EVALUATING FRESH POSTHARVEST KEEPING QUALITY OF BLUEBERRY USING INSTRUMENTAL AND DIFFERENTIAL GENE EXPRESSION ANALYSES

by

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(Under the Direction of Rachel Itle)

ABSTRACT

Georgia's blueberry industry consists of southern highbush (SHB, *Vaccinium corymbosum* L. and *V. darrowii* Camp complex) and rabbiteye (RE, *V. virgatum* Aiton). There exists a subjective bias that SHB has higher fruit quality than RE. Their quality is also compared to northern highbush (NHB, *Vaccinium corymbosum* L.), which is perceived as superior. However, limited information supports this preconceived perception. The objective of this study was to examine the physicochemical postharvest keeping quality of major SHB, RE, and NHB cultivars and examine their differential gene expression relating to their keeping quality. During postharvest storage, the highest texture stability was by SHB, visual appearance by SHB and NHB, and berry weight by RE. Chemical quality traits were generally stable. Gene expression analyses revealed three genes involved in cell wall degradation differed over storage and types. Overall, this may help the industry in selecting and developing new cultivars with superior postharvest keeping quality.

INDEX WORDS: Southern highbush, Rabbiteye, Northern highbush, Physicochemical traits, Texture, Soluble solid content, Total titratable acids, Anthocyanins, Cell wall degradation

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DEDICATION

I would like to dedicate this to my loving mother, friends, and those who have contributed to my success in graduate school. The list of people who I would name is extensive, so if you are reading this please know that I appreciate you and I could never thank you enough for your support. I would also like to dedicate this body of work to the incredible blueberry growers of the state of Georgia.

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CHAPTER 1

INTRODUCTION

Blueberry is a major specialty crop and in the U.S. in 2019. It was the fourth most valuable non-citrus fruit crop with value of utilized production at $909 million, ranking behind strawberry ($2.5 billion), apple ($2.7 billion), and grapes ($5.7 billion) (USDA, 2020). Within Georgia, blueberry ranked number nine in specialty crop value ($308 million) and accounted for over 2.2% of the state's total farm gate value (UGA Center for Agribusiness and Economic Development, 2019). The Georgia blueberry market is made up of two commercial blueberry types: southern highbush (*V. corymbosum* L. x *V. darrowii* Camp) and rabbiteye (*V. virgatum* Aiton) blueberry. Southern highbush and rabbiteye compose approximately 60% and 40% of Georgia acreage, respectively. Both types have been bred to be well adapted and perform excellently in the southeastern U.S., and there are benefits to growing both blueberry types in the state. The early ripening of southern highbush blueberry provide an advantage to growers with price premiums for their early season fruit. However, the early ripening window makes southern highbush highly susceptible to large yield losses due to late spring freezes, and fruit are primarily hand harvested which is costly. Rabbiteye blueberry ripen later and largely avoid late spring freeze damage, are generally more disease tolerant, and are machine harvested which is approximately one seventh the cost of hand harvesting.

One of the major comparisons between southern highbush and rabbiteye blueberry types is their fruit quality and postharvest keeping capability. Southern highbush fruit are viewed to have superior fruit quality over rabbiteye types. In addition, much of the North American

blueberry market consists of a third major commercial blueberry type, northern highbush blueberry (*Vaccinium corymbosum*). This type is well suited for northern latitudes of the U.S., but it is unable to be grown in the Southeastern U.S. due to their adaptability to colder climates. In addition to the fruit quality debate between southern highbush and rabbiteye, northern highbush are viewed to have superior fruit quality over both types. These subjective biases may lead to lowered price points received by Georgia growers, or may lead to type or cultivar exclusion. However, there are few studies evaluating the fruit quality and postharvest keeping capabilities of Georgia-grown southern highbush and rabbiteye blueberry to that of northern highbush blueberry. In addition, this subjectively held industry bias is inconclusive at best due to lack of large studies surveying the major cultivars within the two types.

The overall objective of this study is to provide objective information in order to understand similarities and differences in fruit quality during commercial postharvest cold storage among and within the three blueberry types and cultivars that make up the greater U.S. market. To achieve this, the main objectives of this study are: 1) compare the physical fruit quality aspects of fruit during postharvest storage 2) chemical fruit quality characteristics in early, mid, and late season southern highbush, rabbiteye, and northern highbush cultivars that are representative of the current blueberry market to identify types and cultivars that have superior postharvest keeping quality of physical traits over time, and 3) investigate the gene expression of cell wall degrading enzymes of cultivars of all three blueberry types that differ in postharvest shelf-life. The combination of these evaluations will provide information to growers and marketers with fruit quality specific parameters during the fresh postharvest storage of major rabbiteye, southern highbush, and northern highbush cultivars. These evaluations may also provide to growers and retailers the information needed to cater to niche markets. These findings

will supplement larger studies that will provide important information to the Georgia blueberry industry in order to maintain and increase market share within the U.S. Such information of the overall quality characteristics of southern highbush and rabbiteye cultivars compared to those cultivars that make up the larger blueberry market may help to prevent the lowered price-point of Georgia grown blueberry cultivars.

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CHAPTER 2

LITERATURE REVIEW

Overview

Blueberry (*Vaccinium* sect. *Cyanococcus*) is a major specialty crop. In the U.S. in 2019, blueberry was the fourth most valuable non-citrus fruit crop with value of utilized production at $909 million, ranking behind strawberry ($2.5 billion), apple ($2.7 billion), and grapes ($5.7 billion) (USDA, 2020). Of the 1.5 million tons of berries harvested in the U.S. during the year of 2019, 72 percent was strawberry (~1.1 million), 20 percent blueberry (~340,000), seven percent raspberry (~113,000), and one percent blackberry (~20,000) (USDA, 2020). Blueberry has experienced most of its growth in the past approximately 15-25 years and is now a year-round fruit crop available across the world, able to grow in numerous climate types (Retamales and Hancock, 2018). In addition, from 2015 to 2016, world blueberry consumption rose by 45 percent (Freshuelva, 2018). Consumer health consciousness, demand, and improvements in breeding have heavily contributed to this trend. Ranking high in antioxidant activity, blueberry has been cited as an anti-aging supplement for its nutraceutical properties, which also contributes to its popularity (Joseph et al., 2005; Wu et al., 2004). Blueberry is one of the few fruit crops native to North America. It is a member of the Ericaceae family, which also includes cranberry (*V.* subg. *Oxycoccus*), huckleberry (*V. membranaceum*), and azalea (*Rhododendron* spp.). The Ericaceae family is also commonly known as heath or heather family. Members of the Ericaceae family are able to inhabit nutrient deficient soils, allowing them to thrive in areas where other plants are unable to survive. They tend to thrive in heathlands, which are areas characterized as

lowlands in the montane zone where soils are known to be acidic and low-quality. Most Ericaceous plants require acidic soils for proper growth and development (Pritts and Hancock, 1992). Ericaceae plants can also be found in peat bogs, another low nutrient environment. Main physiological attributes of plants in this family include existing evergreen or deciduous shrubs and having spirally arranged leaves, paired bracteoles, and having considerably more stamens as petals in their inflorescence (Stevens et al., 2004). It is a globally distributed family, containing eight subfamilies with a recorded 129 genera and 4426 species. Ericaceae is similar to other plant families as it is also found in the upper margins of montane forest lands where most plant diversity of the world has evolved (Schwery et al., 2014).

There are four main commercial blueberry types grown 1) northern highbush (*V. corymbosum* L.), 2) rabbiteye (*V. virgatum* Aiton), 3) southern highbush (species complex of *V. corymbosum* L. and *V. darrowii* Camp), and 4) lowbush, also known as Britton Blueberry or wild blueberry, (*V. angustifolium* Aiton) (Jones and Percival, 2003). The area that most differs these blueberry types from one another is the number of chilling requirements and tolerance to cold temperatures. Lowbush blueberries require the most chilling hours (≥1000 hours) and are the most cold hardy, tolerating temperatures down to -30ºC. Northern highbush require between 800 and 1000 chilling hours and can tolerate temperatures down to -20ºC. Southern highbush and rabbiteye types do tolerate below freezing temperatures well, and require approximately 550 and 600 chilling hours, respectively (Retamales and Hancock, 2018). Northern highbush, southern highbush, and rabbiteye blueberry are the three types that make up the main U.S. commercial blueberry market.

Blueberry production

In 2018, there were 270,000 acres harvested world-wide, with 57% in North America

(153,900 acres) 23% in South America (62,100 acres), 11% in Europe (29,700 acres) followed by Asia with 8% (21,600 acres) of the total harvested acreage (FAO, 2018). In the U.S., total value of utilized production of blueberries reached over $909 million in 2019. The top five states were California ($204 million), Washington ($153 million), Oregon ($134 million), Georgia ($133 million), and New Jersey ($85 million). States leading in acres harvested were Georgia (21,700 acres), Michigan (19,700 acres), Washington (16,700 acres), Oregon (13,300 acres), and New Jersey (9,300 acres) (USDA, 2020). In the southeastern U.S., Georgia accounts for the majority of blueberry production with (95 million pounds), next to Florida (24 million pounds) These two states made up approximately 18 percent of the total production in the United States in 2019 (USDA-NASS, 2019).

In 2018, Georgia blueberry production was $308 million. Blueberry ranked number nine in specialty crop value and accounted for over 2.2% of the state's total farm gate value. Blueberry was the highest value fruit crop in the state, ahead of pecan ($218 million), onion ($150 million), bell pepper ($126 million), watermelon ($124 million), cucumber ($84 million), tomato ($51 million), and peach ($48 million) within the state of Georgia (UGA Center for Agribusiness and Economic Development, 2019). Georgia blueberry production has experienced exponential growth in the past five decades (Scherm and Krewer, 2003). In 2000, Georgia had 4,600 bearing-age acres with 90% rabbiteye and 10% southern highbush cultivars and was valued at $18.5 million (Krewer and NeSmith, 2002). More recently, the acreage of rabbiteye blueberry in Georgia has been shifting to around 40 percent of total acreage, and southern highbush with approximately 60 percent (R.A. Itle, personal communication). In 2019 the state's total harvested acreage was over 21,000 acres (USDA, 2020). In Georgia, blueberry is sold as either fresh or frozen product with large price differences between the two. In 2019, 61 million

pounds of commercial fresh market blueberry, and 32 million pounds of processed blueberry were produced (USDA-NASS, 2019). Most of the fruit intended for the fresh market is harvested by hand to achieve high fruit quality, which also makes the cost of production higher. Blueberry is hand-harvested to diminish bruising and fruit injury, prolong post-harvest storage and to maintain their appeal to consumers (Brown et al., 1996). Fruit are also machine harvested, which is less labor intensive but more labor efficient than hand harvesting (Mehra et al., 2013). Market timing and end price point play a large role in determining whether fruit is hand or machine harvested. Southern highbush season harvest lasts until late May or early June, overlapping with rabbiteye which becomes available during this time. Other blueberry producing states in northern latitudes of U.S. begin selling. The market influx of blueberry cultivars from within the state and neighboring states plays a role in the lower price point of rabbiteye blueberry later in the harvest season. When rabbiteye blueberry prices are lower, machine harvesting is utilized for the processing market (NeSmith et al., 2002). Factors such as aggregate productivity and targeted market make it difficult to determine profitability of rabbiteye blueberry and play a role in its market fluctuations. (Fonsah et al., 2011). Weather events that result in the cracking or softening of fruit forces growers to sell their fruit intended for fresh market to frozen market, another contributor to lower grower prices (Scherm and Krewer, 2003).

Georgia blueberry production

Plantings in the southeastern U.S. mostly consist of rabbiteye and southern highbush types. Blueberry is planted in raised beds to improve water drainage in poor soils, and soils can be supplemented with milled pine bark, or bushes can be grown strictly in beds of pure pine bark (Scherm and Krewer, 2003). Rabbiteye blueberry types are native to southern Georgia, north Florida, and southeastern Alabama and are well suited for the lower coastal plain region

(Appling, Bacon, Clinch, Pierce, Wayne, and Ware counties) where 90% of the state's commercial acreage is located. This region is heavily cultivated because of its ideal sandy, acidic soil type that has a high-water table (Scherm and Krewer, 2003). Rabbiteye types generally perform better with higher production and ease of management in the state of Georgia (Krewer and NeSmith, 2006). Rabbiteye types ripen later in the summer, beginning late May and continuing through July (Krewer and NeSmith, 2002). They are most tolerant to pests and diseases, less prone to late spring frost due to their late blooming period and have longer life bush life spans. (Jones and Percival, 2003; Scherm and Krewer, 2003). Rabbiteye types perform well in soils with low organic matter, unlike southern highbush types. Southern highbush are produced for its early harvest which gives a favorable fresh market premium for growers. Southern highbush blueberry are low-chilling requirement blueberry type that were first developed by the University of Florida beginning in the late 1940's, and are derived from interspecific hybridization of highbush, lowbush, rabbiteye, and other wild diploid species. The traits of low chill, heat tolerance, and disease resistance of southern highbush allowed for the wide expansion of planting that constrained highbush blueberry (Ballington, 1990; Draper, 1997). Southern highbush are also favorable to grow in Georgia for its price window for the fresh market. They ripen much earlier than rabbiteye blueberry during mid-April through mid-May in southern Georgia, a window of time that is lucrative when U.S. blueberry supply is low (Fonsah et al., 2007). Southern highbush blueberry production has been increasing because of its early ripening appeal that enables growers to harvest and sell southern highbush before rabbiteye harvest season in the April and May window. Total blueberry acreage went from 3,500 to over 12,000 acres from 1989 to 2009, the majority of this southern highbush plantings (NeSmith, 2009). Experienced growers in Georgia claim southern highbush are a challenging to grow

considering their requirements for wet, yet well drained soils and disease and insect pressure (Fonsah et al., 2007). Another challenge for southern highbush types is being susceptible to early spring freeze damage (Krewer and NeSmith, 2000). The production cost is also much higher for southern highbush blueberries since most of the fruit is hand-harvested (Krewer and NeSmith, 2008).

Northern highbush types are drought sensitive and thrive best in acidic soils that are well drained (Jones and Percival, 2003). They are higher chill (at least 1,000 hours), opposed to lower required chilling hours for southern highbush and rabbiteye. Northern highbush are not cultivated in Georgia mostly because for this reason. Northern highbush make up the greater portion of the U.S. blueberry market, around 70% during the growing season. They are considered vigorous yet are still less vigorous than healthy rabbiteye and southern highbush varieties (Krewer and NeSmith, 2000). Northern highbush are generally self-fertile, but also benefit from cross-pollination to produce larger, earlier ripening berries (Krewer and NeSmith, 2006).

One of the major points of comparison between the three blueberry types is fruit quality Overall, there exists a subjective bias within the blueberry industry that southern highbush have superior fruit quality to rabbiteye, and that northern highbush has the highest fruit quality compared to both southern highbush and rabbiteye types. Currently, differences in fruit quality is not well-understood and information is limited at best. The present studies have compared differences of a few cultivars within each type, or older cultivars that are being phased out of the industry (Ehlenfeldt and Martin, 2008; Saftner et al., 2008; Silva et al., 2005). These have not focused on a broad range of commercially available cultivars in order to compare the types adequately. A larger survey of the major cultivars within each blueberry type over postharvest

storage is needed. A better understanding of fruit quality differences between types would be beneficial to growers and would allow them and their stakeholders to select cultivars that benefit their production and marketing.

Fruit quality

Fruit quality largely consists of the degree of excellence and acceptability based on subjective human sensory evaluation and objective instrumental measurements. Appearance, texture, taste, and aroma are determined by these methods and are thoroughly tested with the consumer's preference in mind. Secondary to these characteristics are chemical composition and nutritive values. Fruit quality can vary greatly based on several factors such as the context in the supply chain, its intended use as a fresh or frozen product, and biased personal preferences and expectations. These attributes are ultimately what gives value to the food crop being eaten by the consumer (Abbott, 1999).

A new shift from producer driven traits such as yield, climatic adaptation, and disease resistance to consumer preference has evolved the breeding goals of blueberry. Fruit quality traits that are of interest to consumers fuel the production and profitability of the blueberry industry (Gilbert et al., 2014). Consumers often cite fruit quality characteristics such as flavor and sweetness as favorable fruit quality attributes, while mealy texture was unfavorable (Galardo et al., 2018; Gilbert et al., 2014). This can influence purchasing decisions and perception of blueberry overall by consumers. Fruit firmness is not only an indication of consumer preference but is also associated with extended shelf-life (Moggia et al., 2017) and improved machine harvestability (Olmstead and Finn, 2014). Many of the traits that overlap production/industry and consumer standards are important to improve and understand within and among blueberry types.

Within the industry there is a bias of a type-hierarchy for fruit quality characteristics. Southern highbush fruit are perceived to have superior fruit quality than that of rabbiteye fruit. In addition to this type comparison, northern highbush are perceived to have superior fruit quality than that of southern highbush. Several studies have examined rudimentary fruit quality attributes between cultivars of highbush types and rabbitye such as fruit firmness, soluble solids, titratable acidity, and flavor attributes (Saftner et al., 2008; Silva et al., 2005). This bias exists for both quality at harvest and throughout postharvest storage. Georgia grown fresh market rabbiteye blueberries may be purchased at a lower price point from third party distributers than other types, or completely excluded, as a result of these biases (R.A. Itle, personal communication). However, there is limited information for industry to support these decisions and sufficiently compare the fruit quality of these three blueberry types. Practices such as this potentially hurt the grower as they receive less money for fruit that otherwise has no evidence for being inferior in quality.

Providing objective instrumental fruit quality information at harvest and throughout storage could help prevent existing bias to certain types within the industry. It would also identify cultivars that would improve storage traits in a breeding program to be used as parental material in improving fruit quality during postharvest storage. Improving blueberry fruit quality traits ensure that the best products are offered to consumers while at the same time help Georgia growers maintain and potentially increase their production in the future.

Postharvest quality

The successful marketing of fresh produce in the U.S. is largely dependent upon maintaining high quality sensory attributes for an acceptable duration in postharvest storage (Gertmenian, 1992). Success is also contingent upon harvest timing, quality control, packaging

and labeling, pricing that is competitive, and quality service at all levels of the distribution system (Allen and Pierson, 1988). After harvesting, fresh fruit commodities are highly susceptible to rapid degradation and quality loss in ambient environment storage. Lightly processed fruits (products that undergo washing, sanitation, and/or packaging for refrigeration) are even more susceptible to this degradation from handling processes that result in damaged plant tissue (Schlimme et al., 1995). Oxidative stress is responsible for fruit quality loss in postharvest production. Factors that influence oxidative damage can occur simultaneously to exacerbate this damage. Typical postharvest disorders caused by oxidative stress include lesions, mutations of nucleic acids, fruit browning, loss of membrane integrity, and inactivation of proteins. Most of these symptoms are directly influenced by water loss, storage duration, temperature, atmosphere, ripening of fruit caused by ethylene, genotype of the commodity, and postharvest handling (Hodges et al., 2004).

Fresh blueberry fruit undergo light processing to preserve their quality for the fresh market. Most fresh market fruit is generally hand harvested to reduce bruising to select the best ripe fruit; however, some growers rely on mechanical harvesting for the fresh market. A mechanical harvester straddles the bush and shakes it to remove the ripe fruit. Berries are caught in plastic lugs that can carry up to 20 pounds of fruit. Once in the packing shed, berries are dumped into conveyors and air cleaned to remove plant debris and underweight berries. Some growers use electronic sorting machines to grade fruit for color and firmness and can remove over or under-ripe fruit. This technology uses a series of cameras to determine blueberry color or bruising. Berries are packaged in plastic clamshells and stored in bulk in cold rooms to avoid berry degradation prior to arriving to the market. Berries destined for the processing market are often dumped into water to sterilize the surface and are then frozen (Longstroth and Hanson,

2012). Blueberry is categorized as a soft fruit along with strawberry, which makes its texture critical in determining its fruit quality (Giongo et al., 2013).). Blueberry fruit firmness is important to withstand shipping and keeping freshness in consumers' homes, affecting consumer acceptance.

In addition to fruit quality at harvest, information pertaining to the postharvest keeping ability of individual fruit quality traits among the three commercially important blueberry types is also limited at best. Researchers have investigated fruit quality traits such as fruit firmness skin toughness, soluble solids content, titratable acidity, and eating quality (soluble solid content to titratable acidity ratio), however most studies rely on a maximum of three to four cultivars within each blueberry type, and often only comparing two types at a time (Ehlenfeldt and Martin, 2008; Saftner et al., 2008). Understanding these differences in storage would help to reduce subjective bias of one type possessing superior fruit quality during postharvest storage. Such information of the overall quality characteristics of the three commercially important blueberry types may help to prevent the lowered price-point of Georgia grown blueberry cultivars.

Instrumental measurements of fruit quality

Produce quality can be assessed by measuring their mechanical and chemical properties which are measurements that use mathematics to objectively categorize quality attributes, usually in favor over sensory evaluations. This is because individuals involved in sensory evaluations have varying judgements, but instrumental measurements can reduce variation by being more precise and providing a common language among researchers and the industry. Texture is generally related to the mechanical properties, taste and aroma to the chemical properties, and appearance (Abbott, 1999). In addition, instrumental measurements are able to record multiple objective measurements that compose a larger quality attribute. Texture analyzers can

objectively measure compression, shearing, extrusion, grit, and fiber. Colorimeters can observe reflectance and transmittance of color. High performance liquid chromatography (HPLC) can determine a more detailed nutritional profile of attributes such as vitamins A, B, C, E, polyphenolics, and carotenoids. Overall, instrumental measurements tend to be more sensitive to small differences between samples that may be undetectable to humans, which make them favorable for quality control and possible detecting trends in quality (Barrett, 2010).

Physical measurements

Ethylene

Ethylene controls several postharvest physiological processes in plants, and is known to regulate senescence, over-ripening, accelerated quality loss, and pathogenic damage. It is an endogenous plant hormone produced by plant tissues in a gaseous form (Martinez-Romero et al., 2007). Generally, fruits can be defined and climacteric and non-climacteric fruit; climacteric fruit show an increase and respiration and autocatalytic ethylene production. In non-climacteric fruit such an increase in respiration and ethylene is not observed and the role of ethylene in ripening is not very well understood (Paul et al., 2012). Climacteric and non-climacteric fruits both possess ethylene-dependent and independent gene regulation pathways (Lelièvre et al.,1997). Unlike climacteric fruit, non-climacteric fruit does not exhibit a positive relationship between autocatalytic ethylene rise and respiration rate during fruit ripening. Depending on exogenous or endogenous levels of ethylene, respiration rate can be reversed or stimulated by ethylene in non-climacteric fruits (Tian et al., 2000). It is hypothesized that there may be a different function for the same ethylene receptors or different ethylene receptors altogether in climacteric and non-climacteric fruits (Yang, 1987). There is currently no consensus on whether blueberries are climacteric or not, which is another reason to investigate its effect on blueberry fruit quality

(Cappai et al., 2018).

In fruit supply chains, the current methods for detecting ethylene gas include gas chromatography, electrochemical sensing, and optical sensing. Gas chromatography is a technique used to separate volatile gas compounds in complex mixtures based on a compound's boiling point, solubility, or polarity. It is a highly sensitive method for detection and consists of a sample passing through a stationary phase that interacts with analyte, pushed by the mobile phase (Hu et al., 2019). Two detection methods are also used, photoionization detection (PID) and flame ionization detection (FID). PID has superior efficiency over FID or since PID's are made to specifically detect aromatic hydrocarbons and sulfur compounds. The second main way to detect ethylene is ectrochemical sensing. Electrochemical sensors typically measure a chemical reaction of the target gas by using a cathode, anode, reference electrode, and electrical current. In amperometric sensors, a current is measured. In chemoresistive sensors, resistance is measured, and in capacitive sensors, a change in capacitance is measured. The third method to detect ethylene concentrations is optical sensing. This is where a light source, usually infrared or laser, and its intensity is measured when passed through an absorption cell. Out of all of the aforementioned methods discussed, gas chromatography with headspace collection is most common in fruit crops (Cristescu et al., 2013).

The inhibition of ethylene in fruits and vegetable crops may act to stabilize titratable acidity, reduce weight loss, and reduce common postharvest pathogens such as gray mold (*Botrytis cinereal*) that contribute to fruit degradation growth during the early weeks of postharvest storage. The ethylene receptor inhibitor 1-methylcyclopropene (MCP) is used as a plant growth regulator to delay the ripening process, which can aid in reducing decay and softening in many fruits and vegetables (Boquete et al., 2004). Ethylene inhibitors are being

examined in blueberry postharvest storage conditions, however, they are not commercially used currently. In one study, 'Lateblue' northern highbush blueberry showed slightly reduced weight loss and significantly lower loss of berries due to fungal growth of *B. cinerea* than control fruit (Chiabrando and Giacalone, 2011). In 1-MCP treated blueberry fruit, titratable acidity was significantly higher than in controls for the first 2-3 weeks, most likely because of the reduced catabolism of organic acids that would otherwise be activated under normal ethylene production (Girardi et al., 2005). Others have sought to use ethylene as an enhancer of certain fruit quality attributes, but success is cultivar dependent. Postharvest application of ethylene enhanced anthocyanin and antioxidant activity in two of three northern highbush cultivars without further depreciating other postharvest quality attributes (Costa et al., 2012). Whether ethylene application can assist other blueberry types is yet to be examined. It is worth noting that since others have noted cultivar specific responses to ethylene and different effects whether as a promoter or inhibitor of ripening, this suggests that ethylene's effect on postharvest blueberry fruit quality should be further investigated.

A caveat of some ethylene treatment studies is that *B. cinerea* is capable of producing ethylene in tandem with fruit. Degradation may not be from a positive feedback mechanism in which ethylene stimulates *B. cinerea* growth, is but exacerbated by the mere presence of *B. cinerea* and its capability to produce ethylene. Infected fruit can also induce softening in uninfected kiwifruit in the same vicinity/ tray / storage compartment (Qadir et al., 1997). This has the potential to skew results of studies that examine the effect of ethylene on postharvest fruit quality.

As ethylene concentration can influence not only rate of postharvest degradation over storage, but also influence pathogen presence and contribute to accelerated decay in neighboring

containers of blueberry (Kwon et al., 2011), it would be highly beneficial to examine the ethylene levels of major cultivars in the three main commercial blueberry types. Additionally, since cultivars can be mixed within a clamshell, it would be highly useful to know which cultivars may degrade faster than others to aid in packaging cultivars with similar ethylene levels which may lengthen postharvest shelf-life. It would also be very useful to examine initial levels of ethylene concentrations at harvest with fruit quality traits at the end of storage to see if initial levels may be indicative of higher fruit quality over storage.

Texture – Fruit firmness and skin strength

Instrumental methods are used to assign objective values to texture profiles in blueberry. However, due to high fruit-to-fruit variability and small fruit size, texture is not easily defined as there have been many techniques and instruments used to study this complex trait and the industry lacks standardized methods to measure texture (Døving and Mage, 2002). In the majority of blueberry texture studies, the methodology follows measuring a force needed to fully puncture, penetrate, or deform fruit (Chiabrando et al., 2009).

Fruits and vegetables possess a visco-elastic behavior. This means that unlike purely elastic materials, the force, distance, and time of loading are determinates in texture tests measurements. Texture tests can be either destructive or non-destructive. In destructive texture tests, an established elastic limit is surpassed and results in permanent tissue damage. Nondestructive tests lie within the elastic limit, but none have been widely adopted in commercial settings. However, they are valuable for research because the same samples can be measured at the beginning and repeatedly again throughout the experiment to reduce variation. These include laser-air puff, impact, and sonic/acoustic tests (Abbott, 2004). The Magness-Taylor test is used to determine fruit firmness in postharvest inspection and harvest maturity in

the commercial setting. There are many variations of the MT test depending on fruit being tested, but all use the same rounded-tip probes, measuring force needed to pierce through flesh at a slow, steady load rate. Samples of intact fruit or tissue specimens are placed between two plates until tissue rupture occurs or a specific force is reached. Maximum force or distance is often reported, although many other indexes can be found on this test. Another firmness test is Kramer Shear which consists of a compression, shear, and extrusion component. Samples are loaded into a cell and dull blades are passed through the sample volume completely. Usually the reported data are the total force required to completely pass through the sample. Tensile/tension tests are the opposite of compression tests and measure the force required to rupture or cause cell separation when attached to two oppositely moving apparatus (Abbott, 2004).

One of the major fruit quality subjective biases that exist is that highbush types have a better, more desirable fruit texture overall compared to rabbiteye types. There is a perception that rabbiteye fruit have firmer, tougher and chewier fruit than highbush types. There have been a limited number of studies examining blueberry fruit firmness across blueberry types at harvest and over cold storage. For studies comparing fruit firmness across blueberry types, it has been reported that rabbiteye cultivars have fruit firmness that was considerably higher than highbush types (Ehlenfeldt and Martin, 2002; Itle and NeSmith, 2016; Makus and Moris, 1987). Ehlenfeldt and Martin (2002) compared pure northern highbush and species introgressed cultivars with southern highbush blueberry cultivars. The results suggested that southern highbush exhibit fruit firmness which were higher than average and all share a common factor of having traces of *V. darrowii*, *V. virgatum* and *V. tennellum* wild species ancestry. Cultivars with *V. angustifolium* ancestry and pure *V. corybosum* tended to produce soft fruit. Overall, rabbiteye (*V. ashei)* berries were firmer than cultivars with *V. corymbosum* and *V. darrowii* ancestry (Ehlenfeldt and Martin,

2002). Itle and NeSmith (2016) found that rabbiteye types did have significantly higher fruit firmness than southern highbush types, however there were no significant differences between rabbiteye and southern highbush skin strength, from a group of seven rabbiteye and seven southern highbush cultivars. Studies have compared differences of a few cultivars within each type (Ehlenfeldt and Martin, 2008; Saftner et al., 2008; Silva et al., 2005), rather than a broad range of commercially available cultivars that are available on the market in order to compare the types adequately. Common perceptions suggest that rabbiteye blueberries have tougher skins than southern highbush blueberries. Silva et al., (2005) measured sensory qualities, chemical composition, color, and texture of two northern highbush and three rabbiteye cultivars. They found the puncture values to be higher for rabbiteye cultivars. Saftner et al., (2008) only compared two rabbiteye cultivars against eight northern highbush cultivars and found northern highbush cultivars to have firmer fruit. Rabbiteye cultivars were harvest in New Jersey, an area where rabbiteye cultivars are not grown commercially.

Other studies have observed how blueberry textural changes occur throughout postharvest cold storage. Over a 35-day period, fruit firmness of 'Coville' and 'Bluecrop' northern highbush cultivars did not significantly change in commercial cold storage (Chiabrando et al., 2009). Studies like these suggest there is a direct relationship between moisture loss/ weight loss and postharvest firmness of blueberries (Paniagua et al., 2013). Shriveling can be observed by just 5 percent of moisture loss in berries (Wills et al., 2007). Moisture loss has been suggested to be the result of loss of turgor, inducing postharvest softening (Allan-Wojtas et al., 2001). Very few studies have measured fruit firmness across multiple blueberry types at multiple timepoints throughout postharvest storage and it would benefit the industry to provide information on the behavior of these traits in storage.

Another important parameter of texture is fruit skin strength, which is used to determine tenderness or toughness. Skin strength can be useful to determine consumer acceptance as how cultivars sustain damage during harvest. Harvesting blueberry includes risks of producing leaky fruit as a result of bruising and thus lowers quality (Takeda et al., 2013). Skin toughness can be determined by measuring the force required for a probe to penetrate the skin of a single blueberry. This test is also common in apple and grape (Grotte et al., 2001; Rolle et al., 2012) and is similar to the test used in other fruit crops.

Skin strength is another major fruit quality comparison point between the blueberry types. Rabbiteye are subjectively perceived to have tough, chewy, and thick skins which would be an undesirable blueberry fruit quality characteristic. Similar to fruit firmness, there have been a limited number of studies examining blueberry skin strength across blueberry types at harvest and over cold storage. For studies comparing fruit firmness across blueberry types rabbiteye and northern highbush blueberry, rabbiteye had firmer skin (Takeda et al., 2013). Silva et al., (2005) observed that of three rabbiteye and two northern highbush cultivars, only one rabbiteye had higher skin strength. Another study reported that there was no difference between southern highbush and rabbiteye cultivars for skin strength, when comparing six cultivars within each blueberry type (Itle and NeSmith, 2016). There are currently no other studies that examine skin strength of multiple blueberry types throughout commercial postharvest cold storage. Examining this would further determine if differences exist in textural fruit quality between these types and alleviate bias that exists throughout the industry.

Overall, results from these studies suggest that there are differences between cultivars and types for fruit textural traits, but a more thorough examination of the major cultivars within each of the three main blueberry types over cold storage has not been conducted over multiple

years. A broader assessment of textural traits from multiple commercial cultivars from each of the three commercially important blueberry types would be a positive step in providing information on key fruit quality attributes. Beyond this, monitoring the behavior of the textural components of firmness and skin strength up to thirty days would be beneficial information to understand what cultivars or types provide good storage quality and/or maintain texture in postharvest. Knowledge of this would be highly beneficial for the blueberry industry to know which cultivars may have longer storage ability and help to prevent lowered price points received due to unfounded subjective biases.

Chemical measurements

Taste – Soluble solids and titratable acidity

Fruit taste is largely dependent on the balance between sweetness, astringency, and acidity/sourness and odor-active volatile compounds/ aroma. Although both taste and aroma contribute to overall flavor, aroma is known to be dominant in flavor attributes (Goff and Klee, 2006). There are recommendations of minimum soluble solids content and maximum titratable acidity to meet standards of consumer acceptability for many fruit crops. Using a refractometer, soluble solid content (SS or SSC) can be measured to quickly estimate sweetness. Soluble solids include sugars, organic acids, soluble pectins, anthocyanins, phenolic compounds, and ascorbic acid, so this method is a rapid yet non-specific method of measuring the concentration of predominant sugars. Sweetness or a sugar profile is the makeup of the predominant sugars of fructose, sucrose and glucose, and is a ranking relative to sucrose.

Sourness, or the acid profile, is the makeup of the predominant organic acids: citric acid, malic acid, and tartaric acid. Overall, it is a ranking relative to citric acid (Kader, 2008). HPLC can be used to approximate individual sugars and acids for a detailed profile. Measuring the

overall total titratable acidity (TA or TTA) is common for fruits and vegetables. It is estimated based on the molecular weight of the predominant acid found in a particular fruit. In blueberries, TA is an estimate of citric acid. TA is most commonly measured by adding 0.1 N sodium hydroxide to the diluted fruit juice and water sample to achieve an endpoint of pH = 8.2 (Mitcham, et al., 1996; Gündüz et al., 2015). Simple estimations of flavor can come from the ratio of the sugar and acid content, or sugar/acid ratio of soluble solids and titratable acidity (Barrett et al., 2010).

In general, there is the subjective perception that highbush types, in particular northern highbush, have a more balanced sugar acid ratio and have a more complex flavor, yet there is limited evidence to support this bias. In a comparison between three northern highbush and three rabbiteye blueberries over three years, rabbiteye generally had higher percentage in soluble solids, pH, and sugar:acid ratio (Makus and Morris, 1993). In a wider comparison between rabbiteye, northern highbush, and southern highbush cultivars over multiple years, there was found to be significant variability for all SS, TA, and pH analyzed among the individual cultivars of all types. Seven rabbiteye cultivars, 11 southern highbush, and 24 northern highbush blueberries were collected. Observing northern highbush cultivars associated with decade of release, there was little change in SS, TA, total phenolic content, total monomeric anthocyanin content, and levels of vitamin C since breeders have not been directly selecting for these particular traits. Comparing SS, TA, and pH between southern highbush and rabbiteye, rabbiteye had significantly higher SS and pH, but lower TA and fruit weight. In the comparison between the three types, southern highbush were lower in acidity than the northern highbush, with the exception of two cultivars. Southern highbush also had SS/TA ratios higher than all northern highbush, with the exception of one cultivar. It is suggested that breeders have been

inadvertently breeding diminished fruit sweetness when selecting for fruit size since there were significant negative correlations between fruit weight and soluble solids (Gündüz et al., 2015). However, this study was not conducted over postharvest cold storage, and it is unknown how these quality traits perform across the three blueberry types.

