{-1}$
Farthing	In	26.89	0.0916	23.14	0.0836
Indigo	In	29.03	0.1151	19.46	0.0580
Legacy	In	13.30	0.0718	16.93	0.1019
Farthing	Out	22.86	0.1178	21.61	0.1678
Indigo	Out	30.19	0.1323	21.22	0.1254
Legacy	Out	21.53	0.1347	21.52	0.1318
Cultivar		A $\mu\text{mol m}^{-2}\text{ s}^{-1}$	gsw $\text{mol m}^{-2}\text{ s}^{-1}$		
Farthing		23.6 a	0.1152		
Indigo Crisp		25.0 a	0.1077		
Legacy		18.3 b	0.1100		
³ Condition		A $\mu\text{mol m}^{-2}\text{ s}^{-1}$	gsw $\text{mol m}^{-2}\text{ s}^{-1}$		
In		21.46	0.087 b		
Out		23.16	0.135 a		
Effects		A	gsw		
Condition*Cultivar*DAT		0.9285	0.7055		
Condition*DAT		0.9791	0.613		
Cultivar*DAT		0.4032	0.6383		
<i>p-value</i>	Condition*Cultivar	0.1146	0.9656		
	Condition	0.3338	0.0275		
	Cultivar	0.0108	0.9535		
	DAT	0.3273	0.9721		

²DAT = Date after transplant. ³Refers to the location as where In = High Tunnel and Out = Open field. This table shows the Maximum photosynthetic response (A) and stomatal conductance (gsw) at a light and CO₂ saturation during two different DATs. P-values indicate the level of significance of the effects and their interactions with photosynthetic rate and stomatal conductance. Tukey grouping shown

4 General Conclusions

It can be concluded that high tunnel production systems have an impact on the growth, development, and photosynthetic efficiency of southern highbush blueberry cultivars during the first year of establishment. The observation of accelerated blooming stages is a point of serious consideration for implementing high tunnels for production. However, the effects of high tunnel conditions on different cultivars vary, with differences observed in biomass accumulation and photosynthetic rate between cultivars. ‘Legacy’ showed to be better adapted to open field conditions as ‘Farthing’ and ‘Inigo Crisp’ showed no differences in growth between conditions. Plants under the high tunnel showed a higher photosynthetic rate during the diurnal measurements. Increases in stomatal conductance in outside conditions resulted in an increase in transpiration. This is a problem considering the known low water uptake blueberry root systems possess, so incorporating methods that decrease water loss through transpiration must be considered. Even though not many significant interactions were observed between cultivar and condition for some parameters for higher values under the high tunnel like biomass and photosynthesis, certain tendencies can be identified. It must be taken into consideration that these differences can become significant once the establishment period is finalized. The results of this study have provided further insight into the influencing factors of photosynthesis under high tunnel production. The ‘Legacy’ cultivar showed higher growth and physiological values in outside conditions. Nonetheless, incorporating high tunnels into blueberry production can potentially improve plant growth and development and, overall plant health, and improve photosynthetic parameters. Overall, the study highlights the potential benefits of high tunnel production for southern highbush blueberry (*V. corymbosum*) cultivars and the need for further research to optimize crop management practices and improve agricultural sustainability.

5 Recommendations

- It is recommended to continue monitoring phenological bloom stage progression and study yield and fruit quality to evaluate the effects of environmental conditions on crop productivity and quality. By analyzing the photosynthetic performance of each cultivar under different systems of production, recommendations for cultivar selection, management practices, and resource allocation can be developed.
- Continue growth and photosynthesis measurements and analysis to study the long-term effects of blueberry production under high tunnel conditions.
- The study recommends increasing irrigation rates and correlating them with stomatal conductance to develop improved irrigation rates for proper blueberry management.
- Include more southern highbush cultivars in future studies to compare growth and development under high tunnel conditions and assess its feasibility compared to open field production systems.