COMPARISON OF CRISP AND STANDARD FRUIT TEXTURE IN SOUTHERN HIGHBUSH BLUEBERRY USING INSTRUMENTAL AND SENSORY PANEL TECHNIQUES

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2013

To my grandmother: Lillian Detweiler

ACKNOWLEDGMENTS

I am especially grateful to my advisor, Dr. James Olmstead, who gave me the opportunity to pursue this degree and participate in the blueberry breeding program. Thank you for allowing me so much freedom with this project while always being available and ready to offer help and a word of encouragement along the way! I thank the members of my committee for their willingness to invest their time and expertise in me and my research: Dr. Don Huber, Dr. Harry Klee, Dr. Steve Sargent and Dr. Wilfred Vermerris. I am also grateful to Dr. Paul Lyrene, Dr. José Chaparro, and Dr. Wayne Sherman: plant breeders and mentors that I have had the privilege and honor to work with since first coming to the University of Florida for my master's degree.

I am grateful to David Norden and Werner Collante for their help in the field and in the laboratory. I thank the many members of my lab and office for their friendship, encouragement, and participation in my research studies: Patricia Hilda-Rodriguez, Rachel Itle, Silvia Marino, Gerardo Nunez, Jessica Gilbert, Sarah Taber, Aparna Krishnamurthy, Elton Goncalves, and Piyasha Ghosh. I have so much enjoyed working along side you all! I am grateful for the assistance of Micah Weiss, Rachel Odom, Elizabeth Thomas, Dana Ciullo, Catherine Cellon, Kyle Guerrero, Alexandra Rucker, Shane Dluzneski, and Ashley Leonard for their help on this project. Many hands make light (and more pleasant) work! For the use of field space and plant material, I thank Alto Straughn and his staff.

The love and care of my church community, dear friends, and wonderful family makes my life truly rich, and I am so grateful for them. And to the Lord Jesus, whose loving-kindness towards me is undeserved, but so gratefully and gladly enjoyed.

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August 2013

Chair: James Olmstead
Major: Horticultural Sciences

A novel texture most often described as “crisp” has been identified in the southern highbush blueberry (SHB, *Vaccinium corymbosum* L. hybrids) germplasm at the University of Florida (UF). Two releases from the UF SHB breeding program, ‘Bluecrisp’, and ‘Sweetcrisp’, possess this crisp fruit texture, and many advanced seedling selections have been subjectively identified. Berries with this crisp texture are of particular interest due to their enhanced eating quality, prolonged postharvest life, and potential value for mechanical harvesting for fresh marketed blueberries. The objective of this research was to use compression and bioyield force measures to characterize crisp and soft-textured SHB genotypes determined by a trained sensory panel, evaluate how genotypes of these texture classes varied in ethylene sensitivity, cellular structure, and cell wall composition. The sensory and instrumental tools developed were then used to phenotype seedling populations from putative crisp parents to determine segregation patterns of crisp texture in SHB.

Instrumental measures of compression and bioyield forces correlated with sensory scores for bursting energy, flesh firmness, and skin toughness. Compression

firmness was then measured in crisp and soft-textured genotypes after preharvest treatment with an ethylene inhibitor, 1-methylecyclopropene, which proved ineffective at increasing firmness in either genotype. Cell type, size, shape, packing, and peel thickness were analyzed by light microscopy in four soft, four crisp, and one intermediate-textured genotype, which were found to vary in cellular structure traits between genotypes, but not between textural classes. Cell wall composition was evaluated in berry skin and flesh from three soft, three crisp, and one intermediate-textured genotype at two maturity stages (pink and ripe fruit) and after three postharvest durations at 3ºC. No differences between texture classes were found for total alcohol insoluble residue which contained primarily cell wall material, uronic acids, or neutral sugars. Sensory and instrumental methods of phenotyping were used to evaluate segregation patterns in five F_1 populations, and four of the five populations fit expected segregation ratios for single gene inheritance with incomplete dominance in an autotetraploid.

CHAPTER 1
INTRODUCTION

Florida Blueberries

Taxonomy

Blueberry is a member of the heath family (*Ericaceae*), and belongs to the genus *Vaccinium*. The *Ericaceae* is the largest family within the Ericales and is composed of 116-125 genera, with as many as 3,500 species worldwide (Walters and Keil, 1996). It is a family of small trees, shrubs, and woody vines that grow well in extreme climates including nutrient poor or acid soils (Walters and Keil, 1996; Fralish and Franklin, 2002). Members of this family have perfect flowers with five fused petals and 10 stamens. Their leaves are simple, entire, and evergreen or deciduous depending on location (Fralish and Franklin, 2002).

The genus *Vaccinium* has traditionally been divided into two subgenera: *Oxycoccus* and *Vaccinium*. Subgenera *Oxycoccus* represents the cranberries and *Vaccinium*, in which cultivated blueberry species are found, is composed of approximately 20 sections that are defined by having thicker, woody shoots and bell-shaped flowers. Commercial blueberries belong to the section *Cyanococcus* that includes approximately 16 species (Uttal, 1987). However, opinion about the division of taxa varies due to the high degree of interbreeding that occurs within these widely diverse populations (Camp, 1942; Vander Kloet, 1983; Uttal, 1987). Ten species are native to Florida: one from section *Polycodium* (*V. stamineum* L.), one from section *Batodendron* (*V. arboreum* Marsh.), and eight species from section *Cyanococcus (V. myrsinites* Lam.*, V. darrowii* Camp*, V. tenellum* Aiton*, V. amoenum* Aiton*, V. virgatum* Aiton (formerly *V. ashei*)*, V. fuscatum* Aiton (or *V. corymbosum* L.)*, V. australe* Small,

and *V. elliottii* Chapm.) (Ward, 1974; Lyrene, 1997). Within the section *Cyanococcus,* species range from diploid (2x) to hexaploid (6x), and cultivated highbush blueberries (*V. corymbosum*) are considered to be autotetraploid (Lyrene, 2003).

Breeding and Early Cultivation

The first breeding efforts toward the cultivation of wild blueberries was begun by Frederick Coville in 1911 using 'Brooks' (a wild *V. corymbosum* selection from the mountains of southern New Hampshire) and 'Russell' (a wild *V. angustifolium* Aiton selection from New Hampshire) to produce the first artificial hybrid (Coville, 1937). The first cultivars from Coville's work were introduced in 1920: 'Pioneer', 'Cabot', and 'Katherine'.

Rabbiteye blueberries (*V. virgatum*) growing wild in the panhandle of Florida were collected and grown commercially in Florida in the 1920s, but with little success due to the small fruit size and lack of uniformity associated with wild seedlings (Moore, 1965). The first breeding efforts in Florida began in 1940 and resulted in the release of two rabbiteye cultivars ('Coastal' and 'Calloway') in 1950. A breeding program was begun at the University of Florida (UF) in 1949 to develop low-chill highbush cultivars with the high quality and short fruit development period (FDP) of northern highbush species and low chill adaptability from Florida native species of several ploidal levels, including *V. myrsinites* (4x), *V. darrowii* (2x), and *V. virgatum* (6x) (Moore, 1965; Sharpe, 1953: Lyrene, 1997). In 1976, 'Sharpblue' was released as the first southern highbush blueberry (SHB, *V. corymbosum* interspecific hybrids) cultivar (Sharpe and Sherman, 1976). After 64 years of breeding at UF, over 30 SHB cultivars have been released and now support a substantial blueberry industry in Florida (U.S. Department of Agriculture (USDA), 2013).

Flowering and Fruit Development

Flower bud initiation occurs during the summer, and buds develop during the fall and winter to produce fruit the following spring (Shutak and Marucci, 1966). Growth slows in the fall in response to lower temperatures and short day lengths, and the plant enters a period of dormancy in which tissues become increasingly acclimated or hardened in areas where temperatures drop below freezing (Gough, 1983; Darnell et al., 1992). After the chilling requirement of dormancy is satisfied, heat units are accumulated that enable buds to swell and bud break to occur (Darnell et al., 1992). In Florida, where prolonged cold temperature periods are not frequent, the chilling accumulation can be less than 300 hours (between 0-7 °C).

Fruit is produced on one-year-old wood, with the general trend of fruit size increasing as wood diameter increases (Shutak and Marucci, 1966). Single flowers are attached by the pedicel to the peduncle to form a cluster (Gough, 1983). Flowers are white or pink in color and consist of five petals fused into a corolla, five fused sepals surrounding an inferior ovary, ten stamens, and a pistil (of greater length than the stamens) which together are inverted and resemble the shape of a bell or urn (Shutak and Marucci, 1966; Gough, 1983). Pollen is shed as tetrads that are able to produce four pollen tubes (Darnell et al., 1992). Honey bees and bumble bees are the principal pollinators of blueberries, and are attracted to nectar produced by nectaries located at the base of the corolla (Shutak and Marucci, 1966). Temperatures below 13 °C, winds above 15 mph, rain, and humidity, are all factors affecting bee activity and pollination (Gough, 1983). Cultivars should be planted in alternating rows or coupled rows to facilitate cross pollination due to reduced yield and berry size that results from the

parthenocarpic fruits of self pollinations (Shutak and Marucci, 1966; Cano-Medrano and Darnell, 1997).

The fruit development period, from petal abscission to berry ripening is variable depending on cultivar and location, but can be as short as 50 to 60 days (Shutak and Marucci, 1966). Blueberry exhibits a double-sigmoid growth pattern characterized by a rapid increase in pericarp size (stage I), rapid embryo development and slowed pericarp growth (stage II), and a final surge in pericarp expansion that coincides with fruit ripening (stage III) (Godoy et al., 2008). The corolla, stamens, and style abscise during the initial stage, leaving a circular scar on the tissue inside the berry calyx (which remains attached to the fruit), along with a dot in its center where the corolla and style respectively were formerly attached (Gough, 1994). Ripening in blueberry begins simultaneous with anthocyanin development or when green fruits initially begin to show pink coloration (Gough, 1994). Shutak et al. (1980) described the stages of ripening according to berry color: immature green, mature green, green pink, blue pink, blue, and ripe. As berries ripen from immature green to the ripe stage, the sugar content increases from 7 to 15%, acidity drops, and size increases due to cell expansion (Gough, 1983). Respiration and ethylene are reported to increase and reach a climacteric peak at the initial stages of coloration and then decrease as berries change from pink to blue (Windus et al., 1976). Ethylene production ranges from 0.5 to 2 μL kg^{-1} h^{-1} for northern highbush to 10 μL kg^{-1} h^{-1} for rabbiteye blueberry (Gross, 2004). Vicente et al. (2007) showed that the greatest change in blueberry firmness also occurred at the onset of color when fruits transitioned from green to 25% blue.

Harvest

At a time when labor costs are increasing and availability is decreasing, the blueberry industry is looking for more affordable ways to harvest their crops while maintaining a high standard of fruit quality that continues to demand a high price when sold for the fresh market (Mehra et al., 2013). The replacement of current hand harvesting practices with machine harvesters offers a substantial economic advantage for growers. Currently, most commercial blueberry cultivars in Florida are not well-suited for mechanical harvest techniques (Mehra et al., 2013). Many factors would need to be considered in order to develop cultivars suitable to mechanical harvest.

Factors that affect the quality of fruit obtained by mechanical harvest include fruit detachment force (FDF), fruit abscission zone, plant architecture, fruit firmness, and the uniformity of fruit ripening. When FDF was measured on mature green, unripe red, and fully ripe blue fruits from ten SHB genotypes, Sargent et al. (2010) found that green fruits have a higher FDF (1.8 to 3.5 N) than blue fruits (0.7 to 1.5 N). Red fruits had a lower FDF than green fruits in two genotypes and a higher FDF in one genotype evaluated (Sargent et al., 2010). In blueberry, fruit abscission occurs primarily at the pedicel-peduncle junction, which can result in “stemmy” fruit that is unmarketable (Vashisth et al., 2012). Variability has been observed among cultivars for both the force required to detach fruits and the degree to which stems are retained in detached fruits using a hand held shaking device (Malladi, 2013). Abscission agents such as methyl jasmonate and ethephon, have been found effective in facilitating fruit detachment in rabbiteye and SHB (Malladi et al., 2012). The goal of these studies was to identify abscission agents in conjunction with cultivars of decreased stem retention and appropriate detachment force that would be suitable for harvest by machine.

Efforts to incorporate architecture and root traits from *V. arboreum* into commercial quality SHB cultivars that could be harvested by machine are being pursued using grafting and hybridization methods (Darnell et al., 2010). *Vaccinium arboreum* (section *Batodendron*), commonly referred to as “sparkleberry”, is a diploid blueberry species that is native to Florida. Plants from this species have a deep root system adapted to the pH of Florida soils and their architecture resembles that of a tree having a monopodial base rather than multiple canes like most SHB cultivars which would make it more conducive to the designs of current machine harvesters (Lyrene, 2011).

Fruit firmness is perhaps the greatest factor affecting the fruit quality of mechanically harvested berries (Mehra et al., 2013). When comparing firmness of rabbiteye blueberries that were hand harvested and those harvested using a machine harvester, NeSmith et al. (2002) found that 20-30% firmness (measured by compression force) was lost in those harvested by machine. When comparing firmness after two weeks cold storage at 1ºC in SHB that were harvested by hand and those with a machine harvester, a study at UF found 6% and 53% soft fruits respectively. The percent of unmarketable soft fruit that was harvested with a machine harvester from 12 SHB genotypes ranged from 1 to 12% (Olmstead, Sargent, and Williamson, personal communication). Appearance, percent shrivel, and percent decay were also measured in SHB harvested by hand and by machine, and showed a decline in each of these fruit quality parameters when harvested by machine (Olmstead, Sargent, and Williamson, personal communication). In the same study, 6 to 30% of fruit harvested by machine was too under-ripe to be marketed, suggesting that plants with increased uniformity in ripening and appropriate FDF may decrease losses due to detachment of immature and under ripe fruits.

Postharvest Storage and Marketing

Berry firmness remains a top priority during postharvest storage. NeSmith et al., (2002) reported a 10-15% loss of firmness in blueberry fruit during the grading and sorting process. Mechanized packing lines are often equipped with a soft berry and color sorter that removes these berries by airflow from the packing line (NeSmith et al., 2002). More targeted detection of soft or damaged fruit has been advanced through the development of sensor technology to nondestructively test fruit firmness and also detect three of the most common postharvest diseases: gray mold, anthracnose, and Alternaria. (Li et al. 2010; Li et al., 2011).

Temperature is well known to affect fruit firmness and postharvest shelf life, and in blueberry, increased benefits to fruit quality are observed as storage temperature is decreased to an optimum low of 1ºC (Ballinger et al., 1978). Blueberry respiration rates range from 2-10 mg CO_2 kg^{-1} h^{-1} at 0ºC to 78-124 mg CO_2 kg^{-1} h^{-1} at 25-27ºC (Gross, 2004). Paniagua et al. (2013) attributed changes in fruit firmness primarily to postharvest moisture loss and suggested that changes in turgor pressure may be the primary cause of fruit softening. To reduce respiration and desiccation, relative humidity should be kept at approximately 95% (Tetteh et al., 2004). Postharvest storage is not recommended to exceed two weeks for low and highbush blueberry and four weeks for rabbiteye cultivars (Gross, 2004).

Elevated carbon dioxide is known to suppress fungal decay, but the levels necessary to suppress decay in blueberry approaches the limit at which excessive carbon dioxide can cause off flavor, odor formation, and even increased decay (Zheng et al., 2008). Oxygen levels are often lowered to suppress ethylene and decrease the rate of ripening, but low oxygen storage has been shown to have very little effect on

blueberry fruits which are harvested when fully ripe (Alsmairat et al., 2011). While others have reported improved quality of blueberry fruit under controlled atmosphere (CA) storage at 8-15 kPa carbon dioxide and 2-4 kPa oxygen (Beaudry et al., 1998), Hancock et al. (2008) reported that CA had little effect on blueberry fruit quality. Controlled atmosphere storage is rarely used in commercial blueberry production, except during extended overseas shipments (Alsmairat et al., 2011).

In 2012, Florida produced 7,756 metric tons of fresh fruit on just over 1,800 ha of land (U.S. Department of Agriculture (USDA), 2013). Florida receives a higher price for fresh market fruit due to the use of early ripening, low-chill cultivars that give Florida growers an essentially unshared market window from 1 April to 10 May. While low-chill and earliness remain important selection criteria in the UF SHB breeding program, other important traits include increased yield and fruit size to maximize the high costs of land and labor. Labor is especially a growing concern for the future of Florida's blueberry industry, and there is a growing interest in the development of cultivars with increased firmness and adaptability to mechanical harvesting (Yu et al., 2012).

Crisp Texture

Germplasm

Two cultivars considered to have a unique crisp texture were selected from SHB germplasm at UF and released in 1997 ('Bluecrisp') and 2005 ('Sweetcrisp') (Okie, 1999; Olmstead, 2011). Only two other blueberry cultivars are known to have been described as crisp ('Dolores' and 'Hortblue Poppins'), but the texture of these cultivars has not been compared with the crisp cultivars from UF (Clark and Finn, 2010; Scalzo et al., 2009). Many unreleased selections in the UF SHB breeding program are also considered to have a crisp phenotype similar to 'Bluecrisp' and 'Sweetcrisp'. Berries

with this crisp texture are of particular interest due to their potential contribution to the development of SHB cultivars able to withstand the impacts of mechanical harvesting, and maintain high fruit quality that can continue to be sold at a high price for the fresh market. The crisp texture has also improved postharvest fruit quality and duration (Mehra et al., 2013), and may appeal to consumer preferences for increased firmness.

Sensory Perception

Texture has been defined as “the sensory and functional manifestation of the structural, mechanical, and surface properties of foods detected through the senses of vision, hearing, touch, and kinesthetics” (Szczesniak, 2002). The structural and mechanical properties of fresh fruits are determined by several factors governing cellular structure, including: fruit anatomy and cellular construction, the mechanical and physiological properties of cells, biochemical changes in the cell wall, turgor pressure, and membrane integrity (Harker et al. 1997). These factors contribute to textural traits such as crispness, hardness, juiciness, and mealiness (Harker et al., 1997). Crisp has been defined as “the amount and pitch of sound generated when the sample is first bitten with the front teeth”, with the low and high reference standards being a ripe banana (*Musa spp.*) and fresh potato chip (*Solanum tuberosum*), respectively. (Harker et al., 1997). The noise produced by crisp fruits is the result of cell rupture and cracking in the tissue (Tunick, 2011). Studies have been performed in grape (*Vitis vinifera* L.) and apple (*Malus domestica* Borkh.) using acoustic vibration to measure the degree of crispness (Iwatani et al., 2011; King et al., 2000).

Sensory evaluations of texture are performed by consumers for hedonic characterizations and trained panels are used for profiling and descriptive analysis (Harker et al., 1997; Worch et al., 2010). While texture contributes to consumer

satisfaction as much as flavor, consumers rarely comment on texture unless they are specifically questioned about it, or unless the texture is found to be displeasing or fails to meet expectations (Tunick, 2011; Szczesniak, 2002). Food quality is also associated with texture, such that crisp fruits and vegetables are indicative of freshness and are therefore more desirable by consumers (Szczesniak, 2002). Crisp and soft-textured genotypes of blueberry were evaluated by an untrained sensory panel that was able to decipher between soft and crisp textured berries and give hedonic assessments about the desirability of crisp texture in blueberry (Padley, 2005). Most panelists preferred crisp blueberries (Padley, 2005).

The aim of sensory analysis by trained panels is to quantify the perception of food traits, which requires both consensus between panelists and reproducibility (Worch et al., 2010). Once texture is quantified by sensory measures, it is often correlated with instrumental measures for the purpose of determining structural and mechanical properties contributing to the food's texture and predicting its sensory perception by consumers (Harker et al., 1997)

Texture Measurement

Fruit texture has been measured in a variety of ways, including point of bioyield tests, compression tests, tactile assessment, shearing tests, beam tests, measures of juice content, and sensory evaluations (Harker et al., 1997). Bioyield, shear cell, and compression tests have been most commonly used to measure firmness in blueberry (Ehlenfeldt and Martin, 2002; Padley, 2005; Silva et al., 2005; Saftner et al., 2008). Bioyield force measures the maximum force (N), required to puncture a berry at a certain speed with a probe and can be measured using an Instron texture analyzer (Instron Corporation, Canton, MA). The Kramer shear cell is a multi-bladed fixture that

can be attached to a texture analyzer. The blades first compress, then extrude, and finally shear the fruit inside a metal box. Compression force can be measured with a Firmtech devise designed by Bioworks (Wamego, KS). It measures the mean force (N) required for a flat bottomed plate (3cm diameter) to compress a berry 2mm.