In order to understand relationships between phytochemical characteristics of sugars and acids and postharvest keeping quality, fruit acidity has been found to play a strong role. In general, fruit acidity has been shown to decrease and soluble solids increase several postharvest studies of blueberry (Angeletti et al., 2010; Chiabrando et al., 2009). Soluble solids content has also repeatably demonstrated to increase in both southern highbush and northern highbush blueberry over storage and is possibly related to moisture loss (Abugoch et al., 2016; Chiabrando et al., 2009) or may be consequences of cell wall degradation as seen in strawberry (Cordenunsi et al., 2003). In addition, blueberry spoilage due to fungal growth in postharvest storage may be related to increases in pH values as a result of the formation of nitrogenous compounds and fungal metabolites (Vieira et al., 2016). Smittle and Miller (1988) found a link between high acidity and general defense mechanisms against organismal decay in 'Woodard' rabbiteye blueberry as acidity increased after 21 days in commercial cold storage. Similar results were found in northern highbush blueberry 'Blueray' (Loyola et al., 1996). Significantly higher titratable acidity values in 'Coville' northern highbush blueberry make it a recommended cultivar for longer postharvest storage life (Galletta et al., 1971). Fruit dehydration over time provoking acid concentration could be a possible explanation to higher acidity levels (Chiabrando and Giacalone, 2011). However, Tournas and Katsoudas (2005) have emphasized that in general, low pH values make fruits susceptible to spoilage from fungal growth, since these favorable conditions eliminate competition from bacterial species.

Currently, there is limited knowledge of the sugar and acid content of a large array of cultivars from the three major commercial blueberry types over postharvest fresh storage. It would be beneficial to understand what blueberry cultivars and types maintain sugar and acid content in storage to identify those that have the best keeping quality of phytochemicals. The potential of examining the relationships of these with percentage of spoilage and berry weight loss would allow researchers to identify cultivars best suited for long term shipments and storage without the need for additional postharvest treatments.

Anthocyanin content

Blueberry is known to be high in bioactive compounds which possess antioxidant activity, making blueberry an attractive fruit for promoting health in humans. Health studies have claimed that antioxidants are helpful in inhibiting oxidation of low-density lipoproteins and preventing oxidative stress. Phenolic compounds which include flavonoids, tannins, anthocyanins, and ascorbic acid are all important bioactive compounds of blueberry that are considered components of nutraceuticals and functional foods to reduce health risks. Phenolic compounds may help to protect against to free radicals, which are known for their oxidative ability and may play a role in cancer and diseases such as heart disease among others. Phenolics can occur in many forms with sugars, acids, and water-soluble and water-insoluble compounds (Ames et al., 1993).

Anthocyanins are of a flavonoid subclass found in the secondary metabolites of blueberries. Anthocyanins are water-soluble glycosides that give fruit, flowers, and vegetables their dark colors, such as in cherry, strawberry, red onions, and grape. There are six anthocyanins that are common in plants: cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin Malvidins, petunidins, delphinidins, and cyanidins are among the anthocyanidins to produce the

highest levels of antioxidant activity and are found in the highest amounts in blueberries (Kong et al., 2003). As opposed to total phenolics, anthocyanins are shown to increase during fruit ripening. Anthocyanins are known antioxidants and are claimed to possess numerous health-promoting qualities, including the attenuation of metabolic complications (DeFuria et al., 2011).

Anthocyanins are normally extracted with polar organic solvents like methanol, acetone, ethanol, or acetonitrile (Barnes et al., 2009). Near-infrared and mid-infrared spectroscopy can be used to analyze total anthocyanin content in blueberry fruit, but with a different extraction method. Sinelli et al., (2008) used an extraction solution of EtOH/HCl/H_2O and diluted the supernatant with acidified ethanol. Total anthocyanin content was then measured as malvidin 3-glucoside at 520 nm with molar absorptivity coefficient of 28,000. Total anthocyanin content is expressed as milligrams per gram of fresh weight. Another method to measure total anthocyanins that is commonly used is using a pH differential method. Blueberry extract in pH buffers are measured at wavelengths of 520 and 700 nm at pH 1.0 and 4.5 and results are expressed as milligrams of cyanidin 3-glucoside equivalent per 100 g of fresh weight. (Cheng and Breen, 1991; Lee et al., 2005).

Currently, it is not largely debated within the blueberry industry which of the three major commercial blueberry types has the highest level of beneficial compounds such as antioxidants and anthocyanins. However, knowledge of this may help the marketing and sale of a particular type or cultivar at an increased price point or in a value-added product. There have been studies that have examined anthocyanin and antioxidant content in blueberry fruit, but not many compare across the three commercial types. Blueberry generally has high phenolic content and antioxidant capacity, but is highly varied among cultivars (Skrovankova et al., 2015). In addition, it has been observed that antioxidant activity is highly correlated to total phenolic

content in four northern highbush blueberry cultivars (Castrejón et al., 2008), so anthocyanin content may be indirectly associated with total antioxidant content. In one study, (Prior et al., 1998) had conducted a comparison of total anthocyanins, total phenolics, and antioxidant capacity between 23 total cultivars of northern and southern highbush, rabbiteye, bilberry (*V. myrtillus* L.), and lowbush blueberry (*V. angustifolium*) types for one year. Thirteen southern highbush and northern highbush cultivars, and four rabbityee cultivars were commercially available; the remaining were not commercially available blueberry types. Bilberry and lowbush blueberries had the highest antioxidant capacity, as well as higher total phenolics. Between southern highbush, northern highbush, and rabbiteye types, southern highbush types had higher average ORAC (oxygen radical absorbance capacity) and average total phenolics, while northern highbush types had highest average anthocyanins. There was a correlation between increased ORAC, anthocyanins, and total phenolics with increased maturity. Another positive correlation was seen between ORAC, anthocyanins, and also total phenolics. Ehlenfeldt and Prior (2001) have also shown that ORAC values have shown to be positively correlated to anthocyanins and to phenolics. In addition, another study reported that growing locations between Oregon, Michigan, and New Jersey had no significant difference on total anthocyanins, total phenolics, and ORAC (Prior et al., 1998).

In a one-year comparison for anthocyanin content between 36 rabbiteye, three rabbiteye-derivatives, and three northern highbush types, rabbiteye had the highest content of malvidins, followed by rabbiteye-derivatives, and northern highbush. Rabbiteye also had highest levels of petunidins, elphinidins, and cyanidins (Wang et al., 2012). Fruit size was found to be highly correlated with total anthocyanin content in 'Summit' highbush blueberries, but not correlated in a series of other *Vaccinium* species: one genotype of *V. angustifolium,* four rabbiteye, one *V.*

constablaei Gray *x V. ashei,* 15 other northern highbush, five *V. membranaceium,* one *V. myrtilloides, V. ovalifolium* Smith*,* two *V. ovatum* Pursh*,* and one *V. parvifolium* Smith (Moyer et al., 2002). Other conflicting evidence suggest that anthocyanins significantly correlated with fruit weight between four highbush blueberry cultivars during the ripening stage (Castrejón et al., 2008). In a comparison study between three southern highbush cultivars and two rabbiteye cultivars during one harvest year, rabbiteye had a significantly higher total anthocyanin content compared to the southern highbush cultivars (Magee, 1999). On the contrary, Prior et al., (1998) showed that total anthocyanins were on average higher in northern highbush cultivars compared to southern highbush and rabbiteye cultivars during one year. In this study, thirteen southern highbush and northern highbush cultivars, and four rabbityee cultivars were commercially available; the remaining were not commercially available blueberry types.

In addition to information being limited for screening blueberry types at a single timepoint for antioxidant and anthocyanin content, it is more so for screening across time points in storage. In one study, antioxidant activity, total phenolic content, and anthocyanin content were stable during postharvest cold storage for at least three weeks. In a comparison between five northern highbush cultivars and one southern highbush cultivar, all had stable quality traits with the exception of one northern highbush cultivar, 'Elliott', which saw an increase in antioxidant activity, total phenolic content, and anthocyanin content over three weeks in commercial cold storage. However, this may have been due to this particular cultivar being harvested when not fully mature blue (Connor et al., 2002). Currently, there is no know literature comparing total anthocyanins among multiple cultivars across blueberry types in extended storage.

With a growing interest in consuming fruits and vegetables rich in nutrients and beneficial compounds, it would be worth investigating the anthocyanin content of multiple cultivars of the three main blueberry types in storage. This would help to determine cultivars that possess superior health promoting qualities and/or superior keeping quality in storage. Currently, there has not been thorough examination of the major cultivars of the three main commercial blueberry types for anthocyanin content over storage. Knowledge of anthocyanin content over time in postharvest fresh storage may also prevent subjective biases for fruit quality among cultivars within types and may help to increase grower profits.

Cell wall structure

Fruit texture is one of the most important traits in fruit quality and is largely discussed in all aspects of fruit crop production from harvesting to packing, shipping, postharvest shelf-life, and eating quality. Overall fruit texture is largely impacted by the structure and integrity of the blueberry fruit cell wall (Goulao and Oliveira, 2008). The primary cell wall of plant tissues typically consist of a matrix of cellulose, hemicellulose, pectin, and structural protein (Vermerris, 2008). In blueberry fruit, primary cell walls are composed of 30-35 percent pectin, and it is suggested that xyloglucans are a principal hemicellulosic component (Vicente et al., 2007). Lignin, a high amount of cellulose, xylans, and glucomannans are highly abundant in the secondary cell wall of blueberry fruits (Knox, 2008). During fruit softening, these cell wall components undergo solubilization and depolymerization of pectin, initiated by cell wall degrading enzymes that lead to swelling of the cell wall, loss of cell-cell adhesion, and diminishing of qualities of crispness (Goulao and Oliveira, 2008). The depolymerization of cell-wall bound pectin and hemicellulosic polymers caused by cell wall degrading enzymes are the main contributions to loss of firmness throughout the stages of ripening in blueberry. This

process is known as cell-wall disassembly and fruit softening throughout the ripening phase (Cappai et al., 2018).

Cell wall degrading enzymes

The degree of degradation of blueberry fruit and loss of firmness most likely depends on the enzyme action of cell wall polysaccharides through a complex process that starts during fruit ripening and extend through fruit maturity. Namely glycoside hydrolases and polysaccharide lyases gene families have been extensively studied for their action during cell wall degradation, pathogen resistance, aromatic acid biosynthesis (Cappai et al., 2018), however these have not been extensively studied across types nor through a large collection of commercially available cultivars in ripened fruit and is likely cultivar dependent. Another leading cause in the loss of firmness is the loss of water and accumulation of osmotic solutes in the apoplast of blueberries in storage (Brummell, 2006). A one year study of two northern highbush cultivars showed positive correlations between urolic acid content at harvest and weight loss and softening during storage has also been shown which could be an area of interest to study cuticular triterpenoid composition (Moggia et al., 2016).

In postharvest cold storage (5°C), the activity of cell wall degrading enzymes such as polygalacturonase, cellulose, ß-galacturonase, and a-galactosidase is greatly suppressed in rabbiteye cultivar ‘Brilliant’, and decreases in water soluble pectin levels are noticeable too compared to fruit stored at 10°C (Chen et al., 2015). Pectin slowly changes from an insoluble substance to that which is more water-soluble throughout ripening (Theuwissen and Mensink, 2008). However, regardless of storage temperature, Chen et al., (2015) observed that all four enzymes showed similar changes. All enzymes’ activity increased slowly for about 28 days, peaked, and then decreased afterwards. Chen et al., (2015) suggest that the delay of softening in

blueberry fruit could be attributed to lower activities of polygalacturnase, cellulose, ß-galacturonase, and a-mannosidase. However, this is the only account of enzyme activity in blueberry during postharvest storage, with only one type and one cultivar as the plant material. The same effect of delaying fruit softening has been found in strawberry through inhibiting cell wall degrading enzymes (Vicente et al., 2005).

The role of calcium in pectin solubilization and postharvest quality has been implicated as well. One finding states that when calcium is present, unesterified regions of homogalacuronan molecules form together to make domains of calcium-pectate gel (Jarvis, 1984). These calcium-pectate gels potentially increase cell-to-cell adhesion due to these calcium-pectate linkages, resulting in firm fruit with increased wall stiffness, preventing polymerization (Thomson et al., 1999). Another study from Vicente et al., (2007) suggests that the main modifications taking place in the cell wall during development was not pectin solubilization, but rather solubilization of hemicellulose. Northern highbush cultivar 'Duke' did not have any changes in pectin size at any point during ripening but had decreasing levels of hemicellulose. These results were similar to what has been found in banana, apple, pepper, and strawberry cultivars (Brummel, 2006; Huber, 1984). Because of this, Vicente et al., (2007) suggests that calcium's positive effects on fruit firmness may be due to an indirect effect on hemicellulose disassembly, rather than preventing pectin depolymerization. Whether cell wall degradation is taking place via pectin solubilization or hemicellulose solubilization, more thorough studies need to be conducted across types in order to understand differences between types.

Overall, a more thorough examination of the major cultivars within each of the three main blueberry types and their enzyme activity and cell wall composition over cold storage should be investigated over multiple years. A broader assessment of enzyme activity from

multiple commercial cultivars from each of the three commercially important blueberry types would be a positive step in providing information on the cell wall degrading enzymes' effect on postharvest keeping quality of blueberry. Any information between type differences in cell wall degrading enzymes and the correlation between firmness is needed to fully exploit favorable breeding material for improved textural quality and shelf-life for consumer acceptability. Knowledge of this would be highly beneficial for the blueberry industry to know.

Cell wall degrading gene expression

An understanding of the gene expression of cell wall degradation would be extremely useful in blueberry. This knowledge could be used to potentially help in amending current postharvest storage conditions to help maximize postharvest shelf-life of fruit. It would also be useful in developing tools to aid in the development of blueberry cultivars in all three types with increased postharvest shelf-life.

The fruit softening gene *polygalcturonase* (*PG*) is highly abundant during ripening in many horticultural crops. It catalyzes the cleavage of homogalacturonan, and some suggest that it could be a fruit softening-rate determining enzyme among strawberry cultivars (Villarreal et al., 2008). Even in blueberry where ethylene may not be a major contributor to fruit ripening, ethylene may play a role in cell wall enzyme gene expression. Since *PG* has been implicated in cell wall degradation in other fruit crops like strawberry (non-climacteric) (Quesada et al., 2009), and tomato (climacteric) (Meli et al., 2010; Orfila et al., 2001; Zhang et al., 2018), pathways of cell wall degrading enzymes may mirror each other and would be interesting to look at the role it plays in blueberry.

Much of the work examining PG activity and expression has been done in response to postharvest treatments aimed at improving or lengthening postharvest shelf-life, such as 1-MCP

which is used to delay the ripening process, which can aid in reducing decay and softening in many fruits and vegetables (Boquete et al., 2004). In one study, treatments of 1-MCP on cv 'Toyonoka' (low-firmness) strawberry fruit during the white stage influenced expression of *PG* by maintaining PG activity at levels similar to non-treatments (Villarreal et al., 2010). In another study, white stage cv 'Camarosa' (high-firmness) fruit treated with ethylene increased *FaPG1* mRNA accumulation but did not modify enzymatic activity, and 1-MCP treatments decreased *PG* expression and total PG activity (Villarreal et al., 2009). Similar results were found with ß-galacturonase, when white stage strawberries were found to have significantly reduced ß-galacturonase enzyme levels when treated with 1-MCP. Total ß-galacturonase activity increased when treated with ethephon as well (Villarreal et al., 2009). *Fa ß-gal1* gene expression in white strawberries appeared to be downregulated when treated with both NAA auxin and ethylene, however other ß-gal genes such as *Fa ß-gal2* and *3* genes or total ß-galacturonase activity was not analyzed (Trainotti et al., 2001). Therefore, other enzymes different from *Fa ß-gal1* could be responsible for the reduced ß-galacturonase enzyme levels when white strawberries were treated with 1-MCP and increase in total levels with ethephon treatment. This indicates that ethylene may play a positive role in regulating *PG* and *ß-galacturonase* expression during strawberry ripening and its role in non-climacteric fruit should thus be reconsidered. Many contradictions in previous literature regarding strawberry's response to ethylene may be a result of different cultivars' sensibility to the hormone, since the cell wall and metabolism is highly variable among cultivars, as well as the ripening stage at which the treatment is applied (Villarreal et al., 2009). 1-MCP has also demonstrated to reduce PG activity in avocado as well (Zhang et al., 2011).

Expansins are known to contribute to the softening of ripening fruits such as tomato (Minoia et al., 2016), strawberry (Dotto et al.,2006), peach (Hayama et al., 2003), and kiwi fruits

(Mitalo et al., 2019). Expansins are a cell wall protein (not an enzyme) that break hydrogen bonds between cellulose and xyloglucan molecules and are the first of other cell wall loosening agents to initiate this process (Brummell et al., 1999). Gene expression of expansin was found not affected by either treatments of ethylene or 1-MCP in white strawberry. Furthermore, *FaEXP2* (encoding the expansin associated with cell wall loosening) expression was significantly increased when treated with ABA and when achenes were removed (removing the endogenous auxin source) (Nardi et al., 2016). Understanding expansin action in blueberry is relevant because other fruits mentioned above go through similar ripening and postharvest processes and could prove to be applicable to blueberry. Overexpression of expansins in tomato fruit has produced softer fruit and silencing their gene action was has correlated to firmer fruit and longer shelf-life (Minoia et al., 2016). This could also show similar results if conducted in blueberry.

Other studies have looked at the interaction of multiple cell wall degrading genes and their diverse functions on improved textural and shelf-life qualities, noting a more complex system of genes involved in cell wall disassembly. In the *Cnr* (colorless, non-ripening) tomato fruit mutant, cell wall swelling and modification of middle lamellae that normally occurs during fruit ripening is not found (Orfila et al., 2001). Orfila et al., (2001) reported that in this mutant fruit, *PG* and *pectin methylesterase* (*PME*) are significantly reduced, but it is possible that these two genes are two of many that contribute to fruit softening in tomato. Orfila et al., (2001) demonstrated that alpha-L-arabinan deposition is disrupted in *Cnr* mutant fruit, meaning it could be a key factor contributing to a lack of pericarp softening. However, modulations of alpha-arabinan have been established to have diverse functions. Alpha-arabinin is what links homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II),

which make up the structural domains of pectin in tomato. Much like tomato, the cell wall matrix of blueberry is also composed of pectin. Peña and Carpita (2004) demonstrate that a selective loss of alpha-L-arabinans proceeds the loss of firm texture in apple, but the loss of rhamnogalacturonan-I branching is closely correlated with the loss of firm texture in 'Gala', 'Red Delicious', 'Firm Gold', and 'Gold Rush' apple cultivars, only after the loss of arabinans. The complex action of these genes on cell wall components of blueberry is not documented. The expression of these cell wall degrading genes in various cultivars and/or types of ripened blueberry fruit would greatly assist in determining the textural as well as keeping quality differences between southern highbush, northern highbush, and rabbiteye blueberry types.

A better understanding of cell wall degrading genes is needed in blueberry. Identifying and studying the gene expression of cell wall degrading genes in ripened blueberry fruit will potentially provide a new screening method to identify cultivars with superior postharvest keeping quality. This would potentially enable the creation of tools to aid in the selection and breeding of new blueberry cultivars with improved postharvest shelf-life.

Main Objectives

The overall focus of this study is to identify the changes in physical and chemical fruit quality traits, and the differential gene expression of cell wall degrading enzymes of multiple cultivars of the three commercially important blueberry types to the overall current U.S. blueberry market. Physical fruit quality characteristics that will be evaluated include fruit firmness, skin strength, berry weight and percent spoilage/ amount of unmarketable fruit throughout a thirty-day storage period length. Chemical fruit quality characteristics that will be evaluated include soluble solids, total titratable acidity, total anthocyanins, and sugar and acid profiles. With the combination of these evaluations, it will provide information to growers and

marketers with fruit quality specific parameters during the fresh postharvest storage of major rabbiteye, southern highbush, and northern highbush cultivars. Depending on the postharvest keeping capabilities and qualities of Georgia cultivars, these evaluations may also provide growers and retailers the information needed to cater to niche markets. These findings will supplement larger studies that will provide important information to the Georgia blueberry industry in order to maintain and increase good market share within the U.S. market. Such information of the overall quality characteristics of southern highbush and rabbiteye cultivars compared to those cultivars that make up the larger blueberry market will prevent the lowered price-point of Georgia grown blueberry cultivars.

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CHAPTER 3

PHYSICAL POSTHARVEST FRUIT QUALITY CHARACTERISTICS OF SOUTHERN HIGHBUSH, RABBITEYE, AND NORTHERN HIGHBUSH BLUEBERRY CULTIVARS THROUGHOUT COLD STORAGE[1]

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Abstract

In 2019, Georgia ranked second in the U.S. for production value of blueberry and first in the southeastern U.S. Two blueberry types are grown in Georgia: southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton). The fruit quality and postharvest storage ability between these types is debated. These types are also compared to northern highbush (NHB, *Vaccinium corymbosum* L.), which is perceived to have the highest fruit quality in the industry. There is limited information available to fully support this. The objective of this study was to examine postharvest keeping quality of SHB, RE, and NHB cultivars that are representative of the current blueberry market. Fresh fruit was collected from commercial blueberry packers in 2018 and 2019. Between the two seasons, examined cultivars were: SHB ('Abundance', 'Camellia', 'Farthing', 'Keecrisp', 'Meadowlark', 'Legacy' from Georgia and Michigan, 'Star', and 'Suziblue'), RE ('Alapaha', 'Austin', 'Brightwell', 'Powderblue', 'Premier', and 'Vernon') and, NHB '(Aurora', 'Bluecrop', 'Draper', 'Elliott', 'Liberty', and 'Nelson'). Fruit were processed at four timepoints (TP) during storage: 1) 3-4, 2) 10-11, 3) 20-21, and 4) 30-31 days after collection. Fruit firmness, skin strength, berry weight, percent healthy fruit, and ethylene concentration were evaluated. SHB types had significantly higher ($P \leq 0.05$) fruit firmness and skin strength than NHB types for both harvest seasons. The data suggest that SHB and NHB types had the best stability of most physical fruit quality characteristics in commercial cold storage, having the least amount of change in traits from TP1-TP4. There was no indication of initial characteristics of physical fruit quality determining long-term keeping quality. This suggests that genotype alone does not account for the differences between these types, as many additional environmental factors need to be considered.

Introduction

In the last 15-25 years, blueberry (*Vaccinium* spp.) has experienced growth as a popular fruit crop in many areas of the world. Demand for blueberry have been driven by improvements in plant breeding and consumer desire for the nutraceutical properties of blueberry, which rank high in antioxidants (Joseph et al., 2005; Wu et al., 2004), in addition to improvements in cultural management techniques. In the U.S. in 2019, blueberry was the fourth most valuable non-citrus fruit crop with value of utilized production at $909 million, ranking behind strawberry ($2.5 billion), apple ($2.7 billion), and grapes ($5.7 billion) (USDA, 2020).

In the southeastern U.S., Georgia accounts for the majority of blueberry production (95 million pounds), next to Florida (24 million pounds). These two states made up approximately 18% of the total production in the U.S. in 2019 (USDA-NASS, 2019). In 2018, within the state of Georgia, blueberry accounted for 2.24% of the state's total farm gate value, ranking number nine at $308 million. This ranks blueberry as the highest value fruit crop, and ahead of other horticultural crops such as pecan, onion, bell pepper, watermelon, cucumber, tomato, and peach within the state of Georgia (UGA Center for Agribusiness and Economic Development, 2019). Georgia's blueberry bearing-age acreage has grown considerably, from 4,600 in the year 2000 to 21,000 acres in 2019 (Krewer and NeSmith, 2002; USDA, 2020) and it is expected to continue growing.

Two major commercial blueberry types are grown in Georgia: southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton). Northern highbush (NHB, *V. corymbosum* L.) types compose the largest portion of the North American fresh market and are unable to grow in Georgia because of their adaptability to the northern latitudes of the U.S. with longer periods of cold temperatures.

Georgia's production has transitioned from approximately 90% rabbiteye and 10% southern highbush cultivation in the year 2000, to about 40% rabbiteye and 60% southern highbush more recently (Krewer and NeSmith, 2002; R. Itle, personal communication May 2018). There are benefits of having both blueberry types on Georgia farms. Southern highbush types are early ripening and extend the state's harvest window at a higher market price, which benefits Georgia by keeping it competitive with early producing locations like Florida and Mexico. However, the early market harvest window makes southern highbush susceptible to yield losses from late spring freeze events, and are generally more susceptible to disease pressures. Rabbiteye ripen later and are less susceptible to late spring freeze yield losses, are generally more disease resistant, and are often machine harvested which is approximately one seventh the price of hand harvesting.

One of the main discussion points between the two blueberry types is fruit quality. There is a subjective bias that is often debated in the blueberry industry that the fruit quality of southern highbush is superior to rabbiteye. In addition to this, the fruit quality of southern highbush and rabbiteye is often compared to the third major commercial blueberry type, northern highbush which is perceived to have the highest fruit quality. However, there is limited information at best to support the superiority of one type's fruit quality over another. This bias exists for both quality at harvest and throughout postharvest storage. Georgia grown southern highbush and rabbiteye blueberry may be purchased at a lower price point from third party distributers or be excluded from purchase entirely as a result of these biases (R. Itle, personal communication May 2018).

Overall, fruit quality of blueberry fruit for all blueberry types is important both at time of harvest and over postharvest storage, and the prolonged storage and shelf-life of blueberry fruit is met with many obstacles. Since blueberry fruit are harvested when fully ripe, they are prone to

rapid postharvest decay. The postharvest marketability of blueberry drastically decreases due to postharvest rotting commonly caused by a form of physiological breakdown, spoilage caused by fungal pathogens, and water loss (Forney, 2008; Schotsmans et al., 2007). Cultivar differences (Miller et al., 1988), harvest methods (Mainland et al., 1975), and ripeness during harvest (Galletta et al., 1971) are all contributing factors to successful postharvest storage of blueberry fruit. Textural traits, such as fruit firmness and skin toughness, are important fruit quality attributes that affect consumer acceptability. Texture also is an index for postharvest storability and susceptibility to injury during handling and storage (Li et al., 2011; Nesmith, 2002). The nature of the postharvest chain and marketing is challenging, as retailers continue to reject fruit below standards of firmness (Prussia et al., 2006), and the nature of postharvest handling compromises the final fruit quality by unavoidable fruit softening (Ehlenfeldt, 2002). Blueberry fruit that are maintained in storage could provide a better eating experience for consumers and sustain the fresh blueberry market.

To date, there are limited studies comparing the fruit quality of southern highbush and rabbiteye fruit grown in Georgia to that of northern highbush blueberry. Common perceptions suggest that rabbiteye blueberry have a less desirable texture than do highbush types, including firmer and chewier fruit over storage, tougher fruit skins and a gritter texture overall. Results on this have been varied, and there have been a limited number of studies examining blueberry fruit firmness across blueberry types at harvest and over postharvest cold storage. For studies comparing fruit firmness across blueberry types, it has been reported that rabbiteye cultivars have fruit firmness that was considerably higher than highbush types (Ehlenfeldt and Martin, 2002; Itle and NeSmith, 2016; Makus and Moris, 1987). Ehlenfeldt and Martin (2002) compared pure northern highbush and species introgressed cultivars with southern highbush blueberry

cultivars. The results suggested that southern highbush exhibit fruit firmness which were higher than average and all share a common factor of having traces of *V. darrowii*, *V. virgatum* and *V. tennellum* wild species ancestry. Cultivars with *V. angustifolium* ancestry and pure *V. corybosum* tended to produce soft fruit. Overall, rabbiteye (*V. virgatum)* berries were firmer than cultivars with *V. corymbosum* and *V. darrowii* ancestry (Ehlenfeldt and Martin, 2002). Itle and NeSmith (2016) found that rabbiteye types did have significantly higher fruit firmness than southern highbush types, however there were no significant differences between rabbiteye and southern highbush skin strength, from a group of seven rabbiteye and seven southern highbush cultivars. Studies have compared differences of a few cultivars within each type (Ehlenfeldt and Martin, 2008; Saftner et al., 2008; Silva et al., 2005), rather than a broad range of commercially available cultivars that are available on the market in order to compare the types adequately. Common perceptions suggest that rabbiteye blueberries have tougher skins than southern highbush blueberries. Silva et al., (2005) measured sensory qualities, chemical composition, color, and texture of two northern highbush and three rabbiteye cultivars. They found the puncture values to be higher for rabbiteye cultivars. Saftner et al., (2008) only compared two rabbiteye cultivars against eight northern highbush cultivars and found northern highbush cultivars to have firmer fruit. Rabbiteye cultivars were harvest in New Jersey, an area where rabbiteye cultivars are not grown commercially.

Other studies have observed how blueberry textural changes occur throughout postharvest cold storage. Over a 35-day period, fruit firmness of ‘Coville’ and ‘Bluecrop’ northern highbush cultivars did not significantly change in commercial cold storage (Chiabrando et al., 2009). Studies like these suggest there is a direct relationship between moisture loss/ weight loss and postharvest firmness of blueberries (Paniagua et al., 2013). Shriveling can be

observed by just 5% of moisture loss in berries (Wills et al., 2007). Moisture loss has been suggested to be the result of loss of turgor, inducing postharvest softening (Allan-Wojtas et al., 2001). Very few studies have measured fruit firmness across multiple blueberry types at multiple timepoints throughout postharvest storage and it would benefit the industry to provide information on the behavior of these traits in storage.

Another important parameter of texture is fruit skin strength, which is used to determine tenderness or toughness. Skin strength can be useful to determine consumer acceptance as how cultivars sustain damage during harvest (Takeda et al., 2013). Similar to fruit firmness, there have been a limited number of studies examining blueberry skin strength across blueberry types at harvest and over cold storage. For studies comparing fruit firmness across blueberry types rabbiteye and northern highbush blueberry, rabbiteye had firmer skin (Takeda et al., 2013). Silva et al., (2005) observed that of three rabbiteye and two northern highbush cultivars, only one rabbiteye had higher skin strength. Another study reported that there was no difference between southern highbush and rabbiteye cultivars for skin strength, when comparing six cultivars within each blueberry type (Itle and NeSmith, 2016). There are currently no other studies that examine skin strength of multiple blueberry types throughout commercial postharvest cold storage. Examining this would further determine if differences exist in textural fruit quality between these types and alleviate bias that exists throughout the industry.

Overall, results from these studies suggest that there are differences among cultivars and types for fruit textural traits, but a more thorough examination of the major cultivars within each of the three main blueberry types over cold storage has not been conducted over multiple years. A broader assessment of textural traits from multiple commercial cultivars from each of the three commercially important blueberry types would be a positive step in providing information on

key fruit quality attributes. Beyond this, monitoring the behavior of the textural components of firmness and skin strength up to thirty days would be beneficial information to understand what cultivars or types provide good storage quality and/or maintain texture in postharvest. Knowledge of this would be highly beneficial for the blueberry industry to know which cultivars may have longer storage ability and help to prevent lowered price points received due to unfounded subjective biases.

The objectives of this study were to 1) compare the postharvest keeping quality of physical fruit quality characteristics in early, mid, and late season SHB, RE, and NHB cultivars that are representative of the current blueberry market to identify types and cultivars that have superior postharvest keeping quality of physical traits over time, and 2) evaluate if any physical postharvest fruit quality traits during early postharvest can predict shelf-life (percent healthy fruit) of fresh blueberry fruit in postharvest cold storage. This information would provide growers with cultivar specific parameters of physical fruit quality during fresh postharvest storage for the rabbiteye, southern highbush, and northern highbush cultivars that are in current commercial production. Growers will be able to identify unique cultivars that are best suited for prolonged postharvest fresh storage.

Materials and Methods

Plant material

Fresh fruit was collected from commercial packers from May to August in 2018 and 2019. In 2018, 18 cultivars were collected including: seven SHB; ‘Camellia’, ‘Farthing’, ‘Keecrisp’, ‘Meadowlark’, ‘Legacy’ from Georgia and Michigan, ‘Star’, and ‘Suziblue’, five RE; ‘Alapaha’, ‘Austin’, ‘Brightwell’, ‘Powderblue’, and ‘Vernon’; and five NHB; ‘Bluecrop’, ‘Draper’, ‘Elliott’, ‘Liberty’, and ‘Nelson’. In 2019, 15 cultivars were collected including seven

southern highbush ('Abundance', 'Camellia', 'Farthing', 'Meadowlark', 'Legacy' from Georgia and Michigan, 'Star', and 'Suziblue'), five rabbiteye ('Alapaha', 'Brightwell', 'Powderblue','Premier', and 'Vernon'), and three northern highbush ('Aurora', 'Elliott', and 'Liberty'). 'Legacy' was collected from both Georgia and Michigan for both years. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan, Indiana, and Canada. The cultivars collected were representative of early, mid, and late season southern highbush and rabbiteye cultivars that make up the Georgia blueberry market, as well as the northern highbush cultivars that make up the larger North American blueberry market.

Fruit collected went through standard commercial harvesting and processing for each season (Table S1). All cultivars were collected during the approximate midpoint of harvest period for each cultivar, and fruit were collected within one week after harvest. Fruit were received sorted and packed in industry standard half-pint clamshells, or came straight from growers' fields after harvest in lugs. If fruit were not previously sorted, fruit were hand-sorted and placed them in half-pint clam shells to the quality of a standard packing line, removing visually defective, under and over ripe fruit. All clam shells were placed into bags, transported on ice in coolers back to campus, and stored in a commercial walk-in cooler at 4°C. Fruit were collected and stored in this manner to mimic how consumers would receive berries from a grocery store.

Research design

Physical fruit quality traits were evaluated at four timepoints (TP): 1) three to four days, 2) 10-11 days, 3) 20-21, and 4) 30-31 days after collection. For each cultivar, seven to eight half-pint clamshells were designated for each timepoint in a completely random design in a

commercial walk-in cooler. Clamshells were placed in the center shelves of the cooler in order to mitigate potential temperature fluctuations caused by the cooler fans or door. At each evaluation, fruit were randomly sampled from all clamshells for each timepoint. Fresh fruit was brought to room temperature (20°C) at benchtop for approximately 2 hours before measurements were taken.

Physical Instrumental Measurements

Fruit firmness

Measurements were taken using a TMS-Pro Texture Analyzer (Food Technology Corporation, Sterling, Virginia) with Kramer Shear press. A 3.0 x 7.1 x 12.6 mm compression cell was filled with a 50.0-51.0 g sample of fresh fruit. The maximum load required for a ten-blade shear equipped with a load range of 2,500 N, travelling at a rate of 100 mm/min to completely shear the berry matrix was determined as fruit firmness. For each cultivar at each of the four timepoints, three reps with three 50.0-51.0g subreps/rep were collected, for a total of nine samples. Units are expressed as [max load (N) Kramer Shear].

Skin strength

Measurements were taken using a TMS-Pro Texture Analyzer (Food Technology Corporation, Sterling, Virginia) with an individual probe. A 1.37mm probe equipped with a load range of 50 N, travelling at a rate of 50 mm/sec punctured individual berries. Berries were placed on their sides, along the equatorial plane, on an indented, plastic platform. Skin strength was determined by the maximum load first required to initially puncture the berry [max load (N) of puncture-in], and by the force required to exit the berry [max load (N) of puncture-out]. For each cultivar at each of the four timepoints, three reps of 12 individual berry subreps/rep were measured, for a total of 36 samples. For each cultivar, the median berry size was selected to

mitigate potential firmness differences due to fruit size. Units are expressed as [max load (N) puncture-in and puncture-out].

Berry weight

For each cultivar at each of the four timepoints, four reps of 20 random berries of similar size were weighed (g) (1501 B MP8-1, Sartorius, Göttingen, Germany). This measurement was used as an indication of water loss.

Percent healthy fruit

Fruit were examined individually for visual imperfections for each cultivar at each of the four timepoints. Visual imperfections included anything that would make a fruit unmarketable including shriveling, leakiness, cracking at the stem or calyx end, dents, bruising, mold, or torn skin. For each cultivar at each of the four timepoints, four reps of 30 random berries were rated. An indication of shelf-life was determined by totaling visual imperfections and dividing by total berry number and this was expressed as percent healthy fruit.

Ethylene concentration

Fresh fruit samples of 25.0 - 26.0g were placed into mason jars with rubber septums and incubated for a minimum of four hours. Head space was mixed with a 1 mL syringe, extracted, and injected into a GC-17A gas chromatographer (Shimadzu, Kyoto, Japan) for ethylene measurements on cultivars. All ethylene measurements took place 1-2 days after fruit collection. For each cultivar at each of the four timepoints, four reps of 25.0 - 26.0g were collected. Peak area at the approximate elution time of 30 seconds was used with a standard curve to calculate ethylene concentration in nL/L, then expressed as (nL/ g x hr) to account for the amount of time for ethylene gas to accumulate in the mason jars.

Data analyses

Data were analyzed using two-way ANOVA with PROC GLM of the SAS v.9.4 (SAS Institute, Cary, N.C.). Differences of means were examined among cultivars and types within each timepoint, and across all four timepoints for all individual cultivars collected during the 2018 and 2019 harvest seasons using Tukey HSD ($P \leq 0.05$). To better describe the overall change in physical quality characteristics, percent change from TP1-TP4 of cultivars and types was calculated [(TP4 mean - TP1 mean)/TP1mean x100] and differences between TP's were analyzed using One-Way ANOVA ($P \leq 0.05$) with PROC GLM. To identify if early postharvest fruit quality traits are indicators of percent healthy fruit during late postharvest storage, Pearson product-moment correlation coefficient were generated using PROC CORR of SAS 9.4 was used to examine the relationship between fruit quality traits during the initial TP1 at 3-4 days after collection and percent healthy fruit at TP4 at 30-31 days after collection for blueberry types collected during each harvest season. To examine the physical quality characteristics during fresh postharvest storage as a whole, a multivariate approach of correlation matrix calculation and principal component analysis (PCA) was conducted in JMP v.14 (SAS Institute, Cary, N.C.) to assess physical quality characteristics of fruit firmness, puncture-in and puncture-out (skin strength), berry weight, and percent healthy fruit during TP1 and TP4, separately. Hierarchical cluster analysis was conducted in JMP v.14 for TP1 and TP4 using Ward's linkage on principal component 1 and principal component 2 score values to determine relatedness among types based on physical quality characteristics. Given there were many significant differences in traits between timepoints and types, PROC CORR of SAS 9.4 was used to generate Spearman's rank correlation coefficients to examine year to year variation and if cultivars ranking similarly between years according to physical quality characteristics,. PROC GLM of SAS 9.4 was used to

determine differences of means of physical quality characteristics between years of subsequent timepoints using One-Way ANOVA ($P \leq 0.05$).

Results and Discussion

Fruit firmness

Within blueberry types and TPs, there was wide variation for fruit firmness, and individual cultivars' fruit firmness varied across types (Table 3.1 and Table 3.2). There were significant differences ($P \leq 0.05$) in fruit firmness for types and cultivars within each of the four TPs of postharvest cold storage for both 2018 and 2019 harvest seasons. There were also significant differences ($P \leq 0.05$) in fruit firmness for nearly all individual cultivars and types across the four TPs of postharvest cold storage. The majority of blueberry cultivars experienced significant overall increases in fruit firmness from TP1 – TP4 for both harvest seasons. With the exception of northern highbush types 'Bluecrop', 'Draper', 'Nelson', these cultivars followed an opposite trend, demonstrating a decrease in fruit firmness during the 2018 harvest season.

During 2018, type comparisons showed that southern highbush cultivars had significantly higher fruit firmness for all four within all four timepoints, followed by northern highbush and rabbiteye types. Percent increase in fruit firmness during the 2018 harvest season (Table 3.14) showed that northern highbush types had the lowest overall change (9%) relative to southern highbush (18.4%) and rabbiteye (26.4%) types. 2018 harvest season southern highbush blueberry cultivars showed variation compared to the 2019 harvest season, as southern highbush types were not consistently significantly higher in fruit firmness for all timepoints. Percent change of fruit firmness from TP1-TP4 during 2019 (Table 3.15) showed southern highbush types to have the lowest percent increase (24.7%) relative to rabbiteye (30.9%) and northern highbush (33.2%) types. This suggests that although southern highbush and northern highbush types' fruit firmness

during fresh postharvest cold storage are influenced by seasonal variation, they may maintain fruit firmness the best of the three commercial types and have higher fruit firmness values compared to rabbiteye types. These results are contrary to subjective biases that rabbiteye have firmer fruit than highbush types.

During both harvest seasons, although the majority of cultivars exhibited an overall significant, linear increase in fruit firmness from TP1-TP4, changes in fruit firmness of several cultivars did not follow a linear trend (Tables 3.1 and 3.2). Rabbiteye cultivar 'Brightwell' during the 2019 harvest season significantly decreased in fruit firmness from TP1-TP2, increased again from TP2-TP3, and finally plateaued at TP4. In 2018, rabbiteye cultivar 'Vernon' showed an opposite trend, increasing at TP1-TP2, decreasing from TP2-TP3, then again increasing from TP3-TP4. Many other cultivars resembled fruit firmness changes in a sigmoidal fashion. These significantly increased through TP3, before decreasing slightly at TP4. During the 2018 harvest season, southern highbush cultivars 'Meadowlark', 'Legacy' MI, 'Keecrisp' and 'Star', and rabbiteye cultivar 'Austin' resembled these characteristics. During the 2019 harvest season, northern highbush cultivar 'Elliott' resembled similar changes. Such fluctuations in fruit firmness may be largely influenced by environmental factors such as variation in pre-harvest environment rather than genetic factors, since results are not consistent for cultivars across years.

In previous studies comparing differences in fruit firmness among types, others reported that rabbiteye cultivars have fruit firmness that was considerably higher than southern highbush and northern highbush types (Itle and NeSmith, 2016; Makus and Moris, 1987). However, these previous studies mention all blueberry types to be universally harvested by hand during sampling. Machine harvesting is known to degrade fruit firmness of rabbiteye blueberries (NeSmith et al., 2000), and several cultivars were machine harvested between the two harvest

seasons in this study. Ehlenfeldt and Martin (2002) have compared between pure northern highbush and species introgressed cultivars, and found that southern highbush blueberry cultivars exhibit fruit firmness that is higher than average and all share a common factor of having traces of *V. darrowii*, *V. virgatum* and *V. tennellum* wild species ancestry. Cultivars with *V. angustifolium* ancestry and pure *V. corybosum* tend to produce soft fruit. Overall, rabbiteye (*V. virgatum)* berries are firmer than cultivars with *V. corymbosum* and *V. darrowii* ancestry (Ehlenfeldt and Martin, 2002). Observing the pedigrees for those cultivars in this study would be useful to determine whether pure or introgressed species may have contributions to their fruit firmness.

Skin strength

Skin strength (puncture-in and puncture-out) of individual cultivars varied across types, and there was wide variation within timepoint and types (Table 3.3 and Table 3.4). Within rabbiteye types, there was much less variation between cultivars compared to cultivars within southern highbush and northern highbush types during all timepoints during the 2018 harvest season. There were significant differences ($P \leq 0.05$) in skin strength for types and cultivars within each of the four TPs of postharvest cold storage for both 2018 and 2019 harvest seasons. In 2018, southern highbush were the highest for fruit skin strength, followed by northern highbush and then by rabbiteye for all four timepoints evaluated. In 2019, both HB types were not different from each other, and they had higher fruit skin strength than rabbiteye at TP1, TP3 and TP4. These results are contrary to subjective biases that rabbiteye have tougher fruit skins and firmer fruit than HB types.

There were also significant differences ($P \leq 0.05$) in skin strength for individual cultivars and types across the four TPs of postharvest cold storage. The majority of blueberry cultivars

experienced significant overall increases in skin strength from TP1 – TP4 for both harvest seasons. Similar to observations in fruit firmness, northern highbush types ‘Draper’ and ‘Nelson’ demonstrated a decrease in skin strength. Overall percent change from TP1-TP4 in skin strength during the 2018 harvest season (Table 3.14) showed that northern highbush and southern highbush types (both non-significant changes for both puncture-in and puncture-out) changed the least relative to rabbiteye types. During the 2019 season, southern highbush types showed the least amount of change in puncture-in (16.9%), followed by northern highbush (17.2%) and rabbiteye (22.5%) types. For puncture-out, rabbiteye types showed the least amount of change in puncture-out (10.7%), followed by southern highbush (15.8%) and northern highbush (18.1%) types. This suggests that northern highbush and southern highbush types maintain skin strength in fresh postharvest cold storage the best overall compared to rabbiteye types.

The majority of cultivars followed a linear increase in skin strength throughout postharvest cold storage, possibly because of moisture loss. However, a few cultivars showed fluctuations in their puncture-in from TP1-TP4. During the 2018 harvest season, southern highbush type ‘Legacy’ GA showed puncture-in to significantly increase from TP1-TP2, then significantly decrease from TP3-TP4. In the same year, rabbiteye type ‘Austin’ followed an opposite fluctuation, significantly decreasing in puncture-in from TP1-TP2, then significantly increasing from TP2-TP3. Unexpectedly, ‘Legacy’ GA followed a similar trend to that of ‘Austin’ during the 2019 harvest season. Variation in skin strength throughout postharvest cold storage and variation between harvest seasons may be a result of differing environmental factors.

Berry weight

There were significant differences ($P \leq 0.05$) in berry weight for individual cultivars and types across the four TPs of postharvest cold storage (Table 3.7 and 3.8). The majority of

blueberry cultivars experienced significant overall decreases in berry weight from TP1 – TP4 for both 2018 and 2019 harvest seasons. Percent change in berry weight from TP1-TP4 showed that rabbiteye types were consistently lowest for both harvest 2018 and 2019 harvest seasons, which had no significant changes in weight for both years, (Table 3.14 and Table 3.15), followed by northern highbush types (-11.2% and -8.5%, respectively) and southern highbush types (-11.6% and -11.5%, respectively). This suggests that rabbiteye cultivars maintain berry weight the best in fresh postharvest cold storage compared to southern highbush and northern highbush types overall. Fluctuations in fruit firmness discussed above may be caused by cultivars' ability to maintain water loss during postharvest storage, which could be influenced by fruit size. This hypothesis is not supported here but has been demonstrated by Paniagua et al, (2013). Weight loss through moisture loss was found to have a causal relationship with loss of postharvest firmness in blueberries, however only one rabbiteye cultivar 'Centurion' was tested.

Percent healthy fruit

There were significant differences ($P \leq 0.05$) for percent healthy fruit for types and cultivars within each of the four TPs of postharvest cold storage, as well as wide variation in percent healthy fruit within timepoint and types (Table 3.9 and Table 3.10). There were also significant differences in percent healthy fruit across timepoints for individual cultivars and for types, with heavy decline by TP4. Type comparisons showed that southern highbush types had significantly higher percent healthy fruit than northern highbush at TP4, followed by rabbiteye for both the 2018 and 2019 harvest seasons. This suggested that southern highbush types maintain visual appearance the best in fresh postharvest cold storage compared to northern highbush and rabbiteye types. Percent change in percent healthy fruit from TP1-TP4 (Table 3.14 and Table 3.15) showed that northern highbush types experienced the least amount of change

during postharvest cold storage (-23%), followed closely by southern highbush types (-24.8%), which were both an almost two-fold difference from rabbiteye types (-45.3%) during the 2018 harvest season. Rabbiteye types again decreased in percent healthy fruit by almost half (-48%) during the 2019 harvest season. During this year, southern highbush types showed the least amount of change in percent healthy fruit (-28.8%), followed by northern highbush types (-34.1%). This suggests that southern highbush and northern highbush types may maintain visual appearance better than rabbiteye types during fresh postharvest cold storage.

Ethylene concentration

There were significant differences ($P \leq 0.05$) for ethylene concentration for types and cultivars 1-2 days after fruit collection during the 2018 harvest season (Table 3.11). There was wide variation for ethylene concentration among and within blueberry types. Southern highbush cultivars 'Keecrisp' and 'Meadowlark' and rabbiteye cultivars 'Austin' and 'Vernon' had relatively high concentrations compared to other cultivars, with 'Meadowlark' among all types to have the highest ethylene accumulation after incubation. This was somewhat unexpected as 'Keecrisp' is an excellent storing berry. In general, higher concentrations of ethylene tend to signal inferior keeping quality in the form of fungal germination decay (Zhu et al., 2012), and further along in the ripening process. Range of ethylene concentration in southern highbush and rabbiteye types was also very high, ranging from 0.03 – 1.2 nL/ g x hr in southern highbush and from 0.04 to 1.03 nL/ g x hr in rabbiteye. Comparison by type showed northern highbush types had significantly lower production of ethylene than southern highbush and rabbiteye 3-4 days after fruit collection.

During the 2019 harvest season, variation in ethylene concentration was not as wide as in 2018 (Table 3.12). There were no significant differences in ethylene concentration between

southern highbush and northern highbush types, and rabbiteye types were significantly higher than both HB types by over eight times. This variation between years could be attributed to differences in temperature during ripening between the two years. Most cultivars have very different forms of ethylene evolution, so this is difficult to determine (Suzuki et al., 1997).

Comparison of years

Spearman's rank correlation coefficients showed that physical quality characteristics had low to moderate correlations for cultivar ranking across years (Table 3.13). Seasonal variation and variation within cultivars make it difficult to pinpoint one early postharvest factor to predict overall shelf-life. Given this, it was expected that within individual cultivars there can be differences in texture when there are differences harvest maturity (Sams, 1999). Factors such as harvest timing (Lobos et al., 2014), handling processes (Bower, 2007), anthesis time (Suzuki and Kawata, 2001), amount of glaucescens or fruit cuticular wax composition (Lara et al., 2014; Chu et al., 2018), changes in secondary cell wall structures, and enzymatic changes in the fruit cell wall (Chea et al., 2019; Chen et al., 2015), and many other environmental factors that affect components of fruit texture may also be contributing to overall postharvest keeping quality.

Cultivar and genetic factors control the majority of postharvest quality factors for perennial fruit crops (Beever and Hopkirk, 1990; Beverly et al., 1993). However, high temperatures combined with rain can cause significant firmness loss to blueberries by delaying harvest and washing off fungicides, and thus manifesting soft berries, moistening stem scars, and causing berries to split (Pritts and Hancock, 1992). Weather events like this could be contributing to the variation seen between the two years. Other cultural factors such as fertilization are a consideration. Levels of phosophorus may affect fruit size, which potentially influence growers' decisions to conduct machine harvesting. Townsend (1973) found that an application of

phosphorus reduced fruit size in one of three years, while Ballinger and Kushman (1969) report that low levels of phosphorus are seen to cause high fruit/ leaf ratio and small fruit in northern highbush blueberry. In this study, site was not a controlled factor and likely varied between years for cultivars. Fertilization rates for southern Georgia blueberries can depend on cultivar and site. Sites which differ in water table proximity to the soil surface, drainage, and levels of cation exchange capacity all play a factor into suggested fertilization rates (Smith, 2019)

Pearson correlation coefficients

There were very few strong correlations between TP1 physical postharvest fruit quality and TP4 percent healthy fruit (Tables 3.16 and 3.17). During the 2018 harvest season, TP1 berry weight of rabbiteye types was the strongest positive correlation with TP4 percent healthy fruit ($r=0.84$), however this was not consistent across all types. TP1 ethylene concentration had a moderate, positive correlation with TP4 percent healthy fruit for rabbiteye type ($r=0.52$). However, TP1 ethylene concentration had no significant correlations with TP4 percent healthy fruit of southern highbush and northern highbush types. During the 2019 harvest season, TP1 ethylene concentration had a moderate, negative correlation with TP4 percent healthy fruit of rabbiteye types ($r= -0.68$), and a strong, negative correlation of northern highbush types ($r= -0.86$. Inconsistencies in correlations between the two harvest seasons suggest that early physical postharvest fruit quality traits may not be reliable indices for postharvest keeping quality of visual appearance or shelf-life of blueberry.

Multivariate analysis

Principal component analysis

During 2018, the first two of the five principal components (PCs) during TP1 have eigenvalues greater than 1.0 (2.9, 1.46) and accounted for about 87% (57.2% and 29.3%) of the

total variance of physical quality characteristics (Figure 3.1). PC1 was associated with puncture-in, puncture-out, and fruit firmness indicated by eigenvectors being dominant in these three traits and their association on the loading plot. PC2 was associated with both berry weight and percent healthy fruit. A threshold of ±0.5 for eigenvector value was used to determine which parameters contributed to PC's. This suggests that PC1 could be interpreted as a textural component and PC2 could be interpreted as a shelf-life component.

Eigenvalues remained relatively unchanged in TP4, however berry weight and percent healthy were more positively connected to PC1 in TP4 than in TP1, but fruit firmness was slightly less positively connected to PC1 in TP4 than in TP1. PCA analysis did not show a discernable grouping of types for either TP (Figure 3.1 and 3.2). Cultivars 'Brightwell', 'Farthing', 'Meadowlark', and 'Star' were omitted from PCA due to missing texture data from 'Meadowlark', 'Farthing', and 'Star' during TP1, and cultivar 'Brightwell' was also omitted for missing texture and percent healthy fruit data during TP4. During TP1, biplot scores showed that rabbiteye cultivars were negatively associated with PC1 and did not show much variation in textural traits. For PC2, there was a wide variation in berry weight and shelf-life for rabbiteye cultivars. In contrast, southern highbush cultivars had a wide variation in textural traits but did not show as much variation in shelf-life and berry weight as rabbiteye cultivars did during TP1. northern highbush cultivars generally were found to lie amongst both southern highbush and rabbiteye types on the biplot. Within northern highbush types, cultivars most likely vary more in textural traits than they do in shelf-life. Most notably in TP4 PC scores, variation in shelf-life was much higher within southern highbush types, while an outlying southern highbush cultivar 'Keecrisp' maintains very high texture trait values. Scores also showed to congregate closer to each other and towards the origin during TP4. This may indicate that variation of physical

quality characteristics between blueberry types may not be as distinctive as time passes in postharvest cold storage. Based on TP1 and TP4 PC scores, the most significant observation was that when comparing types by physical quality characteristics over postharvest storage, rabbiteye and southern highbush types differ the most extremely in textural traits, while northern highbush types are more alike rabbiteye and southern highbush than they are different. Northern highbush cultivar 'Draper' and southern highbush cultivar 'Keecrisp' remained in quadrant 1 during TP1 and TP2 PCA's, which suggests these cultivars maintain favorable physical quality characteristics throughout fresh postharvest storage overall. Southern highbush cultivars 'Camellia' and 'Legacy' GA, and rabbiteye cultivars 'Vernon' and 'Alapaha' maintained scores in quadrant 2, which suggests that although their textural traits may not relatively high postharvest cold storage, they endured postharvest storage without many visual imperfections to a high degree.

The 2019 harvest season PCA for TP 1 (Figure 3.5) showed the first two of the five principal components (PCs) to have eigenvalues greater than 1.0 (2.09, 1.78) and accounted for about 77% (41.8% and 35.6%) of the total variance of physical quality characteristics. Unlike during the 2018 harvest season, fruit firmness, puncture-in, and puncture-out were not as closely associated with one another during the 2019 harvest season as determined by the angles of their loadings. Based on eigenvector values, the variance of puncture-in and puncture-out were more accurately captured in PC1, while PC2 captured variance of fruit firmness, percent healthy fruit, and berry weight best. Therefore, there was less of a distinction in sub-categories of physical fruit quality explained by PC1 and PC2. Much like during the 2018 harvest season, rabbiteye types showed to have negative associations with fruit firmness and skin strength. Rabbiteye cultivars demonstrated wide variation in fruit firmness, berry weight, and percent healthy based

on PC2 scores. Southern highbush also showed wide variation in textural traits but varied less in berry weight and percent healthy fruit. There were no distinct groupings of types in TP1 for the 2019 harvest season, however southern highbush and northern highbush cultivars tended to group in quadrants 1 and 2, suggesting that these types were more associated with high percent healthy fruit and skin strength. Rabbiteye cultivars grouped within quadrants 2 and 3, which demonstrates these cultivars did not associate highly with these high percent healthy fruit and skin strength early in postharvest storage. Variation in puncture-in and percent healthy fruit were most accurately captured in PC1, while Kramer and puncture-out were most accurately captured in PC2. Most notably, several southern highbush and northern highbush types were associated with higher percent healthy fruit and skin strength, while rabbiteye types were negatively associated with both of these quality traits. This again suggests that southern highbush and northern highbush blueberry types may maintain skin strength and visual appearance during postharvest cold storage.

Hierarchical cluster analysis

To further examine differences among and across types, PC1 and PC2 scores were used to cluster cultivars into dendrograms using Ward's method for TP1 and TP4, respectively (Figure 3.5 and 3.6). Three clusters were established for each TP. During each timepoint, cluster analyses placed cultivars with negative PC1 and positive PC2 values (quadrant 2) in cluster 1, cultivars with both negative PC's (quadrant 3) in cluster 2, and cultivars with both PC scores as positive in cluster 3 (quadrant 1). During TP4, cluster 3 showed to have negative values for PC2 and positive values for PC1 (quadrant 4). From TP1 to TP4, several cultivars shifted between clusters, mostly between cluster 1 and cluster 2. All blueberry types were shared between cluster 1 and cluster 2, however no rabbiteye cultivars were included in cluster 3 during either TP. This

suggests that rabbiteye cultivars are negatively associated with physical quality characteristics during initial and late postharvest storage. For the 2019 harvest season, hierarchical cluster analysis evaluated at TP1 grouped cultivars into three clusters based on PC scores: cluster 1 contained cultivars with negative PC1 values and positive PC2 values (quadrant 2), cluster 2 contained cultivars with cultivars with negative values for both PCs (quadrant 3), and cluster 3 contained cultivars with positive values for both PCs (quadrant 1). Most rabbiteye cultivars fell within cluster 1 and 2, while the majority of southern highbush and northern highbush cultivars fell within cluster 3. This suggests that initial physical fruit quality characteristics of southern highbush and northern highbush were more related to one another than to rabbiteye cultivars.

Hierarchical cluster analysis evaluated at TP4 group clusters into three clusters based on PC scores: cluster 1 contained cultivars with negative values for both PCs (quadrant 3), cluster 2 contained cultivars with positive values for both PCs (quadrant 1), and cluster 3 contained cultivars with negative values for PC1 and positive values for PC2 (quadrant 2). Based on loadings that were changed during TP4, rabbiteye cultivars showed to the be the least associated with high puncture-in and puncture-out values and were related to a handful of southern highbush cultivars. The only rabbiteye cultivar to maintain high fruit firmness until TP4 was ‘Alapaha’, along with southern highbush cultivar ‘Legacy’ GA and northern highbush cultivars ‘Aurora’ and ‘Elliott’. Cluster 2 contained a mix of southern highbush and northern highbush cultivars, and these were most highly associated with skin strength and percent healthy fruit. This may suggest that genetic control of fruit firmness and skin strength stability throughout postharvest cold storage may vary within and across types.

Conclusion

Data suggest that southern highbush and northern highbush cultivars have the best stability of most physical postharvest fruit quality characteristics, which was contrary to the subjective bias that northern highbush types always have the highest fruit quality. Rabbiteye cultivars only had the best stability of berry weight in commercial cold storage. Fruit size may be contributing to the fluctuations observed in fruit firmness and skin toughness and water holding ability. In addition, the cultivars presented here with the smallest changes in fruit firmness and skin strength during postharvest cold storage may prove to be suitable candidates for lengthy postharvest storage, as well as mechanized harvesting. The findings also suggest that screening and predicting shelf-life using early postharvest fruit quality characteristics may not be useful. It would be useful to determine the differences in other traits among southern highbush, northern highbush, and rabbiteye blueberry types to further understand structural differences to determine blueberry types best suited to endure long storage times without spoilage. Factors such as number of harvests per bush, location, harvest and processing methods, and on-site cultural practices were not controlled in this study and may have as important an impact on fruit quality differences as genotype. In addition, there was variation in how different blueberry types respond to mechanical harvesting, so it may be beneficial to understand the interaction between cultivar, harvest type, and location for future directions of this project.

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Table 3.1. Fruit firmness [max load (N) Kramer Shear] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Camellia'	***[x]	225.1[w] g[v] C[u]	246.1 g CB	264.9 g B	294.5 gh A
	'Farthing'	NS	.	330.1 ef	349.4 e	345.9 ef
	'Keecrisp'	**	608.0 a C	641.4 a BC	709.8 a A	696.7 a AB
	'Legacy' GA[t]	***	254.3 fg B	322.1 ef A	338.4 e A	331.1 e-g A
	'Legacy' MI	***	361.7 cd B	416.0 c A	452.8 cd A	440.5 cd A
	'Meadowlark'	**	.	468.3 b B	522.6 b A	487.0 b AB
	'Star'	*	.	307.9 f B	332.5 ef A	319.2 fg AB
	'Suziblue'	**	392.4 c B	406.8 c AB	434.8 cd A	417.6 d AB
RE	'Alapaha'	***	239.2 g C	308.9 f A	285.8 fg B	287.3 gh B
	'Austin'	***	361.5 cd B	386.6 cd B	463.5 cd A	454.1 b-d A
	'Brightwell'	***	325.6 de C	384.9 cd B	471.8 c A	.
	'Powderblue'	***	285.3 ef B	303.9 f B	346.6 e A	365.0 e A
	'Vernon'	***	244.3 fg C	315.8 ef A	277.4 g B	322.5 e-g A
NHB	'Bluecrop'	**	306.5 e A	315.1 ef A	312.4 e-g A	271.2 h B
	'Draper'	*	470.6 b AB	504.0 b A	470.7 cd AB	450.0 b-d B
	'Elliott'	***	352.4 cd C	411.2 c B	425.3 cd B	462.2 b-d A
	'Liberty'	***	322.8 de B	354.5 de B	421.9 d A	465.3 bc A
	'Nelson'	**	306.0 e A	314.7 f A	305.3 e-g A	268.5 h B
Type			***	***	***	***
SHB		NS	368.3[s] a	392.4 a	425.7 a	416.6 a
RE		***	291.2 c B	340.0 c A	369.0 c A	357.2 c A
NHB		NS	351.6 b	379.9 b	387.1 b	383.4 b

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***= $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=9 [3 reps of 3(50.0-51.0g-berry sample) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=45 (5 cultivars x 3 reps x 3 subreps/rep) for TP1, N=72 (8 cultivars x 3 reps x 3 subreps/rep) for TP 2-4; RE N=45 (5 cultivars x 3 reps x 3 subreps/rep) for TP 1-3, N=36 (4 cultivars x 3 reps x 3 subreps/rep) for TP4; NHB N=45 (5 cultivars x 3 reps x 3 subreps/rep).

Table 3.2. Fruit firmness [max load (N) Kramer Shear] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Abundance'	***[x]	288.8[w] e-g[v]	C[u]	323.2 e-g	B	384.6 d-g	A	367.3 e	A
	'Camellia'	***	303.2 ef	B	302.6 fg	B	344.1 g	A	362.0 e	A
	'Farthing'	***	350.9 cd	B	377.2 b-d	B	435.5 bc	A	433.6 cd	A
	'Legacy' GA[t]	***	389.5 ab	B	389.5 bc	B	486.5 a	A	511.0 a	A
	'Legacy' MI	**	339.6 d	B	373.4 b-d	A	377.7 e-g	A	371.9 e	A
	'Star'	***	273.8 fg	B	299.9 fg	B	357.4 g	A	369.9 e	A
	'Suziblue'	***	213.2 h	C	440.1 a	B	260.1 h	A	276.7 f	A
RE	'Alapaha'	***	420.6 a	B	341.3 d-f	B	476.5 ab	A	483.6 ab	A
	'Brightwell'	***	379.0 bc	B	383.4 bc	C	425.7 cd	A	434.0 cd	A
	'Powderblue'	***	348.1 cd	C	281.8 g	BC	387.7 c-g	B	454.9 bc	A
	'Premier'	***	268.9 g	C	236.5 h	C	371.6 fg	B	425.1 cd	A
	'Vernon'	***	277.0 fg	C	302.4 fg	B	406.6 c-f	A	419.3 cd	A
NHB	'Aurora'	***	319.3 de	C	390.7 bc	B	425.7 c-e	A	446.6 bc	A
	'Elliott'	***	339.6 d	C	412.2 ab	B	491.4 a	A	446.5 bc	B
	'Liberty'	***	304.5 ef	C	349.8 c-e	B	360.9 fg	AB	389.8 de	A
Type			**		***		***		***	
SHB		***	308.4[s] b	B	328.9 b	B	426.0 a	A	384.6 b	A
RE		***	338.7 a	C	349.8 b	C	413.6 a	B	443.4 a	A
NHB		***	321.1 ab	C	384.2 a	B	378.0 b	A	427.7 a	A

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (**=$P \leq 0.01$, ***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (**=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=9 [3 reps of 3(50.0-51.0g-berry sample) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=63 (7 cultivars x 3 reps x 3 subreps/rep) ; RE N=45 (5 cultivars x 3 reps x 3 subreps/rep); NHB N=27 (3 cultivars x 3 reps x 3 subreps/rep).

Table 3.3. Skin strength [max load (N) puncture-in] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Camellia'	**[x]	0.735[w] fg[v] B[u]	0.758 fg AB	0.878 g-i A	0.879 Ef A
	'Farthing'	NS	.	1.485 b	1.496 b	1.376 B
	'Keecrisp'	NS	1.822 a	1.756 a	1.784 a	1.671 A
	'Legacy' GA[t]	***	1.091 c B	1.340 bc A	1.227 cd AB	1.093 Cd B
	'Legacy' MI	*	0.974 c-e B	1.073 d AB	1.145 de A	1.094 Cd AB
	'Meadowlark'	NS	.	1.481 b	1.531 b	1.589 A
	'Star'	NS	.	1.065 d	1.146 de	1.090 Cd
	'Suziblue'	*	1.448 b AB	1.480 b A	1.404 bc AB	1.369 B B
RE	'Alapaha'	**	0.710 fg B	0.702 fg B	0.803 hi A	0.804 Ef A
	'Austin'	***	0.861 d-f A	0.710 fg B	0.875 g-i A	0.868 Ef A
	'Brightwell'	NS	0.766 fg	0.756 fg	0.807 hi	.
	'Powderblue'	NS	0.646 g	0.613 g	0.689 i	0.701 F
	'Vernon'	NS	0.801 fg	0.868 ef	0.819 hi	0.838 Ef
NHB	'Bluecrop'	NS	1.032 cd	1.055 de	1.017 e-g	0.991 c-e
	'Draper'	***	1.486 b A	1.467 b A	1.374 bc A	1.166 C B
	'Elliott'	**	0.746 fg B	0.744 fg B	0.930 f-h A	0.863 Ef AB
	'Liberty'	***	0.846 ef B	0.815 f B	0.954 e-h AB	1.080 Cd A
	'Nelson'	***	1.117 c A	1.158 cd A	1.092 d-f A	0.950 De B
Type			***	***	***	***
SHB		*	1.214[s] a B	1.305 a AB	1.326 a A	1.270 a AB
RE		**	0.757 c AB	0.730 c B	0.799 c A	0.803 c A
NHB		NS	1.045 b	1.048 b	1.073 b	1.010 b

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***= $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=36 (3 reps x 12 individual berry subreps/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=180 for TP1 (5 cultivars x 3 reps x 12 individual berry subreps/rep), N=288 for TP 2-4 (8 cultivars x 3 reps x 12 individual berry subreps/rep); RE N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep) for TP 1-3, N=144 (4 cultivars x 3 reps x 12 individual berry subreps/rep) for TP4; NHB N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep).

Table 3.4. Skin strength [max load (N) puncture-in] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***[x]	1.176[w] a[v] C[u]	1.265 a BC	1.437 ab A	1.373 Ab AB
	'Camellia'	*	0.858 c-e B	0.929 d-f AB	1.000 de A	1.007 c-f A
	'Farthing'	***	1.174 a B	1.205 ab B	1.460 a A	1.407 A A
	'Legacy' GA[t]	***	1.016 a-c AB	0.897 d-g B	1.121 c-e A	1.098 c-e A
	'Legacy' MI	**	0.987 bc B	1.142 a-c A	1.132 cd A	1.131 Cd A
	'Star'	***	0.859 c-e B	1.013 cd AB	1.127 cd A	1.044 c-f A
	'Suziblue'	**	0.925 b-d B	1.041 b-d AB	1.089 c-e A	1.117 c-e A
RE	'Alapaha'	*	0.853 c-e B	0.942 d-f AB	0.934 ef AB	0.990 d-f A
	'Brightwell'	***	0.777 d-f B	0.729 g B	0.754 fg B	0.899 Fg A
	'Powderblue'	NS	0.723 ef	0.783 e-g	0.721 g	0.761 G
	'Premier'	***	0.659 f B	0.764 fg B	0.772 fg B	0.928 e-g A
	'Vernon'	***	0.771 d-f C	0.867 d-g BC	0.935 ef AB	1.057 c-f A
NHB	'Aurora'	***	1.038 ab B	1.275 a A	1.242 c A	1.092 c-f B
	'Elliott'	***	0.748 ef B	0.958 c-e A	1.112 c-e A	1.032 c-f A
	'Liberty'	**	1.050 ab B	1.209 ab AB	1.251 bc A	1.199 Bc AB
Type			***	***	***	***
SHB		***	0.999[s] a C	1.070 b B	1.195 a A	1.168 a A
RE		***	0.757 b C	0.817 c B	0.823 b B	0.927 b A
NHB		***	0.945 a B	1.147 a A	1.202 a A	1.108 a A

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***= $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=36 (3 reps x 12 individual berry subreps/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=252 (7 cultivars x 3 reps x 12 individual berry subreps/rep); RE N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep); NHB N=108 (3 cultivars x 3 reps x 12 individual berry subreps/rep).

Table 3.5. Skin strength [max load (N) puncture-out] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Camellia'	NS[x]	0.487[w] fg[v]		0.513 f-j		0.556 ef		0.591 b-e	
	'Farthing'	*	.		0.850 bc	B[u]	0.957 bc	A	0.948 a	AB
	'Keecrisp'	*	1.002 a	B	1.072 a	AB	1.149 a	A	1.019 a	AB
	'Legacy' GA[t]	***	0.629 de	C	0.923 ab	A	0.794 cd	B	0.669 cd	C
	'Legacy' MI	NS	0.626 de		0.640 e-g		0.654 d-f		0.687 c	
	'Meadowlark'	**	.		0.796 b-d	B	0.908 bc	AB	1.000 a	A
	'Star'	NS	.		0.601 e-i		0.616 ef		0.683 cd	
	'Suziblue'	***	0.796 bc	B	0.838 bc	B	1.005 ab	A	0.861 ab	B
RE	'Alapaha'	*	0.510 e-g	B	0.596 e-i	AB	0.556 ef	AB	0.610 c-e	A
	'Austin'	NS	0.579 ef		0.570 f-j		0.642 d-f		0.606 c-e	
	'Brightwell'	*	0.516 e-g	AB	0.496 g-j	B	0.641 d-f	A	.	
	'Powderblue'	NS	0.428 g		0.438 j		0.492 f		0.476 e	
	'Vernon'	NS	0.559 e-g		0.617 e-h		0.619 ef		0.610 c-e	
NHB	'Bluecrop'	NS	0.611 d-f		0.651 d-f		0.672 de		0.593 c-e	
	'Draper'	NS	0.931 ab		1.010 a		0.895 bc		0.875 a	
	'Elliott'	**	0.490 fg	B	0.464 ij	B	0.606 ef	A	0.524 de	AB
	'Liberty'	***	0.517 e-g	B	0.474 h-j	B	0.684 de	A	0.704 bc	A
	'Nelson'	***	0.738 cd	A	0.722 c-e	A	0.689 de	A	0.573 c-e	B
Type			***		***		***		***	
SHB		***	0.708[s] a	B	0.779 a	A	0.831 a	A	0.808 a	A
RE		***	0.518 b	B	0.544 c	AB	0.590 c	A	0.576 c	A
NHB		NS	0.658 a		0.664 b		0.709 b		0.654 b	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***= $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=36 (3 reps x 12 individual berry subreps/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=180 for TP1 (5 cultivars x 3 reps x 12 individual berry subreps/rep), N=288 for TP 2-4 (8 cultivars x 3 reps x 12 individual berry subreps/rep); RE N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep) for TP 1-3, N=144 (4 cultivars x 3 reps x 12 individual berry subreps/rep) for TP4; NHB N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep).