Previous studies have surveyed firmness and correlated sensory perceptions of texture with instrumental measurements in blueberry, but none using the crisp cultivars and advanced selections from UF (Silva et al., 2005; Saftner et al., 2008). In a survey of 87 highbush and species-introgressed blueberry cultivars, Ehlenfeldt and Martin, (2002) found that SHB cultivars, having some *V. virgatum* or *V. darrowii* ancestry, were among the highest in firmness based on compression force measurements, suggesting that low chill species introgression could be a potential source of increased blueberry firmness. The relationship between cultivar firmness and release date suggested that the average gain in blueberry firmness per decade was 0.04 $N \cdot mm^{-1}$, and the authors speculated that epidermal thickness might play a role in the measured firmness (Ehlenfeldt and Martin, 2002). Likewise, Silva et al., (2005) found that shear, compression, and bioyield forces were higher in three low chill rabbiteye cultivars compared with two northern highbush cultivars. In 2006, compression firmness was measured for the fruit of 12 blueberry cultivars (10 northern highbush and two rabbiteye) and was compared with sensory ratings corresponding to fruit qualities such as bursting energy (which the authors describe as “crispness”), skin toughness, juiciness, and texture during chewing (Saftner et al., 2008). The compression firmness values best correlated with juiciness ($r = 0.48$), bursting energy ($r = 0.44$), and texture during chewing ($r = 0.33$), but did not correlate

with skin toughness. None of these studies, however included crisp SHB cultivars in their analyses.

In other fruit crops, crisp texture has been more thoroughly explored. Crisp texture is desirable for table grapes, which are cultivated primarily from the two *Vitis* species *V. labrusca* and *V. vinifera*. Sato et al. (1997) showed that sensory perceptions of crispness correlate with a small deformation and large maximum bioyield force measurement. Using a bioyield test to measure crispness in 87 grape cultivars, it was determined that crisp texture was limited to a small pool within *V. vinifera* cultivars (Sato and Yamada, 2003). Crisp texture is also a desirable trait in apple. Apple texture was measured by King et al. (2000) using a trained sensory panel which correlated with penetrometer and acoustic resonance testing to measure stiffness. These results were used to detect marker-trait associations that could be useful for marker assisted breeding of crisp textured fruit in apple (King et al., 2000). Shear and bioyield force measurements have been used to evaluate crisp genotypes from UF, but were not correlated with sensory evaluations by a trained panel (Padley, 2005).

Cell Structure

Several cellular components contribute to overall fruit texture, including cell type, size, number, shape, packing, cell-to-cell adhesion, extracellular space, and cell wall thickness (Harker et al., 1997).

Parenchyma cells are the most numerous type of cells in the flesh of blueberry. Parenchyma cells have a large, mostly water-filled vacuole and thin, non-lignified cell wall that separates them from other parenchyma cells by a pectin rich middle lamella (Harker et al., 1997). Thickened primary cell walls are found in the specialized parenchyma cells of the epidermis and hypodermis which together form the epicarp,

also known as the fruit's skin or peel (Figure 1-1). The parenchyma cells in the epidermis are also unique in that they produce a thick lipid layer of cuticle and waxes which coats the berry surface and functions in water regulation and pathogen resistance (Fava et al., 2006). The epicuticular waxes of blueberry give the otherwise dark pigmented fruit its powdery blue color and have been described to vary in form from amorphous to that of short, narrow rods (Gough, 1994; Fava et al., 2006).

Collenchyma cells and phloem elements also have thickened primary cell walls that provide tensile strength to surrounding tissues. Xylem and sclerenchyma cells such as fibers and sclereids have thick and lignified secondary cell walls, and can be found associated with vascular bundles and stone cells in the berry's flesh (Harker et al., 1997; Gough, 1994).

Cell size varies between fruit species from cross sectional diameters of 40 µm in avocado (*Persea americana* Mill.) to 500-700 µm in watermelon (*Citrullus lanatus* Thunb.) (Harker et al., 1997). Cell size also varies within species and within genotypes. Cano-Medrano and Darnell, (1997) found that differences in blueberry fruit size between GA treated parthenocarpic fruits and hand pollinated fruits of the same rabbiteye blueberry genotype was a result of differences in cell size. However, Johnson et al. (2011) found that differences in blueberry fruit size between 20 genotypes of rabbiteye blueberry were a result of cell number and not significantly related to cell size. Variability in cell size is more likely to play a role in fruit texture than it has been found to contribute to fruit size (Harker et al, 1997). A study by Mann et al. (2005) compared sensory and instrumental measurements to cell number and size in apple, and concluded that fruits with fewer cells per unit area were more crisp than fruits with more cells per unit area. Large cells have a smaller surface area and lower proportion of cell

wall material than small cells, which is considered to decrease firmness and tissue strength (Harker et al., 1997). However, the cells of crisp-textured fruits are thought to burst rather than separate from adjacent cells, in which case increased cell size may increase the likelihood of cell rupture and therefore contribute to crisp texture as was observed by Mann et al. (2005).

Cell size also varies with different cell types during ripening (Harker et al., 1997). Shortly after anthesis, mesocarp cells stop dividing and increase only in size as the fruit continues to develop and enlarge (Darnell et al., 1992). Cell size is much smaller in the epidermal and hypodermal layers that together form the epicarp, where cell division occurs over a longer period of time during fruit expansion (Harker et al., 1997).

Cell shape and packing determine the amount of contact and/or space found between adjacent cells. A comparison between soft and crisp-textured sweet cherries (*Prunus avium* L.) suggested that crisp cherries have a higher frequency of large intercellular spaces than soft-textured cherries (Batisse et al. 1996). Twenty-five percent of fruit volume in apple (also considered a crisp fruit) is reported to be intercellular space (Esau, 1977). The degree to which adjacent cells separate during chewing has an effect on its perceived texture. In the process of chewing, fruit is compressed to the point of fracture, which can occur by separation of adjoining cells – as is the case with soft fruits such as banana – or by individual cell rupture in crisp fruits such as apple and watermelon (Harker et al., 1997). Whether cells separate or rupture is dependent on cell wall strength and the degree of adhesion between cells, which is affected by the amount of cell-to-cell contact, the strength of the pectin rich middle lamella, and the number of plasmodesmata between cells (Harker et al., 1997).

Cell wall thickness and strength may be the greatest overall contributor to fruit firmness and texture (Goulao and Oliveira, 2008; Li et al., 2010). The primary cell wall is a complex matrix composed of approximately 30-40% cellulose, 30% hemicellulose, 15-30% pectin, and 5-10% structural protein (Vermerris, 2008). Cellulose is made up of approximately 36 long linear β-(1-4)-D-glucan chains that are tightly packed in parallel and assembled by hydrogen bonding into crystalline microfibrils that can reach hundreds of micrometers in length (Vermerris, 2008). Hemicelluloses are cross linking glycans that hydrogen bond with cellulose microbrils to form the cell wall matrix and require strong alkali to be extracted from the wall (Brummel, 2006; Vermerris, 2008). Xyloglucans and Glucuronoarabinoxylans (GAXs) are the primary forms of hemicellulose in plant cell walls (Carpita and Gibeaut, 1993). The primary wall of most dicots contains approximately 20% xyloglucan and 5% GAX (Zablackis et al., 1995). Glucuronoarabinoxylans (GAXs) are the primary type of hemicellulose found in graminaceaous species, making up 20-30% of their total cell wall, but can also be found to a lesser degree in the cell walls of dicots (Carpita and Gibeaut, 1993). Pectins are highly hydrated and branched polysaccharides that are rich in D-galacturonic acid and have neutral sugar side chains of rhamnose, galactose, and arabinose (Brummell, 2006). Pectins are especially abundant in the cell walls of fruit where they form a gel in the wall matrix and middle lamella where they are more loosely bound and can be extracted with water and chelating agents (Brummell, 2006). Pectins have been found to comprise 30-35% of the total cell wall of blueberry, but instead of glucose being the primary neutral sugar, xylose and arabinose were detected in greater quantity suggesting that xylan may be the primary form of hemicellulose (Vicente et al., 2007).

Secondary cell walls typically have a higher proportion of cellulose, a lower proportion of pectin, and hemicelluloses that are more abundant in xylans and glucomannans which bind more tightly to cellulose (Knox, 2008). These factors contribute to the fact that primary cell walls are extendable during growth whereas secondary cell walls are non-extendable and only form after growth has occurred and the cell shape is fixed (Lee et al., 2011). Unlike primary cell walls, secondary cell walls contain lignin, which is a complex network of phenylpropanoids that bind tightly to cellulose, making the cell wall rigid, strong, hydrophobic, and protected against pathogens (Hatfield and Vermerris, 2001). Monolignols formed in the cytosol are transported to the plant cell wall where they are polymerized by oxidative coupling (Hatfield and Vermerris, 2001). Lignin biosynthesis occurs in fruit tissue and is suggested to persist in fruits during postharvest storage as a stress response to dehydration and pathogen attack (Bonghi et al., 2012).

Blueberry fruits are known to contain “stone” cells, or sclereids, which are sclerified cells that have thick secondary walls with high lignin content. Gough (1983) found these sclereids just beneath the epidermal cell layer in three highbush blueberry cultivars. All three cultivars contained similar development and distribution of sclereids, but differed in the total number found (Gough, 1983). The average size of stone cells is approximately the same as the surrounding cells, but their wall is three to four times thicker than neighboring parenchyma cells and is reported to increase during ripening and postharvest storage (Gough, 1983; Allan-Wojtas, 2001). Visible pitting in the sclereid cell wall allows for exchange of water and nutrients between cells (Gough, 1983; Tao et al., 2009). Sclereids can be found singly, doubly, or in clusters, and can

bind neighboring parenchyma cells, which is considered to increase structure and firmness in the fruit (Gough, 1983; Allan-Wojtas et al., 2001; Fava et al., 2006).

Modification of Cell Structure

Ripening is a major event in fruit development affecting both texture and firmness. Physiological and biochemical changes that occur during ripening include: conversion of starch to sugar, pigment biosynthesis and accumulation, biosynthesis of flavor and aromatic compounds, cell wall degradation and fruit softening (Brummell, 2006; Goulau and Oliveira, 2008). Textural modifications during fruit softening consist mostly of changes to the mechanical strength of the cell wall and breakdown of cell-to-cell adhesion at the middle lamella. These changes are primarily the result of the enzyme initiated solubilization and depolymerization of pectins and hemicelluloses (Goulao and Oliveira, 2008). Depolymerization of pectins is considered to be one of the most substantial and yet variable factors involved in fruit softening of different fruit species (Brummell, 2006). Depolymerization of ionically bound cyclohexane trans- 1,2-diamine tetraacetate (CDTA)-soluble pectins is evident in avocado, but virtually absent in pepper (*Capsicum annuum* L.), banana, and apple (Brummell, 2006). Sodium carbonate soluble pectins are comprised of ester bound glycans such as homogalacturonan, which is a primary component of the middle lamella where cell-to-cell adhesion is maintained (Brummell, 2006). Pectin solubilization has been related to observed swelling of the cell wall in several melting flesh fruits, but both pectin solubilization and cell wall swelling were diminished in the crisp fruits of apple, watermelon, and pear (*Pyrus communis* L.) (Redgwell et al., 1997).

Fruits are typically divided into two categories based on how they ripen. Climacteric fruits exhibit a peak in both respiration and ethylene production that

correspond with phenotypic changes in color, aroma, texture, flavor, and/or other phenomena associated with ripeness (Lelievre et al., 1997, Rhodes, 1970), while non-climacteric fruits do not exhibit one or all of these characteristics. A small respiratory climacteric (from a baseline of approx. 30 ml to a peak of 75 mL CO_2 $kg^{-1}hr^{-1}$) and peak in endogenous ethylene production (from a baseline of approx. 0.3 µl to a peak of 0.4 µl C_2H_4 $kg^{-1}hr^{-1}$) has been observed at the transition from the mature green to the green-pink stage of ripening in blueberry, which has since been described as a climacteric fruit (Ismail and Kender, 1969; Windus et al., 1976; Suzuki et al., 1997). The climacteric nature of blueberry, however, remains questionable due to the low levels of both CO_2 and ethylene that were detected. Ripening responses have also been reported in blueberry fruits treated with exogenous applications of ethylene. Ban et al. (2007) confirmed earlier reports by Forsyth et al. (1977) and Shimura et al. (1986) that application of ethephon (2-chloroethylphosphonic acid), an ethylene-generating compound, advances the onset of ripening by stimulating a decrease in titratable acidity and an increase in anthocyanin and fruit softening. Blueberries harvested at the green and green-pink stage demonstrated increased respiration when treated with ethylene and acetaldehyde (Janes, 1978). These reports implicate ethylene as a potential factor affecting fruit firmness and texture in blueberry.

Crisp and soft-textured cultivars have been identified in peach (*Prunus persica* L.), and studies have found ethylene to be a major factor contributing to the variability in its fruit texture (Ghiani et al., 2011). Three distinct flesh textures have been identified in peach: melting, non-melting, and stony hard. Melting flesh types have traditionally been preferred by consumers for fresh market consumption, but non-melting and stony-hard types offer increased postharvest quality. It was discovered that melting and non-

melting flesh types are controlled by a single gene, where melting demonstrates complete dominance at a single locus encoding polygalacturonase, which is an enzyme involved in pectin degradation (Haji et al., 2005). Stony hard, however is a result of a mutation in a single gene involved in ethylene production (Tataranni et al., 2010).

In blueberry, cell wall degradation is marked by pectin solubilization in the early and intermediate stages of ripening, and increased solubilization of arabinose from pectins and hemicelluloses in the later stages of ripening (Vicente et al., 2007). The depolymerization of hemicelluloses was found to occur throughout all developmental stages in blueberry (green to ripe fruits), but pectin polymers were not broken down during fruit softening (Vicente et al., 2007). Proctor and Miesle (1991), identified pectinmethylesterase (PME) and polygalacturonase (PG) to be present and increasing in ripening blueberry fruit up to the red-blue stage which coincides with the period when pectin is solubilized, anthocyanins appear, and fruit softens. Mielse et al. (1991), also found increasing levels of peroxidase (POD) activity in ripening blueberry fruits up to the red stage. The degree to which ethylene is involved in and/or responsible for signaling the enzymes involved in fruit softening in blueberry remains unclear.

Ethylene sensitive (climacteric) fruits are expected to show negative responses to ethylene inhibitors such as silver thiosulphate (STS), and 1-methylcyclopropene (1-MCP). The use of 1-MCP as a suppressor of ethylene responses in the ripening of both climacteric and traditionally non-climacteric fruit was summarized by Huber (2008). Climacteric fruit treated with 1-MCP have demonstrated ripening responses such as altered ethylene production and respiration, delayed or suppressed softening, altered or delayed volatile emissions, and/or pigment change (Huber, 2008). Non-climacteric fruits, such as grape and strawberry have also shown delayed or decreased ripening in

response to ethylene inhibitors (Tian et al., 2000; Jiang et al., 2001; Chervin et al., 2004; Bellincontro et al., 2006; Ianetta et al., 2006) Preharvest application of 1-MCP to grape resulted in decreased berry diameter, increased acidity, and decreased anthocyanin accumulation (Chervin et al., 2004). Postharvest applications of 1-MCP also resulted in an initial reduction of ethylene production and delayed anthocyanin breakdown in grape (Bellincontro et al., 2006). Postharvest applications of 1-MCP to strawberry decreased ethylene production, fruit softening and anthocyanin accumulation (Jiang et al., 2001).

The effect of postharvest applications of 1-MCP on blueberry is unclear. DeLong et al., (2003) compared the percent marketable fruit among two highbush blueberry cultivars treated at postharvest with 1-MCP, and found no effect on the shelf life of either cultivar. MacLean and NeSmith (2011) evaluated ethylene production, firmness, TSS, and TA in three rabbiteye cultivars treated with 1-MCP after harvest and found increased ethylene production in all three cultivars, decreased firmness in one cultivar, but no effect on TSS or TA content. There are no published reports on the preharvest application of 1-MCP to blueberry fruit.

Turgor is also thought to play an important role in fruit softening (Thomas et al., 2008). Bruce (2003) suggests that all mechanical properties of plant tissue result from interactions between turgor and the cell wall. Turgor interacts with the cell wall, such that when internal cell pressure is high the cell wall is more taut, stiff, and brittle, and therefore more likely to burst when external pressure is applied (Harker et al., 1997). When external force is applied to tissues with low turgor pressure, however, disruption of cell-to-cell adhesion is more likely (Harker et al., 1997). As discussed previously, cells that burst open as opposed to those that remain intact and separate from neighboring cells have different textures which correspond to crisp and soft tissues

respectively (Harker et al., 1997). Shackel et al. (1991) used a pressure microprobe to measure turgor in ripening tomato (*Solanum lycopersicum* L.), and found that turgor increases prior to the onset of ripening and decreases during ripening, but reaches its maximum 2-4 days before color change occurs, indicating that changes in turgor may precede tissue ripening. Tong et al. (1999) compared differences between apple genotypes that remain crisp or soften during postharvest storage and found that crisp genotypes maintained higher turgor pressure and cell wall integrity than soft genotypes. A study of rabbiteye blueberry demonstrated that fruits stored at a lower relative humidity decreased in firmness as weight loss increased suggesting that water loss is a major cause of decreases in berry firmness (Paniagua et al., 2013).

The plasma membrane regulates the transport of water and solutes in and out of the cell and with turgor, is also closely associated with cell wall structure and degradation (Harker et al., 1997). It remains unclear, however, whether changes in turgor pressure and membrane integrity are prescriptive or descriptive of fruit softening and cell wall degradation.

Current Research

The genetic and physiological basis of crispness in blueberry remains to be uncovered. The objective of this research was 1) to use compression and bioyield force measures to identify crisp and soft-textured genotypes determined by a trained sensory panel, then 2) to evaluate how genotypes of these identified texture classes respond to ethylene inhibition, 3) to investigate differences in cellular structure between genotypes, 4) to quantify differences in cell wall composition between genotypes, and 5) to phenotype seedling populations from putative crisp parents in order to determine segregation patterns and the genetic basis of crisp texture in blueberry.

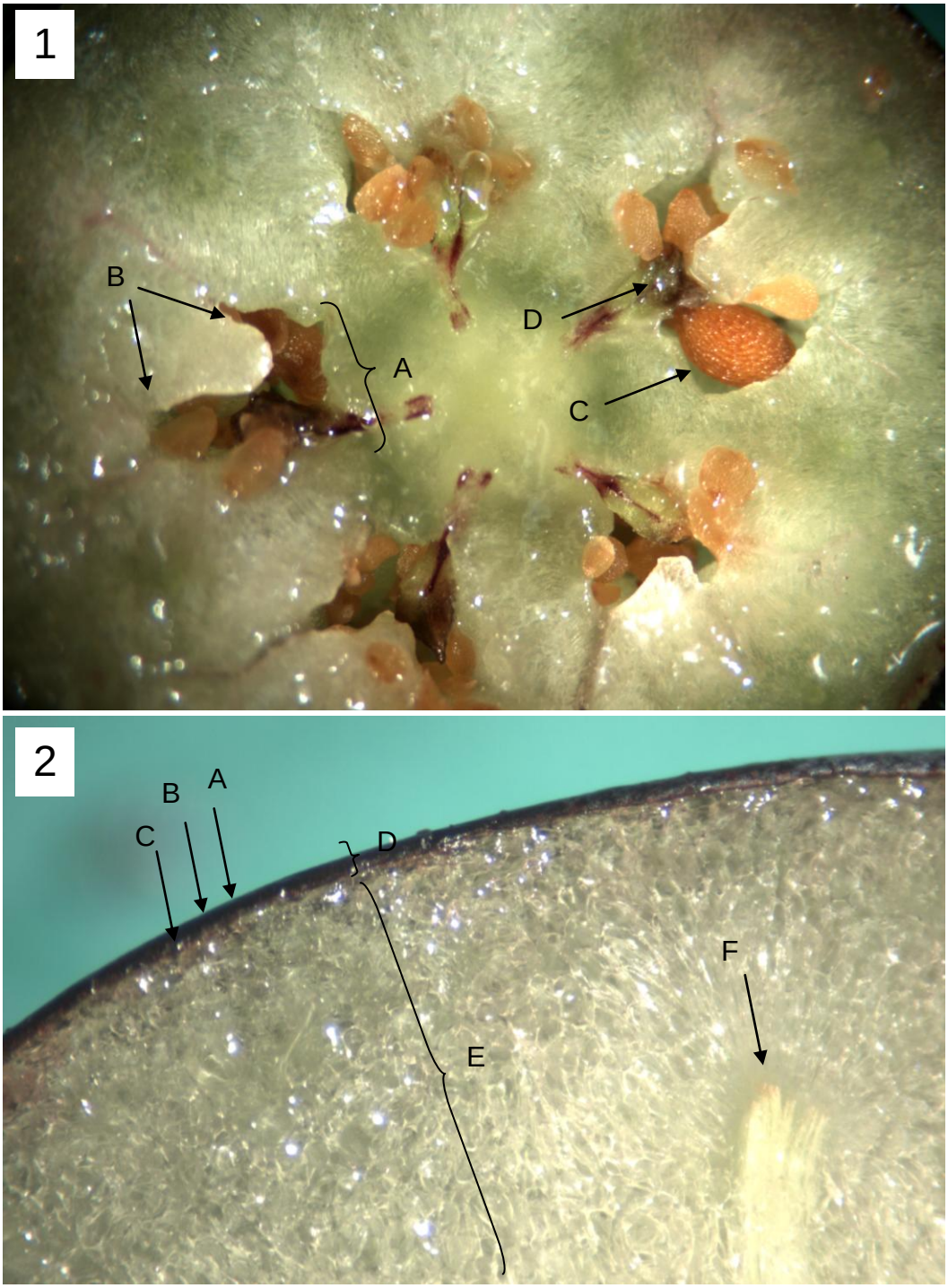

Figure 1-1. Blue fruit of 'Sweetcrisp' (20x magnification) showing the endocarp (1) made up of 5 carpels (A), 10 locules (B), approx. 50 seed (C), and 5 placentae (D). Image 2 shows the cuticle (A), epidermis (B), and hypodermis (C), which together form the epicarp (D). The mesocarp (E) is composed of parenchyma cells, and contains rings of vascular bundles (F). Photos courtesy of Kim Backer-Kelley.