Table 3.6. Skin strength [max load (N) puncture-out] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***[x]	0.635[w] a-c[v] B[u]	0.702 b-d AB	0.785 a-c A	0.789 a-c A
	'Camellia'	NS	0.544 c-e	0.599 d-h	0.616 d-f	0.608 d-g
	'Farthing'	***	0.726 ab C	0.733 b-d BC	0.894 a A	0.862 a AB
	'Legacy' GA[s]	NS	0.636 a-c	0.659 c-f	0.742 b-d	0.736 a-d
	'Legacy' MI	NS	0.638 a-c	0.748 bc	0.672 c-e	0.705 a-e
	'Star'	NS	0.428 e	0.463 h	0.536 e-g	0.523 fg
	'Suziblue'	NS	0.580 cd	0.551 e-h	0.559 e-g	0.625 c-g
RE	'Alapaha'	NS	0.614 b-d	0.631 c-g	0.645 c-e	0.668 c-g
	'Brightwell'	NS	0.546 c-e	0.516 fgh	0.493 fg	0.563 e-g
	'Powderblue'	*	0.493 ed AB	0.496 gh A	0.430 g B	0.455 g AB
	'Premier'	NS	0.488 ed	0.527 fgh	0.481 fg	0.560 e-g
	'Vernon'	***	0.567 cd B	0.627 c-g B	0.610 d-f B	0.754 a-d A
NHB	'Aurora'	**	0.739 ab B	0.904 a A	0.834 ab AB	0.815 ab AB
	'Elliott'	***	0.514 c-e B	0.697 c-e A	0.778 a-c A	0.702 a-e A
	'Liberty'	NS	0.758 a	0.843 ab	0.885 ab	0.858 a
Type			***	***	***	***
SHB		***	0.598[s] b B	0.636 b B	0.686 b A	0.693 b A
RE		***	0.542 c B	0.559 c AB	0.532 c B	0.600 c A
NHB		***	0.670 a B	0.815 a A	0.832 a A	0.792 a A

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=36 (3 reps x 12 individual berry subreps/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[sv]SHB N=252 (7 cultivars x 3 reps x 12 individual berry subreps/rep) ; RE N=180 (5 cultivars x 3 reps x 12 individual berry subreps/rep); NHB N=108 (cultivars x 3 reps x 12 individual berry subreps/rep).

Table 3.7. Berry weight (g) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Camellia'	***[x]	47.3[w] a[v]	A[u]	43.8 b	B	41.5 a	B	38.3 bc	C
	'Farthing'	***	46.9 a	AB	50.6 a	A	42.5 a	BC	45.1 a	C
	'Keecrisp'	*	31.4 e-g	A	27.3 d-f	AB	28.3 b-d	AB	23.7 f-i	B
	'Legacy' GA[t]	***	37.4 cd	B	46.8 ab	A	45.7 a	A	38.4 bc	B
	'Legacy' MI	**	24.1 hi	A	23.3 fg	AB	21.4 ef	BC	21.0 i-k	C
	'Meadowlark'	**	38.8 bc	A	38.8 c	A	32.4 b	B	36.4 c	AB
	'Star'	NS	36.0 c-e		35.4 c		33.3 b		30.8 de	
	'Suziblue'	***	34.2 c-f	A	25.5 ef	B	28.9 b-d	B	27.8 d-f	B
RE	'Alapaha'	NS	30.2 fg		30.8 d		31.4 bc		31.3 d	
	'Austin'	**	29.3 f-h	A	25.7 ef	AB	23.9 de	B	22.2 ij	B
	'Brightwell'	**	27.0 gh	A	27.1 d-f	A	24.0 de	AB	22.8 g-i	B
	'Powderblue'	***	30.9 e-g	A	28.1 de	A	24.0 de	B	22.5 h-j	B
	'Vernon'	***	43.4 ab	BC	47.7 ab	A	45.2 a	AB	41.2 ab	C
NHB	'Bluecrop'	***	32.4 d-g	A	28.6 de	B	26.2 c-e	B	26.7 e-h	B
	'Draper'	NS	28.4 gh		27.4 d-f		28.3 b-d		26.6 e-h	
	'Elliott'	**	21.1 i	A	18.7 h	B	18.4 f	B	18.4 jk	B
	'Liberty'	***	20.6 i	A	19.0 gh	B	18.1 f	BC	16.9 k	C
	'Nelson'	NS	28.0 gh		27.9 de		26.3 c-e		27.1 d-g	
Type			***		***		***		***	
SHB		NS	37.0[s] a		36.4 a		34.2 a		32.7 a	
RE		NS	32.1 b		31.9 b		29.7 b		28.0 b	
NHB		NS	26.1 c		24.3 c		23.5 c		23.2 c	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$

[w]N=4 (4 reps with 20-berry samples/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=32 (8 cultivars x 4 reps with 20-berry samples/rep); RE N=20 (5 cultivars x 4 reps with 20-berry samples/rep); NHB N=20 (5 cultivars x 4 reps with 20-berry samples/rep).

Table 3.8. Berry weight (g) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***[x]	31.78[w] cd[v] B[u]	34.48 cd A	29.85 bc B	31.45 bc B
	'Camellia'	NS	31.52 cd	33.95 c-e	29.53 bc	30.57 cd
	'Farthing'	*	37.73 ab A	35.17 c AB	30.71 b B	32.98 a-c AB
	'Legacy' GA[t]	**	29.32 c-e A	29.39 e-g A	25.95 c-e AB	25.31 ef B
	'Legacy' MI	***	29.36 c-e A	30.18 d-f B	24.93 d-f B	26.71 de B
	'Star'	**	34.08 bc A	42.43 a AB	27.81 b-d B	35.90 a B
	'Suziblue'	**	39.35 ab AB	17.92 i A	38.56 a AB	16.60 h B
RE	'Alapaha'	**	20.42 g A	28.25 fg B	18.23 g AB	27.18 de B
	'Brightwell'	*	27.64 d-f AB	26.10 f-h A	25.74 c-e B	23.38 e-g AB
	'Powderblue'	*	29.08 c-f A	27.41 f-h AB	25.14 d-f B	26.57 de AB
	'Premier'	***	42.69 a A	36.54 bc B	35.83 a B	34.34 a-c B
	'Vernon'	**	41.01 a A	40.25 ab A	35.32 a B	34.74 ab B
NHB	'Aurora'	NS	23.81 fg	25.22 gh	24.46 d-f	24.09 e-g
	'Elliott'	**	24.18 e-g A	23.17 h AB	21.35 fg B	20.56 gh B
	'Liberty'	**	25.08 e-g A	24.85 gh AB	22.74 ef BC	22.24 fg C
Type			***	***	**	***
SHB		***	33.3[s] a A	33.10 a A	29.62 a B	29.47 a B
RE		NS	32.17 a	30.07 a	28.05 a	27.88 a
NHB		**	24.36 b A	24.41 b A	22.85 b AB	22.29 b B

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (**=$P \leq 0.01$, ***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=4 (4 reps with 20-berry samples/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[v]SHB N=28 (7 cultivars x 4 reps with 20-berry samples/rep); RE N=20 (5 cultivars x 4 reps with 20-berry samples/rep); NHB N=12 (3 cultivars x 4 reps with 20-berry samples/rep).

Table 3.9. Percent healthy fruit of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Camellia'	***[x]	93.0[w] b-e[v] AB[u]	95.0 ab A	80.9 bc B	60.8 De C
	'Farthing'	***	91.7 b-e A	97.5 a A	93.3 ab A	80.0 a-d B
	'Keecrisp'	NS	95.9 a-c	93.3 a-c	96.7 a	91.7 A
	'Legacy' GA[t]	**	96.7 ab A	87.5 a-e AB	77.5 bc B	85.0 Ab B
	'Legacy' MI	**	90.9 b-e A	81.7 b-f AB	75.0 b-d BC	64.2 b-e C
	'Meadowlark'	**	90.0 b-e AB	90.9 a-d A	81.7 a-c BC	80.0 a-d C
	'Star'	**	86.7 c-g A	82.5 a-e A	79.2 bc A	60.0 De A
	'Suziblue'	***	94.2 b-d A	61.7 fg B	64.2 c-e B	31.7 Fg C
RE	'Alapaha'	***	100.0a A	92.5 a-c B	78.4 bc BC	65.8 b-e C
	'Austin'	***	70.8 g A	40.0 gh B	10.8 g C	16.7 G C
	'Brightwell'	***	25.0 h A	15.8 h A	0.0 h B	. .
	'Powderblue'	***	91.7 b-e A	70.8 d-f B	21.7 fg C	17.5 G C
	'Vernon'	**	89.2 b-f A	75.0 c-f B	75.9 b-d B	65.0 b-e B
NHB	'Bluecrop'	*	85.0 d-g A	78.4 b-f AB	78.3 bc AB	66.7 b-e B
	'Draper'	NS	95.9 a-d	93.4 a-c	89.2 ab	85.0 a-c
	'Elliott'	**	73.3 fg A	61.7 fg AB	47.5 ef AB	64.2 c-e B
	'Liberty'	NS	71.7 g	65.0 e-g	50.0 de	52.5 Ef
	'Nelson'	***	80.0 e-g A	70.0 d-f A	68.3 c-e A	44.2 Ef B
Type			***	***	***	***
SHB		***	92.0[s] a A	86.3 a AB	81.0 a B	69.2 a C
RE		***	75.3 b A	58.8 c AB	37.3 c B	41.2 c B
NHB		***	81.2 b A	73.7 b AB	66.7 b B	62.5 b B

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$

[w]N=120 (4 reps with 30 individual berry samples/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=960 (8 cultivars x 4 reps x 30 individual berry samples/rep); RE N=600 (5 cultivars x 4 reps x 30 individual berry samples/rep) for TP 1-3, N=480 (4 cultivars x 4 reps x 30 individual berry samples/rep) for TP4; NHB N=600 (5 cultivars x 4 reps x 30 individual berry samples/rep).

Table 3.10. Percent healthy fruit of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***[x]	100.0[w] a[v] A[u]	95.0 a B	91.7 a BA	85.0 a C
	'Camellia'	*	87.5 b-e A	80.0 a-d AB	65.8 bc B	69.2 a-d AB
	'Farthing'	***	98.3 ab A	85.0 ab B	85.0 ab B	70.9 a-c B
	'Legacy' GA[t]	***	97.5 a-c A	73.3 b-d B	52.5 c BC	43.3 d-f C
	'Legacy' MI	*	94.2 a-c A	85.8 ab AB	79.2 a-c AB	72.5 ab B
	'Star'	***	90.0 b-d A	77.5 a-d B	70.8 a-c BC	62.5 a-e C
	'Suziblue'	*	75.0 de A	76.7 a-d A	54.2 c B	55.0 b-f B
RE	'Alapaha'	*	64.2 e AB	72.5 b-e A	63.3 bc AB	46.7 c-f B
	'Brightwell'	***	86.7 b-d A	85.8 ab A	70.8 a-c AB	51.7 b-f B
	'Powderblue'	***	90.0 a-d A	84.2 a-c AB	70.0 a-c BC	55.9 b-f C
	'Premier'	***	90.0 b-d A	54.2 de B	24.2 d C	30.0 f C
	'Vernon'	***	95.0 a-c A	43.3 e B	21.7 d C	35.0 f BC
NHB	'Aurora'	***	87.5 b-e A	81.7 a-d AB	64.2 bc B	38.3 ef C
	'Elliott'	*	82.5 c-e A	57.5 c-e B	72.5 a-c AB	70.0 a-c AB
	'Liberty'	**	91.7 a-d A	88.4 ab A	82.5 ab AB	64.2 a-e B
Type			NS	**	***	***
SHB		***	92.0[s] A	81.9 a B	71.3 a C	65.5 a C
RE		***	85.2 A	68.0 b B	50.0 b C	43.8 b C
NHB		***	87.2 A	75.8 ab AB	73.1 a BC	57.5 a C

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=120 (4 reps with 30 individual berry samples/rep).

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=840 (7 cultivars x 4 reps x 30 individual berry samples/rep); RE N=600 (5 cultivars x 4 reps x 30 individual berry samples/rep). NHB N=360 (3 cultivars x 4 reps x 30 individual berry samples/rep).

Table 3.11. Ethylene concentration (nL/g x hr) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated 3-4 days after collection in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar	Ethylene concentration (nL/g x hr) ***[y]	
SHB	'Camellia'	0.051[x]	e[w]
	'Farthing'	0.153	de
	'Keecrisp'	0.722	bc
	'Legacy' GA[v]	0.176	de
	'Legacy' MI	0.031	e
	'Meadowlark'	1.205	a
	'Star'	0.464	c-e
	'Suziblue'	0.540	cd
RE	'Alapaha'	0.441	c-e
	'Austin'	0.711	bc
	'Brightwell'	0.218	de
	'Powderblue'	0.044	e
	'Vernon'	1.030	ab
NHB	'Bluecrop'	0.041	e
	'Draper'	0.125	de
	'Elliott'	0.107	de
	'Liberty'	0.051	e
	'Nelson'	0.107	de
Type		***	
SHB		0.426[u]	a
RE		0.488	a
NHB		0.086	b

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.
[y]Differences examined using Tukey HSD (***=$P \leq 0.001$).
[x]N=4 [4(25.0-26.0g)samples/rep].
[w]Lower case letters compare between cultivars or types within a timepoint.
[v]'Legacy' was collected from GA and MI.
[u]SHB N=32 (8 cultivars x 4 reps); RE N=20 (5 cultivars x 4 reps); NHB N=20 (5 cultivars x 4 reps).

Table 3.12. Ethylene concentration (nL/g x hr) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated 3-4 days after collection in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar	Ethylene concentration (nL/g x hr) ***[y]
SHB	'Abundance'	0.135[x] b[w]
	'Camellia'	0.000 b
	'Farthing'	0.168 b
	'Legacy' GA[v]	0.001 b
	'Legacy' MI	0.023 b
	'Star'	0.072 b
	'Suziblue'	0.132 b
RE	'Alapaha'	0.072 b
	'Brightwell'	0.223 b
	'Powderblue'	0.134 b
	'Premier'	0.633 a
	'Vernon'	0.637 a
NHB	'Aurora'	0.097 b
	'Elliott'	0.003 b
	'Liberty'	0.026 b
Type		***
SHB		0.076[u] b
RE		0.339 a
NHB		0.042 b

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[y]Differences examined using Tukey HSD (***=$P \leq 0.001$).
[x]N=4 [4(25.0-26.0g)samples/rep].
[w]Lower case letters compare between cultivars or types within a timepoint.
[v]'Legacy' was collected from GA and MI.
[u]SHB N=28 (7 cultivars x 4 reps); RE N=20 (5 cultivars x 4 reps); NHB N=12 (3 cultivars x 4 reps).

Table 3.13. Spearman's rank correlation coefficients of physical fruit quality traits between 2018 and 2019 harvest seasons of all collected timepoints[z] and cultivars[y].

Variable	by Variable	Spearman's ρ	*P*-value
'18 Fruit firmness[x]	'19 Fruit firmness	NS	NS[w]
'18 Skin strength (Puncture-in)[v]	'19 Skin strength (Puncture-in)	0.35	***
'18 Skin strength (Puncture-out)[v]	'19 Skin strength (Puncture-out)	0.35	***
'18 Berry weight[u]	'19 Berry weight	0.58	***
'18 percent healthy fruit[t]	'19 percent healthy fruit	0.34	***
'18 Ethylene[s]	'19 Ethylene	0.48	**

[z]Processing occurred at four TPs: 1) 3-4 days, 2) 10-11 days, 3) 20-21 days, and 4) 30-31 days after collection.
[y]12 common cultivars were collected between 2018 and 2019.
[x]N=864 [12 cultivars x [3 reps of 3(50.0-51.0g-berry sample) subreps/rep/TP/year].
[w]Differences between years examined using One-Way ANOVA (NS, **, ***Nonsiginificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P < 0.001$, respectively).
[v]N=3456 (12 cultivars x 3 reps x 12 individual berry subreps/rep/TP/year).
[u]N=384 (12 cultivars x 4 reps of 20-berry samples/rep/TP/year).
[t]N=11520 (12 cultivars x 4 reps with 30 individual berries/rep/TP/year).
[s]N=384 (12 cultivars x 4 reps of 25.0-26.0g-berry samples/rep/TP/year).

Table 3.14. Percent change in fruit firmness [max load (N) Kramer Shear], skin strength [max load (N) puncture-in and puncture-out], berry weight, and percent healthy fruit between TP1[z] and TP4[y] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) commercial blueberry cultivars in fresh postharvest cold storage (4°C) during the 2018[x] harvest season.

		Percent Change from TP1-TP4				
Type	**Cultivar**	**Fruit firmness[w]**	**Skin strength (P-in)[v]**	**Skin strength (P-out)[v]**	**Berry weight[u]**	**percent healthy fruit[t]**
SHB	'Camellia'	30.8***[s]	19.6*	21.4*	-19.0***	-34.6***
	'Farthing'	.	.	.	NS	-12.7*
	'Keecrisp'	14.6***	-8.3*	NS	-24.7**	NS
	'Legacy' GA[r]	30.2***	NS	NS	NS	-12.1**
	'Legacy' MI	21.8***	12.4*	NS	-13.0**	-29.4**
	'Meadowlark'	.	.	.	NS	-11.1*
	'Star'	.	.	.	NS	-30.8***
	'Suziblue'	6.4***	NS	NS	-18.7**	-66.4***
RE	'Alapaha'	20.1***	13.3**	19.7*	NS	-34.2***
	'Austin'	25.6***	NS	NS	-24.2***	-76.5***
	'Brightwell'	.	.	.	-15.6*	.
	'Powderblue'	27.9***	NS	NS	-27.1***	-80.9***
	'Vernon'	32.0***	NS	NS	NS	-27.2**
NHB	'Bluecrop'	-11.5*	NS	-2.9***	-17.6**	-21.6**
	'Draper'	NS	-21.5***	NS	-6.1*	-11.3***
	'Elliott'	31.2***	15.7*	NS	-12.7***	-12.5*
	'Liberty'	44.2***	27.6***	36.1***	-18.0***	-26.8*
	'Nelson'	-12.2***	-15.0***	-22.4***	NS	-44.8***
Type						
SHB		18.4*	NS	8.1*	-11.6*	-24.8***
RE		26.4***	6.4*	10.9**	NS	-45.3***
NHB		NS	NS	NS	NS	-23.0***

[z]TP1=3-4 days after fruit collection.
[y]TP4=30-31 days after fruit collection.
[x]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.
[w]N=18 [3 reps of 3(50.0-51.0g-berry sample) subreps/rep/TP]
[v]N=72 (3 reps x 12 individual berry subreps/rep/TP).
[u]N=8, 4 (20 berry) reps/TP.
[t]N=120, 4 (30 berry)reps/TP.
[s]Differences between timepoints of individual cultivars or tyes examined using One-Way ANOVA (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).
[r]'Legacy' was collected from GA and MI.

Table 3.15. Percent change in fruit firmness [max load (N) Kramer Shear], skin strength [max load (N) puncture-in and puncture-out], berry weight, and percent healthy fruit between TP1[z] and TP4[y] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) commercial blueberry cultivars in fresh postharvest cold storage (4°C) during the 2019[x] harvest season.

		Percent Change from TP1-TP4				
Type	**Cultivar**	**Fruit firmness[w]**	**Skin strength (P-in)[v]**	**Skin strength (P-out)[v]**	**Berry weight[u]**	**percent healthy fruit[t]**
SHB	'Abundance'	27.2***[s]	16.7***	24.2***	NS	-15.0***
	'Camellia'	19.4***	17.4**	NS	NS	-20.9**
	'Farthing'	23.6***	19.8***	18.8**	NS	-27.9***
	'Legacy' GA[r]	31.2***	NS	NS	-13.7*	-55.6***
	'Legacy' MI	9.5**	14.6**	NS	-9.1**	-23.0*
	'Star'	35.1***	21.5*	22.2*	5.3***	-30.6***
	'Suziblue'	29.7***	20.7***	NS	-57.8*	-26.7*
RE	'Alapaha'	15.0***	16.0**	NS	33.1**	NS
	'Brightwell'	14.5***	15.7**	NS	NS	-40.4**
	'Powderblue'	30.7***	NS	NS	-8.6*	-37.9***
	'Premier'	58.1***	40.7***	NS	-19.6**	-66.7***
	'Vernon'	51.4***	37.1***	33.0***	-15.3***	-63.2***
NHB	'Aurora'	39.9***	NS	NS	NS	-56.2***
	'Elliott'	31.5***	38.1***	36.6***	-15.0**	NS
	'Liberty'	28.0***	14.2*	NS	-11.3**	-30.0**
Type						
SHB		24.7***	16.9***	15.8***	-11.5**	-28.8***
RE		30.9***	22.5***	10.7***	NS	-48.5***
NHB		33.2***	17.2***	18.1***	-8.5**	-34.1***

[z]TP1=3-4 days after fruit collection.
[y]TP4=30-31 days after fruit collection.
[x]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[w]N=18 [3 reps with 3 reps of 3(50.0-51.0g-berry sample) subreps/rep/TP]
[v]N=72 (3 reps x 12 individual berry subreps/rep/TP).
[u]N=8, 4 (20-berry) reps/TP.
[t]N=120, 4 (30 individual berries) reps/TP.
[s]Differences between timepoints of individual cultivars or types examined using One-Way ANOVA (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).
[r]'Legacy' was collected from GA and MI.

Table 3.16. Pearson correlation coefficients (r) between fruit firmness [max force (N) Kramer Shear], skin strength [max force (N) puncture-in and puncture-out], berry weight, and ethylene concentration (nL /g x hr) during timepoint 1 (TP1; 3-4 days after collection), and percent healthy fruit during timepoint 4 (TP4; 30-31 days after collection) of southern highbush (SHB), rabbiteye(RE), and northern highbush (NHB) blueberry types during the 2018[z] harvest season.

		Physical fruit quality at TP1				
		Fruit firmness[y]	Puncture-in[x]	Puncture-out[x]	Berry weight[w]	Ethylene concentration[v]
Percent healthy fruit at TP4[u]	**SHB**					
	r[t]	NS	NS	NS	NS	NS
	N	45	180	180	32	32
	RE					
	r	-0.82**	NS	NS	0.84***	0.52*
	N	45	180	180	20	20
	NHB					
	r	NS	NS	0.52*	NS	NS
	N	45	180	180	20	20

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.
[y]N=SHB 8; RE 5; NHB 5 cultivars, with 3 reps x 3(50-51g) subreps/rep for TP1).
[x]N=SHB 8; RE 5; NHB 5 cultivars, with 3 reps x 12 individual berry subreps/rep for TP1).
[w]N=SHB 8; RE 5; NHB 5 cultivars, with 4 reps x 20-berry samples/rep for TP1).
[v]N=SHB 8; RE 5; NHB 5 cultivars, with 4 reps x 25.0-26.0g-berry samples/rep for TP1).
[u]N=SHB 8; RE 5; NHB 5 cultivars, with 4 reps x 30 individual berries/rep for TP4).
[t]Pearson correlation coefficient (r) (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

Table 3.17. Pearson correlation coefficients (r) between fruit firmness [max force (N) Kramer Shear], skin strength [max force (N) puncture-in and puncture-out], berry weight, and ethylene concentration (nL /g x hr) during timepoint 1 (TP1; 3-4 days after collection), and percent healthy fruit during timepoint 4 (TP4; 30-31 days after collection) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry types during the 2019[z] harvest season.

		Physical fruit quality at TP1				
		Fruit firmness[y]	Puncture-in[x]	Puncture-out[x]	Berry weight[w]	Ethylene concentration[v]
Percent healthy fruit at TP4[u]	**SHB**					
	r[t]	NS	NS	NS	NS	NS
	N	63	252	252	38	28
	RE					
	r	NS	NS	NS	-0.44*	-0.68**
	N	45	180	180	20	20
	NHB					
	r	NS	NS	NS	-0.75**	-0.86**
	N	27	108	108	12	12

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[y]N=SHB 7; RE 5; NHB 3 cultivars, with 3 reps x 3(50-51g) subreps/rep for TP1).
[x]N=SHB 7; RE 5; NHB 3 cultivars, with 3 reps x 12 individual berry subreps/rep for TP1).
[w]N=SHB 7; RE 5; NHB 3 cultivars, with 4 reps x 20-berry samples/rep for TP1).
[v]N=SHB 7; RE 5; NHB 3 cultivars, with 4 reps x 25.0-26.0g-berry samples/rep for TP1).
[u]N=SHB 7; RE 5; NHB 3 cultivars, with 4 reps x 30 individual berries/rep for TP4).
[t]Pearson correlation coefficient (r) (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

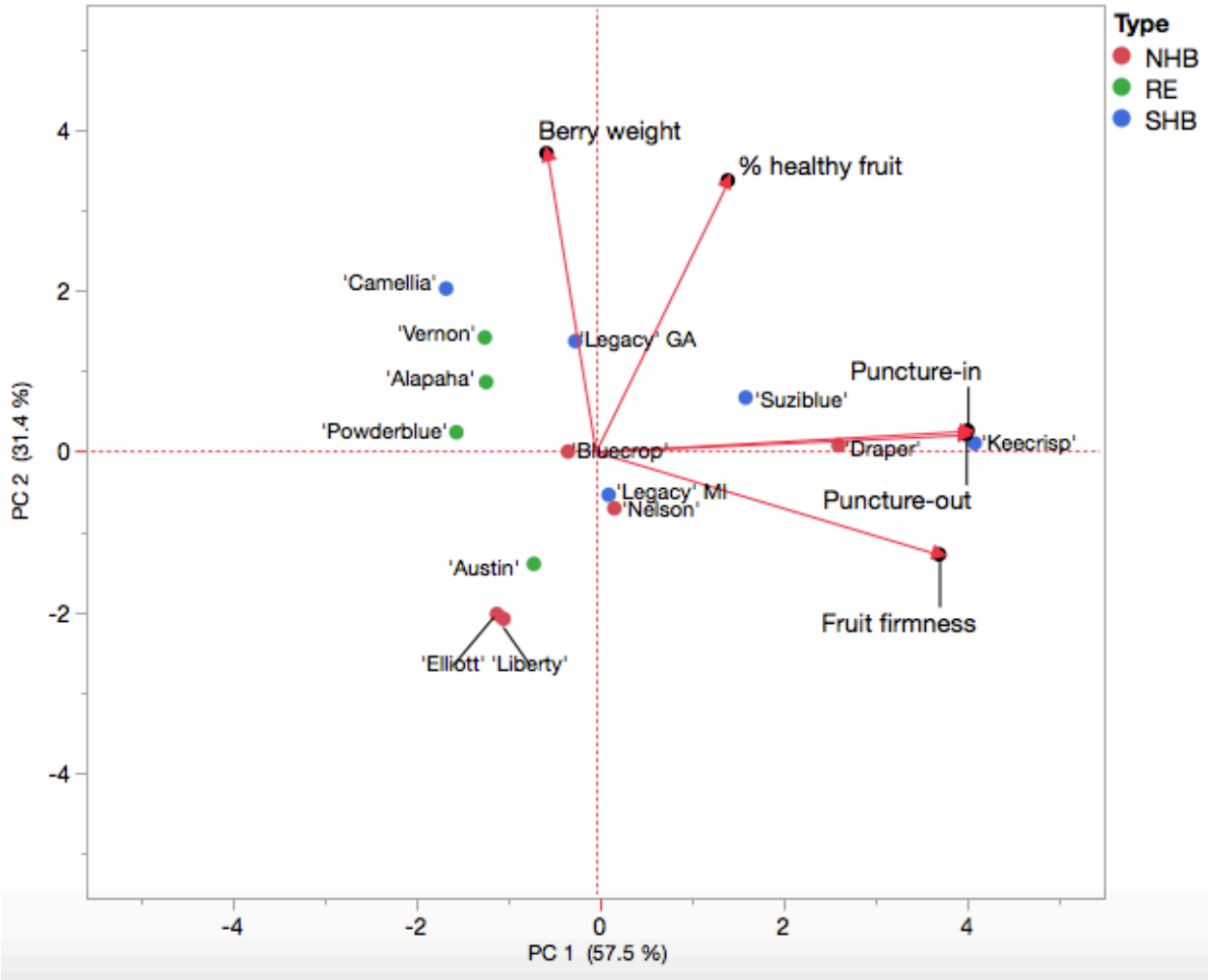

Figure 3.1. Principal component analysis biplot of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 1 (3-4 days after collection) demonstrating variance of physical quality attributes of fruit firmness [max load Kramer Shear(N)], skin strength [max load (N) p-in max and p-out], berry weight, and percent healthy fruit.

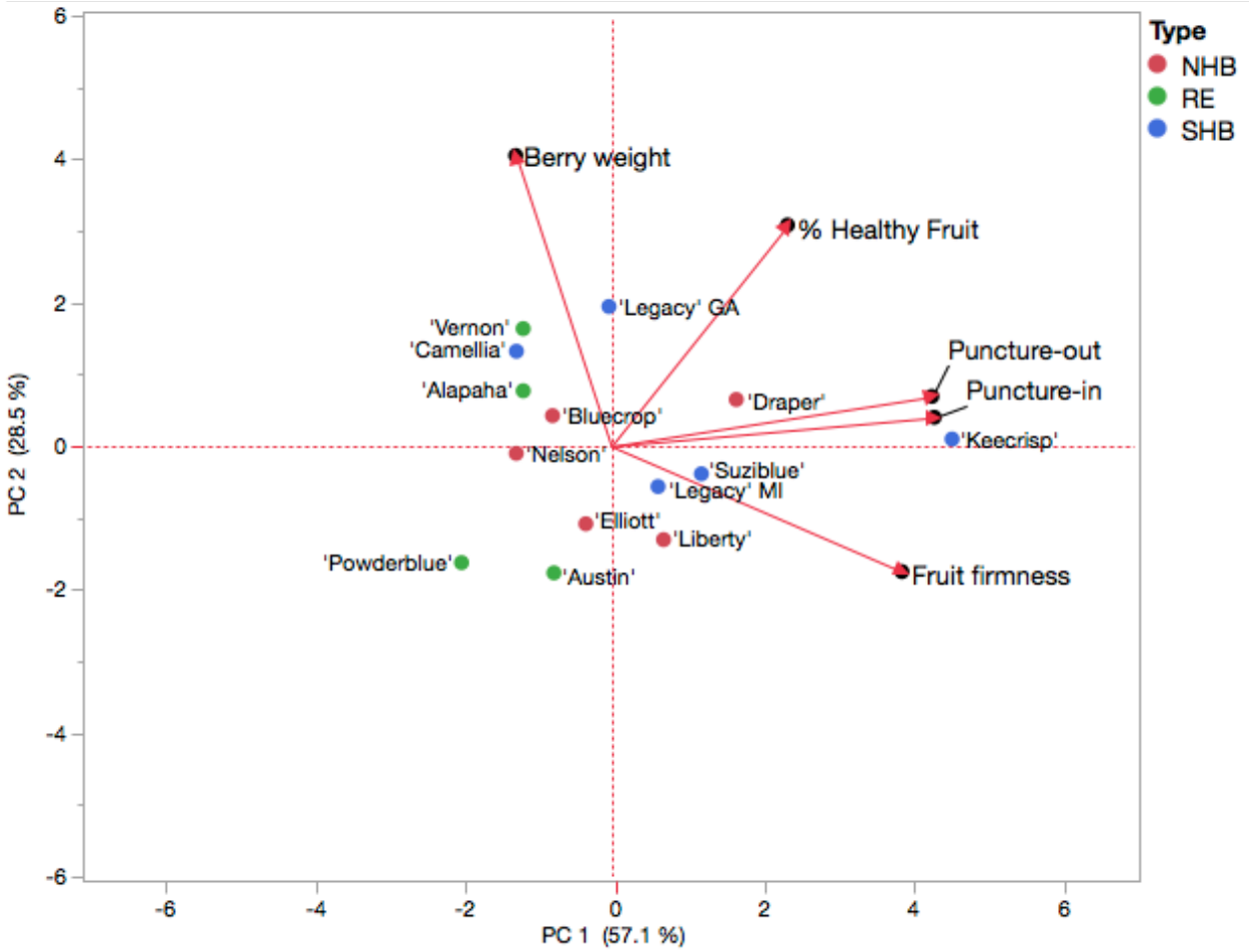

Figure 3.2. Principal component analysis biplot of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 4 (30-31 days after collection) demonstrating variance of physical quality attributes of fruit firmness [max load Kramer Shear(N)], skin strength [max load (N) p-in max and p-out], berry weight, and percent healthy fruit.

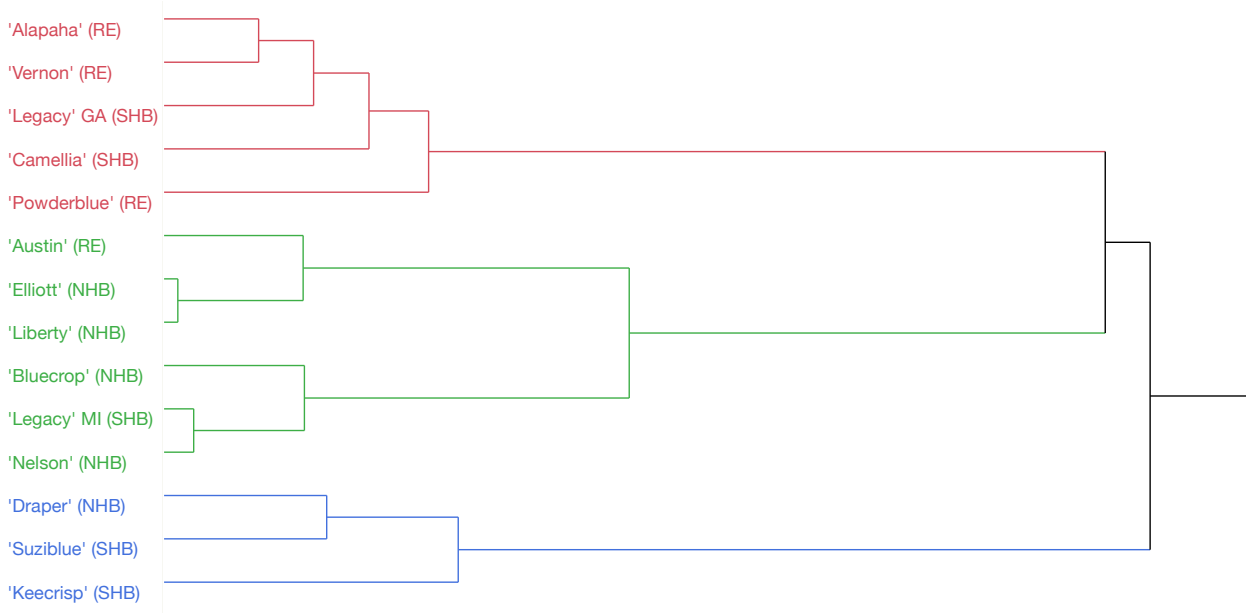

Figure 3.3. Dendrogram from hierarchical cluster analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 1 (3-4 days after collection).

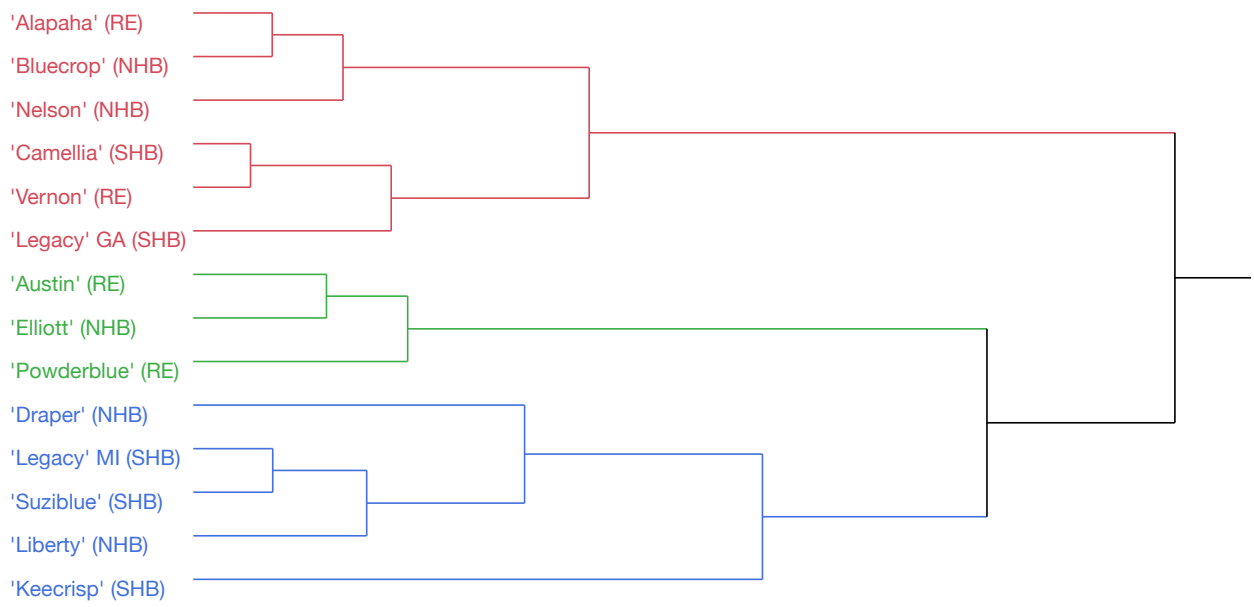

Figure 3.4. Dendrogram from hierarchical cluster analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 4 (30-31 days after collection).