CHAPTER 2
CORRELATION BETWEEN SENSORY AND INSTRUMENTAL MEASUREMENTS OF CRISP TEXTURED BLUEBERRIES

Literature Review

Southern highbush blueberry (SHB, *Vaccinium corymbosum* L. hybrids) production in Florida has increased by 10-fold in industry value and nearly tripled in size of harvested acreage over the last decade. In 2009, Florida ranked second only to Michigan in value of fresh blueberry production (USDA, 2009). The rapid growth of the Florida blueberry industry is the result of increasing demand for fresh blueberry fruit combined with Florida's unique harvest period for fresh blueberry production, from approximately April 1 to May 15. This industry is supported by over 60 years of breeding efforts at the University of Florida (UF) to develop SHB cultivars of commercial fresh market quality that are adapted to Florida's subtropical climate (Lyrene, 2002). These cultivars result from interspecific hybrids between northern highbush (*V. corymbosum* L.) germplasm and sources of low chill traits (usually *V. darrowii* Camp and *V. virgatum* Aiton) (Lyrene, 2002).

As with many horticultural breeding programs, flesh firmness has been a primary fruit quality selection trait. However, in addition to increasing fruit firmness, two cultivars considered to have a unique crisp texture were selected from this SHB germplasm and released from UF in 1997 ('Bluecrisp') and 2005 ('Sweetcrisp') (Okie, 1999; Olmstead, 2011). Previous reports have described a similar fruit texture in other cultivars, and many current selections in the UF blueberry breeding program are also considered to have a crisp phenotype similar to 'Bluecrisp' and 'Sweetcrisp'. Additional cultivars that have been described as crisp are 'Dolores' and 'Hortblue Poppins' (Clark and Finn, 2010; Scalzo et al., 2009). Berries with this crisp texture are of particular interest due to

their enhanced eating quality, prolonged postharvest life, and potential value for mechanical harvesting for fresh marketed blueberries.

Fruit texture is a major factor influencing overall fruit quality. Fruit texture affects both the postharvest life of the fruit, as well as the consumer's eating experience (Harker et al., 1997; Saftner et al., 2008). Additionally, due to rising labor costs and decreasing labor availability for hand harvesting of blueberries, the industry has been looking for ways to mechanically harvest fresh market berries (Strik and Yarborough, 2005). New machine harvesters have been designed and tested for use in blueberry (Peterson et al., 1997; van Dalfsen and Gaye, 1999), and research has been initiated to determine cultural practices and cultivars best suited for mechanical harvesting (Takeda et al., 2008). Several bush and berry traits are thought to be desirable for mechanical harvesting methods, and berry firmness is top among them (Ehlenfeldt, 2005).

Fruit texture is determined by several factors governing cellular structure including: fruit anatomy and cellular construction, the mechanical and physiological properties of cells, biochemical changes in the cell wall, turgor pressure, and membrane integrity (Harker et al., 1997). These factors contribute to textural traits such as crispness, hardness, juiciness, and mealiness (Harker et al., 1997). Fruit texture has been measured in a variety of ways, including bioyield tests, deformation tests, tactile assessment, shearing tests, beam tests, measures of juice content, and sensory evaluations (Harker et al., 1997). Sensory evaluations are performed by consumers for hedonic characterizations and trained panels are used for profiling and descriptive analysis (Worch et al., 2010). Correlating instrumental measures with sensory evaluations is useful for predicting consumer responses while using instrumentation is often desirable for quantitative assessments in breeding.

Previous studies have surveyed firmness and correlated sensory perceptions of texture with instrumental measurements in blueberry, but none using the crisp cultivars and advanced selections from UF (Silva et al., 2005; Saftner et al., 2008). In a survey of 87 highbush and species-introgressed blueberry cultivars, Ehlenfeldt and Martin, (2002) found that SHB cultivars, having some *V. virgatum* or *V. darrowii* ancestry, were among the highest in firmness based on Firmtech 1 (Bioworks, Stillwater, OK) compression measurements, suggesting that low chill species introgression could be a potential source of increased blueberry firmness. Likewise, Silva et al., (2005) found that shear, compression, and bioyield forces were higher in three low-chill rabbiteye cultivars compared with two northern highbush cultivars. Sensory and instrumental correlation studies have been conducted in other crisp-textured fruits such as grape (*Vitis spp.*) and apple (*Malus domestica* Borkh.), but crispness has not been studied in blueberry (King et al., 2000; Mann et al., 2005; Sato et al., 1997; Sato and Yamada, 2003). The ability to objectively phenotype crisp texture in blueberry is important for breeding purposes to identify parents with crisp texture that can be used in developing advanced selections of higher fruit quality and adaptation to mechanical harvest.

The objective of this study was to utilize a broad range of SHB germplasm, including crisp cultivars and selections, to develop descriptors for textural traits using a trained panel, survey the germplasm for firmness differences based on available instrumental measurements, and determine the extent of correlation between trained panel ratings and instrumental measurements of the germplasm.

Methods

Plant Material

Cultivars and selections of southern highbush blueberry were hand harvested from field trials at Straughn Farms, Inc. near Archer, Waldo, and Windsor, FL. Berries were collected on six dates (May 5, 13, 17, 19, and 24) in 2010 from 36 genotypes and on seven dates (April 18, 25, 27, May 2, 5, 9, and 11) in 2011 from 49 genotypes as fruits ripened during the harvest season (Table 2-1). Only mature, fully blue, unblemished berries were harvested. Berries were packed in 170 g plastic vented clamshells (Pactiv, Lake Forest, IL) , stored in coolers filled with ice and transported on the same day to the USDA-ARS research lab in Winter Haven, FL for sensory evaluation and to the blueberry breeding lab at UF in Gainesville, FL for instrumental analyses. At both locations, berries were stored overnight in a cold chamber at 4ºC and brought to room temperature on the next morning before sensory and instrumental analyses were performed.

Sensory Analyses

Eleven to twelve panelists trained to evaluate fruit and fruit products met in four (2010) and six (2011) one-hour sessions to discuss texture descriptors. Descriptors were adapted from Saftner et al. (2008). A consensus was reached to define descriptors: “bursting energy” = impression from the first bite, from mushy to crunchy; “firmness during chewing” = firmness between the molars, from soft to firm; “skin toughness” = amount of residual skin that needs chewing after the flesh is gone, from thin to tough; “graininess” = texture from stone cells or seeds, from smooth to gritty/grainy; “juiciness” = amount of juice from the flesh, from not juicy to juicy;

“mealiness” = pasty, dry feeling in the mouth, from not mealy to mealy; “overall flavor intensity” = blueberry, fruity flavor, from low to high.

Each descriptor was rated on an 11-point scale (0 to 10). To compensate for fruit-to-fruit variability, panelists were instructed to taste two berries at a time, and repeat at least twice. Six to eight berries were presented in 120 mL soufflé cups with lids (SOLO® Cup Company, Urbana, IL), labeled with 3-digit number codes and served at room temperature. Six and five samples were presented per session in 2010 and 2011, respectively, with two sessions per day. Tasting took place in booths under red lighting; spring water and unsalted crackers were provided to panelists to rinse their mouth between samples. To assess panelist and cultivar reproducibility within a harvest season and between years, five cultivars and one numbered selection were evaluated on two days with three and two replications on each day in 2010 and 2011, respectively. Data were collected using Compusense® 5.0 data acquisition and analysis software (Compusense Inc., Guelph, Ontario, Canada).

Instrumental Analyses

Compression and bioyield force were measured on 25 berries from each cultivar in 2010 and 2011. For compression measurements, berries were oriented equatorially upright (Ehlenfeldt and Martin, 2002), on a FirmTech 2 (Bioworks, Wamego, KS) fitted with a 3 cm diameter flat bottom plate load cell. The point of compression was marked with a permanent marker, and the same berries were rotated 90º along the equatorial plane and punctured with a 4 mm probe in 2010 and a 3 mm probe in 2011 using an Instron texture analyzer (Instron Corporation, Canton, MA). Compression firmness ($N \cdot mm^{-1}$) measured the average force required to compress the berry two mm. Bioyield

force (N) was measured as the maximum force required to puncture a berry at a speed of 50 mm·min^{-1}.

In 2011, additional berries from the pooled samples of each genotype were stored at -20ºC to measure soluble solids content (SSC, °Brix), pH, total titratable acidity (TTA), and to assess seed and placentae weight. The total weight of 10 frozen berries and their extracted seed were recorded to determine percent seed weight. Approximately 15 additional frozen berries were processed using an immersion blender (General Electric, model 898683). The mixture was centrifuged at 12,000 rpm for 20 min and the supernatant was filtered through cheese cloth into a 15 mL plastic tube. SSC was measured with a digital refractometer (Atago, Bellevue, WA); pH and TTA (citric acid equivalent) were measured using an automated end-point titrator, titrating 6 mL of juice with 0.1 N NaOH to an endpoint of pH 8.2 (Mettler Toledo, Schwerzenbach, Switzerland).

Data Analyses

Panelist discrimination, reproducibility, and consensus with panel were assessed using the data from the replicated samples and using Senpaq 4.1 sensory software (QiStatistics, Ruscombe, Reading, UK). A general Procrustes analysis (GPA) was also performed to assess panel agreement (Meullenet et al., 2007) using XLStat (Addinsoft, Paris, France). After removing two (2010) and three (2011) panelists for lack of discrimination for some attributes, lack of reproducibility, or not attending all sessions, the means across replications (for replicated samples) and panelists were used to perform a principal components analysis (PCA) using XLStat. PCA was performed using the covariance (n-1) option.

Sensory and instrumental measurements of genotypes replicated on two different harvest dates in one season and between years were analyzed using the mixed procedure (SAS 9.2) with dates as a fixed effect of sensory and instrumental measures and panelists as a random factor of sensory measures.

ANOVA was performed for all genotypes in 2010 and 2011 using the GLM procedure (SAS 9.2) with genotype as a fixed effect of instrumental force measurements and using the GLIMMIX procedure and Kenward-Roger method (SAS 9.2) with genotype as a fixed effect and panelists as a random factor of sensory measurements. Tukey's honestly significant difference (HSD) test was used to determine significant differences ($P \leq 0.05$) between genotype means. Correlation analyses were performed using the correlation procedure (SAS 9.2).

Results

Genotypes

The genotypes selected for use in these experiments represented a wide range of germplasm utilized by the UF SHB breeding program and included recent cultivar releases, standard cultivars, and advanced selections still under trial (Table 2-1). Because a primary goal was to develop descriptors for the crisp texture phenotype, approximately equal numbers of crisp and non-crisp genotypes were selected for analyses each year (18 crisp and 18 non-crisp, and 26 crisp and 23 non-crisp in 2010 and 2011, respectively). For this initial grouping, the determination between crisp and non-crisp was a subjective decision made by the blueberry breeders after several years of observation.

Sensory Analyses

In general, SHB genotypes will ripen over a four to six week period. To evaluate the potential changes in sensory evaluations on multiple harvest dates, six genotypes replicated on two different harvest dates within the 2010 and 2011 season were compared (Table 2-2). There were significant differences in the sensory evaluation of juiciness in 'Emerald', 'Farthing' and 'Springhigh' and in the bursting energy of 'Springhigh' when evaluated on different harvest dates in 2010, but no differences in sensory evaluation due to harvest date in 2011 (Table 2-2). There was no significant year interaction in the sensory evaluation of bursting energy, firmness, skin toughness, juiciness, and mealiness of the six replicated genotypes that were evaluated in 2010 and 2011.

Significant differences between genotypes were observed for all sensory traits evaluated by the trained panels in 2010 and 2011 (Tables 2-3 and 2-4). Bursting energy demonstrated the broadest range of trait variability among cultivars in both 2010 (1.7 to 6.8) and 2011 (1.6 to 8.3). Eleven (2010) and fourteen (2011) Tukey groupings were identified. Selection FL 07-449 had the highest score for bursting energy in both 2010 and 2011. Panelists were able to differentiate genotypes by firmness, skin toughness, juiciness, mealiness, grittiness, and overall flavor but observed less variability in range for these traits and fewer Tukey groupings were identified. Principal components analysis was used as an exploratory technique to identify correlations among variables, to identify groups among samples and to identify potential outliers. The first two principal components explained 94.59 % and 81.81 % of the total variation in 2010 and 2011, respectively. The plot of the first two components showed that juiciness was negatively correlated with mealiness, and there were no correlations with the descriptor indicators

of firmness (firmness, bursting energy and skin toughness) (Figures 2-1 and 2-2). In 2011, adding the variables “graininess” and “blueberry flavor” did not change how juiciness, mealiness, bursting energy, firmness and skin toughness related to each other (compare Figures 2-1 and 2-2), however “blueberry flavor” correlated positively with “juiciness” and negatively with “mealiness”. Likewise, the distribution of genotypes in the PCA plots were similar both years. Most named commercial cultivars, except ‘Raven’, ‘Kestrel’ (2010) and ‘Southern Belle’ (2011), were on the negative side of PC1, indicating low firmness and bursting energy, while most numbered hybrids and ‘Sweetcrisp’ were on the positive side of PC1 (Figures 2-1 and 2-2). ‘Rebel’, ‘Millennia’ and ‘Emerald’ tended to have higher mealiness (or lesser juiciness) both years, as indicated by their position on the F2 axis. Genotypes receiving the highest scores for perceived bursting energy, firmness, and skin toughness were also the same cultivars subjectively identified by breeders at UF to have a unique crisp texture prior to this study (Figures 2-1 and 2-2).

Instrumental Analyses

FirmTech 2 (compression force) and Instron (bioyield force) measures of six genotypes were repeated on two different dates during 2010 and 2011. There was a significant year x genotype interaction ($P < 0.05$), so results within each year were analyzed separately (Table 2-2). Among the cultivars replicated within the season in 2010, compression force measurements were significantly different between the two dates of evaluation for FL 98-325, ‘Emerald’, and ‘Farthing’, but not significantly different for ‘Sweetcrisp’, ‘Springhigh’, and ‘Star’ (Table 2-2). Compression force measurements were likewise significantly different between evaluation dates for FL 98-325 and ‘Emerald’ in 2011, and not significantly different for ‘Springhigh’ and ‘Star’ in 2011.

Bioyield force measurements in 2010 were significantly different between evaluation dates for two cultivars (‘Farthing’ and ‘Star’), but not significantly different for ‘Emerald’ and ‘Sweetcrisp’. In 2011, ‘Emerald’ was the only cultivar for which bioyield force measurements were significantly different between evaluation dates.

There were significant differences between genotypes for compression and bioyield force measurements in 2010 and 2011 (Tables 2-3 and 2-4). Compression force ranged from 1.58 to 3.03 N in 2010 and 1.71 to 2.93 N in 2011, with twenty-two and twenty Tukey groupings identified in 2010 and 2011 respectively. Bioyield force ranged from 1.74 to 5.04 N in 2010 and 1.00 to 2.48 N in 2011, with eighteen and twenty-eight Tukey groupings identified in 2010 and 2011 respectively. The scale and range of bioyield force measurements was different in 2010 and 2011 due to the use of different sized probes, but the relationship of bioyield forces between genotypes within a year was unaffected and therefore correlations of bioyield force with compression force and sensory scores in 2010 and 2011 were comparable. Selection FL 07-449 required the greatest bioyield force in both 2010 and 2011. ‘Bobolink’ had the lowest bioyield and compression force in 2010, and ‘Snowchaser’ had the lowest bioyield and compression force in 2011. Cultivars having the greatest bioyield and compression force measurements were also the same cultivars subjectively identified by breeders at UF to have crisp texture prior to this study.

Seed weight, placenta weight, SSC, pH, and TTA were measured in 2011, but none were significantly different between genotypes. Seed weight varied from 0.0005 to 0.0149% fresh fruit weight and mean placenta weight ranged from 0.2 to 7 mg. SSC ranged from 10.1 to 15.9%, pH ranged from 2.8 to 4.3, and TTA ranged from 0.09 to 1.2%.

Sensory x Instrumental Correlations

Correlations between sensory measurements of bursting energy, firmness and skin toughness were significant at $P < 0.001$ in 2010 and 2011 (Tables 2-5 and 2-6). Mealiness and juiciness were negatively correlated ($P < 0.001$) in 2010 and 2011. In 2011, the additional sensory categories of graininess and flavor were added to panel evaluations. Juiciness was found to be negatively correlated with graininess ($P < 0.01$) and to be positively correlated with flavor ($P < 0.01$) (Table 2-6). Compression and bioyield force measurements of all cultivars and selections were correlated with an R value of 0.78 ($P < 0.001$) and 0.71 ($P < 0.001$) in 2010 and 2011, respectively (Tables 2-5 and 2-6). Individually, compression and bioyield force were highly correlated to sensory perceived bursting energy, firmness, and skin toughness, but poorly correlated to perceived juiciness, mealiness, graininess, and flavor (Tables 2-5 and 2-6). Measurements made in 2011 for seed and placenta weight were not correlated with the sensory evaluation of graininess. Similarly, there were no strong correlations between sensory evaluation of flavor and measured SSC, pH, or TTA.

Discussion

Using previous definitions for texture adopted for consumer evaluations of blueberry fruit (Saftner et al., 2008), we developed blueberry texture descriptors by a trained panel. In the first year, the focus was to describe blueberry texture, and in particular, include subjectively identified crisp-textured blueberry fruit, as this texture had not been analyzed previously. Subsequently, graininess and flavor were developed as additional descriptors by the trained panel based on comments in the first year. Because of the short harvest window in Florida blueberry production (April-May, with an approximately four to six week harvest period for a given genotype), and the limited

number of plants available for many of the advanced selections within the breeding program, the number of replicated genotypes within a growing season that could be provided to a trained panel was limited. Therefore, we adopted a strategy that allowed multiple genotypes to be evaluated by the trained panel while including standard cultivars and selections that could be evaluated multiple times by the panel. For the most part, panelist reproducibility for the replicated cultivars and selections was excellent. The only exceptions were in the category of bursting energy in 2010 for the cultivar 'Springhigh' and in the category of juiciness in 2010, where three cultivars ('Emerald', 'Farthing', and 'Springhigh') were significantly different. The differences between those replications could be due to panelists' inability to measure bursting energy and juiciness in those cultivars that year, or that those cultivars were more variable in bursting energy and juiciness between evaluation dates. It is possible that irrigation could have been a factor affecting perceived juiciness between replication dates. 'Emerald' and 'Springhigh' were irrigated by overhead sprinklers on a three day rotation, while 'Farthing' received drip irrigation daily. It is unlikely that rain was a factor affecting juiciness as rainfall was minimal between evaluation dates, and juiciness increased in 'Emerald' but decreased in 'Farthing' after these light rains occurred.

With PCA, the subjectively identified crisp-textured cultivars and selections form a relatively large group that is most closely associated with bursting energy, firmness, and skin toughness (Figures 2-1 and 2-2). The grouping of these traits may be due to the panelists' inability to differentiate between them, or due to these traits being biologically linked with one another. As one might expect, juiciness and mealiness were inversely proportional to one another. Collectively, there was considerable overlap between Tukey groupings (Tables 2-3 and 2-4), which could have resulted from the panelists'

inability to perceive crispness in a background of other varying textural traits such as berry firmness and skin toughness. Supporting this observation is the relatively broad distribution of subjectively identified crisp genotypes by sensory analyses, and the overlap of the cultivars ‘Kestrel’ and ‘Raven’ with the subjectively identified crisp genotypes. Breeder evaluations of both ‘Kestrel’ and ‘Raven’ have not included them in the crisp category, but the results of this study warrant further examination. It remains unclear whether crispness is in fact a new trait, or the extreme expression of already characterized traits in blueberry such as firmness and skin toughness. Observing segregation patterns from putative crisp parents would help to elucidate the genetic basis of these cultivars considered to have a unique texture.

On the same day that the trained panel evaluations were performed, compression and bioyield forces were measured on fruit harvested from the same plants and genotypes. When these instrumental measures were analyzed, there was a significant year x genotype interaction (Table 2-2). Compared to 2011, the 2010 harvest was delayed by approximately three weeks due to unusually cool spring temperatures that year, which may have been exhibited as instrumentally measured differences, while the relative yearly differences were not apparent to the trained panel. Additionally, significant differences were found between replicated genotypes within a season using these precise compression and bioyield force instruments (Table 2-2). These differences may simply result from changes in management and environmental conditions that can occur rapidly within a growing season. That these significant differences are not evident in the panel evaluations may not be surprising. Ross et al. (2009) found that an analytical value differing by 0.39 $N \cdot mm^{-1}$ using a similar compression force instrument was required before a trained sensory panel could

determine a significant difference in cherry firmness. Given this potential lack of congruence between panel evaluations and instrumental measures, we used a correlative approach to align trained panel results with common instrumental measurements.

In a 2008 study of 12 highbush blueberry cultivars, compression firmness, also measured with a FirmTech 2, best correlated with juiciness (R = 0.48), bursting energy (R = 0.44) and texture during chewing (R = 0.33), but was not associated with skin toughness (Saftner et al., 2008). The reason for lower correlations observed by Saftner et al. (2008) could be due to differences among panels or experimental design, but probably due to the narrow range of cultivar textures evaluated, which did not include several crisp cultivars as was surveyed in this study.

Many of the subjectively identified crisp blueberry cultivars and selections were perceived as having a sweeter flavor, although the correlation between rated flavor and SSC was low (R = 0.27). Saftner et al. (2008) found similarly low correlations between perceived flavor traits and SSC in 12 highbush blueberry cultivars and cited Kader et al. (2003), who reported that anthocyanins (known to be rich in blueberry fruit) could interfere with SSC measures and inaccurately represent total sugars and therefore perceived sweetness. Rosenfeld et al., (1999), however, found strong correlations between SSC and perceived sweetness by trained panelists evaluating blueberries. In the present study TTA and pH were inversely correlated (R = -0.80) but individually were poorly correlated to perceived flavor. Because overall blueberry flavor was the trait evaluated by panelists in this study, low correlations with SSC, TTA, SSC/TTA ratio, and pH may be due to other flavor components besides sweetness and acidity.

Blueberries contain five woody placentae and up to 65 seeds, both of which vary in size by genotype (Gough, 1994). It was speculated that perceived graininess would be related to the amount of seed and size of placentae, but correlations between perceived graininess and measured seed weight (R = 0.28) and placentae weight (R = 0.21) were low in 2011. Like pear, the mesocarp of blueberry contains stone cells known to give fruit a grainy texture, so it is possible that perceived graininess depends more on the number of stone cells than the amount of seed or placentae in the fruit tissue (Tao et al., 2009). Stone cells, also called sclereids, are cells with thickened cell walls containing lignin. Gough (1983) found sclereids just beneath the epidermal cell layer in three highbush cultivars, and thought these structures might contribute to berry firmness. Sclereids can occur singly, doubly, or in clusters, and bind neighboring parenchyma cells and serve to strengthen this tissue (Allan-Wojtas et al., 2001; Fava et al., 2006). The correlation between perceived graininess and compression firmness (R = -0.05) in this study, however, was low. Future work correlating number of stones cells with perceived graininess would be necessary to determine if these lignified cells contribute to sensory perceptions of graininess in blueberry as they have been shown to in pear.

The objective of this study was to develop descriptors for textural traits in blueberry using a trained sensory panel, and survey a broad range of germplasm, including crisp cultivars and selections, to detect differences in firmness and the extent of correlation between trained panel ranking and instrumental measurements of blueberry texture. We found three descriptors that align sensory evaluation of fruit texture and firmness with instrumental measures that could be used for quantitative measurements during breeding selection. Instrumental measures of compression and

bioyield forces were significantly different among cultivars and correlated with sensory scores for bursting energy, flesh firmness, and skin toughness. The results of sensory and instrumental measures support the distinction of crisp and non-crisp cultivars in blueberry, and suggest that crispness is related to both higher compression and bioyield force measurements and to sensory perception of increased bursting energy, flesh firmness, and skin toughness.

The genetic and physiological basis of crispness in blueberry remains to be discovered. Using compression and bioyield force measures developed in this study to identify genotypes of crisp and non-crisp texture could be used to further investigate differences in cellular structure and/or composition between these fruit types. These instrumental measurements could also be used to phenotype seedling populations from putative crisp parents in order to determine segregation patterns and the genetic basis of crisp texture in blueberry.

Table 2-1. Parents of genotypes of southern highbush blueberry cultivars and advanced selections evaluated by sensory panel and instrumental analysis in 2010 and/or 2011.

Genotype	Female Parent	Male Parent
FL 01-15	FL 98-14	FL 98-50
FL 01-25	FL 97-27	FL 92-236
FL 02-22	Bluecrisp	FL 97-139
FL 03-161	FL 96-138	Corindi 95-115
FL 05-252	Sweetcrisp	O.P.
FL 05-256	FL 02-07	FL 98-325
FL 06-244	FL 02-37	FL 00-19
FL 06-245	FL 02-37	FL 00-19
FL 06-300	FL 03-98	FL 90-4
FL 06-552	Sweetcrisp	FL 98-325
FL 06-553	Sweetcrisp	FL 98-325
FL 06-556	Sweetcrisp	FL 98-325
FL 06-558	Sweetcrisp	FL 98-325
FL 06-561	FL 98-325	FL 03-61
FL 06-562	FL 98-325	FL 03-61
FL 06-571	Bluecrisp	FL 02-22
FL 06-572	FL 98-325	FL 03-61
FL 06-80	Sweetcrisp	FL 98-325
FL 06-88	FL 98-325	FL 03-61
FL 07-100	FL 04-60	Farthing
FL 07-160	FL 04-34	Sweetcrisp
FL 07-164	Sweetcrisp	FL 00-180
FL 07-176	FL 03-49	Sweetcrisp
FL 07-23	FL 03-34	Sweetcrisp
FL 07-30	FL 98-325	FL 00-180
FL 07-31	FL 03-49	Sweetcrisp
FL 07-32	FL 03-49	Sweetcrisp
FL 07-38	FL 04-64	Sweetcrisp
FL 07-43	FL 04-21	FL 00-180
FL 07-449	Sweetcrisp	FL 97-136
FL 07-452	Sweetcrisp	Bluecrisp
FL 07-453	Sweetcrisp	FL 98-325
FL 07-87	FL 03-10	FL 00-200
FL 98-325	FL 96-27	Windsor
Bobolink	FL 00-28	FL 98-365
Emerald	FL 91-69	NC1528
Farthing	FL 92-27	Windsor

Table 2-1. Continued

Genotype	Female Parent	Male Parent
Flicker	FL 93-51	FL 93-46
Jewel	Unknown	
Kestrel	FL 95-54	FL 97-125
Meadowlark	FL 84-33	FL 98-133
Millennia	FL 85-69	O'Neal
Primadonna	O'Neal	FL 87-286
Raven	FL 01-26	Windsor
Rebel	Primadonna	O.P.
Southern Belle	Unknown	
Scintilla	Flicker	FL 96-26
Snowchaser	FL 95-57	FL 89-119
Springhigh	FL 91-226	Southmoon
Star	FL 80-31	O'Neal
Sweetcrisp	Southern Belle	FL 95-3
Windsor	FL 83-153	Sharpblue

Table 2-2. Comparison of sensory and instrumental P-values of replicated southern highbush blueberry genotypes evaluated on two harvest dates in 2010, 2011, and between years. ($P < 0.001$***, $P < 0.01$**, $P < 0.05$*).

Year	Sensory/Instrumental Measure	Genotype												All Genotypes	
		FL 98-325		Emerald		Farthing		Sweetcrisp		Springhigh		Star			
2010															
	Bursting Energy	0.613		1.000		1.000		0.604		0.011	*	0.477			
	Firmness	0.794		0.289		0.479		0.771		0.108		0.368			
	Skin toughness	0.572		0.771		0.554		0.534		0.340		0.287			
	Juiciness	0.179		0.032	*	0.027	*	0.760		0.016	*	0.492			
	Mealiness	0.358		0.554		0.744		0.522		0.785		0.800			
	Compression Force	0.005	**	0.001	***	0.029	*	0.935		0.053		0.732			
	Bioyield Force	-		0.943		0.002	**	0.955		-		<0.0001	***		
2011															
	Bursting Energy	0.566		0.764		0.588		0.909		0.051		0.634			
	Firmness	0.848		0.479		0.423		0.986		0.065		0.314			
	Skin toughness	1.000		0.411		0.361		0.639		0.280		0.631			
	Juiciness	0.809		1.000		0.474		0.356		0.736		0.830			
	Mealiness	0.683		0.929		0.684		0.200		0.215		0.563			
	Blueberry Flavor	0.253		0.928		0.308		0.100		0.861		0.132			
	Graininess	0.829		0.699		0.106		0.804		0.811		0.438			
	Compression Force	0.008	**	0.011	*	-		-		0.098		0.210			
	Bioyield Force	0.491		<0.0001	***	0.793		0.071		0.061		0.161			
2010 x 2011															
	Bursting Energy													0.370	
	Firmness													0.645	
	Juiciness													0.979	
	Mealiness													0.723	
	Skin toughness													0.935	
	Compression Force													<0.0001	***

Table 2-3. Mean scores for sensory and instrumental measurements of southern highbush blueberry genotypes evaluated in 2010.

	Sensory					Instrumental	
Genotype	Bursting Energy[z]	Firmness	Skin Toughness	Mealiness	Juiciness	Compression Force ($N \cdot mm^{-1}$)	Bioyield Force (N)
FL 01-25	3.3 d-k	2.7 d-j	3.2 b-e	1.0 c	4.2 a-f	2.12 m-s	1.88 q-r
FL 05-252	6.2 ab	4.8 ab	4.1 a-e	0.9 c	3.8 a-f	2.50 g-l	2.97 f-j
FL 05-256	5.4 a-d	5.0 ab	5.0 ab	1.9 bc	3.1 a-f	2.46 g-l	2.95 f-k
FL 06-244	5.1 a-e	4.2 a-g	4.3 a-e	1.7 bc	3.1 b-f	2.53 f-l	-
FL 06-300	2.8 f-k	2.6 e-j	3.2 b-e	1.5 bc	3.4 a-f	1.81 t-v	-
FL 06-552	5.0 a-f	4.0 a-g	3.6 a-e	1.7 bc	3.4 a-f	2.63 c-h	3.99 bc
FL 06-553	5.0 a-f	4.9 ab	4.2 a-e	1.9 bc	3.5 a-f	2.55 d-j	-
FL 06-556	4.6 a-h	4.2 a-g	4.5 a-e	2.5 a-c	2.9 c-f	2.85 a-d	2.95 f-k
FL 06-558	6.0 a-c	4.9 ab	4.9 a-c	1.2 bc	3.8 a-f	2.84 a-e	-
FL 06-561	6.2 ab	4.7 a-c	4.3 a-e	1.1 c	5.3 ab	2.55 e-j	-
FL 06-562$_1$	4.5 a-i	3.2 b-j	3.3 b-e	1.5 bc	4.3 a-f	2.30 i-o	2.95 f-k
FL 06-562$_2$	4.6 a-h	3.5 a-j	3.8 a-e	0.9 c	4.5 a-e	2.29 i-p	2.50 k-o
FL 06-571$_1$	4.1 b-j	3.9 a-h	4.4 a-e	1.7 bc	3.9 a-f	2.51 g-l	3.76 c
FL 06-571$_2$	4.3 b-j	3.7 a-i	3.7 a-e	1.8 bc	4.0 a-f	2.55 e-k	3.30 d-f
FL 06-572	5.0 a-f	4.9 ab	4.1 a-e	1.5 bc	3.9 a-f	2.82 a-f	-
FL 06-80	6.3 ab	5.3 a	4.2 a-e	1.4 bc	4.0 a-f	3.03 a	3.56 c-e
FL 06-88	6.6 a	5.2 a	4.4 a-e	1.8 bc	2.7 d-f	2.93 ab	3.85 bc
FL 07-100	6.1 ab	4.7 a-d	4.4 a-e	1.0 c	4.9 a-c	2.97 a	4.26 b
FL 07-30	6.0 a-c	4.7 a-d	4.4 a-e	1.5 bc	4.6 a-e	2.64 b-h	3.68 cd
FL 07-449	6.8 a	4.8 a-c	5.4 a	1.2 bc	4.6 a-e	2.62 d-h	5.04 a
FL 98-325$_1$	4.7 a-g	4.5 a-e	4.2 a-e	1.4 bc	3.0 c-f	2.27 j-p	2.74 h-m
FL 98-325$_2$	4.8 a-g	4.3 a-f	4.3 a-e	1.7 bc	3.5 a-f	2.49 g-l	-
Bobolink	1.7 k	1.7 i	3.4 b-e	4.2 a	2.1 f	1.58 v	1.74 r
Emerald$_1$	3.5 d-k	3.0 b-j	3.8 a-e	2.5 a-c	3.6 a-f	2.11 m-s	2.37 l-p
Emerald$_2$	3.5 d-k	3.4 a-j	3.9 a-e	2.8 a-c	2.8 c-f	2.35 h-n	2.38 l-p
Farthing$_1$	4.2 b-j	3.4 a-j	4.3 a-e	1.6 bc	3.4 a-f	2.58 d-i	3.17 e-i
Farthing$_2$	4.2 b-j	3.6 a-j	4.0 a-e	1.5 bc	4.2 a-f	2.36 h-m	2.72 i-n

Table 2-3. Continued.

	Sensory					Instrumental	
Genotype	Bursting Energy[z]	Firmness	Skin Toughness	Mealiness	Juiciness	Compression Force ($N \cdot mm^{-1}$)	Bioyield Force (N)
Flicker	3.2 d-k	3.1 b-j	3.9 a-e	1.9 bc	3.5 a-f	2.10 m-t	1.99 p-q
Jewel	2.4 h-k	2.0 h-j	2.8 de	1.4 bc	4.6 a-d	1.94 r-u	1.97 p-q
Kestrel	5.3 a-d	3.5 a-j	4.0 a-e	1.2 c	4.5 a-e	1.95 q-u	3.24 d-g
Meadowlark	3.7 c-k	2.8 c-j	3.7 a-e	1.7 bc	4.0 a-f	2.28 j-p	2.79 g-l
Millennia	2.3 i-k	2.5 f-j	3.1 b-e	3.4 ab	2.1 f	2.05 n-t	2.12 o-r
Primadonna	2.6 g-k	2.4 f-j	2.5 e	2.2 a-c	3.8 a-f	2.03 o-t	1.91 q-r
Raven	4.7 a-g	4.1 a-g	4.9 a-c	1.9 bc	4.1 a-f	2.92 a-c	3.20 e-h
Rebel	3.1 e-k	2.5 f-j	3.0 c-e	2.2 a-c	2.4 ef	2.25 k-q	1.98 p-q
Scintilla	2.8 f-k	2.7 c-j	3.8 a-e	1.3 bc	4.5 a-e	2.40 h-m	2.66 j-n
Snowchaser	2.1 jk	1.9 ij	2.7 e	1.7 bc	3.0 c-f	1.72 uv	1.91 q-r
$Springhigh_1$	3.4 d-k	2.8 c-j	3.3 b-e	1.1 c	4.1 a-f	1.99 p-u	2.29 m-q
$Springhigh_2$	2.4 h-k	2.3 g-j	2.7 e	1.0 c	5.3 a	2.12 m-s	-
$Star_1$	2.7 f-k	2.9 b-j	3.4 a-e	1.4 bc	3.6 a-f	2.26 j-p	2.67 j-n
$Star_2$	2.8 e-k	2.5 f-j	2.9 c-e	1.4 bc	4.2 a-f	2.24 l-r	2.19 o-r
$Sweetcrisp_1$	6.0 a-c	4.9 ab	4.8 a-d	1.3 bc	4.1 a-f	2.75 a-g	3.93 bc
$Sweetcrisp_2$	6.2 ab	5.0 ab	4.8 a-c	1.2 c	4.0 a-f	2.76 a-g	3.93 bc
Windsor	2.3 i-k	2.7 c-j	2.9 c-e	1.5 bc	3.6 a-f	1.93 s-u	2322.28 n-q

[z]Different letters within a column indicate significant differences between genotypes using Tukey's test ($P \leq 0.05$).

Table 2-4. Mean scores for sensory and instrumental measurements of southern highbush blueberry genotypes evaluated in 2011.

	Sensory					Instrumental	
Genotype	Bursting Energy[z]	Firmness	Skin Toughness	Mealiness	Juiciness	Compression Force ($N \cdot mm^{-1}$)	Bioyield Force (N)
FL 01-15	4.1 g-n	2.8 f-i	3.0 de	1.6 b-d	4.4 a-c	2.50 c-j	1.26 s-A
FL 01-25	3.3 j-n	2.5 f-i	3.8 a-e	1.6 b-d	4.6 ab	2.08 m-s	1.20 y-B
FL 02-22	3.4 j-n	2.5 f-i	3.6 a-e	2.5 a-d	4.1 a-c	2.25 h-p	1.25 t-A
FL 03-161	4.5 f-l	3.1 e-i	3.3 c-e	2.9 a-d	3.8 a-c	2.19 i-q	1.36 r-z
FL 05-252	6.6 a-g	5.1 a-f	4.9 a-e	2.0 a-d	5.1 a	2.38 e-m	1.50 m-s
FL 05-256	6.9 a-f	4.9 a-g	5.6 a-d	2.5 a-d	4.1 a-c	2.27 g-p	1.48 n-u
FL 06-244	5.8 a-j	5.1 a-g	5.0 a-e	2.1 a-d	3.6 a-c	2.53 c-h	1.78 g-l
FL 06-245	2.5 k-n	2.4 f-i	3.3 a-e	2.8 a-d	2.9 a-c	1.81 st	1.03 AB
FL 06-552	7.5 a-c	6.3 a-c	3.9 a-e	1.4 b-d	4.6 ab	2.89 ab	1.95 d-i
FL 06-553	6.5 a-h	5.9 a-e	4.2 a-e	1.6 b-d	3.8 a-c	2.54 c-h	1.54 l-r
FL 06-556	6.6 a-g	5.4 a-f	3.7 a-e	2.4 a-d	2.9 a-c	2.88 ab	1.87 e-j
FL 06-558	7.4 a-d	6.5 a	6.0 ab	2.4 a-d	3.4 a-c	2.40 d-m	1.71 j-n
FL 06-561	7.3 a-d	6.1 a-c	5.4 a-e	0.8 d	4.8 ab	2.51 c-i	1.70 j-o
FL 06-562	6.5 a-h	4.6 a-g	4.2 a-e	1.4 b-d	5.6 a	2.24 h-p	1.42 p-w
FL 06-571	6.1 a-i	3.9 a-i	4.2 a-e	1.3 b-d	4.6 a-c	2.34 f-n	1.71 i-n
FL 06-572	7.8 ab	6.5 a	5.6 a-d	1.6 b-d	4.1 a-c	2.67 a-e	2.01 d-g
FL 06-80	7.1 a-e	6.0 a-d	4.0 a-e	1.6 b-d	4.3 a-c	2.62 a-f	1.80 f-k
FL 06-88	7.4 a-d	5.1 a-f	5.0 a-e	3.0 a-d	4.1 a-c	2.90 a	1.78 g-l
FL 07-100	7.3 a-d	5.8 a-e	6.1 ab	1.3 b-d	4.3 a-c	- -	2.18 b-d
FL 07-160	5.8 b-j	4.5 a-g	4.4 a-e	1.3 b-d	4.5 a-c	2.27 g-p	1.85 f-k
FL 07-164	6.4 a-h	4.8 a-g	5.8 a-d	1.8 b-d	4.6 ab	2.06 n-s	1.99 d-h
FL 07-176	6.4 a-h	5.1 a-f	4.6 a-e	1.8 b-d	3.9 a-c	2.49 d-j	1.76 h-l
FL 07-23	5.8 b-j	4.8 a-g	4.3 a-e	1.9 a-d	4.5 a-c	2.42 d-l	1.49 m-t
FL 07-30	7.3 a-d	5.0 a-g	5.5 a-e	1.0 c-d	4.6 a-c	2.81 a-c	2.10 b-e
FL 07-31	3.9 h-n	3.6 c-i	4.3 a-e	2.5 a-d	4.5 a-c	2.03 n-s	1.41 q-y
FL 07-32	3.8 i-n	2.6 f-i	4.5 a-e	4.0 ab	1.9 c	2.30 g-o	1.73 i-m
FL 07-38	6.3 a-h	4.5 a-g	4.3 a-e	1.0 c-d	4.9 ab	2.12 l-s	1.61 k-q
FL 07-43	6.6 a-g	4.7 a-g	5.5 a-e	1.0 c-d	5.0 ab	- -	1.99 d-h

Table 2-4. Continued.