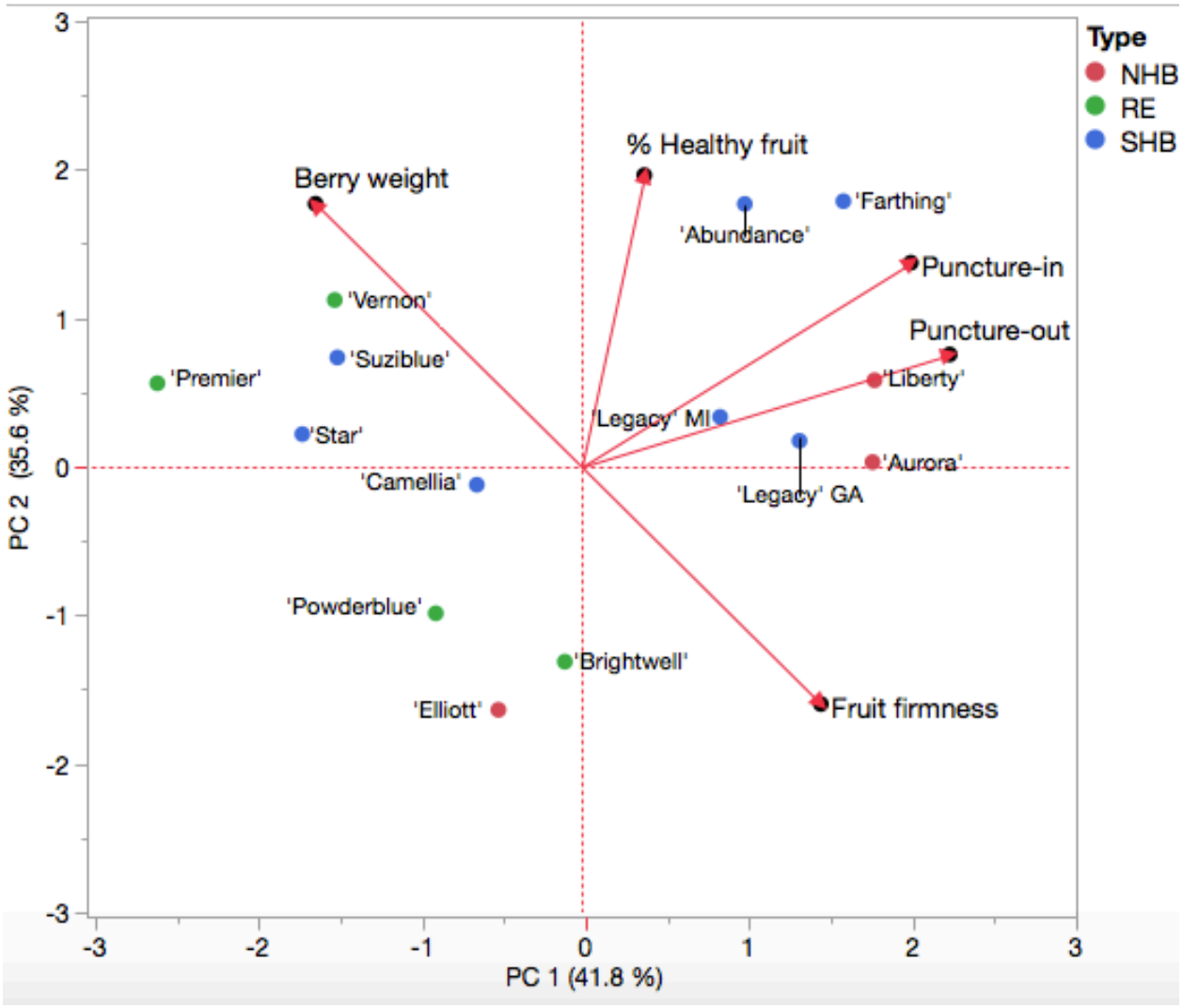

Figure 3.5. Principal component analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 1 (3-4 days after collection) demonstrating variance of physical quality attributes of fruit firmness [max load Kramer Shear(N)], skin strength [max load (N) p-in max and p-out], berry weight, and percent healthy fruit.

Figure 3.6. Principal component analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 4 (30-31 days after collection) demonstrating variance of physical quality attributes of fruit firmness [max load Kramer Shear(N)], skin strength [max load (N) p-in max and p-out], berry weight, and percent healthy fruit.

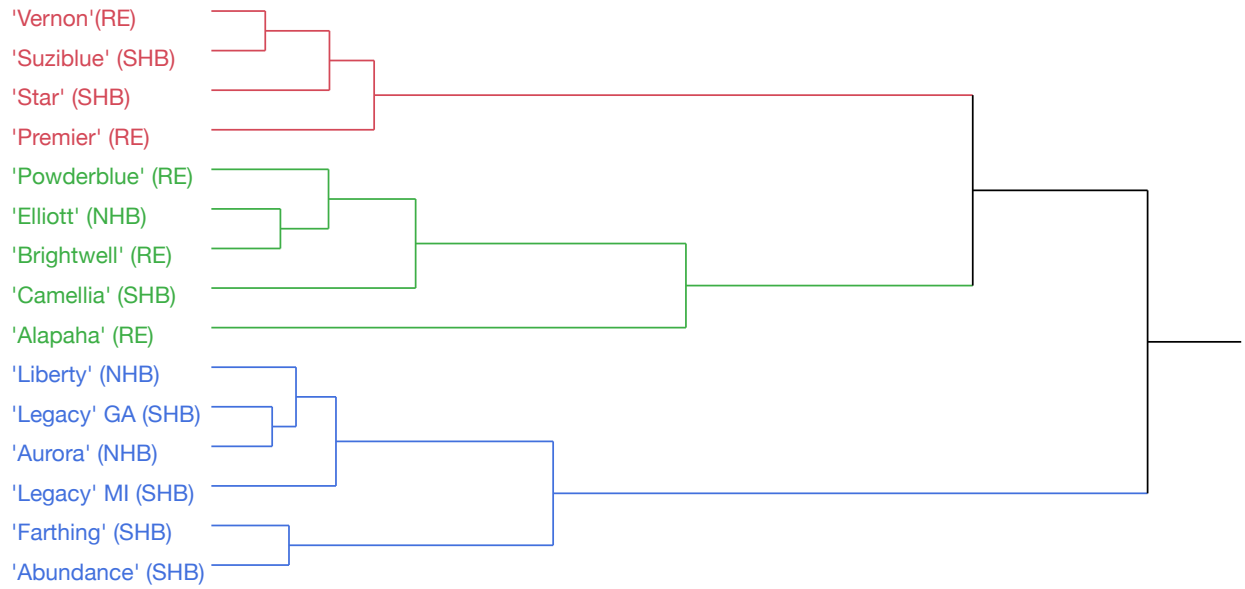

Figure 3.7. Dendrogram from hierarchical cluster analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 1 (3-4 days after collection).

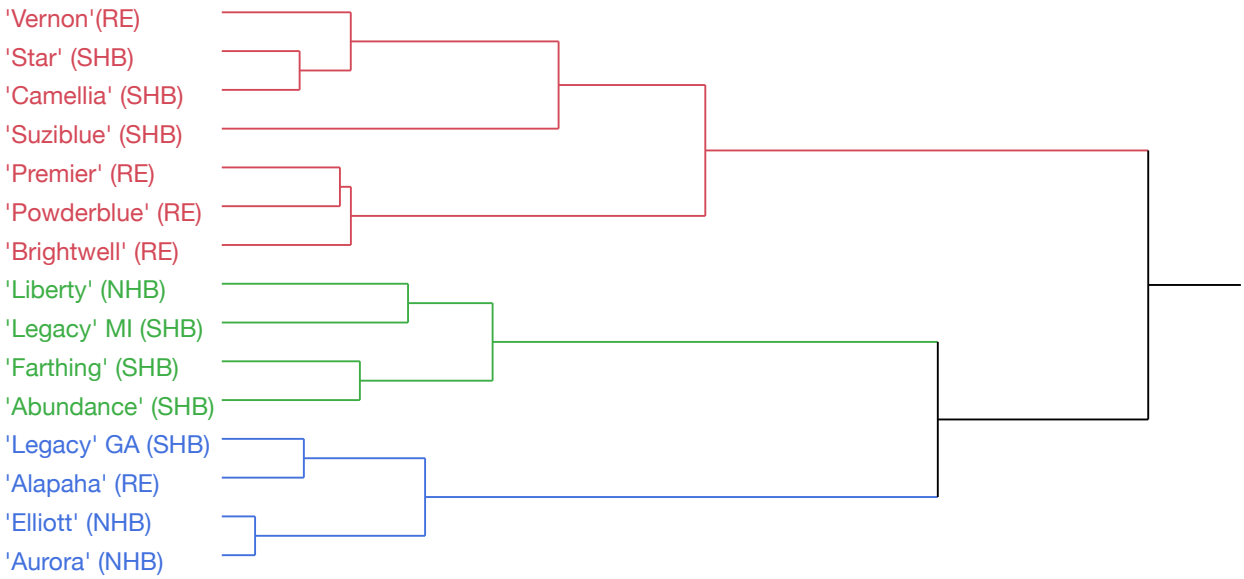

Figure 3.8. Dendrogram from hierarchical cluster analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 4 (30-31 days after collection).

S.1. Harvest and handling types for 2018 and 2019 harvesting seasons. Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia for both 2018 and 2019 seasons, and northern highbush cultivars were collected from commercial packers in Michigan and Canada in the 2018 harvest season, and from Michigan and Indiana in the 2019 harvest season.

		2018			2019		
Cultivar	**Type**	**Commercial hand-harvested**	**Commercial machine-harvested**	**Lab hand-harvested**	**Commercial hand-harvested**	**Commercial machine-harvested**	**Lab hand-harvested**
'Bluecrop'	NHB	X			-	-	-[z]
'Draper'	NHB	X			-	-	-
'Elliott'	NHB	X			X		
'Liberty'	NHB	X			X		
'Nelson '	NHB	X			-	-	-
'Alapaha'	RE			X		X	
'Austin'	RE		X		-	-	-
'Brightwell'	RE		X			X	
'Powderblue'	RE		X			X	
'Vernon'	RE			X		X	
'Abundance'	SHB	-	-	-	X		
'Camellia'	SHB			X		X	
'Farthing'	SHB	X			X		
'Keecrisp '	SHB	X			-	-	-
'Legacy' GA	SHB	X				X	
'Legacy' MI	SHB	X			X		
'Meadowlark'	SHB	X			-	-	-
'Star'	SHB	X				X	
'Suziblue '	SHB	X				X	

[z]Indicates cultivars that were not harvested for harvest season

CHAPTER 4

CHEMICAL POSTHARVEST FRUIT QUALITY CHARACTERISTICS OF SOUTHERN HIGHBUSH, RABBITEYE, AND NORTHERN HIGHBUSH BLUEBERRY CULTIVARS THROUGHOUT COLD STORAGE[1]

[1]Mooneyham, R., S.U. Nambeesan, D.S. NeSmith, R.M. Allen, D.J. Chavez, and R.A. Itle. To be submitted to *HortScience*.

Abstract

In 2019, Georgia ranked second in the U.S. for production value of blueberry and first in the southeastern U.S. Two blueberry types are grown in Georgia: southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton). The fruit quality and postharvest storage ability between these types is debated. These types are also compared to northern highbush (NHB, *Vaccinium corymbosum* L.), which is perceived to have the highest fruit quality in the industry. There is limited information available to fully support this. The objective of this study was to examine the chemical postharvest keeping quality of SHB, RE, and NHB cultivars that are representative of the current blueberry market. Fresh fruit was collected from commercial packers from May to August in the 2018 and 2019 blueberry harvest seasons. Between the two seasons, examined cultivars were: SHB; 'Abundance', 'Camellia', 'Farthing', 'Keecrisp', 'Meadowlark', 'Legacy' from Georgia and Michigan, 'Star', and 'Suziblue', RE; 'Alapaha', 'Austin', 'Brightwell', 'Powderblue', 'Premier', and 'Vernon', and NHB; 'Aurora', 'Bluecrop', 'Draper', 'Elliott', 'Liberty', and 'Nelson'. Fruit were processed at four timepoints (TP) during storage: 1) 3-4, 2) 10-11, 3) 20-21, and 4) 30-31 days after collection. Fruit were processed at four timepoints (TP) during storage: 1) 3-4, 2) 10-11, 3) 20-21, and 4) 30-31 days after collection. Chemical characteristics evaluated included: total soluble solids, total titratable acidity, sugar:acid ratio, total anthocyanins, and percent healthy fruit. The data suggest that no type was superior than the other in maintaining chemical quality characteristics during postharvest commercial cold storage.

Introduction

Blueberry (*Vaccinium* spp.) has experienced considerable growth as a popular fruit crop in many areas of the world. Production worldwide was at approximately 42,000 hectares in 2005 and rose to reaching over 109,000 hectares in 2014 (Brazelton, 2016). In a single year, worldwide blueberry consumption rose by 45 percent from 2015 to 2016 (Freshuelva, 2018). Availability of blueberry has been possible through new cultivar development and consumer interest in the high number of antioxidants that blueberry offers (Joseph et al., 2005; Wu et al., 2004). In the U.S. in 2019, blueberry was the fourth most valuable non-citrus fruit crop with value of utilized production at $909 million, ranking behind strawberry ($2.5 billion), apple ($2.7 billion), and grapes ($5.7 billion) (USDA, 2020). Florida and Georgia make up the majority of blueberry production in the southeastern U.S., producing 24 million and 95 million pounds respectively, making up 18% of the total U.S. production in 2019 (USDA-NASS, 2019). Blueberries comprised 2.24% of Georgia's total farm gate value, ranking 9 out of all commodity crops with $308 million (UGA Center for Agribusiness and Economic Development, 2019). Georgia acreage grew from 4,600 acres in 2000 to 21,000 acres in 2019 (Krewer and NeSmith, 2002; USDA, 2020).

The Georgia blueberry market is made up of southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton). A third major type, northern highbush (NHB, *V. corymbosum* L.) dominates the North American fresh market and are unable to grow in Georgia because of their adaptability to the northern latitudes of the U.S. These three make up the main commercial types of the U.S. market today. The biggest difference between these types is their chilling requirement and tolerance to cold temperatures. northern highbush require between 800 and 1000 chilling hours and can tolerate

temperatures down to -20ºC. Southern highbush and rabbiteye types do tolerate below freezing temperatures well, and require approximately 550 and 600 chilling hours, respectively (Retamales and Hancock, 2018).

Eating quality and taste is an important aspect of blueberry fruit quality, among appearance and fruit texture. Eating quality is also synonymous with consumer acceptability. Fruit quality traits that are of interest to consumers fuel the production and profitability of the blueberry industry (Gilbert et al., 2014). Soluble solids content (SS), total titratable acidity (TTA), and sugar:acid ratio make up the basic indices of taste. Improvement of blueberry fruit quality in the area of shelf-life stability is of high importance with blueberry now entering into a global market and continuing to increase in consumption. Shelf-life quality can heavily influence perceptions of consumers and their purchasing decisions when considering purchasing blueberries. In order to successfully market fresh produce in the U.S., maintaining high quality sensory attributes for an acceptable duration in postharvest storage is a must (Gertmenian, 1992). Blueberries are harvested when ripe, prone to rapid degradation and quality loss. During postharvest storage, fruit acidity decreases and soluble solids increase related to moisture loss (Abugoch et al., 2016; Angeletti et al., 2010; Chiabrando et al., 2009). These changes in chemical fruit quality throughout postharvest certainly influence consumer acceptance and industry standards and expectations of blueberry types.

There are benefits of having both southern highbush and rabbiteye on farms in Georgia. Southern highbush types are early ripening and extend the state's harvest window, which benefits Georgia by keeping it competitive with early producing states like Florida. Market timing and berry size make rabbiteye types useful for more labor efficient machine harvesting when blueberry fruit prices from Georgia are lower, as more states enter into the fresh market.

There is a bias of a type-hierarchy of fruit quality characteristics within the industry. Southern highbush fruit are perceived to have superior fruit quality than that of rabbiteye fruit. This bias exists for both quality at harvest and throughout postharvest storage. Georgia grown fresh market rabbiteye types are sometimes purchased at a lower price point from third party distributers than other types as a result of these biases (R. Itle, personal communication May 2018). However, there is limited information for the industry to support these decisions and sufficiently compare the fruit quality of these three blueberry types. The postharvest quality between the three types is debated as well.

Beyond the debate between southern highbush and rabbiteye, northern highbush fruit quality is perceived to have superior fruit quality than that of southern highbush and rabbiteye. In terms of differences in fruit quality among the three blueberry types of southern highbush, northern highbush, and rabbiteye, not much is understood, and information is limited at best. A better understanding of fruit quality differences between types is beneficial to growers and would allow them and their stakeholders to select cultivars that benefit their production and marketing.

Few studies have simultaneously evaluated the postharvest keeping quality of all three types of blueberry that are most commercially important to the U.S. fresh market – northern highbush, southern highbush, and rabbiteye blueberry types. Studies that address differences between the three do so only sampling a small subset of cultivars from each type. In a comparison between northern highbush and rabbiteye types, rabbiteye generally had higher percentage in soluble solids, pH, and sugar:acid ratio (Makus and Morris, 1993). In a wider comparision between rabbiteye, northern highbush, and southern highbush cultivars Gündüz et al., (2015) found there was significant variability for all traits analyzed among the individual cultivars of all types. Comparing soluble solids, titratable acidity, and pH between southern

highbush and rabbiteye, rabbiteye had significantly higher soluble solids and pH, but lower titratable acidity and fruit weight. In the comparison between the three types, southern highbush were lower in acidity than the northern highbush, with the exception of two cultivars. Southern highbush had sugar/acid ratios higher than all northern highbush, with the exception of one cultivar. Prior et al., (1998) compared anthocyanins between 23 total cultivars of northern and southern highbush, rabbiteye, bilberry (*V. myrtillus* L.), and lowbush blueberry (*V. angustifolium*) types for one year. Thirteen southern highbush and northern highbush cultivars, and four rabbityee cultivars were commercially available; the remaining were not commercially available blueberry types. Between southern highbush, northern highbush, and rabbiteye types, northern highbush types had highest average anthocyanins. Others have found conflicting results that find rabbiteye types to have higher anthocyanin content. Wang et al., (2012) compared anthocyanin content between 36 rabbiteye, three rabbiteye-derivatives, and three northern highbush types, rabbiteye had the highest content of malvidins, followed by rabbiteye-derivatives, and northern highbush. Rabbiteye also had highest levels of petunidins, elphinidins, and cyanidins. In a comparison study between three southern highbush cultivars and two rabbiteye cultivars during one harvest year, rabbiteye had a significantly higher total anthocyanin content compared to the southern highbush cultivars (Magee, 1999).

Fruit acidity has been found to play an important role in the postharvest keeping quality of blueberry. Blueberry spoilage due to fungal growth in postharvest storage may be related to increases in pH values as a result of the formation of nitrogenous compounds and fungal metabolites (Vieira et al., 2016). Smittle and Miller (1988) found a link between high acidity and general defense mechanisms against organismal decay in ‘Woodard’ rabbiteye blueberry. Similar results were found in northern highbush ‘Blueray’ (Loyola et al., 1996). Significantly higher

titratable acidity values in ‘Coville’ northern highbush make it a recommended cultivar for longer postharvest storage life (Galletta et al., 1971). It would be beneficial to investigate the stability of pH and titratable acidity of blueberry types and cultivars without postharvest treatments to identify those that are superior in this area without any additional inputs and handling.

A more robust sample from more southern highbush and rabbiteye cultivars from multiple years would help bring clarity to the fruit quality debate between these types and be useful to growers and stakeholders in Georgia. Growers will be better able to identify unique cultivars that are best suited for prolonged postharvest storage with this information. The objective of this study was to determine differences in postharvest keeping of chemical quality characteristics during commercial cold storage between types and cultivars that make up the current U.S. blueberry fresh market, by collecting a large subset of cultivars from each type.

Materials and Methods

Plant material

Fresh fruit were collected from commercial packers from May to August in 2018 and 2019. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan, Indiana, and Canada. ‘Legacy’ SHB was collected from both Georgia and Michigan for both yea. In 2018, seven southern highbush (‘Camellia’, ‘Farthing’, ‘Keecrisp’, ‘Meadowlark’, ‘Legacy’, ‘Star’, and ‘Suziblue’), five rabbiteye (‘Alapaha’, ‘Austin’, ‘Brightwell’, ‘Powderblue’, and ‘Vernon’) and five northern highbush (‘Bluecrop’, ‘Draper’, ‘Elliott’, ‘Liberty’, and ‘Nelson’), were collected, a total of eighteen cultivars. In 2019, seven southern highbush (‘Abundance’, ‘Camellia’, ‘Farthing’, ‘Meadowlark’, ‘Legacy’, ‘Star’, and

‘Suziblue’), five rabbiteye (‘Alapaha’, ‘Brightwell’, ‘Powderblue’,’Premier’, and ‘Vernon’), and three northern highbush (‘Aurora’, ‘Elliott’, and ‘Liberty’) were collected, a total of fifteen cultivars. The cultivars collected were representative of early, mid, and late season southern highbush and rabbiteye cultivars that make up the Georgia blueberry market, as well as the northern highbush cultivars that make up the larger North American blueberry market. Fruit that was collected went through processes similar to how they would reach the consumer market, and harvest types changed accordingly to market timing and available resources (Table S1). All cultivars were collected during the midpoint of harvest period for each cultivar, and all fruit was obtained within one week after harvest. Commercial packers provided sorted and packed fruit in half-pint clamshells, or otherwise came straight from growers’ fields. If fruit was not previously sorted, the Itle lab hand-sorted berries and placed them in half-pint clam shells. Green or damaged berries were culled during this hand-sorting process. All clam shells were placed into coolers over ice and transported to the UGA Pilot Plant in the Melton Building (Griffin, GA) where they were stored in a commercial walk-in cooler at 4°C. Fruit was collected and stored in this manner to mimic how consumers would receive berries from a grocery store. The manner in which berry samples were received from commercial packers was dependent on growers’ available cultivars, and circumstances surrounding labor availability. Thus, harvest type, cultivars, handling processes, and location were not consistent between both years of data presented here and are suspected to be contributing to the variation in fruit quality traits seen between harvest seasons. A complete list of cultivars and harvest type have been included in the supplementary material (Table S1).

Research design

Seven to eight half-pint clamshells per cultivar were designated for each timepoint in a complete random design in a commercial walk-in cooler. Clamshells were placed in the center shelves of the cooler in order to account for differences in temperature between the wall closest to the cooler fan and the cooler door/entrance. On days of sampling, fruit was randomly sampled from all seven-eight clamshells that were assigned for each timepoint. Sampling of the respective group of clamshells only occurred on days of data collection for the appropriate timepoint in order to minimize handling of fruit. On days of sampling, fresh fruit was brought to room temperature at benchtop for approximately 2 hours before collecting data. Chemical quality traits were evaluated at four timepoints: 1) three to four days, 2) ten to eleven days, 3) twenty to twenty-one, and 4) thirty to thirty-one days after collection (TP1, TP2, TP3, and TP4, respectively).

Chemical Instrumental Measurements

Total soluble solids and total titratable acidity

A purified juice sample was prepared. Four replications of a 55.0-56.0g berry sample were pureed with a Ninja® Ultima Blender (Model BL810 30, Newton, MA) for approximately one minute, until thoroughly homogenized. Homogenate was weighted to approximately 40.0g and poured into clear 50 mL Oak Ridge centrifuge tube (Thermo Scientific Nalgene, Waltham, MA). Samples were centrifuged with the Centrifuge 5810 R (Eppendorf, Hamburg, Germany) for 20 minutes at 12,100 rpm, at 5˚C. The supernatant was filtered through cheesecloth and frozen immediately in 15 mL tubes. Juice tubes were thawed on benchtop at room temperature for approximately one hour, vortexed, and kept on ice. A 300 µL juice sample was placed on a Pal-1 pocket refractometer (Atago, Saitama, Japan) to measure soluble solids expressed as °Brix.

The Easy PRO, Easy plus™ Titrator (Mettler Toledo, Greifensee, Switzerland) with an EG11-BNC sensor pH aqueous was used for auto-titration with 0.1N NaOH titrant. The initial pH, the volume of mL of 0.1N NaOH (VEP), and the %TTA was calculated until an endpoint titration of pH=8.2 using an acid milliequivalent factor of 0.064 (citric acid). For each respective cultivar, two sub-replications for a total of four replications were used for both total soluble solids and total titratable acidity measurements. Sugar:acid ratio was calculated by using °Brix/%TTA.

Total monomeric anthocyanins

A modified protocol from Lee et al., (2005) was used to determine total monomeric anthocyanin content. Purified juice samples were thawed at benchtop for approximately one hour. Total anthocyanin content was determined using a pH differential method as described by Giusti and Wrolstad (2001), with 2 mL of sodium acetate buffer (pH 4.5) and 2 mL potassium chloride buffer (pH 1.0) diluted with 500 µL of purified blueberry juice and equilibrated for approximately 20 minutes before transferring 200 µL of the anthyocyanin sample into a Immulon 96 well plate (Dynatek, Galena, MO). Samples were analyzed on a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT). Absorbance was calculated using the following equation:

Absorbance = (A520nm – A 700nm) pH 1.0 – (A520nm – A700nm) pH 4.5.

Monomeric anthocyanin pigment was determined using the following equation:

Monomeric anthocyanin content = $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$,

where MW (molecular weight)=449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu), DF (dilution factor)=1 cm, and ε (molar absorptivity)=26900 L x mol^{-1} x cm^{-1} for cyd-3-glu. Total anthocyanins were expressed as mg/L cyanidin-3-glucoside equivalents.

Percent healthy fruit

Fruit were examined individually for visual imperfections for each cultivar at each of the four timepoints. Visual imperfections included anything that would make a fruit unmarketable including shriveling, leakiness, cracking at the stem or calyx end, dents, bruising, mold, or torn skin. For each cultivar at each of the four timepoints, four reps of 30 random berries were rated. An indication of shelf-life was determined by totaling visual imperfections and dividing by total berry number and this was expressed as percent healthy fruit.

Data analyses

Chemical quality characteristic data were analyzed using two-way ANOVA with PROC GLM of SAS v.9.4 (SAS Institute, Cary, N.C.). Differences of means were examined 1) between cultivars and types within each timepoint and 2) across all four timepoints for all individual cultivars collected during the 2018 and 2019 harvest seasons using Tukey HSD ($P \leq 0.05$). To better describe the overall change in chemical quality characteristics, percent change from TP1-TP4 of cultivars and types was calculated [(TP4 mean - TP1 mean)/TP1mean x100] and differences between TP's were subjected to One-Way ANOVA ($P \leq 0.05$). To identify if early postharvest fruit quality traits are indicators of percent healthy fruit during late postharvest storage, Pearson product-moment correlation coefficients between fruit quality traits during 3-4 days after collection (TP1, independent variable) and percent healthy fruit 30-31 days after collection (TP4, dependent variable) for all cultivars collected during the 2018 and 2019 harvest seasons were generated using PROC CORR of SAS 9.4. To best explain the variance of chemical quality characteristics during fresh postharvest storage as a whole, a multivariate approach of correlation matrix calculation and principal component analysis (PCA) was conducted in JMP v.14 (SAS Institute, Cary, N.C.) to assess chemical quality characteristics of total soluble solids, total titratable acidity, and percent healthy fruit during TP1 and TP4,

separately. Hierarchical cluster analysis was conducted in JMP v.14 for TP1 and TP4 using Ward's linkage on principal component 1 and principal component 2 score values to determine relatedness among types based on chemical quality characteristics. To assess year to year variation and if cultivars ranked similarly for both years according to chemical quality characteristics, the PROC CORR model of SAS 9.4 was used to generate spearman's rank correlation coefficients. The PROC GLM model of SAS 9.4 was used to determine differences of means of chemical quality characteristics between years of subsequent timepoints using One-Way ANOVA ($P \leq 0.05$).

Results and Discussion

Total soluble solids

There were significant differences ($P \leq 0.05$) in total soluble solids between types and cultivars within each of the four TPs of postharvest cold storage for both 2018 and 2019 harvest seasons (Table 4.1 and Table 4.2). Within all timepoints, rabbiteye types showed to have the lowest total soluble solids compared to southern highbush and northern highbush types in 2018. Southern highbush types were significantly higher than northern highbush types with the exception of TP1 and TP4. However, in the 2019 season rabbiteye types' total soluble solids were not significantly different from southern highbush types. Both rabbiteye and southern highbush maintained highest total soluble solids values throughout postharvest cold storage during 2019. Across TP comparisons also showed there were significant differences ($P \leq 0.05$) in total soluble solids between the four timepoints of postharvest cold storage for individual cultivars, but not for types. Percent change from TP1 to TP4 during the 2018 harvest season (Table 4.10) showed that very few cultivars from each blueberry type had overall significant changes in total soluble solids. This information was contrary to other studies stating that

rabbiteye has significantly higher soluble solids than southern highbush or northern highbush types.

All changes that occurred were positive increases in total soluble solids. Within southern highbush, only ‘Camellia’ and ‘Keecrisp’ had significant increases of 4.3 and 3.6%, respectfully. Within rabbiteye, ‘Powderblue’ had a relatively high percent increase of 8.5%, comparable to northern highbush type ‘Elliott’ of 8.8%. The 2019 harvest season presented different results with cultivars both significantly increasing and decreasing in total soluble solids from TP1-TP4 (Table 4.11). Cultivars ranged from decreasing by 21.8% (rabbiteye type ‘Premier’) to increasing by 11.7% (southern highbush type ‘Farthing’). Both southern highbush and rabbiteye types had cultivars that both significantly increased and decreased in total soluble solids during the 2019 harvest season, however within northern highbush types, only a single cultivar ‘Aurora’ had a significant increase of 5.9%. Given that the changes in total soluble solids for each harvest season seemed to differ, and the existing variation in rankings between types within each TP, these results suggest that environmental factors may play a more predominant role in changes of total soluble solids during postharvest cold storage rather than genotypic differences. Because there were no significant differences across timepoints when comparing by type for either harvest season, the data do not suggest that any one type was superior at maintaining total soluble solids during postharvest cold storage. It would be most beneficial to growers to select those individual cultivars that maintain their quality throughout postharvest storage, such as ‘Legacy’, ‘Elliott’, and ‘Liberty’, which maintained soluble solids throughout postharvest for both years.

Total titratable acidity

There were significant differences ($P \leq 0.05$) in total titratable acidity between types and cultivars within each of the four TPs of postharvest cold storage during 2018 and 2019 harvest seasons (Table 4.3 and Table 4.4). Type comparisons showed that northern highbush types consistently had significantly higher values of total titratable acidity during all timepoints compared to rabbiteye and southern highbush types, for both harvest seasons. During the 2018 harvest season, rabbiteye types had significantly lowest total titratable acidity values during TP1and TP2 but was not significantly different from southern highbush during TP3 and TP4. The 2019 harvest season showed that rabbiteye types did not significantly differ in total titratable acidity to southern highbush types for all timepoints. Across timepoint comparisons showed that there were significant differences ($P \leq 0.05$) between timepoints for individual cultivars. During both harvest seasons, southern highbush types' total titratable acidity values significantly changed from timepoint to timepoint, but there were no significant changes in total titratable acidity for rabbiteye and northern highbush types throughout the four timepoints. Percent change from TP1-TP4 (Table 4.10 and Table 4.11) showed that many cultivars decreased in total titratable acidity throughout postharvest cold storage, all except rabbiteye types 'Austin' and 'Brightwell' and northern highbush type 'Nelson' which saw significant increases during the 2018 harvest season. This has contributed to northern highbush and rabbiteye to not show significant changes in total titratable acidity from TP1-TP4 on the type comparison level. Evaluation by type showed that southern highbush was the only type to have significant overall changes in titratable acidity from TP1-TP4 in 2018. Southern highbush cultivars 'Legacy' MI and 'Star', and rabbiteye cultivars 'Alapaha' and 'Vernon' had significant decreases in total titratable acidity from TP1-TP4 during the 2018 harvest season but had no significant changes

during the 2019 harvest season. Rabbiteye cultivar 'Brightwell' saw an overall increase in total titratable acidity by 13.2 percent from TP1-TP4 in the 2018 harvest season, however decreased by 10.7% in the 2019 harvest season. These results suggest that regardless of type, the majority of blueberry cultivars decrease in total titratable acidity during postharvest cold storage. Also, because there are instances of cultivars both increasing and decreasing in total titratable acidity during postharvest cold storage, it was unexpected and may be affected by variables not considered in this experiment. The data also suggest that the stability of total titratable acidity may be best in rabbiteye and northern highbush types since there were no significant changes in these types for both harvest seasons. This also suggests that these types may also be better suited for defense against organismal and fungal decay, as increases in acidity during storage may result in higher spoilage rates (Galletta et al., 1971; Loyola et al., 1996; Smittle and Miller 1988). In order to verify this, it might be necessary to collect mold and yeast counts expressed in log colony forming units (CFU) on these same cultivars as have Abugoch et al. (2015) have done.

Sugar:acid ratio

Because significant changes in total titratable acidity and total soluble solids throughout postharvest cold storage were observed, this also affected sugar:acid ratios. There were significant differences ($P \leq 0.05$) between individual cultivars and types within all four timepoints throughout postharvest cold storage. Rabbiteye and southern highbush types had significantly higher sugar:acid ratio values compared to that of northern highbush types within all four timepoints for both years (Table 4.5 and Table 4.6). In the 2018 harvest season, southern highbush types had significantly higher sugar:acid ratios than rabbiteye types with the exception of TP1. The 2019 harvest season showed there were no significant differences between rabbiteye and southern highbush sugar:acid ratio values within all four timepoints. This was apparent

based on observations of high total soluble solid values of rabbiteye and southern highbush types, while total titratable acidity remained highest in northern highbush types during the 2019 harvest season. Across timepoint comparisons among types showed that only southern highbush types had significant changes in sugar:acid values from timepoint to timepoint, while rabbiteye and northern highbush types did not see any significant changes. This was true for both harvest seasons. This may suggest that rabbiteye and northern highbush types may maintain sugar:acid ratios better than that of southern highbush types, most likely as a direct result of significant changes in total titratable acidity values among southern highbush types throughout postharvest cold storage. These findings corroborate with other studies that found southern highbush to have higher sugar:acid ratios than northern highbush types.

Total anthocyanins

There were significant differences ($P \leq 0.05$) in total monomeric anthocyanins between cultivars within each of the four TPs of postharvest cold storage during 2018 and 2019 harvest seasons (Table 4.7 and Table 4.8). Type comparisons in 2018 showed that northern highbush types maintained the highest total monomeric anthocyanin content from timepoints 1-3, followed by southern highbush, and rabbiteye. By timepoint 4, southern highbush types had the highest total monomeric anthocyanins, however there was no significant change in across four timepoints individually for any of the blueberry types. Very few cultivars showed changes in anthocyanin concentration throughout postharvest storage, with most having only one letter grouping change at a singular timepoint. The 2019 type comparisons showed that there were no significant differences in total monomeric anthocyanins at any of the four postharvest timepoints. Percent change of total monomeric anthocyanins during 2018 (Table 4.10) showed mostly decreases in concentrations, as seen in rabbiteye types ‘Austin’, and northern highbush type

‘Liberty’. In the 2019 harvest season, ‘Liberty’ was the only cultivar, and northern highbush type to have a large percent change in total monomeric anthocyanin content (Table 4.11). For both harvest seasons, ‘Liberty’ anthocyanin concentration changed with a 60% decrease in 2018 and nearly 50% decrease in 2019. Both large changes occurred between timepoints three and four. Overall, these data suggest that total monomeric anthocyanin concentration during postharvest storage does not change as much as other fruit quality traits such as total titratable acidity and total soluble solids, and may be more stable. These findings do not corroborate with earlier studies by Prior et al. (1998) that found northern highbush highbush types to have higher anthocyanin concentrations than that of southern highbush or rabbiteye types. This also goes against the findings of Lohachoompol et al. (2008) stating that rabbiteye types have higher total anthocyanin content than southern highbush and northern highbush types at harvest.