			Sensory						Instrumental			
Genotype	Bursting Energy[z]		Firmness		Skin Toughness		Mealiness		Juiciness		Compression Force ($N \cdot mm^{-1}$)		Bioyield Force (N)	
FL 07-449	8.3	a	6.1	a-c	5.9	a-c	1.0	c-d	5.1	a	2.49	d-k	2.48	a
FL 07-452	7.3	a-d	6.0	a-d	5.3	a-e	3.4	a-d	3.3	a-c	2.71	a-d	2.32	ab
FL 07-453	6.9	a-f	5.9	a-e	5.5	a-e	2.5	a-d	4.5	a-c	2.58	b-g	1.99	d-h
FL 07-87	4.1	g-n	3.3	e-i	5.8	a-d	1.1	b-d	4.6	a-c	-	-	2.04	c-f
FL 98-325$_1$	6.4	a-h	5.0	a-g	5.0	a-e	1.9	a-d	3.9	a-c	2.19	j-r	1.65	j-p
FL 98-325$_2$	6.1	a-i	4.9	a-g	5.0	a-e	2.1	a-d	4.1	a-c	2.32	f-n	1.62	k-q
Bobolink	3.3	j-n	2.7	f-i	4.1	a-e	3.5	a-d	3.3	a-c	-	-	1.22	v-B
Emerald$_1$	4.3	g-m	3.5	d-i	4.4	a-e	2.9	a-d	3.1	a-c	1.96	p-t	1.44	p-w
Emerald$_2$	4.4	g-l	3.9	b-i	4.1	a-e	2.9	a-d	3.1	a-c	2.10	m-s	1.22	v-B
Farthing$_1$	4.7	e-l	4.1	a-h	4.6	a-e	1.1	c-d	5.3	a	2.17	k-r	1.47	n-u
Farthing$_2$	5.1	c-k	3.9	a-i	5.3	a-e	1.2	b-d	5.5	a	-	-	1.46	o-v
Jewel	2.4	l-n	1.6	h-i	3.1	de	1.3	b-d	5.1	a	2.17	l-r	1.17	y-B
Meadowlark	4.9	d-l	3.5	c-i	4.4	a-e	1.5	b-d	4.4	a-c	-	-	1.81	f-k
Millennia	3.9	h-n	3.0	f-i	4.0	a-e	3.8	a-c	3.3	a-c	2.16	l-r	1.23	u-B
Primadonna	4.3	g-m	2.5	f-i	4.6	a-e	2.0	a-d	3.9	a-c	1.87	r-t	1.17	y-B
Raven	6.1	a-i	5.4	a-f	6.1	a	2.9	a-d	3.9	a-c	2.93	a	1.65	j-p
Rebel	3.5	i-n	2.6	f-i	3.9	a-e	4.9	a	2.4	bc	2.30	g-o	1.21	w-B
Southern Belle	6.0	a-i	4.3	a-g	5.0	a-e	3.3	a-d	3.6	a-c	-	-	-	
Scintilla	2.9	k-n	2.8	f-i	4.0	a-e	2.0	a-d	4.8	ab	2.21	i-q	1.47	n-u
Snowchaser	1.6	n	1.5	i	2.8	de	2.0	a-d	4.6	ab	1.71	t	1.00	B
Springhigh$_1$	1.8	mn	1.5	i	3.3	b-e	2.3	a-d	5.0	ab	1.90	q-t	1.06	AB
Springhigh$_2$	2.5	k-n	2.1	g-i	3.8	a-e	1.8	a-d	4.5	a-c	2.04	n-s	1.15	z-B
Star$_1$	3.1	j-n	2.5	f-i	3.3	a-e	2.2	a-d	3.7	a-c	2.10	m-s	1.07	AB
Star$_2$	3.5	i-n	2.9	f-i	3.6	a-e	2.3	a-d	3.7	a-c	2.18	k-r	1.13	z-B
Sweetcrisp$_1$	7.2	a-e	6.3	a-c	4.8	a-e	0.8	c-d	5.2	a	-	-	2.10	b-e
Sweetcrisp$_2$	7.4	a-d	6.3	ab	5.0	a-e	1.3	b-d	4.8	ab	2.57	c-g	2.26	a-c
Windsor	4.0	g-n	3.5	d-i	4.5	a-e	1.6	b-d	4.3	a-c	2.01	o-t	1.27	s-A

[z]Different letters within a column indicate significant differences between genotypes using Tukey's test ($P \leq 0.05$).

Table 2-5. R values ($P < 0.001$***, $P < 0.01$**, $P < 0.05$*) for correlation between sensory and quantitative scores for all southern highbush blueberry genotypes evaluated in 2010.

	Firmness		Skin Toughness		Mealiness		Juiciness		Compression Force		Bioyield Force	
Bursting Energy	0.94	***	0.83	***	-0.41	**	0.27		0.81	***	0.86	***
Firmness			0.86	***	-0.31	*	0.15		0.85	***	0.82	***
Skin Toughness					-0.16		0.10		0.75	***	0.78	***
Mealiness							-0.75	***	-0.28		-0.37	*
Juiciness									0.20		0.38	*
Compression Force											0.78	***

Table 2-6. R values ($P < 0.001$***, $P < 0.01$**, $P < 0.05$*) for correlation between sensory and instrumental scores for all southern highbush blueberry genotypes evaluated in 2011.

	Firmness		Skin Toughness		Mealiness		Juiciness		Graininess		Blueberry Flavor		Compression Force		Bioyield Force	
Bursting Energy	0.96	** *	0.70	***	-0.32	*	0.18		0.24		0.01		0.75	***	0.82	** *
Firmness			0.68	***	-0.30	*	0.14		0.26		-0.03		0.74	***	0.80	** *
Skin Toughness					-0.19		0.13		0.33	*	-0.01		0.46	**	0.72	** *
Mealiness							-0.80	-** *	0.19		-0.32	*	-0.07		-0.35	**
Juiciness									-0.35	* *	0.36	* *	-0.03		0.18	
Compression Force															0.71	** *

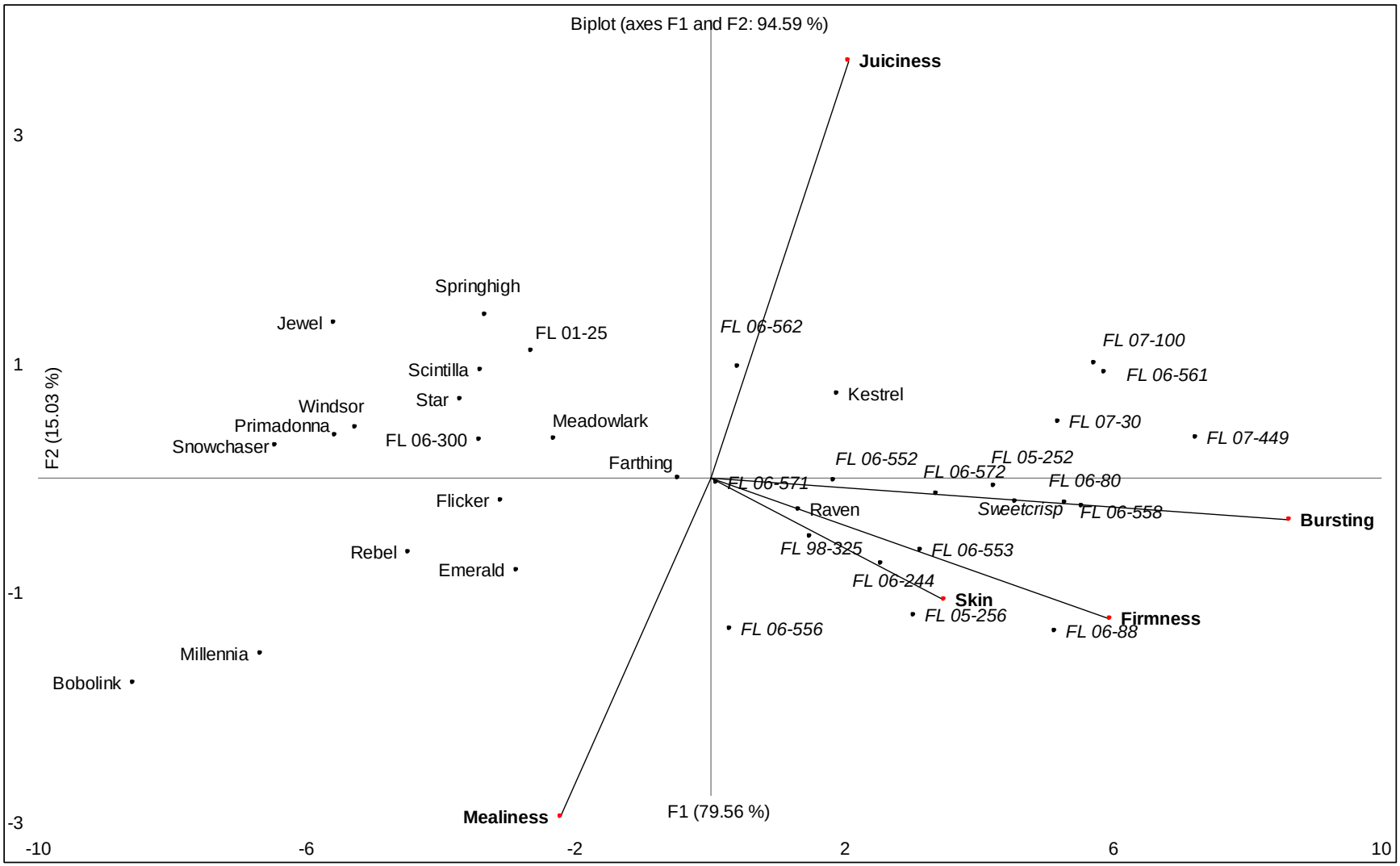

Figure 2-1. Principal component analysis (PCA) biplot of sensory evaluation of 36 southern highbush blueberry cultivars and hybrids harvested from 5-24 May, 2010. Genotypes subjectively evaluated as having crisp texture are in italics.

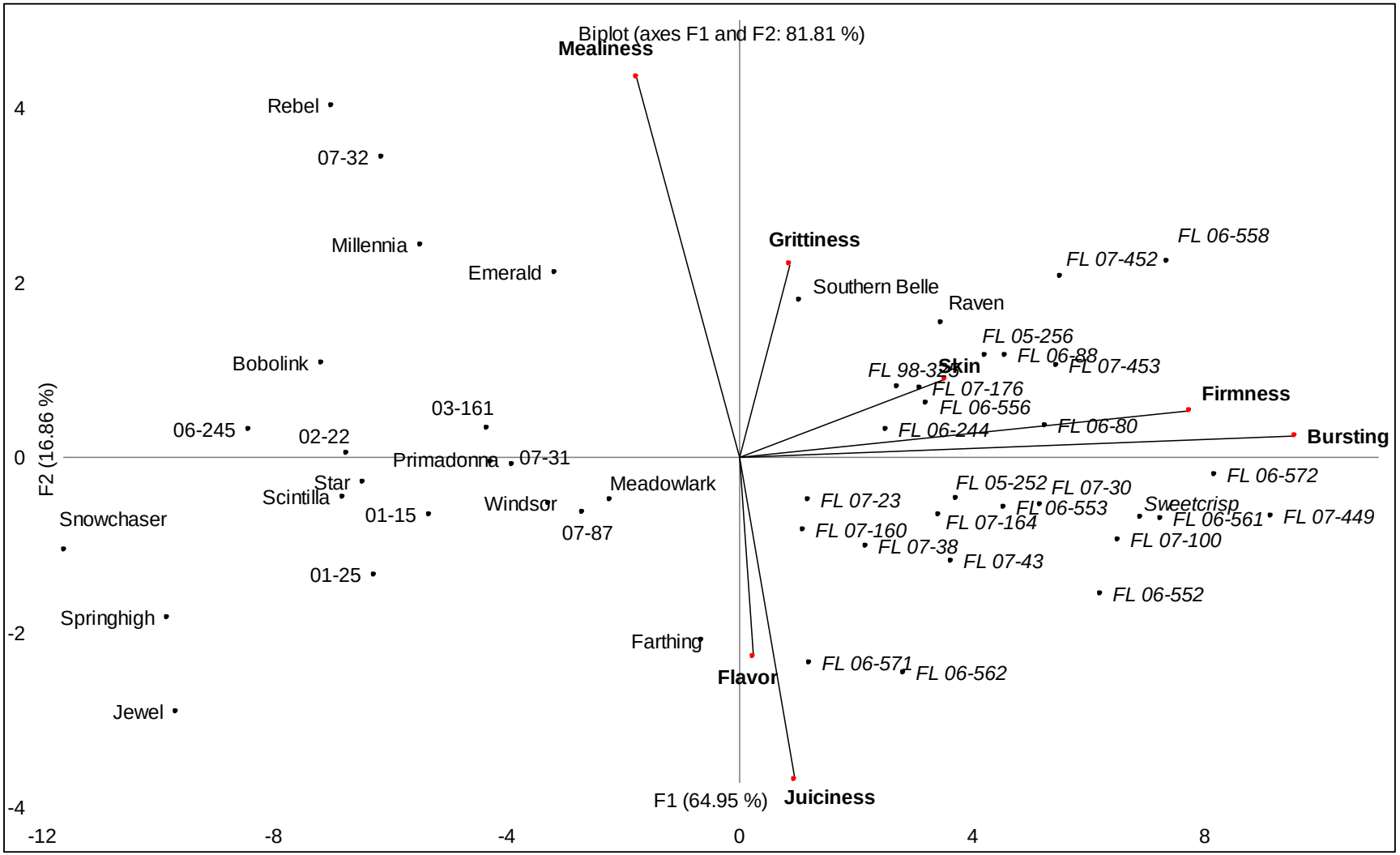

Figure 2-2. Principal component analysis (PCA) biplot of sensory evaluation of 49 southern highbush blueberry cultivars and hybrids harvested from 18 April to 9 May, 2011. Genotypes subjectively evaluated as having crisp texture are in italics.

CHAPTER 3
EFFECTS OF PREHARVEST APPLICATIONS OF 1-METHYLCYCLOPROPENE ON FRUIT FIRMNESS IN SOUTHERN HIGHBUSH BLUEBERRY

Literature Review

The University of Florida (UF) blueberry breeding program has been developing southern highbush blueberry (*Vaccinium corymbosum* L. hybrids) cultivars for over 60 years. During this period, fruit firmness has been a primary selection trait, and a novel texture most often described as “crisp” has recently been identified. Two releases from the program, ‘Bluecrisp’, and ‘Sweetcrisp’, possess this crisp fruit texture, and many advanced seedling selections have been identified (Okie, 1999; Olmstead, 2011). This unique texture characteristic is not only promising for harvesting purposes, but also for improving berry quality and storage potential that would keep Florida blueberries competitive with other markets. The mechanism responsible for crisp texture remains unclear.

Ripening is a major event in fruit development affecting both texture and firmness. Fruits are typically divided into two categories based on their mode of ripening. Climacteric fruits exhibit a peak in respiration and ethylene production that correspond with phenotypic changes in color, aroma, texture, flavor, and/or other phenomena associated with ripeness (Lelievre et al., 1997, Rhodes, 1970), while non-climacteric fruits do not exhibit one or all of these characteristics. Blueberry has been described as a climacteric fruit due to observations of a respiratory climacteric and peak in endogenous ethylene production at the transition from the mature green to green-pink stage of ripening (Ismail and Kender, 1969; Windus et al., 1976; Suzuki et al., 1997). This designation implicates ethylene as a potential factor affecting firmness and softening in blueberry. Crisp and soft-textured cultivars have been identified in other

climacteric fruits, and in peach (*Prunus persica* L.), ethylene has been found to be a major factor in the variability of its fruit texture (Ghiani et al., 2011). The degree to which ethylene is involved in the variability among fruit textures in blueberry and the overall ripening process of blueberry, however, remains unclear.

Ethylene sensitive (climacteric) fruits are expected to show positive and negative ripening responses to exogenous applications of ethylene and ethylene inhibitors such as silver thiosulphate (STS), and 1-methylcyclopropene (1-MCP). Ripening responses have been reported in blueberry fruits treated with ethylene. Preharvest application of ethephon (2-chloroethylphosphonic acid), an ethylene generating compound, advances the onset of ripening in blueberry as evidenced by a decrease in titratable acidity (TA) and an increase in total soluble solids (TSS), anthocyanins, and fruit softening (Ban et al., 2007; Eck, 1970; Forsyth et al., 1977; Warren et al., 1973). Blueberries harvested at the green and green-pink stage demonstrated increased respiration when treated with ethylene and acetaldehyde (Janes, 1978). The use of 1-MCP as a suppressor of ethylene responses in the ripening of both climacteric and traditionally non-climacteric fruit was summarized by Huber, (2008). Climacteric fruit treated with 1-MCP have demonstrated ripening responses such as altered ethylene production and respiration, delayed or suppressed softening, altered or delayed volatile emissions, and/or pigment change (Huber, 2008). Non-climacteric fruits, such as grape (*Vitis vinifera* L.) and strawberry (*Fragaria x ananassa* Duchesne) have also shown delayed or decreased ripening in response to ethylene inhibitors (Tian et al., 2000; Jiang et al., 2001; Chervin et al., 2004; Bellincontro et al., 2006; Ianetta et al., 2006) Preharvest application of 1-MCP to grape resulted in decreased berry diameter, increased acidity, and decreased anthocyanin accumulation (Chervin et al., 2004). Postharvest applications of 1-MCP

also resulted in an initial reduction of ethylene production and delayed anthocyanin breakdown in grape (Bellincontro et al., 2006). Postharvest applications of 1-MCP to strawberry decreased ethylene production, fruit softening and anthocyanin accumulation (Jiang et al., 2001).

The response of blueberries to postharvest applications of 1-MCP has been mixed. DeLong et al., (2003) observed no differences in the percent marketable fruit among two highbush blueberry cultivars treated at postharvest timing with 1-MCP, and found no effect on the shelf life of either cultivar treated. MacLean and NeSmith (2011) evaluated ethylene production, firmness, TSS, and TA in three rabbiteye blueberry (*Vaccinium virgatum* Aiton) cultivars treated with 1-MCP after harvest and found increased ethylene production in all three cultivars, decreased firmness in one cultivar, but no effect on TSS or TA content. There are no published reports on the preharvest application of 1-MCP to blueberry fruit. The objective of this study was to determine if the preharvest application of 1-MCP to pre-climacteric blueberry fruit affects fruit firmness in two southern highbush cultivars having soft and crisp fruit texture.

Materials and Methods

Two southern highbush blueberry cultivars with soft and crisp fruit texture were selected from Straughn Farms, Inc. in Windsor, FL for use in this study. 'Star' and 'Sweetcrisp' were developed at UF and have been evaluated by a trained sensory panel that identified 'Sweetcrisp' as having more firm, crisp fruit than 'Star' (see Chapter 2). Plants were established in 2009 and spaced at 0.76 m in rows 3 m apart. Any fruit that had initiated ripening (as determined by color change) were removed from the plants prior to the first treatment application.

A proprietary formulation of 1-MCP (3.8% a.i.; Harvista, AgroFresh Inc., Spring House, PA) was applied at a rate of 160 mg/L using a double boom backpack sprayer calibrated to supply ~60g a.i./acre. Silwet L-77 organosilicone surfactant (Helena Chemical Co., Collierville, TN) was added at 0.1% of the total volume.

Three replications (blocks) of a split plot cultivar x treatment design were used to evaluate two genotypes ('Star' and 'Sweetcrisp') in the whole plots and three 1-MCP treatments (9 day preharvest, 5 day preharvest, untreated control) in the split plots. There were two guard plants between each set of three treated plants. Ten unblemished fully ripe berries were harvested from each plant and transported on ice to the research lab at UF in Gainesville, FL where they were stored at 7ºC overnight. On the next day, berries were brought to room temperature and compression firmness ($N \cdot mm^{-1}$) was measured using a FirmTech 2 (Bioworks, Inc., Wamego, KS).

Statistical analysis was performed using the GLIMMIX procedure (SAS9.2) with cultivar and treatment as fixed factors and block as a random factor. Compression firmness measurements were transformed by log10. Tukey's HSD test was used to determine significant differences ($P \leq 0.05$) between cultivar and treatment means.

Results and Discussion

There were significant differences in firmness for both cultivars and treatments *(P* < 0.05) but not for the cultivar x treatment interaction (*P* = 0.089). For all treatments, 'Sweetcrisp' was significantly firmer than 'Star' (Figure 3-1). Firmness of the untreated control was not significantly different from the nine day preharvest 1-MCP treatment (*P* = 0.808), and the two 1-MCP treatments (9 day and 5 day) were not statistically different from one another (*P* = 0.058) (Figure 3-1). However, plants that did not receive 1-MCP had firmer berries than plants treated with 1-MCP five days prior to harvest (*P* = 0.011).