Seasonal variation

Spearman's rank correlation coefficients showed that chemical quality characteristics had low correlations for cultivar ranking, and even negative rankings in total soluble solids (Table 4.9). This would corroborate with the observation of variation in ranking of types’ total titratable acidity levels and total soluble solids within individual timepoints between the 2018 and 2019 harvest seasons. The study presented here demonstrates that seasonal variation may have played a significant role in the postharvest keeping quality of chemical traits in all blueberry types. Several cultivars saw overall increases one year, yet decreases in the other. This also addresses the claim that northern highbush or southern highbush types possess superiority to rabbiteye in stability of chemical fruit quality in postharvest storage may not be substantiated. Increased soluble solids content and total titratable acidity during postharvest cold storage has been repeatedly demonstrated (Abugoch et al., 2016; Chiabrando et al., 2009) and could be related to

moisture loss/ fruit dehydration (Chiabrando and Giacalone 2011). Decreased fruit acidity paralleled with increase of soluble solids of cultivars has also been reported by Angeletti et al. (2010) and Chiabrando et al. (2009), as seen in cultivars in this study. When it comes to relationships between shelf-life and fruit acidity and soluble solids, Galletta et al. (1971) suggested that the significantly higher titratable acidity values in 'Coville' northern highbush blueberry make it a recommended cultivar for longer postharvest storage life. However, the only correlations between fruit acidity and percent healthy fruit were only observed in rabbiteye fruit, which was a moderate, negative relationship (Tables 4.12 and Tables 4.13).

A higher number of southern highbush cultivars collected from the 2019 harvest season were mechanically harvested during than the previous year. It was difficult to determine if harvest method was a direct result of the differences observed in postharvest keeping quality of acidity and sugars in storage. Few studies have investigated the effect of mechanical harvesting on the chemical postharvest keeping quality of blueberries. A study in the response of lowbush blueberry quality parameters to mechanical harvesting showed that titratable acidity increased with higher impact damage, but it was not certain if fruit moisture content had a direct effect (Sanford et al., 1991). A study by Sargent et al. (2013) demonstrated that total soluble content, total titratable acidity, and pH remained unchanged between hand and mechanical harvesting methods. Of the literature investigated detailing mechanical vs. hand harvesting methods, none have considered fruit moisture in the evaluation to the knowledge of the author. It may provide useful for future studies to expand on the effects of harvest type, and to collect fruit moisture by drying small samples of fruit in vacuum ovens at subsequent timepoints to confirm relationships between chemical quality traits, moisture, and percent healthy fruit.

Pearson correlation coefficients

Pearson correlation coefficients showed very little strong relationships between TP1 chemical quality traits and TP4 percent healthy fruit (Table 4.12 and 4.13). There were moderate, negative correlation correlations between TP1 total titratable acidity and TP4 percent healthy fruit only within RE types for both harvest seasons (r= –0.46). This may suggest that specifically to rabbiteye types, cultivars that possess relatively lower total titratable acidity values may be more susceptible to instances of high levels of visual imperfections and physical damage. The extremely poor percent healthy fruit of outlier 'Brightwell' may be contributing to this significant correlation. During the 2018 harvest season, there were conflicting correlations between total monomeric anthocyanin concentration at TP1 and percent healthy fruit at TP4. There was a negative, moderate correlation for southern highbush types (r= –0.46), but a positive, moderate correlation for rabbiteye types (r= 0.66). Inconsistencies in correlations between the two harvest seasons suggest that early chemical postharvest fruit quality traits may not be reliable indices for postharvest keeping quality of visual appearance or shelf-life of blueberry.

Principal component analyses

The first two of the four principal components (PCs) during TP1 of the 2018 harvest season have eigenvalues greater than 1.0 (1.99, 1.46) and accounted for about 69% (39.8% and 29.2%) of the total variance of chemical quality characteristics. A threshold of ±0.5 for eigenvector value was used to determine which parameters contributed to PC's. PC1 had a high association with total titratable acidity, and sugar:acid ratio. PC2 showed high associations to both total soluble solids and total monomeric anthocyanins (Figure 4.1). Eigenvalues remained relatively unchanged in TP4. PCA analysis showed that there was wide variation in both PCs,

and there were discernable groupings of types (Figure 4.2). Rabbiteye types were most negatively associated with total soluble solids and percent healthy fruit for both timepoints, while many southern highbush and northern highbush types were higher in percent healthy fruit and total soluble solids. The variation in both PCs seemed to be higher in southern highbush and northern highbush types as well, as opposed to rabbiteye types. There are clear outliers from all types in early storage, such as northern highbush types 'Elliott' and 'Draper', southern highbush type 'Keecrisp', and rabbiteye type 'Brightwell'. Overall, these outliers as well as other cultivars maintained their positions within the same quadrants from TP1-TP4 and suggests that these would be most suitable for maintaining chemical traits during postharvest cold storage.

During the 2019 harvest season, the first two of the four principal components during TP1 showed to have eigen values greater than 1.0 (2.44, 1.31) and accounted for about 75.2% (48.9% and 26.2%) of the total variance of chemical quality characteristics (Figure 4.5). PC1 was highly associated to total titratable acidity and sugar:acid ratio. PC2 was highly associated with percent healthy fruit and total monomeric anthocyanin concentration. This can be interpreted as PC1 being the chemical component, while PC2 makes up the shelf-life component. Eigenvector values were relatively unchanged in TP4 (Figure 4.6). The 2018 harvest season did not see this same change in eigen vectors from TP1 to TP4, and also did not see negative correlations between percent healthy fruit and anthocyanin concentration in TP4. In 2018, there is a clear distinction that northern highbush types clustered around quadrants 2 and 3, having higher association with total titratable acidity, while southern highbush types clustered in quadrants 1 and 4, with higher association with total soluble solids. In 2019 northern highbush types congregated in quadrants 1 and 4. There is also a very wide variation in PC1 for rabbiteye types in 2019. These differences became more pronounced in TP4, as northern highbush types

remained in quadrant three, and southern highbush moving towards quadrants one and four. In 2019, by TP4, those cultivars that had the highest percent healthy fruit were strongly, negatively associated with total monomeric anthocyanin values, namely cultivars such as 'Abundance', 'Legacy' MI, and 'Star', and 'Suziblue'. This was somewhat different from the 2018 harvest season, which showed that cultivars that performed well in percent healthy fruit also had high values of total soluble solids and total monomeric anthocyanins.

Hierarchical cluster analyses

PC1 and PC2 scores were used to generate hierarchical cluster analysis with Ward's method into dendrograms for TP1 and TP4 respectively to further simplify differences among and across types (Figure 4.3 and 4.4, 4.7 and 4.8). During the 2018 harvest season, four clusters were formed for both timepoints. Clusters 4 during TP1 included outlying cultivars, such as 'Austin', 'Keecrisp', and 'Legacy' MI. By the end of the postharvest cold storage period during 2018, the distance between individuals in clusters had changed slightly. Also, by TP4 most rabbiteye types fell into clusters 3 and 4, which negatively associated with percent healthy fruit and total soluble solids and confined in quadrants three and four (Figure 4.2). Although Pearson's correlation coefficients only show negative relationships between total titratable acidity values and percent healthy fruit in rabbiteye types, cultivars that maintained their positions in quadrants 1 and 2 with positive values for both PC1 and PC2 could have other chemical characteristics not measured in this study that contribute to relatively higher percent healthy fruit. A clear outlier, 'Keecrisp', is the only cultivar to have high association with total soluble solids and percent healthy fruit, so it is inconclusive to state that any chemical quality characterizes measured here contributed to superior percent healthy fruit or shelf-life.

During the 2019 harvest season, five clusters were formed for both timepoints (Figures 4.7 and 4.8). There were no clear groupings by type or discernable clusters during this year. During TP1, cluster 2 containing 'Abundance', 'Legacy' GA, 'Premier', 'Star', and 'Vernon' were within quadrant one of the PCA, being highly associated with higher total anthocyanins and percent healthy fruit. These cultivars did not stay in the same cluster/ quadrant however, as total monomeric anthocyanin concentration and percent healthy fruit became negatively associated in TP4, which only occurred in the 2019 harvest season. By TP4, many cultivars clustered together in cluster 5, becoming more similar to one another in chemical fruit quality late in postharvest storage. This may be because of changes within individual sugar and/or acid composition throughout postharvest storage not measured in this study. Further investigation into the details of the sugar, acid, and anthocyanin profiles of these cultivars may lend to better understanding of chemical quality characteristics and their relation to postharvest shelf-life.

Conclusion

Data suggest that there were no significant differences between types for total soluble solids, but northern highbush and rabbiteye had better stability of total titratable acidity throughout postharvest cold storage. It would be useful to determine the differences in other traits among southern highbush, northern highbush, and rabbiteye blueberry types to further understand structural differences to determine blueberry types best suited for postharvest storage. Sugar and acid profiles, along with total antioxidants could reveal individual sugar and/or acid components that lend to superior postharvest keeping quality. Correlations between percent healthy fruit and antioxidant capacity would also be useful in determining cultivars and types that contain more nutraceuticals, but those high in antioxidants may lend to superior postharvest keeping quality as well. Overall, these findings would allow growers to choose individual

cultivars and types that maintain their total soluble solids and total titratable acidity throughout postharvest cold storage.

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Table 4.1. Total soluble solids (°Brix) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars individually across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Camellia'	***[x]	14.5[w] c-f[v] B[u]	15.0 bc A	15.2 b-d A	15.1 cd A
	'Farthing'	***	13.0 gh B	14.9 bc A	13.6 f-h AB	12.1 hi B
	'Keecrisp'	**	18.7 a B	19.3 a AB	19.7 a A	19.4 a AB
	'Legacy' GA[t]	NS	12.3 hi	12.4 gh	12.5 h	12.4 hi
	'Legacy' MI	NS	15.0 b-d	14.7 bc	14.5 df	15.0 c-e
	'Meadowlark'	***	12.2 hi B	14.1 c-e A	13.5 f-h A	12.5 hi B
	'Star'	**	14.0 d-g B	14.8 bc A	14.5 d-f AB	13.8 fg B
	'Suziblue'	*	13.6 e-h A	14.5 cd A	14.8 c-e A	13.7 fg A
RE	'Alapaha'	NS	12.7 gh	12.5 gh	13.0 gh	13.0 gh
	'Austin'	**	14.6 b-e B	14.5 cd B	15.6 b-d A	14.4 d-f B
	'Brightwell'	***	11.3 ij B	12.2 h A	10.9 i B	11.4 ij B
	'Powderblue'	***	12.2 hi B	13.3 e-g A	12.9 gh AB	13.3 f-h A
	'Vernon'	NS	10.1 j	11.0 i	10.6 i	10.8 j
NHB	'Bluecrop'	NS	14.4 c-f	13.6 d-f	13.5 f-h	14.3 d-f
	'Draper'	NS	15.9 b	15.7 b	16.0 b	15.9 bc
	'Elliott'	NS	12.7 gh	12.9 f-h	13.2 gh	13.8 e-g
	'Liberty'	NS	15.7 bc	15.8 b	15.7 bc	16.3 b
	'Nelson'	**	13.2 f-h B	14.1 c-e A	14.0 e-g A	13.8 fg AB
Type			***	***	***	***
SHB		NS	14.2[s] a	15.0 a	14.8 a	14.3 b
RE		NS	12.2 b	12.7 c	12.6 c	12.5 c
NHB		NS	14.4 a	14.4 b	14.5 b	14.8 a

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(300µL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=64 [8 cultivars x 4 reps with 2(300µL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(300µL of juice) subreps/rep]; NHB N=40 [5 cultivars x 4 reps with 2(300µL of juice) subreps/rep].

Table 4.2. Total soluble solids (°Brix) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Abundance'	***	14.5[w] b-e[v]	BC[u]	15.7 a	A	15.2 bc	AB	14.0 de	C
	'Camellia'	*	13.6 c-f	B	14.6 bc	AB	15.5 b	A	14.1 d	AB
	'Farthing'	***	13.5 c-f	B	14.3 cd	AB	15.2 bc	A	15.1 bc	A
	'Legacy' GA[t]	NS	16.8 ag		16.0 a		15.7 b		15.4 b	
	'Legacy' MI	**	12.9 ef	B	12.9 fg	B	13.3 e-g	AB	13.7 de	A
	'Star'	**	15.8 a-c	A	14.5 b-d	B	14.3 c-e	B	14.4 cd	B
	'Suziblue'	***	12.0 f	AB	12.6 f-h	A	12.6 gh	A	11.6 g	B
RE	'Alapaha'	***	17.4 a	A	16.0 a	B	17.3 a	A	17.2 a	A
	'Brightwell'	*	13.6 c-f	B	14.2 c-e	A	14.0 d-f	AB	14.2 d	AB
	'Powderblue'	***	13.4 def	C	15.3 ab	A	14.6 b-d	AB	14.4 cd	B
	'Premier'	***	16.1 ab	A	13.2 ef	B	12.6 gh	B	12.6 f	B
	'Vernon'	***	15.3 a-d	A	13.5 d-f	B	13.0 f-h	B	13.7 de	B
NHB	'Aurora'	**	11.8 f	B	11.5 h	B	12.1 h	AB	12.5 f	A
	'Elliott'	NS	12.2 f		11.9 gh		12.3 gh		12.4 fg	
	'Liberty'	NS	13.3 d-f		13.1 f		12.7 gh		13.2 ef	
Type			***		***		***		***	
SHB		NS	14.2[s] b		14.4 a		14.5 a		14.0 a	
RE		NS	15.2 a		14.5 a		14.3 a		14.4 a	
NHB		NS	12.4 c		12.2 b		12.4 b		12.7 b	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(300µL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=56 [7 cultivars x 4 reps with 2(300µL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(300µL of juice) subreps/rep]; NHB N=24 [3 cultivars x 4 reps with 2(300µL of juice) subreps/rep].

Table 4.3. Total titratable acidity (%TTA) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Camellia'	*	0.649[w] de[v]	AD[u]	0.589 d	AB	0.569 cd	B	0.598 ef	AB
	'Farthing'	***	0.483 fg	BC	0.546 de	A	0.531 c-e	AB	0.456 g-i	C
	'Keecrisp'	***	0.524 f	A	0.375 i	B	0.373 f	B	0.350 j	B
	'Legacy' GA[t]	NS	0.545 f		0.525 d-f		0.478 d-f		0.509 fg	
	'Legacy' MI	**	0.383 h	AB	0.368 i	B	0.424 ef	A	0.406 h-j	AB
	'Meadowlark'	***	0.486 fg	A	0.476 e-h	A	0.386 f	B	0.333 j	C
	'Star'	*	0.754 c	A	0.746 c	AB	0.724 b	AB	0.669 de	B
	'Suziblue'	***	0.505 fg	A	0.436 g-i	B	0.398 f	C	0.401 h-j	BC
RE	'Alapaha'	*	0.416 gh	AB	0.419 hi	A	0.403 f	AB	0.370 ij	B
	'Austin'	**	0.368 h	B	0.396 hi	AB	0.408 f	A	0.409 h-j	A
	'Brightwell'	**	0.551 f	B	0.573 d	B	0.586 c	AB	0.624 de	A
	'Powderblue'	**	0.479 fg	B	0.531 de	A	0.471 d-f	B	0.483 gh	B
	'Vernon'	***	0.566 ef	A	0.505 d-g	B	0.566 cd	A	0.521 fg	B
NHB	'Bluecrop'	*	0.708 cd	AB	0.692 c	B	0.794 b	A	0.704 cd	AB
	'Draper'	*	1.045 b	AB	1.094 a	A	1.070 a	AB	0.987 b	B
	'Elliott'	***	1.150 a	AB	1.001 b	B	1.065 a	B	1.282 a	A
	'Liberty'	***	0.535 f	A	0.445 f-i	B	0.449 ef	B	0.451 g-i	B
	'Nelson'	***	0.667 cd	C	0.739 c	B	0.813 b	A	0.788 c	AB
Type			***		***		***		***	
SHB		**	0.541[s] b	A	0.508 b	AB	0.485 b	AB	0.465 b	B
RE		NS	0.476 c		0.485 c		0.487 b		0.481 b	
NHB		NS	0.821 a		0.794 a		0.838 a		0.842 a	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(6 mL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=64 [8 cultivars x 4 reps with 2(6 mL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(6 mL of juice) subreps/rep]; NHB N=40 [5 cultivars x 4 reps with 2(6 mL of juice) subreps/rep].

Table 4.4. Total titratable acidity (%TTA) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***	0.380[w] hi[v] A[u]	0.281 h C	0.315 gh BC	0.342 bc AB
	'Camellia'	*	0.633 cd A	0.516 d B	0.573 d AB	0.569 a-c AB
	'Farthing'	***	0.531 ef A	0.375 fg C	0.425 ef BC	0.449 a-c B
	'Legacy' GA[t]	***	0.604 de A	0.475 de B	0.454 ef B	0.588 a-c A
	'Legacy' MI	NS	0.456 fgh	0.400 ef	0.439 ef	0.380 a-c
	'Star'	***	0.376 hi A	0.294 gh B	0.329 gh B	0.390 bc A
	'Suziblue'	***	0.434 fgh A	0.352 f-h B	0.281 h C	0.302 bc BC
RE	'Alapaha'	NS	0.280 i	0.280 h	0.271 h	0.268 c
	'Brightwell'	**	0.401 gh A	0.372 fg AB	0.378 fg AB	0.358 bc B
	'Powderblue'	NS	0.487 fg	0.495 d	0.473 e	0.497 a-c
	'Premier'	***	0.636 cd A	0.536 d B	0.584 d AB	0.613 ab A
	'Vernon'	***	0.711 c A	0.546 d B	0.681 c A	0.688 a-c A
NHB	'Aurora'	NS	0.994 b	0.993 b	0.956 b	0.983 a-c
	'Elliott'	NS	1.345 a	1.437 a	1.354 a	1.391 a
	'Liberty'	**	0.720 c A	0.692 c AB	0.664 c BC	0.636 a-c C
Type			***	***	***	***
SHB		*	0.488[s] b A	0.385 b B	0.402 b B	0.4876 b B
RE		NS	0.503 b	0.446 b	0.477 b	0.484 b
NHB		NS	0.983 a	1.041 a	0.991 a	0.967 a

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(6 mL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=56 [7 cultivars x 4 reps with 2(6 mL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(6 mL of juice) subreps/rep]; NHB N=24 [3 cultivars x 4 reps with 2(6 mL of juice) subreps/rep].

Table 4.5. Sugar:acid ratio (°Brix/%TTA) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Camellia'	*[x]	22.6[w] fg[v] B[u]	25.5 f-h AB	26.9 d A	25.5 cd AB
	'Farthing'	NS	26.8 de	27.5 e-g	26.0 d	26.7 c
	'Keecrisp'	***	35.9 b C	51.5 a B	52.9 a AB	55.7 a A
	'Legacy' GA[t]	NS	22.8 fg	24.0 g-i	26.4 d	24.7 c-e
	'Legacy' MI	**	39.3 ab A	40.3 b A	34.3 bc B	37.1 b AB
	'Meadowlark'	***	25.2 ef C	29.9 d-f B	35.2 bc A	37.6 b A
	'Star'	NS	18.7 hi	20.0 ij	20.0 e	20.8 d-f
	'Suziblue'	***	26.9 de C	33.3 cd B	37.4 b A	34.4 b AB
RE	'Alapaha'	**	30.6 c B	30.3 de B	32.4 c A	35.2 b AB
	'Austin'	*	39.8 a A	36.8 bc AB	38.4 b AB	35.2 b B
	'Brightwell'	***	20.6 gh A	21.3 h-j A	18.7 ef B	18.2 f B
	'Powderblue'	*	25.6 ef A	25.1 gh A	27.5 d A	27.6 c A
	'Vernon'	***	17.8 hi C	21.8 h-j A	18.7 ef BC	20.8 d-f AB
NHB	'Bluecrop'	***	20.5 gh A	19.7 ij A	17.2 ef B	20.4 ef A
	'Draper'	**	15.3 i AB	14.4 k B	15.0 fg AB	16.2 f A
	'Elliott'	*	11.1 j A	13.0 k A	12.6 g A	10.9 g A
	'Liberty'	***	29.7 cd B	35.7 c A	35.0 bc A	36.3 b A
	'Nelson'	**	19.7 gh A	19.2 j AB	17.2 ef C	17.6 f BC
Type			***	***	***	***
SHB		**	27.3[s] a B	31.5 a AB	32.4 a A	32.8 a A
RE		NS	26.9 a	27.0 b	27.1 b	27.4 b
NHB		NS	19.3 b	20.4 c	19.4 c	20.3 c

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(300µL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=64 [8 cultivars x 4 reps with 2(300µL/6mL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(300µL/6mL of juice) subreps/rep]; NHB N=40 [5 cultivars x 4 reps with 2(300µL/6mL of juice) subreps/rep].

Table 4.6. Sugar:acid ratio (°Brix/%TTA) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
			***[y]	***	***	***
SHB	'Abundance'	***[x]	38.4[w] bc[v] C[u]	56.2 a A	48.6 b B	41.3 b C
	'Camellia'	***	22.0 f-h B	28.3 ef A	27.4 de A	24.8 ef AB
	'Farthing'	***	25.9 e-g B	38.1 c A	36.1 c A	33.9 cd A
	'Legacy' GA[t]	**	29.0 de AB	33.9 c-e A	34.7 cd A	26.6 e B
	'Legacy' MI	*	28.5 d-f B	33.4 c-e AB	31.0 b AB	36.7 bc A
	'Star'	***	42.4 b BC	49.5 b A	44.0 b AB	37.0 bc C
	'Suziblue'	***	27.8 d-g C	36.8 cd B	45.5 a A	38.9 bc AB
RE	'Alapaha'	**	62.3 a AB	57.3 a B	64.3 c A	64.3 a A
	'Brightwell'	***	34.0 cd B	38.5 c A	37.2 c AB	39.7 b A
	'Powderblue'	**	27.7 d-g B	31.0 d-f A	30.9 cd A	29.1 de AB
	'Premier'	***	25.4 e-g A	24.7 fg A	21.6 ef B	20.6 f B
	'Vernon'	***	21.6 gh AB	25.0 fg A	19.2 fg B	20.0 f B
NHB	'Aurora'	NS	11.9 ij	11.7 h	12.7 gh	12.8 g
	'Elliott'	NS	9.1 j	8.3 h	9.1 h	9.0 g
	'Liberty'	*	18.5 hi B	19.1 g AB	19.2 fg AB	20.8 f A
Type			***	***	***	***
SHB		***	30.6[s] a C	39.4 a A	38.2 a AB	34.2 a BC
RE		NS	34.2 a	35.3 a	34.6 a	34.7 a
NHB		NS	13.1 b	13.0 b	13.7 b	14.2 b

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (***Significant at $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).

[w]N= 8 [4 reps with 2(300µL/6 mL of juice) subreps/rep].

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=56 [7 cultivars x 4 reps with 2(300µL/6 mL of juice) subreps/rep]; RE N= 40 [5 cultivars x 4 reps with 2(300µL/6 mL of juice) subreps/rep]; NHB N=24 [3 cultivars x 4 reps with 2(300µL/6 mL of juice) subreps/rep].

Table 4.7. Total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2018[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***y		***		***		***	
SHB	'Camellia'	NS[x]	482.7[w] a[v]		429.2 ab		408.3 b		448.4 ab	
	'Farthing'	**	224.2 cd	B[u]	305.7 b-d	A	239.9 cd	AB	204.7 ef	B
	'Keecrisp'	*	265.5 cd	A	315.1 a-d	A	269.7 b-d	A	343.4 b-d	A
	'Legacy' GA[t]	NS	165.7 d		214.2 cd		168.9 d		212.9 ef	
	'Legacy' MI	NS	185.9 cd		130.0 d		267.4 b-d		90.8 f	
	'Meadowlark'	NS	164.0 d		289.2 bcd		322.5 b-d		202.6 ef	
	'Star'	*	195.7 cd	B	333.5 a-c	A	340.0 bc	A	275.9 c-e	AB
	'Suziblue'	*	315.9 b-d	AB	340.6 a-c	AB	269.8 b-d	B	389.7 a-c	A
RE	'Alapaha'	NS	185.1 cd		216.2 cd		190.5 cd		219.0 ef	
	'Austin'	***	339.0 a-c	A	280.1 b-d	A	282.5 b-d	A	210.4 ef	B
	'Brightwell'	*	195.7 cd	A	180.8 cd	AB	185.6 cd	AB	145.6 f	B
	'Powderblue'	NS	230.6 cd		265.7 b-d		219.5 cd		230.0 d-f	
	'Vernon'	NS	212.7 cd		216.6 cd		233.6 cd		279.5 c-e	
NHB	'Bluecrop'	NS	308.0 b-d		227.7 cd		275.0 b-d		275.9 c-e	
	'Draper'	NS	241.3 cd		279.3 b-d		242.3 cd		272.3 de	
	'Elliott'	NS	433.9 ab		495.3 a		581.3 a		501.9 a	
	'Liberty'	***	344.2 a-c	A	349.9 a-c	A	269.4 b-d	A	137.8 f	B
	'Nelson'	NS	234.4 cd		230.1 cd		306.2 b-d		276.3 c-e	
Type			*		*		**		*	
SHB		NS	250.0[s] ab		294.7 ab		285.8 ab		289.7 a	
RE		NS	232.6 b		231.9 b		222.3 b		216.9 b	
NHB		NS	312.4 a		316.5 a		334.8 a		292.8 ab	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (*=$P \leq 0.05$,**=$P \leq 0.01$,***= $P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$).

[w]N=4 reps/ cultivar/TP

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=36 (8 cultivars x 4 reps); RE N=45 (5 cultivars x 4 reps); NHB N=45 (5 cultivars x 4 reps).

Table 4.8. Total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars within and across four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) in fresh postharvest cold storage (4°C) in 2019[z].

Type	Cultivar		Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4	
			***[y]		***		***		***	
SHB	'Abundance'	NS[x]	239.9[w] ab[v]		276.7 a-d		274.1 bc		226.2 a-e	
	'Camellia'	NS	289.0 a		375.4 a		387.0 ab		316.8 a	
	'Farthing'	NS	272.7 a		305.2 a-c		306.7 a-c		283.5 a-d	
	'Legacy' GA[t]	NS	273.5 a		331.1 a-c		313.9 a-c		308.6 ab	
	'Legacy' MI	NS	39.7 c		33.6 f		42.1 e		32.8 f	
	'Star'	NS	211.6 ab		234.8 cd		209.9 cd		185.4 b-e	
	'Suziblue'	***	204.0 ab	BC[u]	179.0 de	C	245.9 cd	A	241.1 a-e	AB
RE	'Alapaha'	*	116.3 bc	AB	89.0 ef	B	125.1 de	AB	158.3 d-f	A
	'Brightwell'	NS	220.5 ab		189.5 de		252.8 c		232.6 a-e	
	'Powderblue'	NS	224.7 ab		234.5 cd		292.6 a-c		248.6 a-e	
	'Premier'	NS	231.9 ab		289.2 a-d		270.2 bc		288.0 a-c	
	'Vernon'	*	277.5 a	AB	359.7 ab	AB	388.1 ab	A	272.7 a-d	B
NHB	'Aurora'	NS	213.3 ab		247.3 b-d		285.7 bc		168.6 c-e	
	'Elliott'	NS	201.6 ab		329.0 a-c		412.0 a		215.2 a-e	
	'Liberty'	***	257.5 a	A	218.9 cd	AB	291.5 a-c	A	129.6 ef	B
Type			NS		NS		NS		NS	
SHB			217.8[s] a		248.0 a		329.7 a		227.8 a	
RE			214.2 a		232.4 a		265.8 a		240.0 a	
NHB			224.7 a		269.3 a		254.2 a		171.2 a	

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.

[y]Differences between cultivars or types within each timepoint examined using Tukey HSD (NS=Nonsignificant, ***=$P \leq 0.001$).

[x]Differences between timepoints of individual cultivars or types examined using Tukey HSD (NS=Nonsignificant, *=$P \leq 0.05$, ***=$P \leq 0.001$).

[w]N=4

[v] Lower case letters compare between cultivars or types within a timepoint.

[u]Upper case letters compare between timepoints of an individual cultivar or type.

[t]'Legacy' was collected from GA and MI.

[s]SHB N=28 (7 cultivars x 4 reps); RE N=45 (5 cultivars x 4 reps); NHB N=12 (3 cultivars x 4 reps).

Table 4.9. Spearman's rank correlation coefficients of fruit quality traits between 2018 and 2019 harvest seasons of all collected timepoints[z] and cultivars[y].

Variable	by Variable	Spearman ρ	*P*-value
'18 Total titratable acidity[x]	'19 Total titratable acidity	0.39	*** [w]
'18 Total soluble solids[v]	'19 Total soluble solids	-0.21	**
'18 Sugar:acid ratio[u]	'19 Sugar:acid ratio	0.25	***
'18 Total monomeric anthocyanins[t]	'19 Total monomeric anthocyanins	0.28	***

[z]Processing occurred at four TPs: 1) 3-4 days, 2) 10-11 days, 3) 20-21 days, and 4) 30-31 days after collection.

[y]12 common cultivars were collected between 2018 and 2019.

[x]N=768 ,4 reps [2 (6 mL of juice) subreps/rep] /TP/year.

[w]Differences between years examined using One-Way ANOVA (**, ***Significant at $P \leq 0.01$ or $P \leq 0.001$, respectively).

[v]N=768 ,4 reps [2 (300µL of juice) subreps/rep] /TP/year.

[u]N=768 ,4 reps [2 (300µL/6mL of juice) subreps/rep] /TP/year.

[t]N=768 ,4 reps/TP/year.

Table 4.10. Percent change in total soluble solids (°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA), and total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) between TP1[z] and TP4[y] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) commercial blueberry cultivars in fresh postharvest cold storage (4°C) during the 2018[x] harvest season.

		Percent Change from TP1-TP4			
Type	**Cultivar**	**Total soluble solids[w]**	**Total titratable acidity[v]**	**Sugar:acid ratio[u]**	**Total monomeric anthocyanins[t]**
SHB	'Camellia'	4.3 ***[s]	NS	12.7 *	NS
	'Farthing'	NS	NS	NS	NS
	'Keecrisp'	3.6 *	-33.2 ***	55.2 ***	29.3 **
	'Legacy' GA[r]	NS	NS	NS	NS
	'Legacy' MI	NS	NS	NS	NS
	'Meadowlark'	NS	-31.6 ***	49.0 ***	23.5 ***
	'Star'	NS	-11.3 *	NS	NS
	'Suziblue'	NS	-20.5 ***	28.0 ***	NS
RE	'Alapaha'	NS	-11.1 **	15.0 ***	NS
	'Austin'	NS	11.2 **	-11.5 **	-37.9 ***
	'Brightwell'	NS	13.2 ***	-11.4 ***	-25.6 *
	'Powderblue'	8.5 **	NS	NS	NS
	'Vernon'	NS	-7.9 *	17.0 *	NS
NHB	'Bluecrop'	NS	NS	NS	NS
	'Draper'	NS	NS	NS	NS
	'Elliott'	8.8 *	NS	NS	NS
	'Liberty'	3.6 *	-15.8 **	22.3 ***	-60.0 ***
	'Nelson'	NS	18.0 ***	-10.8 *	NS
Type					
SHB		NS	-14.0 ***	20.1 ***	NS
RE		NS	NS	NS	NS
NHB		NS	NS	NS	NS

[z]TP1=3-4 days after fruit collection.
[y]TP4=30-31 days after fruit collection.
[x]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Canada.
[w]N=16 [4 reps with 2(300µL of juice) subreps/rep/TP].
[v]N= 16 [4 reps with 2(6mL of juice) subreps/rep/TP].
[u]N=16 [4 reps with 2(300µL/6mL of juice) subreps/rep/TP].
[t]N=4 reps/cultivar/TP.
[s]Differences between timepoints of individual cultivars examined using One-Way ANOVA (NS=Nonsignificant, *=P ≤0.05, **=P ≤0.01, ***=P ≤0.001).
[r]'Legacy' was collected from GA and MI.

Table 4.11. Percent change in total soluble solids (°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA), and total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) between TP1[z] and TP4[y] of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) commercial blueberry cultivars in fresh postharvest cold storage (4°C) during the 2019[x] harvest season.

		Percent Change from TP1-TP4			
Type	**Cultivar**	**Total soluble solids[w]**	**Total titratable acidity[v]**	**Sugar:acid ratio[u]**	**Total monomeric anthocyanins[t]**
SHB	'Abundance'	-3.7 *[t]	-9.9 *	NS	NS
	'Camellia'	NS	NS	NS	NS
	'Farthing'	11.7 **	-15.5 *	31.0 ***	NS
	'Legacy' GA[r]	NS	NS	NS	NS
	'Legacy' MI	6.2 **	-16.6 **	28.6 **	NS
	'Star'	-9.0 *	NS	-12.8 *	NS
	'Suziblue'	NS	-30.5 ***	40.3 ***	NS
RE	'Alapaha'	NS	NS	NS	NS
	'Brightwell'	4.5 *	-10.7 ***	16.9 ***	NS
	'Powderblue'	7.3 **	NS	NS	NS
	'Premier'	-21.8 ***	NS	-18.8 **	NS
	'Vernon'	-10.5 *	NS	NS	NS
NHB	'Aurora'	5.9 ***	NS	NS	NS
	'Elliott'	NS	NS	NS	NS
	'Liberty'	NS	-11.6 ***	12.6 **	-49.7 **
Type					
SHB		NS	NS	11.7 *	NS
RE		NS	NS	NS	NS
NHB		NS	NS	NS	NS

[z]TP1=3-4 days after fruit collection.
[y]TP4=30-31 days after fruit collection.
[x]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[w]N=16 [4 reps with 2(300µL of juice) subreps/rep/TP].
[v]N= 16 [4 reps with 2(6mL of juice) subreps/rep/TP].
[u]N=16 [4 reps with 2(300µL/6mL of juice) subreps/rep/TP].
[s]Differences between timepoints of individual cultivars examined using One-Way ANOVA (NS,*,**,***Nonsignificant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$, respectively).
[r]'Legacy' was collected from GA and MI.

Table 4.12. Pearson correlation coefficients (r) between total soluble solids (°Brix), total titratable acidity (%TTA) sugar:acid ratio (°Brix:%TTA), and total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) during timepoint 1 (TP1; 3-4 days after collection), and percent healthy fruit during timepoint 4 (TP4;30-31 days after collection) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry types during the 2018[z] harvest season.

		Chemical fruit quality at TP1			
		Total soluble solids[y]	Total titratable acidity[x]	Sugar:acid ratio[w]	Total monomeric anthocyanins[v]
Percent healthy fruit at TP4[u]	**SHB**				
	r[t]	NS	NS	NS	-0.46**
	N	64	64	64	36
	RE				
	r	NS	-0.46*	NS	0.66**
	N	40	40	40	20
	NHB				
	r	NS	NS	NS	NS
	N	40	40	40	20

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[y]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(300µL of juice) subreps/rep for TP1).
[x]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(6mL of juice) subreps/rep for TP1).
[w]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(300µL/6mL of juice) subreps/rep for TP1).
[v]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps for TP4).
[u]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps x 30 individual berries/rep for TP4).
[t]Pearson correlation coefficient (r) (NS=Nonsignificant, *=$P \leq 0.05$).

Table 4.13. Pearson correlation coefficients (r) between total soluble solids (°Brix), total titratable acidity (%TTA) sugar:acid ratio (°Brix:%TTA), and total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents) during timepoint 1 (TP1; 3-4 days after collection), and percent healthy fruit during timepoint 4 (TP4;30-31 days after collection) of southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry types during the 2019[z] harvest season.