The results of this study suggest that 1-MCP application five days prior to harvest may decrease fruit firmness of southern highbush blueberries at the time of harvest. MacLean and NeSmith (2011) also observed decreased firmness in rabbiteye blueberry fruits treated with a postharvest application of 1-MCP. Preharvest 1-MCP treatments were applied to the whole plant, whereas postharvest treatments were only applied to the detached fruits. Pre and postharvest treatments were also applied to the fruits at different stages of maturity and may therefore have had different effects on fruit softening. When postharvest applications of 1-MCP were compared with preharvest applications of 1-MCP in apple (*Malus domestica* Borkh), preharvest treatments applied closer to the harvest date demonstrated responses more similar to those of postharvest treatments than preharvest treatments applied several days or weeks prior to harvest (Elfving et al., 2007; McArtney et al., 2009). In this study, plants treated with 1-MCP nine days prior to harvest did not differ in berry firmness from the untreated control, but plants treated five days prior to harvest showed decreased firmness. McArtney et al. (2009) suggested that fruits remaining attached to the plant may be capable of creating new ethylene receptors uninhibited by previous 1-MCP treatments that would restore ethylene response. It remains unclear, however, why ethylene inhibition would result in decreased firmness. Regardless, it does not appear that variability in ethylene response plays a central role in crisp blueberry texture, as neither preharvest treatment of ‘Star’ with 1-MCP resulted in a significant increase in firmness. Rather, it may be anatomical differences that lead to crisp texture in certain blueberry genotypes.

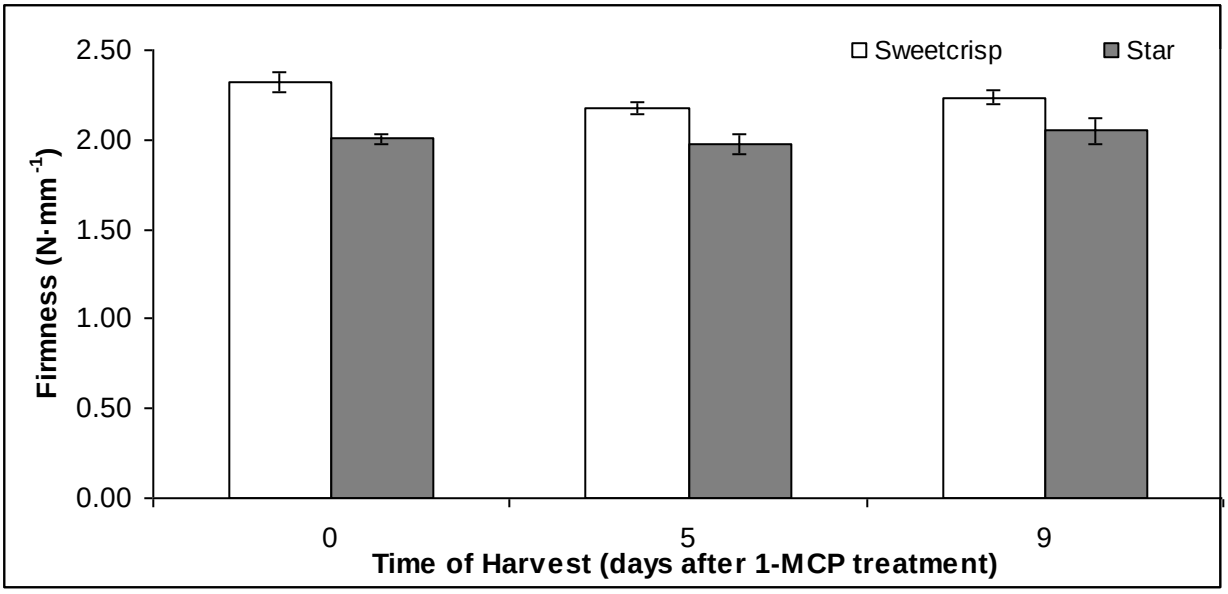

Figure 3-1. Average fruit firmness and standard error of ‘Sweetcrisp’ and ‘Star’ blueberry fruit harvested after untreated control (0 day), 5 day, and 9 day preharvest treatments of 1-MCP.

CHAPTER 4
STONE CELL FREQUENCY AND CELL SIZE VARIATION OF CRISP AND SOFT TEXTURED FRUITS FROM NINE SOUTHERN HIGHBUSH BLUEBERRY CULTIVARS

Literature Review

Several fresh market fruit species have textures that range from soft to crisp, including apple (*Malus domestica* Borkh.), grape (*Vitis vinifera* L.), peach (*Prunus persica* L.), and sweet cherry (*Prunus avium* L.) (Tong et al., 1999; Sato et al., 2006; Ghiani et al., 2001; Batisse et al., 1996). More recently, two southern highbush blueberry cultivars (*Vaccinium corymbosum* L. hybrids) considered to have a unique crisp texture were released by the University of Florida (UF) in 1997 ('Bluecrisp') and 2005 ('Sweetcrisp') (Okie, 1999; Olmstead, 2011). Previous reports have described a similar fruit texture in the blueberry cultivars 'Dolores' and 'Hortblue Poppins' (Clark and Finn, 2010; Scalzo et al., 2009), and many current selections in the UF blueberry breeding program are also considered to have a crisp phenotype similar to 'Bluecrisp' and 'Sweetcrisp' (see Chapter 2). Berries with this crisp texture are of particular interest due to their enhanced eating quality, prolonged postharvest life, and potential value for mechanical harvesting for fresh marketed blueberries (Padley, 2005; Mehra et al., 2013, and Takeda et al., 2013).

Several cellular components contribute to overall fruit texture, including cell type, size, shape, packing, cell to cell adhesion, extracellular space, and cell wall thickness (Harker et al., 1997). Parenchyma cells are the most numerous type of cells in the flesh of blueberry fruit and have thin, non-lignified cell walls and a large, mostly water-filled vacuole (Harker et al., 1997). The epidermis is composed of specialized parenchyma cells that have thickened primary cell walls and are covered by a cuticle consisting of cutin and associated waxes (Esau, 1977). Collenchyma cells and phloem elements

have thickened primary cell walls that provide tensile strength to surrounding tissues. Xylem and sclerenchyma cells such as fibers and sclereids have thick and lignified secondary cell walls that are dead at maturity and give support (Harker et al., 1997).

Cell size varies with different cell types during ripening. Fruit development in blueberry follows a double-sigmoid growth pattern in which the pericarp initially increases in volume (stage I), then the embryo develops while pericarp growth slows down (stage II), and ripening occurs in conjunction with a final expansion in pericarp size (stage III) (Godoy et al., 2008). Shortly after anthesis, mesocarp cells stop dividing and increase only in size as the fruit continues to develop and enlarge (Darnell et al., 1992). Cell size is much smaller in the epidermal and hypodermal layers that together form the epicarp, where cell division occurs over a longer period of time during fruit expansion (Harker et al., 1997). A study by Mann et al., (2005) compared instrumental and sensory measurements to cell number and size in apple, and concluded that fruits with fewer cells per unit area in the apple cortex (mesocarp) were crisper than fruits with more cells per unit area. Smaller sized cells have an increased surface area and higher proportion of cell wall material, which has been suggested to translate into greater firmness and tissue strength, but Mann et al. (2005) suggests that larger sized cells contribute to crispness in apple, which may be due to an increased likelihood for larger cells to burst rather than separate from neighboring cells as is believed to occur in crisp-textured fruits (Harker et al., 1997).

The amount of contact and/or space between neighboring cells is influenced by the shape and packing of cells (Harker et al., 1997). Batisse et al. (1996) observed that crisp-textured sweet cherries have more large intercellular spaces than soft-textured sweet cherries. The degree to which adjacent cells separate during chewing also has

an effect on its perceived texture. In the process of chewing, force is applied to the fruit tissue until it fractures, which can occur by cell separation – as is the case with soft fruits such as banana (*Musa spp.*) – or by individual cell rupture in crisp fruits such as apple and watermelon (*Citrullus lanatus* Thunb.) (Harker et al., 1997). Cell wall strength and cell-to-cell adhesion also contribute to whether cells separate or rupture (Harker et al., 1997).

It is important to consider blueberry fruit anatomy when searching for the basis of crisp fruit texture. Blueberry fruits develop from an inferior ovary. The epidermis of the berry, having originated from the flower's calyx, is covered by a cuticle and associated waxes that give the otherwise dark pigmented fruit its blue color (Gough, 1994). Together, the epidermis and hypodermal layers contain pigmentation from anthocyanins and form the epicarp, commonly referred to as the peel or skin (Gough, 1994). The endocarp is composed of five carpels with 10 locules and five highly lignified placentae which are attached to approximately 50 seeds (Gough, 1994). The mesocarp is located between these layers and contains mostly parenchyma cells, along with rings of vascular bundles and occasional sclerified "stone cells" that can be found approximately 460 to 920 µm below the epidermis (Gough, 1983). These lignified cells with thick secondary cell walls can occur singly, doubly, or in clusters, and bind neighboring parenchyma cells that serve to strengthen the flesh tissue (Gough, 1983; Allan-Wojtas et al., 2001; Fava et al., 2006). Potential increased firmness just beneath the epidermal layer where initial rupture of the berry fruit takes place suggests that stone cells may have a role in the crispness detected in some southern highbush blueberry cultivars. Results of a trained sensory panel that evaluated texture of several genotypes of UF blueberry germplasm ranging from soft to crisp also suggested that the crisp texture

may be related to the epidermal region (see Chapter 2). Genotypes receiving high sensory scores for crisp texture by the panel were often also rated for having a high level of skin toughness. Together these findings suggest that crisp texture is likely associated with differences in or near the epidermal layer of the berry. The objective of this study was to perform a histological analysis of cell type, size, and structure of the outermost cell layers of soft and crisp-textured fruits from nine southern highbush blueberry genotypes.

Methods

Plant Material

Fruits were harvested from nine southern highbush blueberry genotypes grown on commercial farms in Windsor and Waldo, FL. Genotypes were selected based on results from sensory and instrumental measures of soft and crisp fruit texture (see Chapter 2). Four crisp-textured genotypes (FL 06-244, FL 98-325, FL 07-100, and 'Sweetcrisp') and four soft-textured genotypes (FL 06-245, 'Windsor', Springhigh', and 'Star') were harvested at the mature green and ripe stages of development as described by Shutak et al. (1980). 'Raven' was also included in the study as it has very firm texture, but had not been subjectively evaluated as crisp prior to trained panel evaluations. Genotypes of unique genetic background were preferentially selected; however, one full sib pair (FL 06-244 and FL 06-245) was evaluated to compare cellular structure of a crisp and non-crisp genotype from the same genetic background.

Microscopy

A 0.23 mm width steel razor was used to remove the calyx and stem end of each fruit before being immersed and stored in FAA solution (10 formaldehyde : 5 acetic acid

: 35 alcohol). Fruits were stored in fixative for 1-3 months and the fixative was refreshed several times during this period.

Radial sections (approximately 3 mm width) were taken using a 0.23 mm width steel razor and sections were dehydrated in a graded ethanol series (30, 40, 50, 60, 70, 80, 90, 95, and 100% for 45 min.) followed by paraffin infiltration and embedding using *tert*-butyl alcohol as an intermediate solvent (Ruzin, 1999).

Sections of 12-14 µm were obtained using a 0.25 mm steel microtome blade on a rotary microtome and were mounted on glass slides. The mounted sections were de-parafinized with Histoclear II, stained with Safranin O and Aniline Blue, and were permanently mounted with a cover glass using DePex Mounting Medium.

A Leitz Ortholux light microscope (Leica Microsystems, Wetzlar, Germany) was used to visualize samples using the 10x and 40x objectives. Images were captured with a Moticam 1000 1.3 M pixel camera (Motic, Inc., Hong Kong, China) and visualized using Motic Images Plus 2.0 ML software.

Image Analysis

The total number of stone cells within 1,200 µm of the epidermis was counted in a whole section of four berries from each maturity stage and genotype. Stone cells were identified as cells with a thick cell wall that was darkly stained with Safranin O. Cell size was measured using the ruler function in Adobe Photoshop CS5 (Adobe Systems, Inc., San Jose, CA). Cell height and width were used to calculate the cell area of 40 cells in the outer four cell layers of mature green fruits from each genotype and 40 cells in the outer three cell layers of ripe fruits from each genotype.

Statistical Analysis

Data was analyzed for ANOVA and means separation with SAS 9.2 (SAS Institute, Inc., Cary, NC) using Proc GLM and Tukey's HSD test ($P \leq 0.05$).

Results and Discussion

There was a visible difference between genotypes in the number of cell layers that formed the epicarp of mature green and ripe fruits (Figure 4-1, 4-2, 4-3, 4-4). 'Star' was unique in having a very thin peel that appeared to consist only of the epidermal cell layer. The other eight genotypes had an epicarp consisting of the epidermis and one or two hypodermal cell layers. In other textural studies involving the separation of the peel from the berry flesh, it was noted that 'Star' was more difficult to peel than the other genotypes, which is consistent with the histological findings that its epicarp contains fewer cell layers.

Cell shape appeared to vary by genotype as well. The biggest change in cell shape between the epidermis and first layer of hypodermis of ripe fruits was detected in 'Springhigh' and 'Sweetcrisp' (Figure 4-1, 4-2). Despite 'Springhigh' cells in the first layer of hypodermis becoming much longer than they were observed to be in the epidermis, these cells were still more round in shape than any other genotype within those two cell layers. The shape of 'Sweetcrisp' cells also changed dramatically between the epidermal and first hypodermal layer, such that these cells were more round/square than most other genotypes in the epidermal layer, but more long and rectangular than any other genotype in the first layer of hypodermis. The least change in shape within the outer three cell layers of ripe fruits was observed in 'Star'. The cells of 'Star' maintained the same basic proportions as they increased in size between cell layers. The two cell layers below the epidermis, which were not considered hypodermis

in ‘Star’ whose peel consisted of a single cell layer, were more round in shape than other genotypes. The epidermis of ‘Star’, however, had the longest cells when compared to the epidermal layer of all other genotypes.

Evidence of intercellular spaces was observed in all genotypes at the mature green and ripe stage of development (Figure 4-5). Genotypes appeared to vary in the amount and size of space between cells, and was most evident in the numerous and large intercellular spaces detected in ‘Star’. The large round shape of cells in ‘Star’ may account for the increased space observed between cells. ‘Star’ also demonstrated a less structured pattern of cell packing than other genotypes whose cells had a more layered pattern of organization. The lack of layered structure in ‘Star’ may be due to the subepidermal cells beneath its single layered epicarp, which are considered to belong to the mesocarp and have completed cell division sooner and undergone a longer period of cell expansion than cells in the epicarp.

Average cell size ranged from 436 μm^2 to 718 μm^2 in the outermost cell layer of mature green fruit and from 429 μm^2 to 668 μm^2 in the outermost cell layer of ripe blue fruit (Table 4-1). This suggests that there is not a dramatic increase of cell size in the epidermal layer of berries as they ripen from mature green to fully ripe fruits, which is consistent with previous results suggesting that cell division persists in the epicarp, while mesocarp cells stop dividing and increase only in size during the latter stages of ripening (Harker et al., 1997).

For all genotypes, average cell size successively increased in the second, third, and fourth outer cell layers of both mature green and ripe blue fruits (Figure 4-1, 4-2, 4-3, 4-4; Table 4-1). The berries of FL 06-244 and ‘Star’, when compared with all other genotypes of mature green and ripe fruits, respectively, had the largest difference in

average cell size between the epidermis and first layer of hypodermis. However, differences in cell size between outermost cell layers measured did not appear to correspond to soft and crisp textured genotypes. For example, the crisp genotype FL 07-100 was grouped with genotypes having the smallest cell size in the two outermost cell layers of mature green fruits and the outermost cell layer of ripe fruits, while the crisp genotype FL 06-244 was grouped with genotypes having the largest cell sizes (Table 4-1) There was a significant difference in cell size between cultivars, but there was no significant difference between the cell size of crisp and soft-textured genotypes in any cell layer of either fruit maturity stage.

The difference in cell size between ripe blue and mature green fruits is indicative of cell expansion during the ripening process. While cell size could not be measured in the same fruit during ripening, we observed that the largest differences in the epidermal cell layer between mature green and ripe fruits were in three soft-textured genotypes (FL 06-245, 'Springhigh' and 'Star'). By contrast, the average size of epidermal cells in mature green fruits of three crisp genotypes (FL 06-244, FL 07-100, and 'Raven') was greater than in ripe fruits, suggesting prolonged cell division and a lesser degree of cell expansion in these genotypes during ripening (Table 4-1). 'Raven' was included in the study as a genotype having a texture somewhere between soft and crisp. A trained sensory panel, however, found it to be as equally crisp as the crisp genotype FL 98-325, and firmness measurements of 'Raven' exceeded those of FL 98-325 (see Chapter 2). While these observations are not conclusive, they offer a possible explanation of how cell division and cell expansion in the epidermal layer of crisp and soft-textured blueberry occurs during ripening, and may be worth further exploration.

Stone cells were observed in the mesocarp tissue of some southern highbush genotypes (‘Springhigh’, FL06-245, ‘Windsor’, ‘Raven’, FL 06-244, and ‘Sweetcrisp’) that we evaluated (Table 4-2). Stone cells were found singly or in pairs as previously reported (Gough, 1983; Allan-Wojtas et al., 2001; Fava et al., 2006), but no clusters were detected (Figure 4-6). The average number of stone cells in a single berry ranged from 0 to 95 (Table 4-2). Two crisp genotypes (FL 98-325 and FL 07-100) did not have any stone cells, while ‘Sweetcrisp’ had a moderate number of stone cells (an average of seven per green fruit and 17 per ripe fruit). The crisp genotype FL 06-244 had more stone cells/berry than any other genotype evaluated. Two full sibs, FL 06-244 (crisp) and FL 06-245 (soft textured), both demonstrated a high frequency of stone cells (Table 4-2), which suggests that this trait is genetically regulated, but may not be correlated with crisp texture. As a whole, the crisp genotypes that were evaluated here did not have a higher frequency of stone cells than non-crisp genotypes, suggesting that stone cells are not correlated with crisp texture in blueberry. With the lack of obvious anatomical differences that correlate with crisp fruit texture, a more detailed examination of the composition of epicarp cells is warranted.

Table 4-1. Average cell area (μm^2) for each cell layer of soft and crisp-textured genotypes at the mature green and ripe blue stages of development. The epidermal cell layer is represented as 1, the 2nd outermost cell layer is marked 2, and the 3rd and 4th layers are 3 and 4 respectively.

		Average Cell Area (μm^2)						
		Mature Green				Blue Ripe		
Texture	Genotype	1	2	3	4	1	2	3
Soft	Springhigh	558 ^{BZ}	1148 ^{B}	1429 ^{B}	2483 ^{BC}	661 ^{A}	1147 ^{B}	1380 ^{C}
	FL 06-245	436 ^{D}	1083 ^{BC}	1901 ^{A}	3555 ^{A}	614 ^{AB}	1302 ^{B}	1653 ^{BC}
	Star	576 ^{B}	972 ^{BCD}	1563 ^{AB}	2164 ^{C}	689 ^{A}	1706 ^{A}	2610 ^{A}
	Windsor	536 ^{BC}	998 ^{BC}	1393 ^{B}	2715 ^{BC}	582 ^{AB}	1256 ^{B}	1689 ^{BC}
	Raven	561 ^{B}	929 ^{CD}	1307 ^{B}	2224 ^{C}	521 ^{BC}	1126 ^{B}	1837 ^{B}
Crisp	FL 06-244	718 ^{A}	1417 ^{A}	1782 ^{A}	3157 ^{AB}	668 ^{A}	1306 ^{B}	1866 ^{B}
	FL 98-325	584 ^{B}	1112 ^{BC}	1586 ^{AB}	2698 ^{BC}	610 ^{AB}	1264 ^{B}	1873 ^{B}
	Sweetcrisp	525 ^{BC}	1130 ^{BC}	1570 ^{AB}	2453 ^{BC}	595 ^{AB}	1312 ^{B}	1681 ^{BC}
	FL 07-100	470 ^{CD}	774 ^{D}	1418 ^{B}	2703 ^{BC}	429 ^{C}	787 ^{C}	1275 ^{C}

[Z]Tukey-Kramer grouping for Least Square Means (α=0.05). LS means with the same letter within a column are not significantly different.

Table 4-2. Mean number of stone cells per fruit at the mature green and ripe blue stages of maturity for genotypes with soft and crisp-textured berries.

Texture	Genotype	No. Stone Cells Mature Green		Blue Ripe	
Soft	Springhigh	12	D[Z]	13	BC
	FL 06-245	67	AB	22	B
	Star	0	D	0	C
	Windsor	17	CD	3.5	BC
	Raven	51	BC	23	B
Crisp	FL 06-244	95	A	56	A
	FL 98-325	0	D	0	C
	Sweetcrisp	7	D	17	BC
	FL 07-100	0	D	0	C

[Z]Tukey-Kramer grouping for Least Square Means (α=0.05). LS means with the same letter within a column are not significantly different.