		Chemical fruit quality at TP1			
		Total soluble solids[y]	Total titratable acidity[x]	Sugar:acid ratio[w]	Total monomeric anthocyanins[v]
Percent healthy fruit at TP4[u]	**SHB**				
	r[t]	NS	NS	NS	NS
	N	56	56	56	28
	RE				
	r	NS	-0.49*	NS	NS
	N	40	40	40	20
	NHB				
	r	NS	NS	NS	NS
	N	24	24	24	12

[z]Fresh fruit was collected from commercial packers. Southern highbush and rabbiteye cultivars were collected from commercial packers throughout southern Georgia, and northern highbush cultivars were collected from commercial packers in Michigan and Indiana.
[y]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(300µL of juice) subreps/rep for TP1).
[x]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(6mL of juice) subreps/rep for TP1).
[w]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps with 2(300µL/6mL of juice) subreps/rep for TP1).
[v]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps for TP4).
[u]N=(SHB 7; RE 5; NHB 3 cultivars, with 4 reps x 30 individual berries/rep for TP4).
[t]Pearson correlation coefficient (r) (NS=Nonsignificant, *=$P \leq 0.05$).

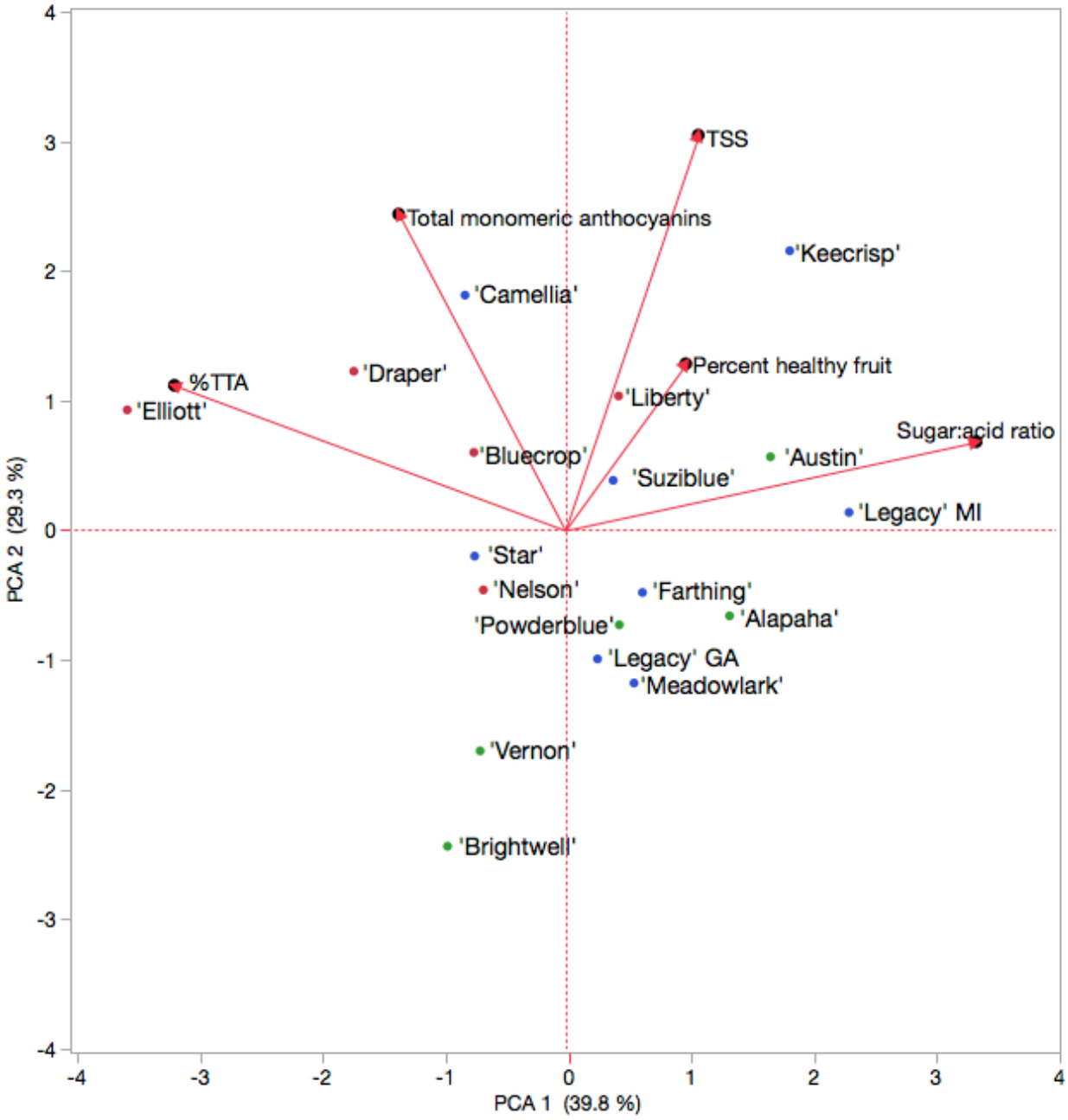

Figure 4.1. Principal component analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 1 (3-4 days after collection) demonstrating variance of chemical quality attributes of total soluble solids (TSS,°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA), total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents), and relation to percent healthy fruit.

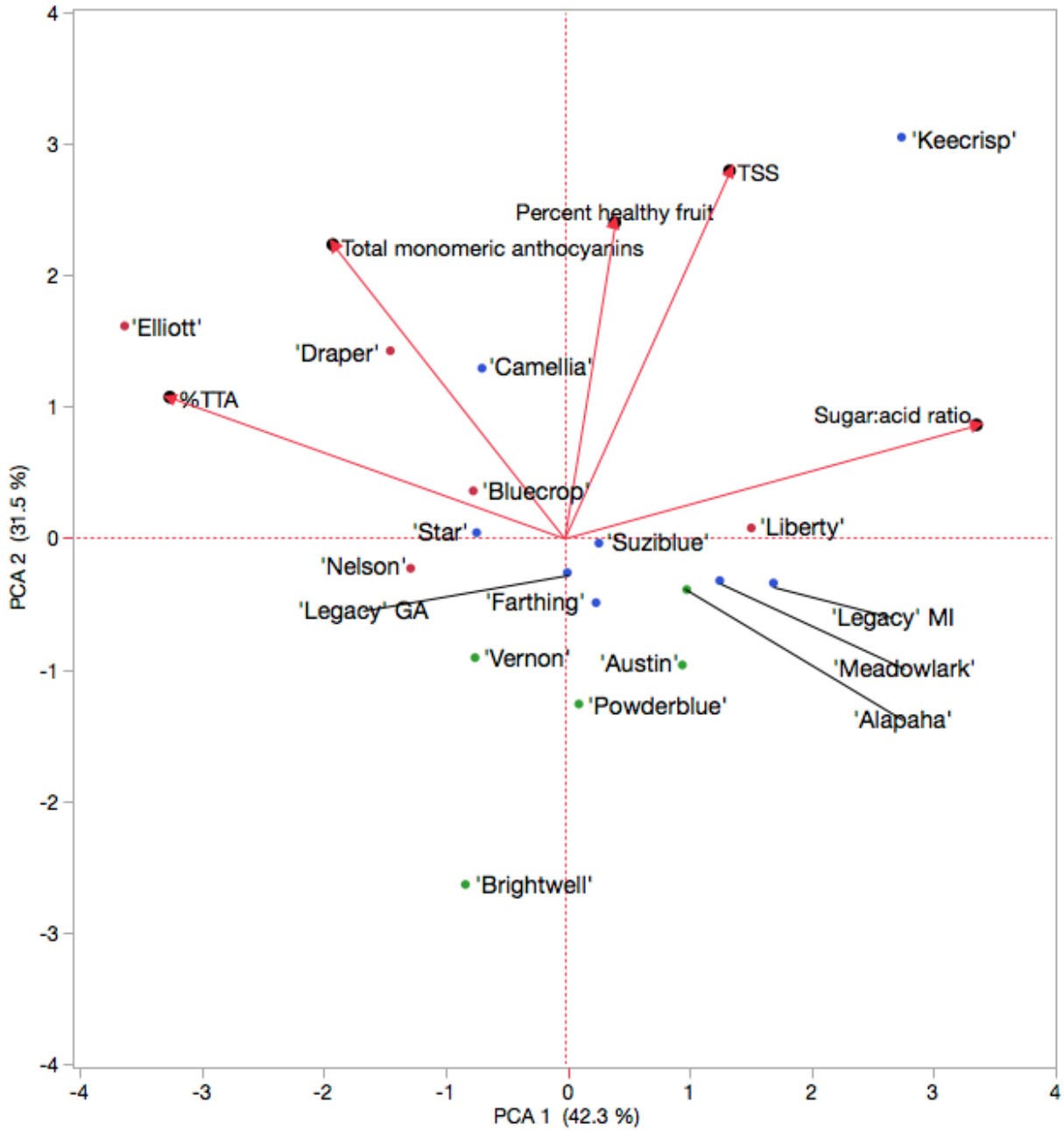

Figure 4.2. Principal component analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 4 (3-4 days after collection) demonstrating variance of chemical quality attributes of total soluble solids (TSS,°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA), total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents), and relation to percent healthy fruit.

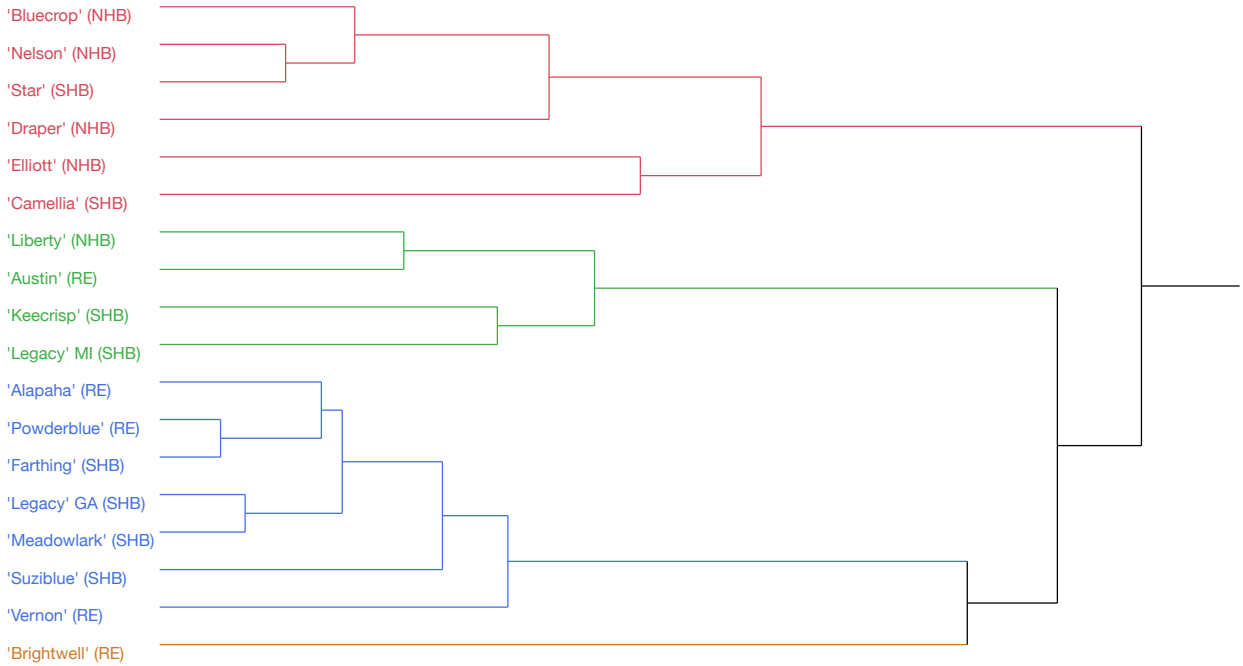

Figure 4.3. Dendrogram from hierarchical cluster analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 1 (3-4 days after collection).

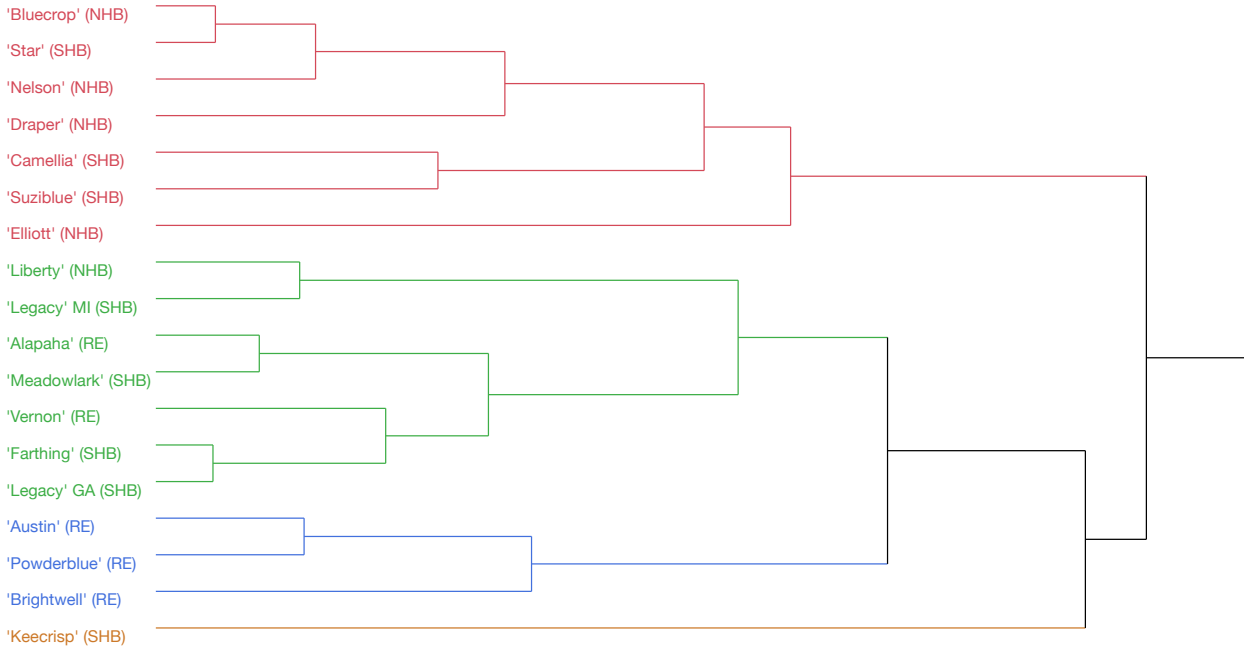

Figure 4.4. Dendrogram from hierarchical cluster analysis of 2018 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 4 (3-4 days after collection).

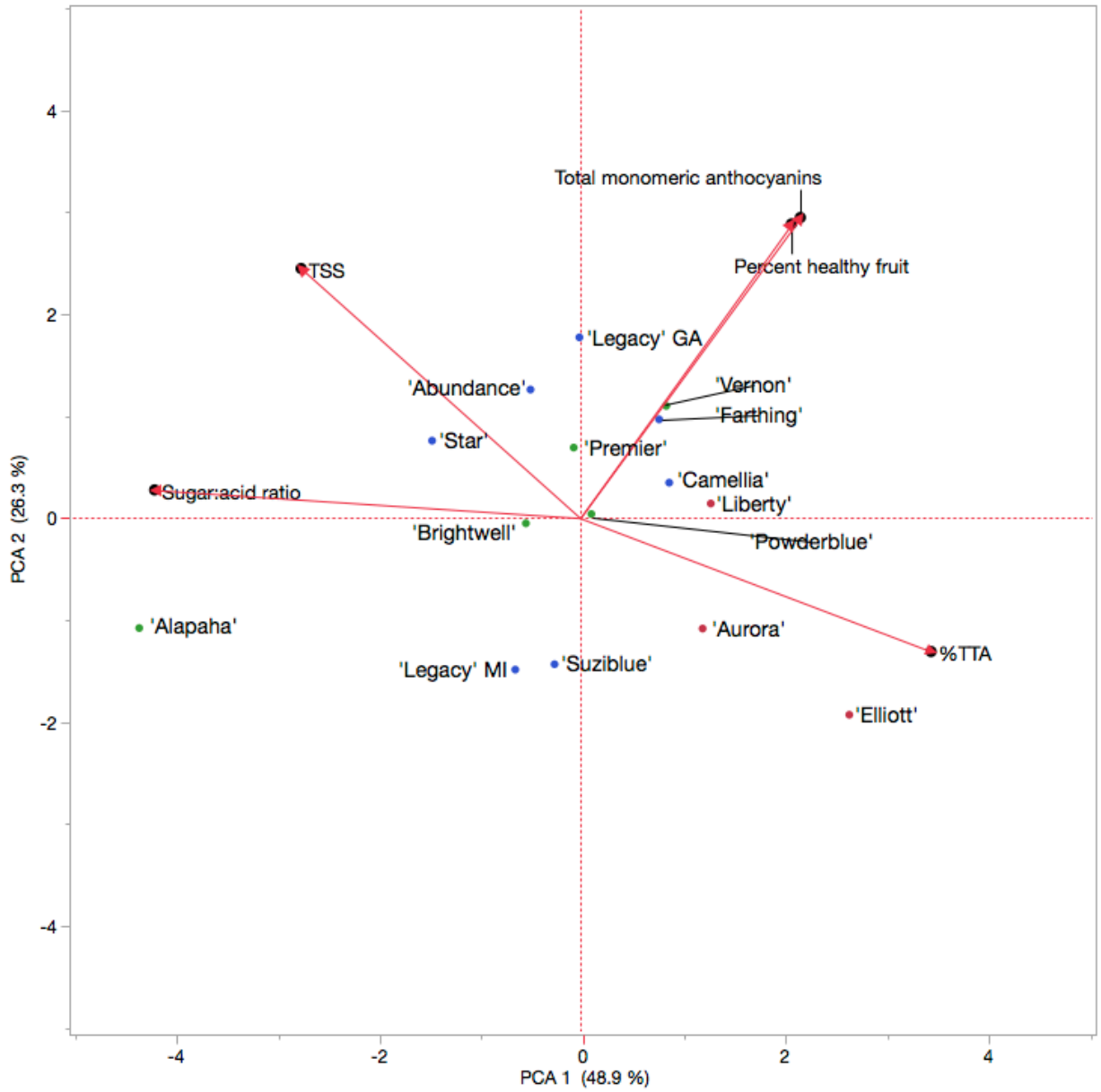

Figure 4.5. Principal component analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 1 (3-4 days after collection) demonstrating variance of chemical quality attributes of total soluble solids (TSS,°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA), total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents), and relation to percent healthy fruit.

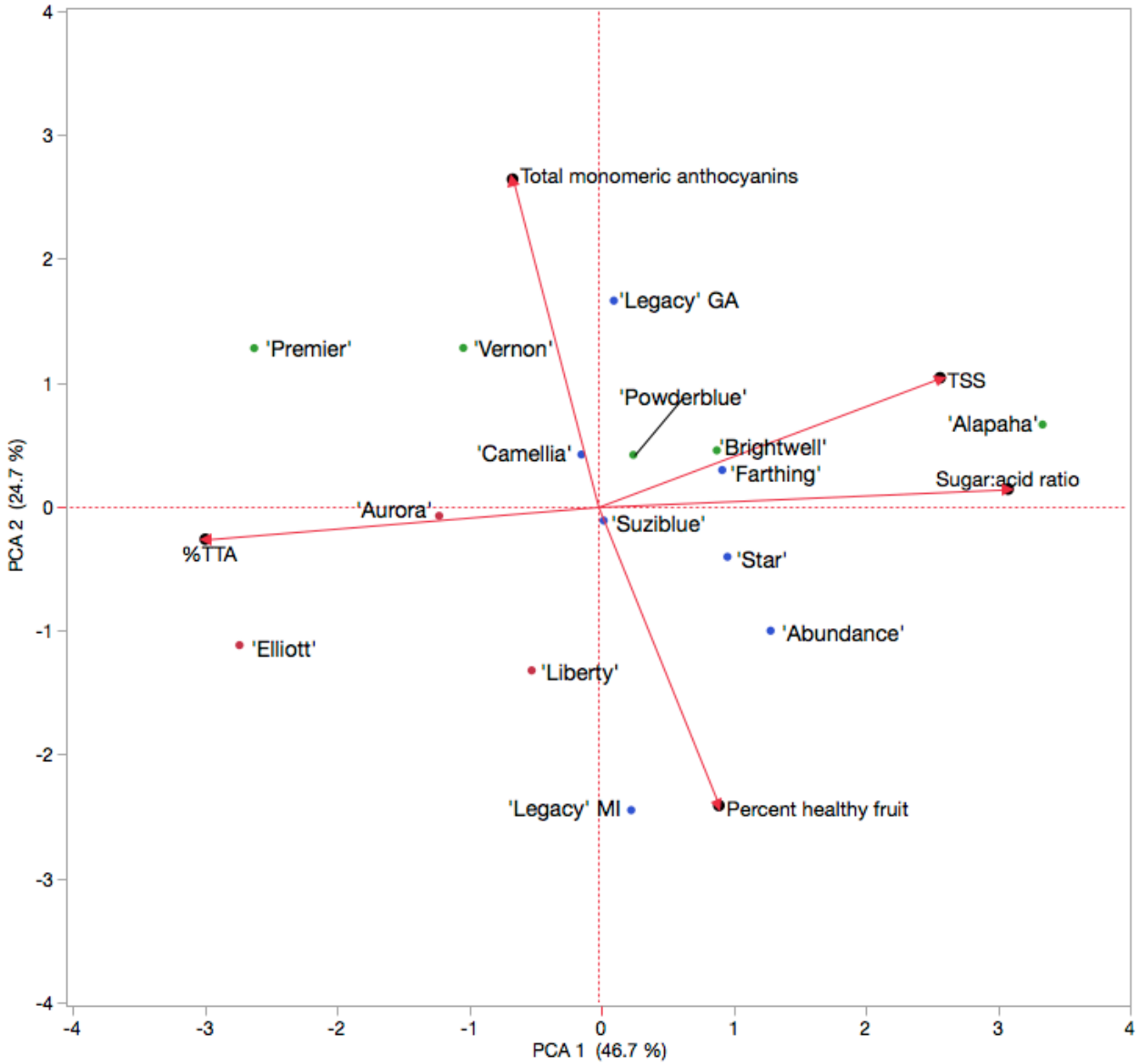

Figure 4.6. Principal component analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars evaluated during Timepoint 4 (30-31 days after collection) demonstrating variance of chemical quality attributes of total soluble solids (°Brix), total titratable acidity (%TTA), sugar:acid ratio (°Brix:%TTA) total monomeric anthocyanins (mg/L cyanidin-3-glucoside equivalents), and relation to percent healthy fruit.

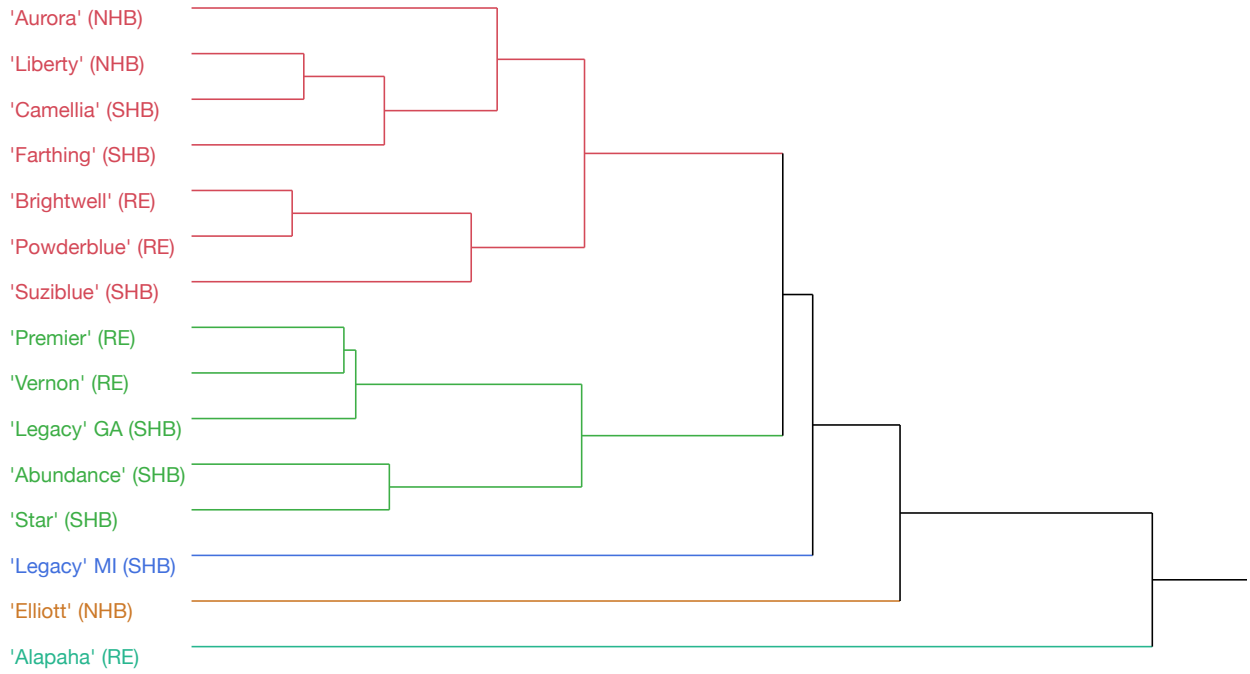

Figure 4.7. Dendrogram from hierarchical cluster analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 1 (3-4 days after collection).

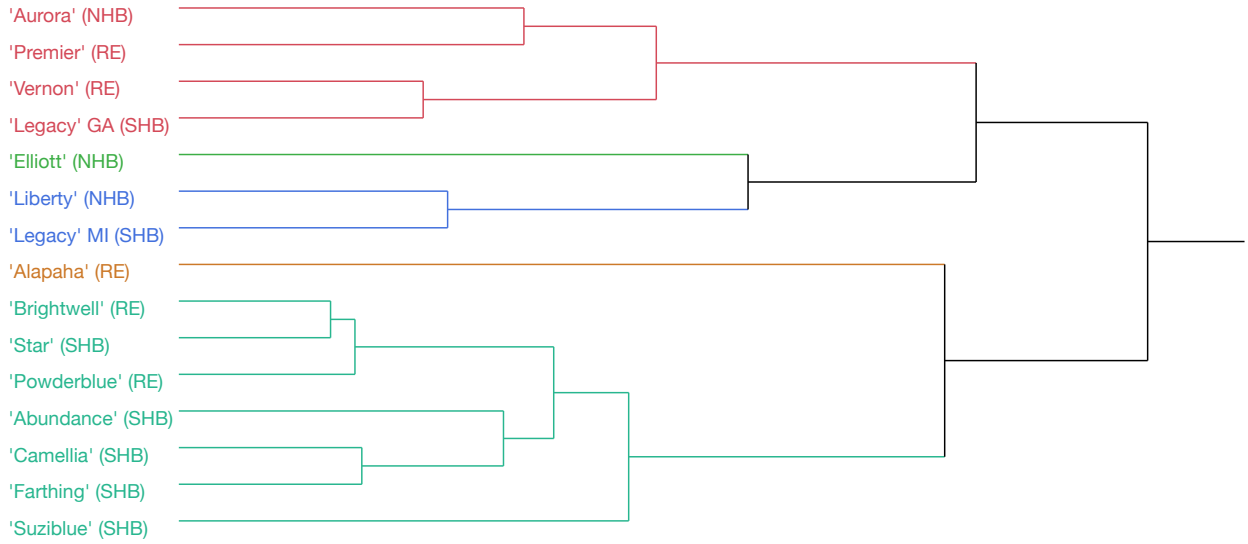

Figure 4.8. Dendrogram from hierarchical cluster analysis of 2019 southern highbush (SHB), rabbiteye (RE), and northern highbush (NHB) blueberry cultivars grouped based on principal component 1 and principal component 2 values evaluated during Timepoint 4 (30-31 days after collection).

CHAPTER 5

GENE EXPRESSION OF CELL WALL DEGRADING ENZYMES DURING POSTHARVEST COMMERCIAL COLD STORAGE[1]

[1]Mooneyham, R., S.U. Nambeesan, Yi-Wen Wang, D.J. Chavez, and R.A. Itle. To be submitted to *HortScience*.

Abstract

Fruit quality after harvest is important for consumer satisfaction. Losses in postharvest fruit quality can occur due to excessive fruit softening, shriveling and pathogen susceptibility. In blueberry, the fruit quality of southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton) types grown in Georgia is debated. Furthermore, northern highbush (NHB) (*Vaccinium corymbosum* L.) grown in northern states, is perceived to have the highest quality. Thus, it is important to evaluate postharvest keeping quality among the three types. Cell wall degradation is an important process in fruit ripening and subsequent fruit softening. Higher gene expression of genes associated with cell wall degradation may lead to undesirable fruit softening. The objective of this study was to determine the expression of cell wall degrading genes among blueberry types differing in quality during cold storage. Differential gene expression analyses of 12 cell wall degrading genes were examined with one cultivar that maintained a relatively high percent healthy fruit, and another that steadily plummeted over a 30-day cold storage period. These cultivars included 'Draper', 'Nelson' (NHB); 'Alapaha', 'Austin' (RE); and 'Keecrisp', 'Suziblue' (SHB), the former cultivar for each type maintained fruit quality during postharvest storage. Overall, differential gene expression showed that 'Suziblue' had higher transcript abundance of *β-galactosidase (β-Gal)* than 'Keecrisp'. 'Keecrisp' had unexpectedly highest transcript abundance of *β-D-N-acetylhexosaminidase/ β-hexosaminidase* 1 (*β-Hex1*) than all cultivars. 'Keecrisp' and 'Suziblue' had highest transcript abundance of *pectinesterase* (*PE)* compared to other types, and 'Nelson' had higher transcript abundance of *xyloglucan endotransglucosylase/hydrolase 1 and 2* (*XTH1 and XTH2*) than 'Draper'. Further studies screening more cultivars that vary in shelf-life in each of these types would provide stronger evidence for association of cell wall degrading genes to

post harvest shelf-life. Further, future studies looking at specific cell wall components will be important to understand the roles of cell wall degrading genes in stability of blueberry shelf-life.

Introduction

Blueberry (*Vaccinium* spp.) has experienced considerable growth as a popular fruit crop in many areas of the world. Production worldwide was at approximately 42,000 hectares in 2005, and reaching over 109,000 hectares in 2014 (Brazelton, 2016). In a single year, worldwide blueberry consumption rose by 45% from 2015 to 2016 (Freshuelva, 2018). Availability of blueberries has been possible through new cultivar development and consumer interest in the high number of antioxidants that blueberries offer (Joseph et al., 2005; Wu et al., 2004). In the U.S., blueberry was the fourth most valuable non-citrus fruit crop, with value of utilized production at $909 million, behind strawberry ($2.5 billion), apple ($2.7 billion), and grapes ($5.7 billion) in the year 2019 (USDA, 2020). Florida and Georgia make up the majority of blueberry production in the southeastern U.S., producing 24 million and 95 million pounds respectively, making up 18% of the total U.S. production in the year 2019 (USDA-NASS, 2019). Blueberries consisted of 2.24 percent of Georgia's total farm gate value, ranking it number nine at 308 million dollars worth of production out of all commodity crops (University of Georgia Center for Agribusiness and Economic Development, 2019). As world production has also increased, so has Georgia's bearing acreage. In the year 2000, Georgia grew 4600 acres as opposed to approximately 21,000 acres in the year 2019 and this is expected to grow (Krewer and NeSmith, 2002; United States Department of Agriculture, 2020).

The Georgia blueberry market is made up of southern highbush (SHB, species complex of *Vaccinium corymbosum* L. and *V. darrowii* Camp) and rabbiteye (RE, *V. virgatum* Aiton). A third major type, northern highbush (NHB, *V. corymbosum* L.) dominates the North American fresh market and are unable to grow in Georgia because of their adaptability to the northern latitudes of the U.S. These three make up the three main commercial types of the U.S. market today. The

biggest difference between these types is the number of chilling requirements and tolerance to cold temperatures. NHB require between 800 and 1000 chilling hours and can tolerate temperatures down to -20ºC. SHB and RE types do tolerate below freezing temperatures well, and require approximately 550 and 600 chilling hours, respectively (Retamales and Hancock, 2018).

Within the industry there is a bias of a type-hierarchy of fruit quality characteristics. SHB fruit are perceived to have superior fruit quality than RE fruit. Beyond this, NHB fruit quality is perceived to have superior fruit quality than that of SHB. This bias exists for both quality at harvest and throughout postharvest storage. Georgia grown fresh market RE blueberries are sometimes purchased at a lower price point from third party distributers than other types as a result of these biases (personal communication with Dr. Rachel Itle). However, there is limited information for industry to support these decisions and sufficiently compare the fruit quality of these three blueberry types.

The main obstacles to prolonged blueberry shelf-life are spoiling caused by fungal pathogens (Schotsmans et al., 2007) and oxidative stress caused by postharvest handling (Hodges et al., 2004). Blueberry is categorized as a soft fruit which makes texture a critical factor in determining the fruit quality (Giongo et al., 2013). Since blueberries are harvested when ripe, they are susceptible to rapid degradation and quality loss. Therefore, fruit firmness of blueberry also needs to be held at a standard to withstand shipping to markets and staying fresh in consumers' homes.

Most of the fruit intended for the fresh market is harvested by hand to achieve high fruit quality, which also makes the cost of production higher. Blueberries are hand-harvested to diminish bruising and fruit injury, prolong post-harvest storage and to maintain their appeal to consumers (Brown et al., 1996). Fruit are also machine harvested, which is less labor intensive

than hand harvesting (Mehra et al., 2013). A downside to machine harvesting is the loss in quality and higher susceptibility to oxidative stress as a result of excessive handling (Hodges et al., 2004). Market timing and end price point play a large role in determining whether fruit is hand or machine harvested. When SHB season harvest ends in May, RE becomes available. During this time, other blueberry producing states in northern latitudes of U.S. begin selling. The market influx of blueberries plays a role in the lower price point of RE blueberries later in the harvest season. When RE blueberry prices are lower, machine harvesting is utilized for the processing market (NeSmith et al., 2002). Factors such as aggregate productivity and targeted market make it difficult to determine profitability of RE blueberry and play a role in its market fluctuations. (Fonsah et al., 2011). Weather events that result in the cracking or softening of fruit forces growers to sell their fruit intended for fresh market to frozen market, another contributor to lower grower prices (Scherm and Krewer, 2003).

Throughout the ripening phase a process known as cell-wall disassembly leads to fruit softening (Cappai et al., 2018). Fruit firmness is not only an indication of consumer preference but is also associated with extended shelf-life (Moggia et al., 2017) and improved machine harvestability (Olmstead and Finn, 2014). Fruit firmness of blueberry is held at a standard to handling, withstand shipping to markets and staying fresh in consumers' homes and has a large effect on consumer acceptance. Therefore, future studies are required to investigate traits that overlap with the production/industry and consumer standards and if changes associated with fruit firmness are common among blueberry types. The depolymerization of cell-wall bound pectin and hemicellulosic polymers caused by cell wall degrading enzymes are the main contributions to loss of firmness throughout the stages of ripening in blueberry. Pectin slowly changes from an insoluble substance to that which is more water-soluble throughout ripening (Theuwissen and Mensink,

2008). The loss of intercellular adhesion is involved as well, with an increase in intercellular spaces (Brummell, 2006). Postharvest cold storage of blueberries may delay the progression of fruit softening due to lower activities of cell wall degrading enzymes (Chen et al., 2015). In postharvest cold storage at 5°C, the activity of cell wall degrading enzymes such as polygalacturonase, cellulose, ß-galacturonase, and α-galactosidase is greatly suppressed, and decreases in water soluble pectin levels are noticeable too compared to fruit stored at 10°C (Chen et al., 2015). The role of calcium in pectin solubilization and postharvest quality has been implicated as well. Using a calcium chloride application during the water immersion process commonly used to separate less-dense blueberries has shown to enhance firmness and postharvest storage. However, the commercial application is not feasible since concentrations of 2-4% of calcium chloride presented objectionable, salty taste of frozen berries to sensory panelists (Hanson et al., 1993). This corroborates with findings that state when calcium is present, unesterified regions of homogalacuronan molecules form together to make domains of calcium-pectate gel (Jarvis, 1984). These calcium-petate gels potentially increase cell-to-cell adhesion due to these calcium-pectate linkages, resulting in firm fruit with increased wall stiffness, preventing polymerization (Thomson et al., 1999). In my previous study (Chapter 1), I identified cultivars that differ in postharvest shelf-life within the three blueberry types. The objective of this study was to investigate the gene expression of cell wall degrading enzymes of cultivars of all three blueberry types that differ in postharvest shelf-life. This information will identify important cell wall degrading genes that are associated with fruit softening and cell wall degradation that lead to loss of quality of blueberry fruit in postharvest storage.

Materials and Methods

Plant material

In 2018, seven SHB ('Camellia', 'Farthing', 'Keecrisp', 'Meadowlark', 'Legacy' 'Star', and 'Suziblue'), five RE ('Alapaha', 'Austin', 'Brightwell', 'Powderblue', and 'Vernon') and five NHB ('Bluecrop', 'Draper', 'Elliott', 'Liberty', and 'Nelson'), were collected from commercial packers from May to August 2018. . SHB and RE cultivars were collected from commercial packers throughout southern Georgia, and NHB cultivars were collected from commercial packers in Michigan and Canada. The cultivars collected were representative of early, mid, and late season SHB and RE cultivars that make up the Georgia blueberry market, as well as the NHB cultivars that make up the larger North American blueberry market. Fruit collected went through processes similar to how they would reach the consumer market, and harvest types changed accordingly to market timing and available resources. Harvest type of all cultivars is mentioned in Table S1. All cultivars were collected during the midpoint of harvest period for each cultivar, and the study was initiated within one week after harvest for all the cultivars. Commercial packers provided sorted and packed fruit in half-pint clamshells, or otherwise came straight from growers' fields. If fruit was not previously sorted, the berries were hand-sorted and placed in half-pint clam shells. Green or damaged berries were culled during this hand-sorting process. All SHB and RE were placed into clamshells, into coolers over ice and transported to the UGA Pilot Plant in the Melton Building (Griffin, GA) where they were stored in a commercial walk-in cooler at 4°C. The cultivars from Michigan, and Canada were received on a refrigerated truck and stored in the same walk-in cooler.

Research design

Seven to eight half-pint clamshells were designated for each timepoint in a complete random design in a commercial walk-in cooler. Clamshells were placed in the center shelves of

the cooler in order to account for differences in temperature between the wall closest to the cooler fan and the cooler door/entrance. Fruit was evaluated at four timepoints during postharvest storage: 1) three to four days, 2) ten to eleven days, 3) twenty to twenty-one days, and 4) thirty to thirty-one days after collection (TP1, TP2, TP3, and TP4, respectively). For each timepoint fruit from one clamshell was randomly selected in order to minimize handling of fruit. On days of sampling, fresh fruit was brought to room temperature at benchtop for approximately 2 hours before collecting data.

Percent healthy fruit and cultivar selection

Percent healthy fruit was a composite measurement to determine how well cultivars performed in postharvest storage through integrity of skins and visual assessment. Four replications of thirty random berries were examined individually for visual imperfections such as leakiness, dents, tears, mold, or any signs of postharvest decay was assessed at four timepoints (3-4; 10-11; 20-21; and 30-31 days after collection) during postharvest cold storage (4°C) in 2018. An indication of shelf-life was determined by totaling visual imperfections and dividing by total berry number. This is expressed as percent healthy fruit. Of all the cultivars evaluated in 2018, two cultivars from each blueberry type that differed significantly in shelf-life were selected. One cultivar that maintained a relatively high percent healthy fruit from TP1 – TP4, and the other one that steadily plummeted over the 30-day period. The following cultivars were chosen that fit these criteria: SHB, ‘Keecrisp’ and ‘Suziblue’; RE, ‘Alapaha’ and ‘Austin’; NHB, ‘Draper and Nelson’ (Figure 5.1). The first cultivar named for each type maintained high percent healthy fruit throughout the study. At every time point fruit samples were frozen in liquid N_2 to be used for gene expression analysis.

Identification of cell wall degrading genes

A total of 8 genes were identified: *α-mannosidase* (*α-Man*), *1,4-β-mannosidase (β-Mann), β-galactosidase (β-Gal), β-D-N-acetylhexosaminidase/ β-hexosaminidase* (*β-Hex*), *β-D-xylosidase/α-L-arabinofuranosidase (XYL), pectinesterase* (*PE*), *polygalacturonase* (*PG*), and *xyloglucan endotransglucosylase/hydrolase* (*XTH*). Meli et al. (2010) and Zhang et al. (2018) demonstrated that *XTH*, *β-Mann*, *β-Gal*, *α-Man*, and *β-Hex* showed significant contributions to changes in cell wall composition of tomato (*Solanum lycopersicum*), and Orfila et al. (2001) of *XYL*, and *PE* in tomato as well. Villarreal et al. (2008) showed *PG* contributed to changes in cell wall composition of strawberry (*Fragaria* x *ananassa*). These were chosen for primer design and investigation in ripened blueberry fruit. In some cases, two members of one gene family were identified, both of which were highly expressed in ripe blueberry fruit tissue. The two members also were present in all the three blueberry types 'Draper', 'Powderblue' and 'Suziblue' with high identity (≥ a score of 1000 when conducting BLASTn between types). Both genes belonging to the same gene family were indicated as a different member through annotated descriptions from BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/) (as described below) and not being identical. Four of the 8 genes selected had two transcripts representing members of the same gene family. These genes were *α-Man, β-Hex, XYL*, and *XTH*. In total gene expression analyses were conducted with 12 genes.

Primer design

Genes of interest were searched using the genome assembly of cv. 'Draper' available at the Genome Database for Vaccinium (www.vaccinium.org). Expression data according to tissue type available via the *GigaScience* database Giga DB (gigadb.org/dataset/view/id/100537) was used to determine the candidate cell wall degrading genes that are highly expressed in ripe blueberry fruit.

The blueberry candidate genes from the 'Draper' genome was used to conduct a BLASTn search for hits between 'Draper' sequences and previously acquired PacBio transcriptome data from cvs. 'Powderblue' (RE) and 'Suziblue' (SHB) (Made available from Dr. Savithri Nambeesan) using a custom script in NCBI (http://www.ncbi.nlm.nih.gov/BLAST/). Hits between 'Draper', 'Powderblue' and 'Suziblue' with high identity (≥ score of 1000 when conducting BLASTn between types) were chosen for subsequent primer design. This helped in identification of the same candidate gene in all three types of blueberry. Sequences from all candidate genes from the three types of blueberry was used to perform BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/) to ensure that annotated descriptions were consistent with predicted gene function. Primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), using 'Draper' target sequences. Primers were designed specifically in order to amplify target genes across all three blueberry types. Genes belonging to the same gene family as candidate genes but having lower transcript abundance in ripe fruit was also retrieved from the Draper genome. These genes were used to ensure that primers were specific for the selected target genes and did not amplify multiple genes from the same gene family. Multiple sequence alignment using the EMBL-EBI Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used to determine sequences common to all three blueberry types for a given target genes but dissimilar to the low abundance genes in the same gene family. Sequences listed in Table 5.2 were aligned in Clustal Omega for this purpose. Four reference genes were used for qRT-PCR normalization (Vashisth et al., 2011) to normalize the expression of cell wall degrading genes: CLATHRIN ADAPTOR COMPLEXES SUBUNIT FAMILY PROTEIN (CACSa, NCBI accession: DR067098), POLYUBIQUITIN 3 (UBQ3b , NCBI accession: CV091027), UBIQUITIN-CONJUGATING ENZYME 28 (UBC28, NCBI accession: CF811189), and RNA HELICASE-LIKE 8 (RH8, NCBI

accession: DR067965). Later, UBQ3b did not amplify well with pooled cDNA, so only three reference genes were used for normalization. Table 5.1 lists forward and reverse primer sequences with primer concentration.

RNA extraction, cDNA synthesis, and qRT-PCR

Ripe fruit was removed from cold storage at every time point when fruit quality analyses were performed, warmed to room temperature at benchtop for approximately two hours, then immediately frozen in liquid N_2 and stored at -80°C until time of extraction. RNA was extracted in four replications at TP1 and TP3 using the protocol described by Vashisth et al. (2011). Timepoint 3 (TP3) was chosen as the latter timepoint to examine differences in gene expression over time to anticipate change in phenotype as a result of expression. RNA quality was assessed using a 2000/2000c Nanodrop (Thermo Scientific, Waltham, MA) using the 260/280 absorbance ratio, where all RNA samples had a ratio between 1.9 and 2.1. RNA quality was further assessed by visualization on a 1.2% agarose mixture with 0.5X Tris-Borate-EDTA buffer by using aTfm-30V High Performance 302Nm UV Transilluminator (UVP, Analytik Jena US LLC, Upland, CA). 1 µg of prepared RNA was used to synthesize cDNA and diluted to 100 µL following protocol established by Vashisth et al. (2011).

qRT-PCR reactions were set up using 1 µL of cDNA, 0.15-0.2 uM Primer and Power UP SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in a 12 µL reaction in a Stratagene MX 3005P qRT-PCR System (Agilent Technologies, CA) following: 50 °C for 2 minutes, 95 °C for 5 minutes, followed by 95 °C for 30 seconds, and 60 °C for 1 minute repeated for 40 cycles, followed by a melting curve analysis of 95 °C for 1 min, 55 °C for 30 s, 95 °C for 30 s. Primer specificity was ensured by making sure that a single dissociation curve was observed for every gene. PCR reaction efficiency was determined using LinRegPCR (v. 11.0)

Relative gene expression was determined using mean primer efficiency correction as described by Vashisth et al. (2011).

Statistical analyses

JMP Pro v.14 (SAS Institute, Cary, NC, USA) was used to examine differences of means of relative gene expression using the log 2 transformation of the normalized relative quantity (NRQ) between all cultivars and types, across both timepoints [Tukey HSD ($P \leq 0.05$)].

Results and discussion

Cell wall degrading genes were selected from literature detailing the progress made in tomato, as it has shown to be an excellent model for fruit ripening and softening. Gene expression varied within and across types for several cell wall degrading genes, however overall no noticeable changes were observed between time-points TP1 and TP3.

The NHB cultivar, 'Nelson', which displayed low percent healthy fruit during postharvest storage, showed higher transcript abundance of *XTH 1* and *XTH2*, especially at TP1 compared with 'Draper' that had a better shelf-life (Fig. 5.2 k and l). Xyloglucans are the most abundant hemicellulose in primary cell walls of most plants. XTH has two distinct enzymatic activities – to catalyze cleavage of the xyloglucan polymer backbone and to act as hydrolase (Saladié et al., 2006). These two actions distinguish XTH's into XET (endotransglucosylase) and XEH (endohydrolase) respectively with their own action on cell wall integrity. XTH's further complicate cell wall degradation depending on acceptor substrates (Rose et al., 2002). The overexpression of a tomato ripening specific GRAS protein named *SlFSR* subsequently (*fruit shelf-life regulator*) upregulated a multitude cell wall degrading genes, including *XTH*, and shortened fruit shelf-life (Zhang et al., 2018). Overexpression of *XTH* in apple (*Malus pumila*) cultivars 'Golden Delicious' and 'Fuji' resulted in faster softening than control fruit as well (Ma et al.,

2020). If higher *XTH* expression in 'Nelson' compared to 'Draper' is associated with higher self-life in NHB, this warrants more studies.

β-Mannosidase is an enzyme that cleaves the mannan backbone, a hemicellulosic network that serves as structural support for hemicellulose-cellulose bonds, similar to xyloglucans. *β-Man* is said to have a similar action as *XTH* acting as a transglycosylase during fruit softening (Schröder et al., 2009). In this study, no differences in transcript abundance of this gene was observed between cultivars that varied in postharvest shelf-life and between the three types of blueberry (Fig. 5.2 f).

XYL is responsible for hydrolyzing arabinoxylans and xylans, which are both widely distributed in the plant hemicellulose network, and are subject to modification during early fruit development and ripening (Itai et al., 2003). The transcript abundance of *XYL1*was higher in 'Suziblue' compared with 'Keecrisp' at TP1; there were no differences between cultivars of NHB and RE. No differences between types or cultivars were observed for *XYL2* (Fig. 5.2 g and h) It would be further interesting to investigate if this gene is important in other SHB cultivars that vary in shelf-life. Interestingly in peach *XYL* is thought to play a predominant role in cell-wall reorganization rather that degradation and is even detected before fruit softening occurs when ethylene levels are low. Other factors like auxins are thought to regulate transcription of *XYL,* as they are activated before the climacteric onset (Di Santo et al., 2009).

PG is highly abundant during ripening in many horticultural crops. It catalyzes the cleavage of homogalacturonan, and some suggest that it could be a fruit softening-rate determining enzyme among strawberry cultivars (Villarreal et al., 2008). PG is thought to greatly affect pectin degradation even in extremely low levels. In tomato, even an 80% reduction in PG activity through antisense fruit had little impact on pectin structure (Smith et al., 1990), meaning pectin disassembly

may be dependent on PG activity even in low levels (Hadfield and Bennett, 1998). However, there were no differences in transcript abundance of *PG* among cultivars used in this study. Further, it is interesting to observe the expression of *PG* was lowest in RE types (Fig. 5.2 j). It would be interesting to investigate more RE cultivars to determine if this is a general trend in RE cultivars.

PE activity is ubiquitous throughout plant tissues, as it is known to be responsible not only for fruit development and ripening (Tucker, 2004), but also during stem elongation (Micheli, 2001), pollen tube development (Bosch and Hepler, 2005), and abscission (Wang et al., 2005), and exists in multiple isoforms with unique modes of action (Phan et al., 2007). Phan et al., (2007) suggest that deesterified pectin, the product of a specific *PE* isoform *PMEU1* (found in both leaf and fruit tissue), helps to strengthen the cell wall by resisting softening during the ripening process in tomato. Silencing *PMEU1* in fruit tissue showed to increase the rate of softening in transgenic tomato fruit, compared to wild-type. Although the predominant isoform in fruit tissue is *PMEU2* (Tucker et al., 1982), antisense of *PMEU2* decreased PE activity in ripe fruit, but fruit developed without any phenotypic differences than wild types and rate of softening was no different (Hall et al., 1993). With this, blueberry-specific *PE*, its isoforms, and action on multiple plant tissues should be further investigated. In this study, *PE* was expressed highest in SHB cultivars 'Keecrisp' and 'Suziblue' during TP1, and had lower transcript abundance during TP3 for both types (Fig 5.2 i). Although primers were designed relying on the target with highest transcript abundance in ripe fruit from the 'Draper' genome, this specific isoform may also either have variation by blueberry type or may not have a strong influence on postharvest cell wall degradation.

There are at least seven different *β-Gal* genes that are expressed during tomato fruit development, of which *TBG4* has shown to be involved in cell wall degradation by fruit softening (Smith and Gross, 2002). β-Gal activity is known to reduce galactosyl levels in the cell wall of

tomato. Down regulation of *TBG4* resulted in decreased fruit softening, which could be the result of intact galactosyl-containing side chains obstructing cell wall degrading enzymes to other wall components by reduction of cell wall porosity (Redgwell et al., 1997). Transcript abundance of *β-Gal* was higher during TP1 than TP3 across all cultivars and types. SHB type of lower percent healthy fruit 'Suziblue' during TP1 had highest expression of *β-Gal*. Further, RE type 'Alapaha' showed high transcript abundance of *β-Gal* during TP1 as well, however 'Alapaha' is the RE type of higher percent healthy fruit (Fig. 5.2 c). This may indicate a type difference of *β-Gal* expression and requires further studies.

β-Hex and *α-Man* jointly accelerate glycoprotein and glycolipid degradation by cleaving terminal N-acetyl-D-hexosamine residues and α-mannosidic linkages, respectively, to form free *N*-glycans (Jagadeesh et al., 2004; Meli et al., 2009). These resulting *N*-glycan products of hydrolysis have biological activity that stimulate fruit ripening in tomato (Priem et al., 1992) and metabolism (Handa et al., 1985), and could also play a role in blueberry. Especially the importance of these two genes, have been shown to play important toles in cell wall disassembly. In *α-Man* and *β-Hex* RNAi tomato fruit, these showed a down-regulation of *β-Mann*, and *β-Gal* that are involved in cell-wall degradation (Meli et al., 2009). Based on the results presented in this study, 'Keecrisp' having a higher transcript abundance of *β-Hex1* than all other cultivars in addition to higher percent healthy fruit than others cultivars is unexpected. There were no differences observed in *β-Hex2* (Fig. 5.2 d and e). *α-Man 1* and *α-Man 2* did not change across cultivars and blueberry types (Fig. 5.2 a and b). 'Keecrisp' has been described as having "crisp" and firm texture (Williamson et al., 2019). mRNA accumulation can sometimes not reflect modifications in enzyme activity (or vise-versa), as seen when ethylene treated strawberry increased in mRNA accumulation *FaPG1* but not PG activity (Villarreal et al., 2009). Histologically, crispy genotypes

have smaller cell area on average, with no difference in stone cell layer frequency (Blaker and Olmstead, 2014). It would be interesting to investigate the enzyme activity and gene expression, comparing genotypes that are considered "crispy" and that of standard firmness.

As such, comparison of gene expression between types in this study is challenging, due to differences in ploidy. SHB and NHB blueberry types are autotetraploid (2x = 4n = 48) and demonstrate tetrasomic inheritance segregation ratios determined from isozyme (Krebs and Hancock., 1989) and RAPD markers (Qu and Hancock, 1997, 1998). RE types are hexaploid (2n = 6x = 72) (Lyrene et al., 2002). Autotetraploids are unique in that they have random chromosome segregation during meiosis, as well as double reduction, a process where alleles are delivered to the same gamete (Milbourne et al., 2008), making the inheritance and thus expression of cell wall degrading genes of autotetraploid northern and SHB different from hexaploid RE. Hexaploids have a possible six possible alleles at each locus and can have more allelic and non-allelic interactions than tetraploids. The same for tetraploids, they have more allelic or non-allelic interactions than diploids. In maize, global studies of gene expression between tetraploid hybrids, diploid, and triploid lines, have shown that gene expression differs between lines, ploidy levels, and hybrids (Riddle et al., 2010).

Gene expression of cell wall degrading enzymes in ripe blueberry during postharvest storage is extremely complex, given the results of this study. Physiologically, both blueberry (El-Agamy et al., 1982; Shimura et al., 1986) and tomato (Adams-Phillips et al., 2004) have been described as climacteric fruit. Blueberry has also shown to respond to ethephon, a ethylene releasing plant growth regulator (PGR) by accelerating fruit ripening in RE types (Wang et al., 2018). Ethylene treatments have also shown to promote sucrose metabolism and fruit softening in blueberries (Wang et al., 2020). Further, genotype has been implicated as a factor to ethylene

response (Costa et al., 2018). However, there is currently no consensus on whether blueberries are climacteric or not, as others have described blueberry fruit softening (Sun et al., 2013) and anthocyanin accumulation (Oh et al., 2018) to occur after application of abscisic acid (ABA). Buran et al., (2012) found that ABA had an opposite effect by delaying ripening of blueberry. Regardless, both tomato and blueberry are similar in being soft fruit that rapidly undo changes in firmness throughout the ripening process, and the cause for decrease in shelf-life can be attributed to fruit softening.

Conclusion

Overall, there were few significant findings of gene expression differences between cultivars that varied in shelf-life of each type. The lower percent healthy fruit NHB cultivar 'Nelson' showed higher transcript abundance of *XTH 1* and *XTH2*, especially at TP1 compared to 'Draper' that had a better shelf-life. If the expression of these *XTH* genes in 'Nelson' compared to 'Draper' is associated with higher self-life in NHB, this warrants more studies. Transcript abundance of *β-Gal* was higher during TP1 than TP3 across all cultivars and types. SHB type of lower percent healthy fruit 'Suziblue' had higher transcript abundance of *β-Gal* at TP1 compared to 'Keecrisp' which displayed a longer shelf-life and compared to all other cultivars. If the expression of these *β-Gal* genes in 'Suziblue' compared to 'Keecrisp' is associated with higher self-life in SHB, this also warrants more studies. Last and unexpectedly, higher shelf-life SHB cultivar 'Keecrisp' had a higher transcript abundance of *β-Hex1* than all other cultivars. In future studies, it would be interesting to jointly examine differential expression of genes in multiple cultivars with a type that varies in shelf-life, Moreover, relating transcript abundance of cell wall degrading genes to ethylene and/or ABA biosynthesis and cell wall degradation may help to understand the link between fruit softening and hormonal interactions.

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Table 5.1. Candidate cell wall degrading genes displaying high transcript abundance in ripe fruit, from the 'Draper' genome and corresponding 'Powderblue' and 'Suziblue' PacBio (PB) transcriptome sequences showing highest identity using BLASTn.

Gene of interest	'Draper' target	'Powderblue' PB Sequence	'Suziblue' PB Sequence
α-Man 1	VaccDscaff17-snap-gene-362.37	PB.9647.3	PB.5689.1
α-Man 2	VaccDscaff32-processed-gene-325.3	PB.9167.1	PB.9974.1
β-Hex 1	VaccDscaff22-augustus-gene-75.22	PB.2232.2	PB.2287.2
β-Hex 2	VaccDscaff29-snap-gene-158.28	no hits	PB.11132.1
β-Gal	VaccDscaff2-augustus-gene-431.29	PB.6708.4	PB.7211.6
β-mann	VaccDscaff32-processed-gene-198.25	PB.9045.1	PB.9858.1
PE	VaccDscaff34-augustus-gene-2.29	PB.5490.3	PB.10624.1
PG	VaccDscaff43-augustus-gene-258.27	PB.13151.1	PB.14290.2
XTH 1	VaccDscaff4-augustus-gene-250.24	PB.14933.1	PB.8662.1
XTH 2	VaccDscaff20-augustus-gene-157.23	PB.14860.1	PB.12986.1
XYL 1	VaccDscaff27-snap-gene-85.30	PB.5253.1	PB.10304.4
XYL 2	VaccDscaff31-augustus-gene-30.33	PB.15667.1	PB.925.1

Table 5.2. List of genes of interest and reference genes, sequences of primers, and primer concentration used in qualitative RT-PCR.

Gene Name	Forward Primer Sequence [5'-3']	Reverse Primer Sequence [5'-3']	Primer concentration (µL)
Gene of Interest			
α-Man 1	GCGAGTCTATCCTGGCAGATTCTG	GTCCCCCATGGCCCCAAAAGTT	0.2
α-Man 2	TACCTCCTCTTTGGGGACAACA	TTTGGCGGCAGTAGAAGTCTCA	0.15
β-Hex 1	TCAACCCCTTGAAGCCAAAGAC	TCAAGAAGTTGGCTGAGGGTTCCA	0.2
β-Hex 2	TGTGGTGCATAACTGGTTGGGT	TTGCTCCACAAACGCTCTGCAGCT	0.15
β-Gal	CATATACAGACAGCTGCGAGGGTT	GGCAACATCTGCGTCTTGGATGTT	0.2
β-mann	ATGTGGCTTCAGACCCATCGCAGA	CCATACCTTCTTGCCTCCGCTATA	0.2
PE	GCGGGAGTGTATGTGGAGAATGTG	AGCCTCTGCCTACAACAGCGAAAG	0.2
PG	ACGGATAATCGGTTCCTCCCCTTT	TTCAAGTGGATGCCGACTCCCATG	0.15
XTH 1	TCTGGAACAATGGCTGCTACAC	CCAAGGATAGCTGAATCTCGGA	0.2
XTH 2	TATCGAAATGCGACCACGCATC	CACACATTCGGGTGGAGGAACTTT	0.2
XYL 1	CCCAAGCCCCTAACCAAGTCTCAA	ACCCTCAAATGGACGTCGAAAGCT	0.15
XYL 2	ATGTTGATGGGTGCTGGTGATGGG	GCAGCTCGCTTTACCTTGTTGT	0.2
Reference Gene			
CACSa	TTGGATGGCGAAGAGAGGGTCTT	CCCAACTTCAAATCAGGCATTCCAG	0.2
UBC28	CCATCCACTTCCCTCCAGATTATCCAT	ACAGATTGAGAGCAGCACCTTGGA	0.2
RH8	GGTGAATCGAGTAGAACTGCTGGC	AGATTCCTGCATGCACCATTCCGA	0.2

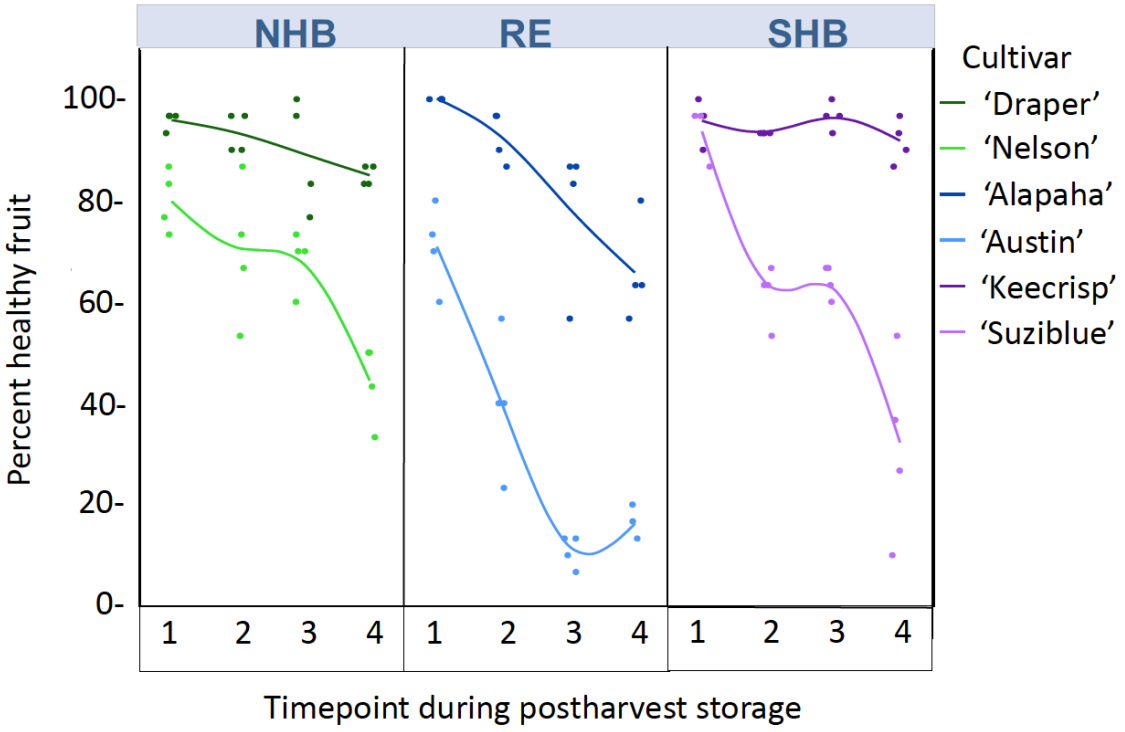

Fig. 5.1. Fresh fruit from cultivars within three commercial blueberry types [northern highbush (NHB), rabbiteye (RE) and southern highbush (SHB)] were collected during the 2018 season and were evaluated for percent healthy fruit at four timepoints in cold storage: 1) 3-4 days, 2) 10-11 days, 3) 20-21 days, 4) 30-31days after fruit collection. To study the gene expression relating to cell wall degradation, cultivar extremes were selected within each type: 'Draper' and 'Nelson' (NHB), 'Alapaha' and 'Austin' (RE), and 'Keecrisp' and 'Suziblue' (SHB).

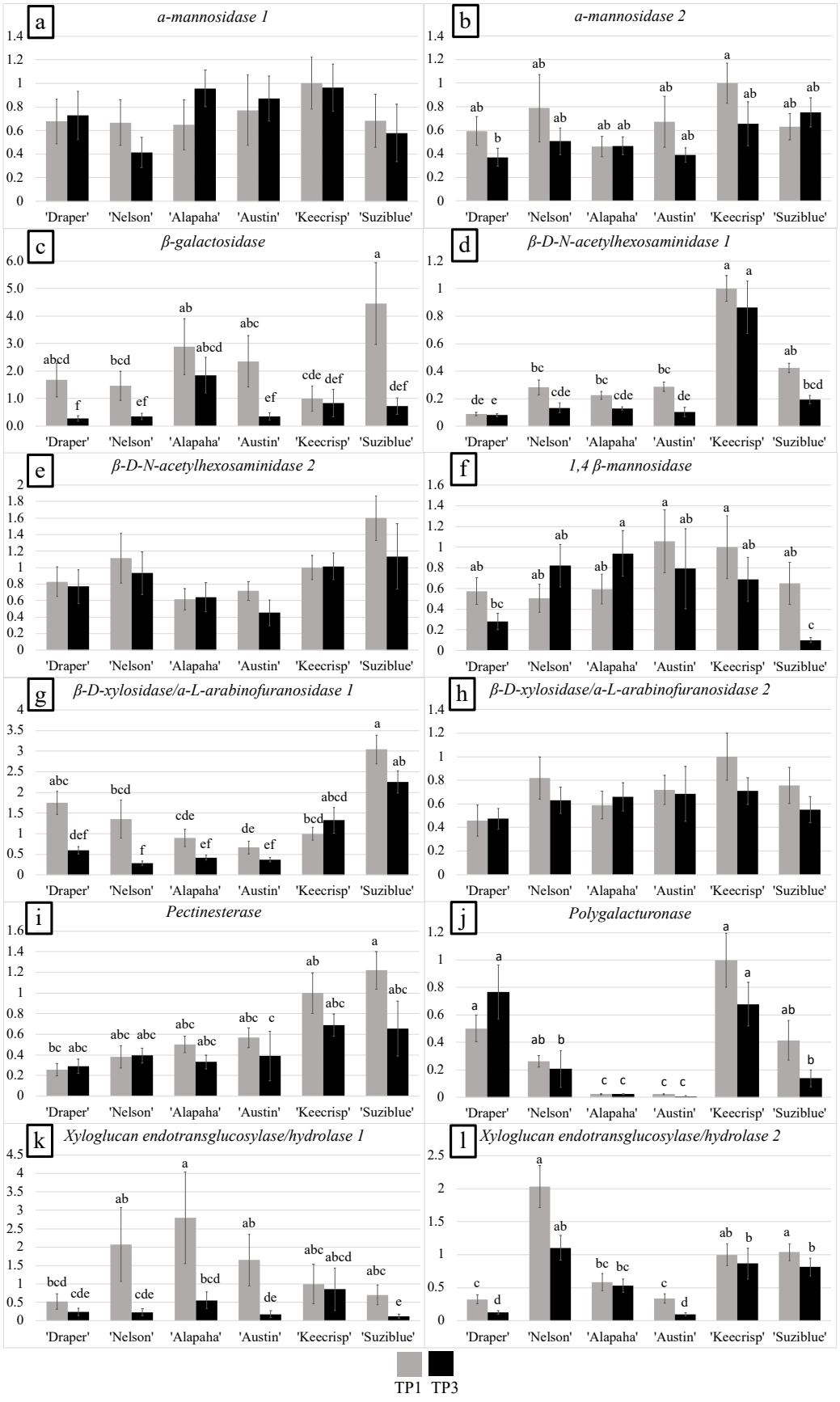
a
a-mannosidase 1
b
a-mannosidase 2
c
β-galactosidase
d
β-D-N-acetylhexosaminidase 1
e
β-D-N-acetylhexosaminidase 2
f
1,4 β-mannosidase
g
β-D-xylosidase/α-L-arabinofuranosidase 1
h
β-D-xylosidase/α-L-arabinofuranosidase 2
i
Pectinesterase
j
Polygalacturonase
k
Xyloglucan endotransglucosylase/hydrolase 1
l
Xyloglucan endotransglucosylase/hydrolase 2
'Draper'
'Nelson'
'Alapaha'
'Austin'
'Keecrisp'
'Suziblue'
TP1
TP3

Fig. 5.2. Relative gene expression of 12 cell wall degrading genes examined in cultivar extremes for postharvest keeping quality within each blueberry type. High and low keeping quality, respectively, were identified in ‘Draper and ‘Nelson’ (NHB), ‘Alapaha’ and ‘Austin‘ (RE), and ‘Keecrisp’ and ‘Suziblue’ (SHB) during the 2018 harvest season during timepoint 1 (TP1;3-4 days after collection) and timepoint 3 (TP3; 20-21 days after collection) of postharvest cold storage. The first listed cultivar of each type is that of higher percent healthy fruit compared to the second listed. Means separation was performed using Tukey’s HSD using ANOVA ($P \leq 0.05$). Means followed by a different lowercase letter are significantly different.

CHAPTER 6

CONCLUSIONS

There is a subjective bias that is often debated in the blueberry industry that the fruit quality of southern highbush is superior to rabbiteye. In addition to this, the fruit quality of southern highbush and rabbiteye is often compared to the third major commercial blueberry type, northern highbush which is perceived to have the highest fruit quality. This bias exists for both quality at harvest and throughout postharvest storage. In this study, a comprehensive comparative study of multiple cultivars and types during 2018 and 2019 was conducted to alleviate bias that exists throughout the industry. Physical and chemical fruit quality characteristics of early, mid, and late season southern highbush, rabbiteye, and northern highbush cultivars that are representative of the current blueberry market were compared in postharvest cold storage. Gene expression of cell wall degrading enzymes of cultivars of all three blueberry types that differ in postharvest shelf-life were also investigated.

Physical quality characteristic comparison showed that southern highbush types had significantly higher fruit firmness and skin strength than northern highbush types for both harvest seasons. The data suggest that southern highbush and northern highbush types had the best stability of most physical fruit quality characteristics in commercial cold storage, having the least amount of change in traits from TP1-TP4. This suggests that genotype alone does not account for the differences between these types, as many additional environmental factors need to be considered. These results are contrary to subjective biases that rabbiteye have firmer fruit than highbush types. Chemical quality characteristic comparison showed data that suggest that

no type was superior than the other in maintaining chemical quality characteristics during postharvest commercial cold storage. Differential gene expression showed that southern highbush type 'Suziblue' had higher transcript abundance of *β-galactosidase* (β-Gal) than southern highbush type 'Keecrisp', 'Keecrisp' had unexpectedly highest transcript abundance of *β-D-N-acetylhexosaminidase/ β-hexosaminidase 1* (*β-Hex1*) than all cultivars, 'Keecrisp' and 'Suziblue' had highest transcript abundance of *pectinesterase* (*PE*) compared to other types, and northern highbush type 'Nelson' had higher transcript abundance of *xyloglucan endotransglucosylase/hydrolase 1* and *2* (*XTH1 and XTH2*) than northern highbush type 'Draper'. These results suggest that the industry bias against certain blueberry types may be outdated and needs further research. The textural, visual, chemical, and genetic changes throughout postharvest cold storage included in this study will provide growers and retailers with the specific keeping quality of individual types and cultivars for their designated markets. In addition, cultivars with the best shelf-life and quality parameters have been identified for prolonged shipping or marketing.

In addition to the research conducted, it would be beneficial to examine other potential sources of year to year variation present. Pre-harvest factors and environmental factors could be underestimated when considering the stability of good fruit quality in postharvest and would be worth further investigation. Although genetic differences may exist between and within blueberry types, it is equally as important to avoid excessive handling and process fruit destined for the fresh market in the most efficient manner to prolong shelf-life. In a general sense, for every hour that passes after harvest without chilling, this decreases the shelf-life by a full day (D. Picha, personal communication May 2015). Cultural practices such as the immediate removal of field heat should continue to be prioritized in order to prolong shelf-life. New tools that are

available to growers and packers in order to improve the quality of fruit that consumers receive include highly advanced sorting machines. These provide consistent quality, high throughput operations, and favorable labor requirements. Unfortunately, although handling may be minimized, it is difficult to be completely removed. In larger packing operations, it is also difficult to maintain warehouses at optimum temperatures due to constant influx and volume of fruit entering packing houses during the busy harvest season.

As reported in chapters 3-5, physical and chemical keeping quality of individual cultivars was expectedly inconsistent between the 2018 and 2019 harvest seasons, so it may be difficult to pinpoint any single pre-harvest factors that contribute to superior shelf-life and keeping quality. More information about the impacts of pre-harvest and environmental factors on fruit quality and how they may affect different blueberry types may be necessary to better understand how fruit quality is best maintained in postharvest commercial cold storage. Directing a focus to understanding of harvest timing, anthesis time, and handling processes would greatly benefit to the understanding of blueberry shelf-life. In conclusion, there are differences in physical, chemical, and genetic keeping quality between and within the three major blueberry types during postharvest commercial cold storage.