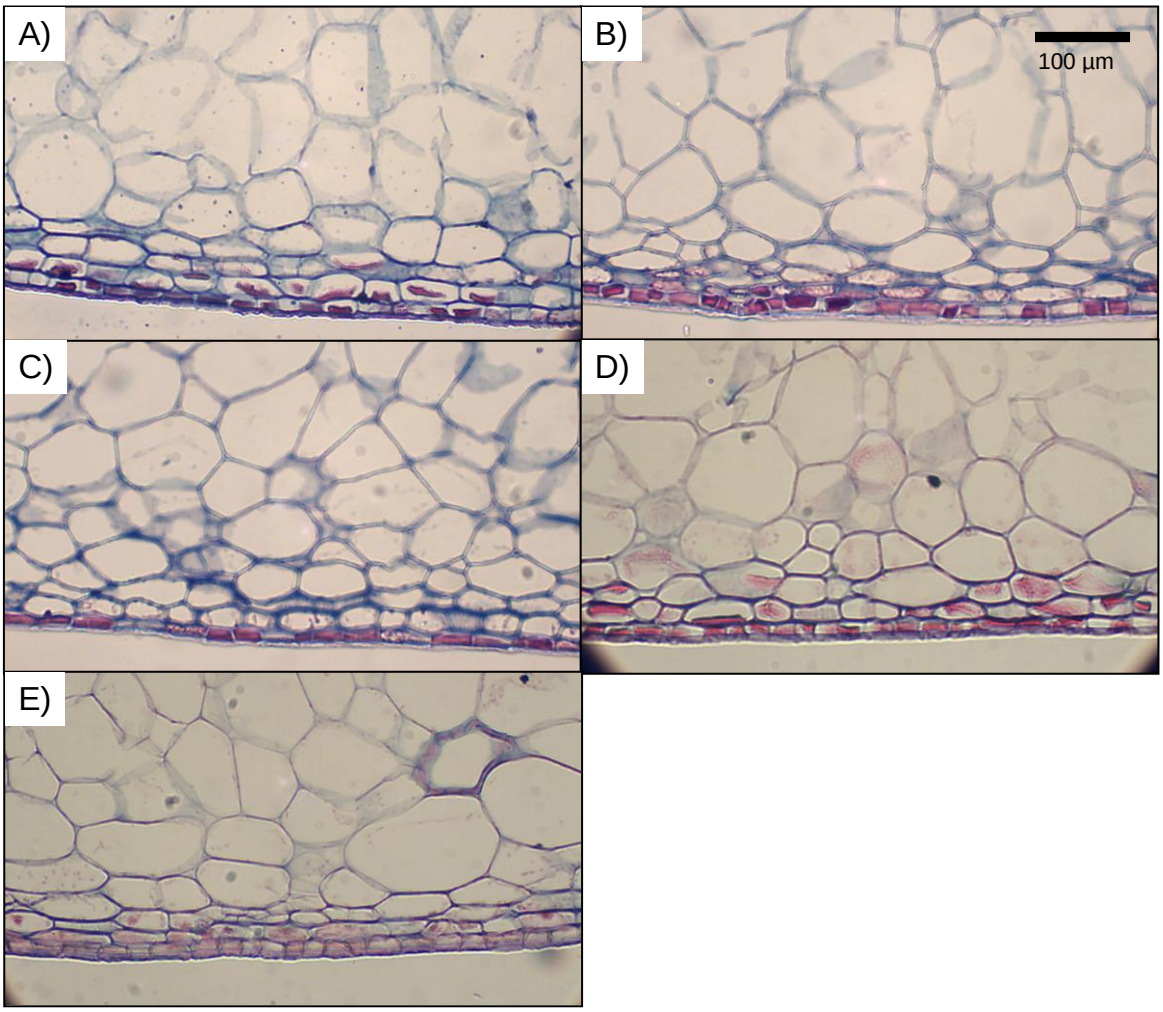

Figure 4-1. Images of mature green fruits from soft-textured genotypes (100x magnification). A) ‘Springhigh’, B) ‘Windsor’, C) ‘Star’, D) FL 06-245, E) ‘Raven’. Photos courtesy of Kendra Blaker.

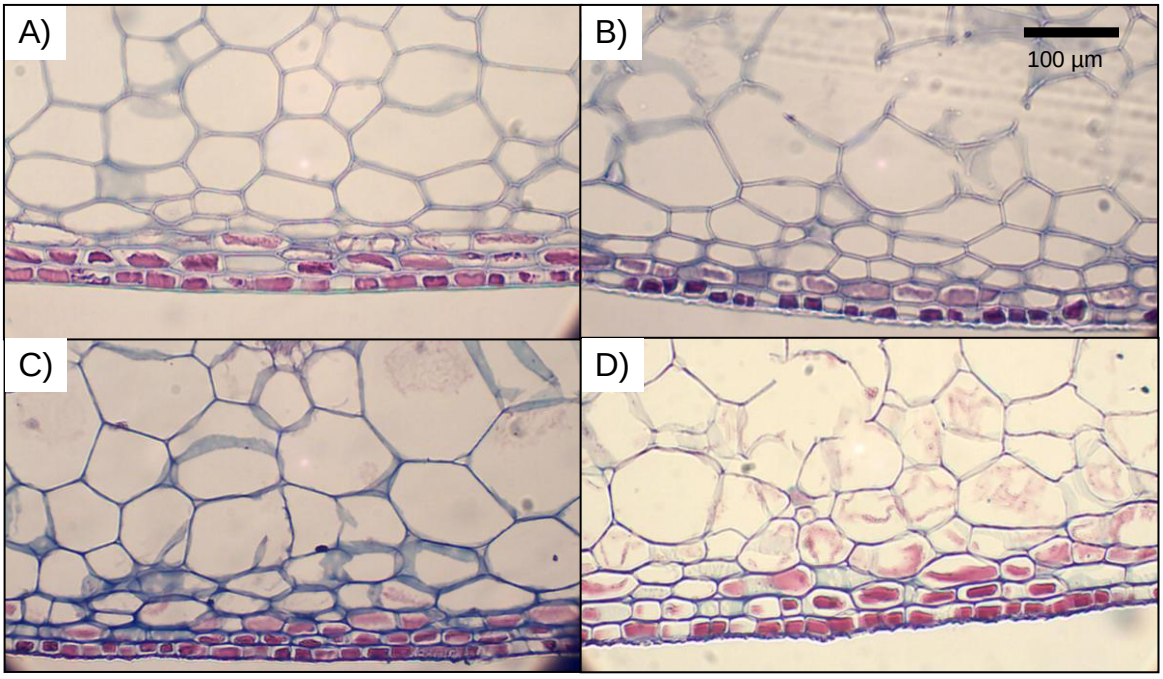

Figure 4-2. Images of mature green fruits from crisp-textured genotypes (100x magnification): A) FL 98-325, B) 'Sweetcrisp', C) FL 07-100, D) FL 06-244. Photos courtesy of Kendra Blaker.

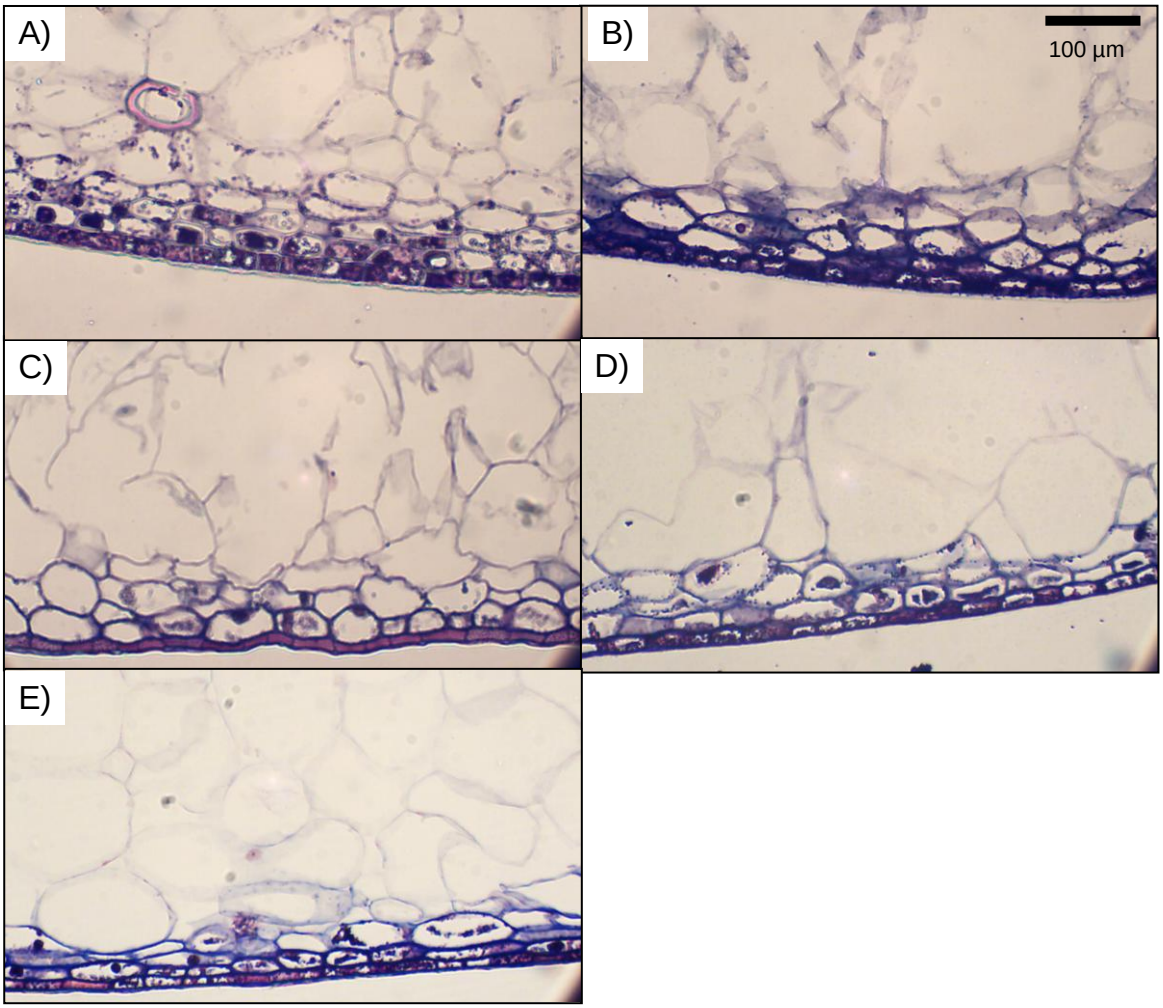

Figure 4-3. Images of ripe blue fruits from soft-textured genotypes (100x magnification). A) 'Springhigh', B) 'Windsor', C) 'Star', D) FL 06-245, E) 'Raven'. Photos courtesy of Kendra Blaker.

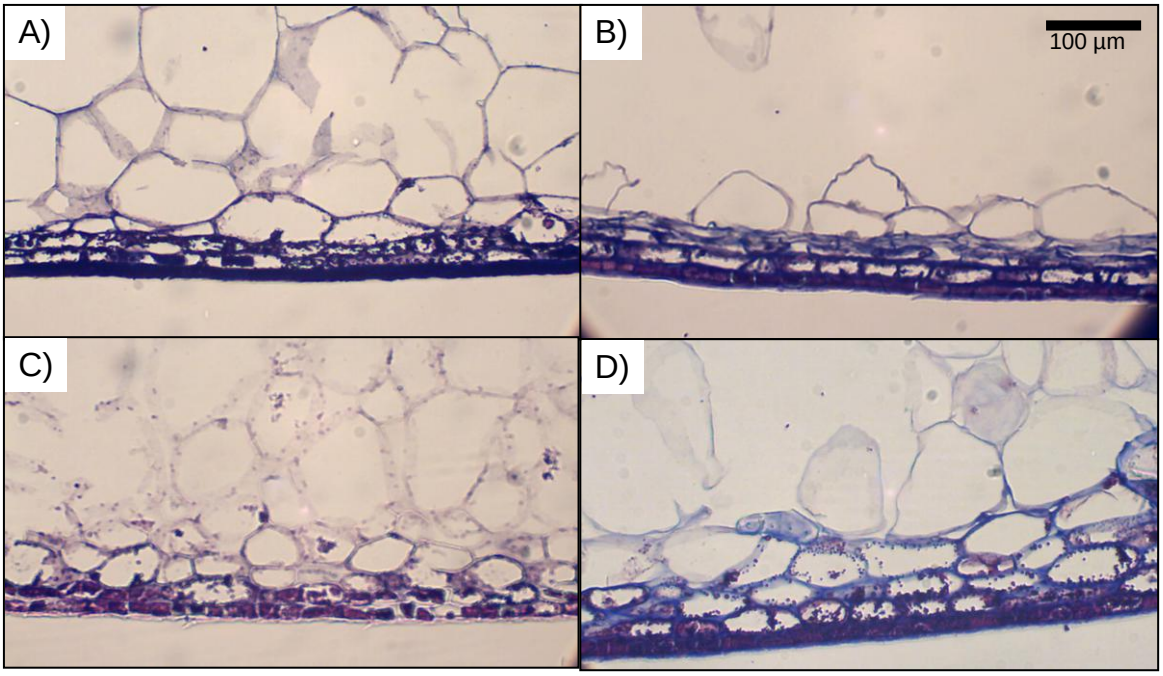

Figure 4-4. Images of ripe blue fruits from crisp-textured genotypes (100x magnification). A) FL 98-325, B) 'Sweetcrisp', C) FL 07-100, D) FL 06-244. Photos courtesy of Kendra Blaker.

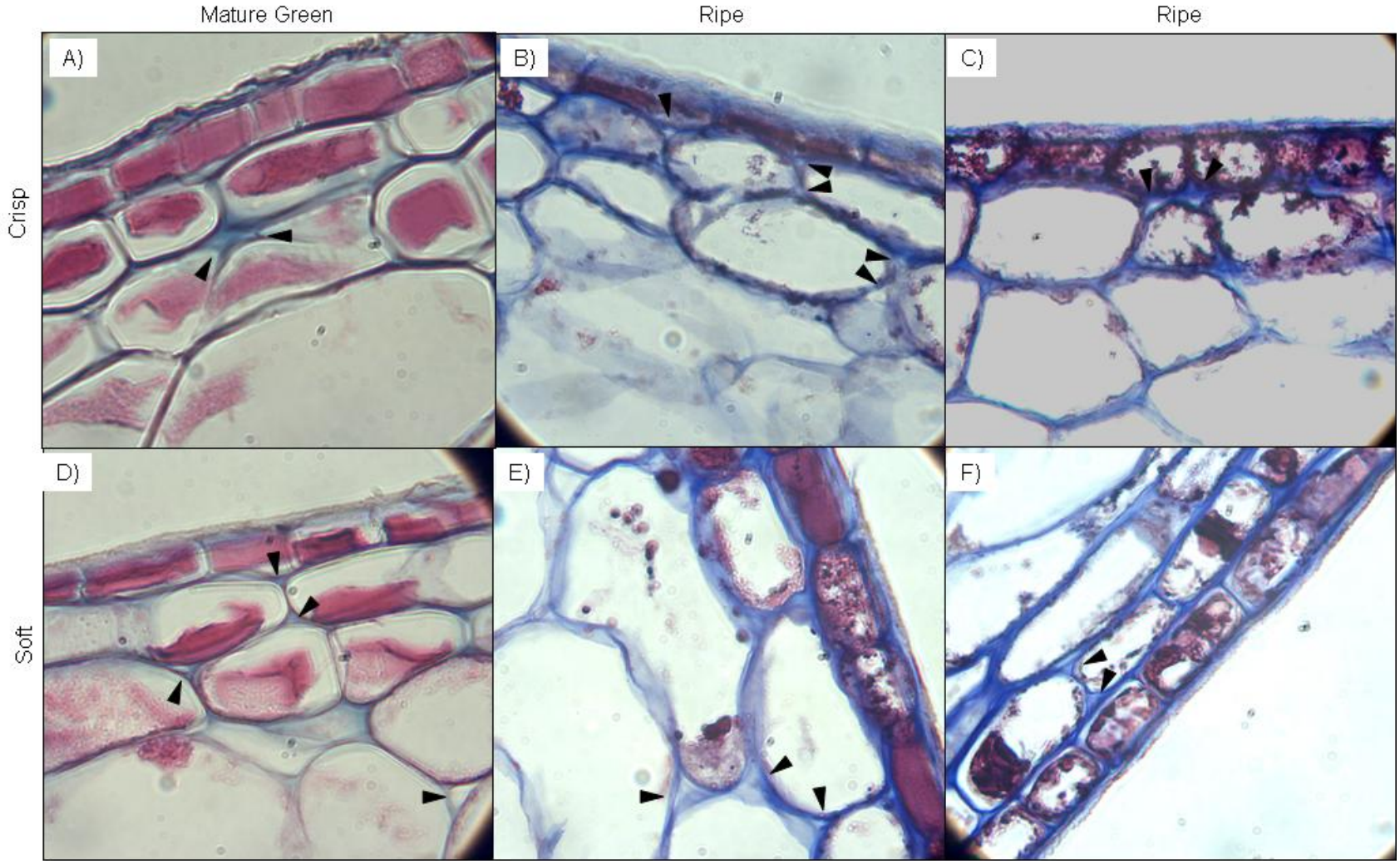

Figure 4-5. Images of mature green (A and D) and ripe blue (B, C, E, F) fruits from crisp (A-C) and soft-textured (D-F) genotypes (400x magnification). A) FL 06-244, B) FL 07-100, C) 'Sweetcrisp', D) FL 06-245, E) Star, F) Springhigh. Arrows indicate intercellular space. Photos courtesy of Kendra Blaker.

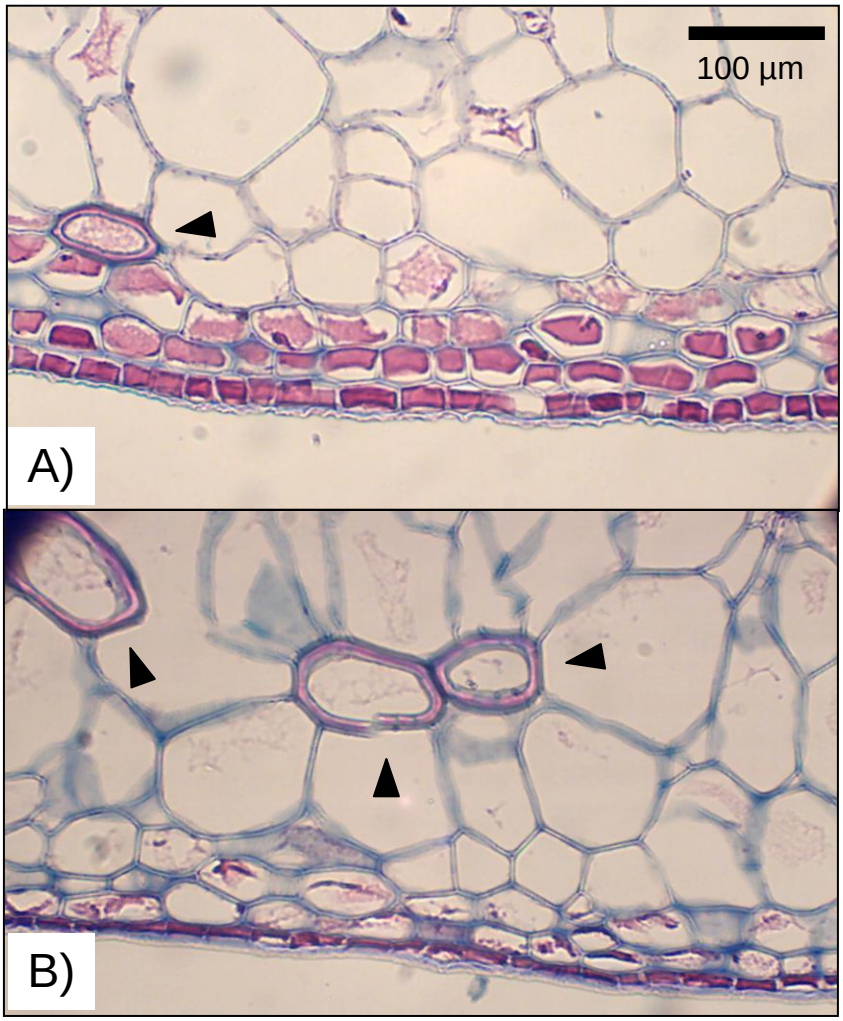

Figure 4-6. Images of stone cells in crisp and non-crisp genotypes. A) Crisp genotype FL 06-244. B) Non-crisp genotype FL 06-245, a full sib of FL 06-244 (100x magnification). Arrows indicate stone cells. The thickened cell walls of stone cells are pink after staining with Safranin O. Note that the stone cells occur singly or in pairs, and are located just below the epidermal cells of the fruit. Photos courtesy of Kendra Blaker.

CHAPTER 5
CELL WALL COMPOSITION OF THE MESOCARP AND EPIDERMAL TISSUE OF CRISP AND SOFT TEXTURED BLUEBERRY GENOTYPES DURING POST HARVEST STORAGE

Literature Review

Two southern highbush blueberry (*Vaccinium corymbosum* L. hybrids) cultivars – 'Bluecrisp' and 'Sweetcrisp' – released by the University of Florida (UF) in 1997 and 2005, respectively, are considered to have a unique crisp texture (Okie, 1999; Olmstead, 2011). Many current selections in the UF blueberry breeding program are also considered to have a similar crisp phenotype (see Chapter 2). Berries with this crisp texture are of particular interest due to their enhanced and perhaps novel eating quality, prolonged postharvest life, and potential value for mechanical harvesting for fresh marketed blueberries (Padley, 2005; Mehra et al., 2013; Takeda et al., 2013). Understanding the physiological basis of this trait would therefore be useful for predicting the potential benefits that crisp texture may contribute to blueberry production, and potentially aid in selection and improvement of the trait through breeding efforts.

The strength and thickness of cell walls have been considered to have the greatest overall impact on fruit firmness and texture (Goulao and Oliveira, 2008; Li et al., 2010), and the crisp phenotype in blueberries may be related to some aspect of cell wall architecture. Parenchyma cells are the most numerous type of cell in the flesh of blueberry fruit and have thin, non-lignified cell walls (Gough, 1994). The epidermis is composed of specialized parenchyma cells that have thickened primary cell walls infused with other substances such as cutin, waxes, suberin, and lignin (Gough, 1994; Fava et al., 2006). Xylem and sclerenchyma cells such as fibers and sclereids have

thick and lignified secondary cell walls, and can be found associated with vascular bundles and stone cells in blueberry flesh (Gough, 1994).

Primary cell walls form a complex matrix of approximately 30-40% cellulose, 30% hemicellulose, 15-30% pectin, and 5-10% structural protein (Vermerris, 2008). Carpita and Gibeaut (1993) have described two types of primary cell walls. Most non-commelinoid flowering plants have Type I cell walls composed of xyloglucan-rich hemicelluloses with pectin-rich matrices, while members of the grass family (*Poaceae*) have Type II cell walls made of glucuronoarabinoxylan (GAX) rich hemicelluloses and lesser amounts of pectin. In blueberry, pectins were found to comprise 30-35% of the total cell wall as would be expected of most non-graminaceous plants, but xylose exceeded glucose content enough to suggest the presence of xylans rather than xyloglucans as the principal hemicellulosic component (Vicente et al., 2007).

Secondary cell walls typically have a higher proportion of cellulose, a lower proportion of pectin, and hemicellusloses that are more abundant in xylans and glucomannans which bind more tightly to cellulose (Knox, 2008). These factors contribute to the fact that primary cell walls are extendable during growth whereas secondary cell walls are non-extendable and only form after growth has occurred and the cell shape is fixed (Lee et al., 2011). Unlike most primary cell walls, secondary cell walls contain lignin, which is a complex network of phenylpropanoids that confers rigidity, strength, hydrophobicity, protection against pathogens, facilitation of water transport, and also prevents further enlargement of the cell (Hatfield and Vermerris, 2001).

Blueberry is known to contain stone cells with thick secondary walls and a high content of lignin which may provide increased firmness to the berry fruit tissue (Gough,

1983; Allan-Wojtas et al., 2001; Fava et al., 2006). Gough (1983) found sclereids just beneath the epidermal cell layer in three highbush blueberry cultivars. All three cultivars contained similar development and distribution of sclereids, but differed in the total number of sclereids (Gough, 1983). Sclereids bind neighboring parenchyma cells and can occur individually, in pairs, or in clusters, (Gough, 1983; Allan-Wojtas et al., 2001; Fava et al., 2006). Because secondary cell walls can be rich in xylan, it is possible that stone cells may account for the increased levels of xylose observed by Vicente et al. (2007) in the walls of ripening blueberry fruits (Knox, 2008). However, our previous experiments did not find any association between number or location of sclereids and the crisp blueberry texture (see Chapter 4).

Physiological and biochemical changes that occur during ripening include: conversion of starch to sugar, pigment biosynthesis and accumulation, biosynthesis of flavor and aromatic compounds, cell wall degradation and fruit softening. (Brummell, 2006; Goulau and Oliveira, 2008). Textural modifications during fruit softening consist mostly of changes to the mechanical strength of the cell wall and adhesion between cells at the middle lamella (Goulao and Oliveira, 2008). These changes are primarily the result of the enzyme initiated solubilization and depolymerization of pectins and hemicelluloses (Goulao and Oliveira, 2008). Depolymerization of hemicelluloses is considered to be one of the most influential and yet variable factors involved in fruit softening of different fruit species (Brummell, 2006; Vermerris, 2008). For example, depolymerization of ionically bound cyclohexane diamine tetraacetic acid (CDTA)-soluble pectins is evident in avocado (*Persea americana* Mill.), but virtually absent in pepper (*Capsicum annuum* L.), banana (*Musa spp.*), and apple (*Malus domestica* Borkh.) (Brummell, 2006). Sodium carbonate soluble pectins are composed of ester

bound glycans such as homogalacturonan, which is a primary component of the middle lamella where cell-to-cell adhesion is maintained (Brummell, 2006). Pectin solubilization has been related to swelling of the cell wall in several melting flesh fruits, but both pectin solubilization and cell wall swelling were diminished in the crisp fruits of apple, watermelon (*Citrullus lanatus* Thunb.), and pear (*Pyrus communis* L.) (Redgwell et al., 1997).

In blueberry, cell wall degradation is marked by pectin solubilization in the early and intermediate stages of ripening, and increased solubilization of arabinose from pectins and hemicelluloses in the later stages of ripening (Vicente et al., 2007). The depolymerization of hemicelluloses was detected in all ripening stages (green to ripe fruits), but Vicente et al. (2007) did not find evidence of pectin depolymerization during fruit softening.

Several fresh market fruit species having textures that range from soft to crisp, have been identified and studied, including apple, grape (*Vitis vinifera* L), peach (*Prunus persica* L.), and sweet cherry (*Prunus avium* L.) (Tong et al., 1999; Sato et al., 2006; Ghiani et al., 2001; Batisse et al., 1996). Little is known, however, about the physiological basis of crisp texture in blueberry. The objective of this study was to compare bioyield force, dry weight, total cell wall material, total pectins, and neutral sugars between crisp and soft-textured blueberry genotypes at two stages of developmental maturity and after three durations of postharvest storage.

Methods

Plant Material

Seven southern highbush blueberry genotypes having soft (‘Springhigh’, ‘Star’, Windsor’) and crisp (‘Sweetcrisp’, FL 06-561, FL 06-562, and FL 98-325) fruit texture

were selected for use in this study. The texture of these genotypes was determined from a previous study in which bioyield force measurements using a texture analyzer were correlated with sensory score ratings made by a trained sensory panel to identify soft and crisp textured genotypes (see Chapter 2).

Experiments followed an incomplete block design in which three replications from five developmental and postharvest stages were collected from seven genotypes at two field locations (out of five possible locations). Location varied by genotype due to limited availability of multiple plants for the selections identified for use based on texture. Cultivars and selections were hand harvested from three field trials at Straughn Farms, Inc. near Waldo and Windsor, FL, and from two fields at the University of Florida Plant Science Research and Education Unit near Citra, FL.

Each replication consisted of approximately 250 g of fruit. Unblemished berries were collected at two stages of maturity. Berries having uniformly pink and blue color represented fruits at the onset and fully ripe stage of maturity, respectively. Berries were harvested in white plastic 4 L buckets, stored in coolers filled with ice, and transported on the same day to the blueberry breeding lab at the University of Florida in Gainesville, FL for postharvest treatments and instrumental analyses. All berries were frozen in liquid nitrogen and stored at -80ºC for later analysis.

Postharvest Storage Treatment

The weight (within closest proximity to 150 g) of unblemished ripe fruits was recorded, and berries were packed in 170 g plastic clamshells (Pactiv, Lake Forest, IL) for postharvest storage at 3ºC. Berries were removed from the cooler after 7, 14, and 32 days. Weight loss, counts of soft/moldy fruit, and bioyield force were measured.

Instrumental Analysis

Bioyield was measured on 25 berries from each replicate. Individual berries were oriented equatorially upright (Ehlenfeldt and Martin, 2002) and punctured with a 4 mm probe using a TA.HD plus Texture Analyzer (Texture Technologies, Corp., Scarsdale, New York). Bioyield (N) was measured as the maximum force required to puncture a berry at a speed of 50 $mm \cdot min^{-1}$.

Sample Preparation

Fruits were peeled by hand and endocarp was removed from the flesh. Skins and flesh were stored at -80ºC prior to freeze drying with a Freezone 1 freeze drier (Labconco Corporation, Kansas City, MO). Dry weight was calculated from freeze dried samples and powders were obtained by grinding with a mortar and pestle.

Alcohol Insoluble Residue (AIR) Isolation

The isolation was performed using the procedure described by Vicente et al. (2007) with the following changes. Approximately 30 mL of 95% ethanol was added to approximately 2 and 6 g of powder from berry skins and flesh respectively. Suspensions were boiled for 45 min to inactivate enzymes, centrifuged (17,000 g, for 20 min), and the insoluble residue was washed 3x with approximately 30 mL of 95 % ethanol, followed by chloroform/methanol (1:1 v/v), and finally acetone. The weight of the alcohol insoluble residue (AIR) was recorded after drying overnight at 37ºC.

Uronic Acid (UA) and Neutral Sugar (NS) Measurement

Following the basic methods of Ahmed and Labavitch (1977), 2.5 mg of AIR was weighed into 16x100mm disposable glass culture tubes and placed in ice on a shaker. Two mL of chilled 95% H_2SO_4 was added to each tube by small increasing amounts over the course of 30 minutes.

The amounts of uronic acids (UA) and neutral sugars (NS) were measured using microtiter plate methodology as described by Van den Hoogen et al (1998) and Laurentin and Edwards (2003), respectively. For the measurement of UA, a 96 well microtiter plate (Costar, Corning, NY) was kept on ice, and each well was filled with 40 µL of sample and 200 µL of 95% H_2SO_4 containing 120 mM sodium tetraborate that had been mixed overnight using a stir bar and plate. Each plate contained 24 samples, a blank, and seven standards of galacturonic acid (0.5, 1, 1.5, 2, 4, 6, and 8 µg) in triplicate. The plate was covered with an adhesive plate sealer and placed in a water bath for one hour at 85ºC. The plate was cooled and centrifuged at low speed for 30 s. Background absorbance was measured at 520 nm using a Synergy HT microplate reader and Gen5 microplate software for Windows (BioTek, Winooski, VT). The reaction was initiated with 40 µL of *m*-hydroxydiphenyl solution (100 µL of 100 mg/mL *m*-hydroxydiphenyl in dimethyl sulfoxide stored away from light at 4ºC was mixed with 4.9 mL 80% H_2SO_4 just prior to use). The plate was covered with adhesive plate sealer and inverted several times to mix the sample, and centrifuged at low speed for 30 s. Absorbance was measured after 10 min at 520 nm.

For the measurement of NS, 40 µL of sample was added to each well of a 96 well microtiter plate with removable 1x8 strip assemblies (Immulon 4 HBX, Milford, MA) while on ice. Each plate contained 24 samples, a blank, and seven glucose standards (0.5, 1, 1.5, 2, 4, 7, and 10 µg) in triplicate. The reaction was initiated with 100 µL anthrone solution (2 $g \cdot L^{-1}$ anthrone in 95% H_2SO_4), the plate was covered with plate sealer, and mixed gently using a vortex. The wells were removed from the plate and suspended in a water bath at 92ºC for 3 min., and then cooled in a water bath at room temperature for 5 min. The plate was centrifuged for 30 s at low speed and after 20 min from when the

anthrone solution was added, the absorbance was read at 630 nm. The amount of UA and NS (µg mg^{-1} AIR) of each sample was calculated from the absorbance readings of standards used to form a calibration curve.

Statistical Analysis

ANOVA was performed using the GLIMMIX procedure and Kenward-Roger method (SAS 9.2) with genotype and treatment as fixed effects and location as a random effect. Tukey's HSD (honestly significant difference) test was used to determine significant differences ($P \leq 0.05$) between genotypes and treatments.

Results and Discussion

The percent of weight loss during storage increased for all genotypes ($P < 0.0001$) (data not shown). The weight loss of 'Springhigh' was significantly greater than 'Sweetcrisp', 'Star', and 'Windsor' after seven and 14 days of storage (Figure 5-1). After 32 days of storage, no significant differences in weight loss were detected between genotypes. Magnetic resonance imaging (MRI) was used by Paniagua et al. (2013) to visualize water distribution in blueberry fruits during postharvest storage and demonstrated that water loss occurs in all tissues of the fruit, but primarily around the stem scar. The number of soft and/or moldy fruits counted after 14 and 32 days of postharvest storage was significantly greater among 'Springhigh' berries which averaged two ($P = 0.006$) and 15 ($P<0.0001$) fruits, respectively. These results, in which 'Springhigh' demonstrated greater water loss and incidence of soft and/or moldy berries than other genotypes during postharvest storage, are consistent with field reports that 'Springhigh' has a soft fruit and "leaky" berry that has a large stem scar which tends to tear when harvested. The increased water loss and deterioration observed in 'Springhigh' fruits may be the result of its large stem scar, which provides a wider

pathway for both water and pathogens to leave and enter the fruit. These results suggest that water loss increases during berry storage and that while there was variability between genotypes for the amount of water loss that occurred, crisp-textured genotypes were not less susceptible to desiccation than soft fruits, and that differences between soft and crisp-textured blueberries were not the result of differences in water loss during ripening and postharvest storage.

In this experiment, the largest reduction in firmness as measured by bioyield force (BF), occurred between those fruits harvested at pink and ripe stages of development for all genotypes (Figure 5-2). The BF of ripe fruits was less than stored fruits for all genotypes except 'Springhigh', whose firmness did not change during storage (Figure 5-2). Vicente et al. (2007) showed that the greatest change in firmness measured in ripening blueberry fruits (green to ripe blue) occurred at the onset of color change when fruits transitioned from green to 25% blue, which is also recognized as the stage at which ripening begins in blueberry (Gough, 1994). Vicente et al. (2007) also found that berries continued to decrease in firmness as color increased (25, 75, and 100% blue), but that firmness remained the same for 100% blue and blue ripe fruits. Those results are consistent with our findings of decreased firmness as berries developed color from pink to blue. Others have reported that blueberry firmness decreased during postharvest storage, however, which is inconsistent with the increased firmness that we observed in all genotypes except 'Springhigh' (Angeletti et al., 2010; Tetteh et al., 2004; Paniagua et al., 2013). Greater weight loss was reported in those studies, however, and Paniagua et al. (2013) suggest that firmness can increase in stored blueberry fruits when weight loss is less than 4%, which may explain the increased firmness that we observed during postharvest storage.

BF measurements were significantly different ($P < 0.0001$) between genotypes that were compared at each of five maturity stages (Figure 5-3). The BF of pink fruits ranged from 3.72 to 6.70 N and ripe fruits ranged from 2.62 to 4.83 N. 'Sweetcrisp' required a greater bioyield force measurement than any other genotype at every developmental stage (Figure 5-3). When crisp ('Sweetcrisp', FL 06-561, FL 06-562, and FL 98-325) and soft textured genotypes ('Springhigh', 'Star', 'Windsor') were analyzed for variance, a significant difference was found between these two texture categories at every developmental stage (Figure 5-4). These results are consistent with those in chapter 2, where BF of several crisp and soft-textured genotypes were highly correlated with sensory evaluations of texture by a trained panel.

The dry weight of berry flesh increased during the ripening of pink to ripe fruits in four genotypes ('Sweetcrisp', FL 98-325, 'Star', and 'Windsor') and the dry weight of skin tissue from two genotypes (FL 06-561 and FL 06-562) also increased in fruits evaluated from the pink and ripe stage of development. This increase is most likely due to continued fruit growth. Blueberry follows a double-sigmoid pattern of growth in which berry size increases during an initial stage, followed by a period of minimal growth and rapid embryo development, and a final stage of fruit expansion (accounting for approx. 60% of final berry size) that coincides with ripening (Godoy et al., 2008). Fruit expansion consists primarily of cell enlargement rather than division, however, cells forming the epicarp continue to divide (Darnell et al., 1992; Harker et al., 1997). The dry weight of 'Windsor' continued to increase up to two weeks after storage (data not shown). This increase may be due to water loss during postharvest storage, which would have an effect on the calculation of dry weight.

The dry weight of flesh tissue from ripe berries of each genotype ranged from 14.1 to 17.1% fresh weight (FW) and from 21.9 to 27.5% FW for skin tissue (Table 5-1). Variation of flesh dry weight was observed between genotypes, but no difference between skin dry weights was detected (Table 5-1). Flesh dry weight of 'Sweetcrisp' was greater than other genotypes and supported expectations that crisp fruit would have more dry matter accounting for their increased firmness. However, the flesh dry weight of crisp genotypes FL 06-561 and FL 06-562, were the same or significantly less than those of soft-textured genotypes (Table 5-1). Skin dry weights were not significantly different between genotypes at the pink, ripe, and 7 day postharvest maturity stages. The skins of FL 98-325 and 'Springhigh', representing crisp and soft genotypes, respectively, had higher dry weights than other genotypes after 14 days postharvest storage. The dry weight of flesh and skin tissue was not significantly different between classes of crisp and soft texture.

AIR was expressed in milligrams per 100 mg FW and ranged from 1.31 to 1.56 $mg \cdot 100mg^{-1}$ in the flesh of ripe fruits and from 5.83 to 10.13 $mg \cdot 100mg^{-1}$ in the skins of ripe fruit (Table 5-1). The amount of AIR in whole blueberry fruits from the cultivar 'Duke' was measured by Vicente et al. (2007) to be approximately 3 to 4 $mg \cdot 100mg^{-1}$ and is therefore considered to be consistent with the AIR values we obtained from flesh and skin tissue separately (Vicente et al., 2007). Flesh tissue from 'Star', and skin tissue from FL 98-325, were the only examples from this study in which a difference in AIR could be detected between pink, ripe, and postharvest stored fruits. However, Vicente et al. (2007) found that AIR decreased over five ripening stages (green to ripe) in the cultivar 'Duke'.

Genotypes differed in the amount of AIR from flesh tissue at the pink, ripe, and 32 day storage period, and in AIR from skin tissue at all developmental stages except pink. The AIR from the berry flesh of crisp-textured FL 98-325 and soft-textured Windsor was greater than the soft-textured 'Springhigh', and the AIR from the berry skin of 'Springhigh' was greater than FL 98-325 and soft-textured 'Star'. These results suggest that genotypes vary in their amount of cell wall material, but differences between genotypes were unrelated to the textural categories of soft and crisp.

The contents of uronic acids (UA) and neutral sugars (NS) were measured as micrograms per milligram of AIR. UA ranged from 187 to 251 $\mu g \cdot mg^{-1}$ in the flesh of ripe fruit and from 191 to 360 $\mu g \cdot mg^{-1}$ in the skins of ripe fruit. These values were lower than those obtained from the measure of UA in whole blueberry fruits from the cultivar 'Duke', which ranged from 311 to 344 $\mu g \cdot mg^{-1}$ AIR (Vicente et al., 2007). Color production by uronic acids is reduced in the presence of interfering neutral sugars, and despite efforts to minimize interference by reducing the reaction temperature and measuring background absorbance, browning from neutral sugars may have interfered with UA absorbance readings (van den Hoogen et al., 1998). NS ranged from 464 to 620 $\mu g \cdot mg^{-1}$ in the flesh of ripe fruits and from 395 to 488 $\mu g \cdot mg^{-1}$ in the skins of ripe fruit. These values are consistent with those measured by Vicente et al. (2007) in the blueberry cultivar 'Duke'. The amount of UA and NS in the flesh and skins of fruit did not change during fruit development from pink to 32 days postharvest storage in any of the genotypes that we assayed (Table 5-2). Similarly, UA and NS did not change during the ripening of 'Duke' (Vicente et al., 2007). Variability between ripening stages of 'Duke' was detected, however, after further and more specific analysis of non-cellulosic neutral sugars (Vicente et al., 2007). Differences in the UA content of genotypes was detected

in the flesh of berries after 14 days, and in the skin of berries after 32 days of storage, but genotypes did not vary in the amount of NS measured from flesh and skin tissue.

Dry weight, AIR, UA, and NS content were measured in the separated flesh and skin tissues of each genotype at each developmental stage, and compared between crisp and soft-textured genotypes (Table 5-1). There were differences in the dry weight and AIR between genotypes, but these differences did not correspond to crisp and soft texture classes (Table 5-1).

Together, these results confirm that there is a phenotypic difference between crisp and soft textured blueberry genotypes that can be detected with bioyield force measurements, but that gross quantitative measures of total cell wall material, pectins, and neutral sugars are not descriptive enough to detect the physiological basis of these differences. To further pursue an explanation of these differences, the AIR could be separated into fractions based on the solubility of cell wall components in which polymer sizes are measured and specific neutral sugars are identified (Brummell, 2006). Further in depth studies could also be pursued with the use of monoclonal antibodies which bind specific cell wall polymers that may help identify structural differences between crisp and soft-textured berries (Willats et al., 2006). Atomic force microscopy (AFM) was used to image hemicelluloses in Chinese cherry (*Prunus pseudocerasus* L.), and revealed that the branching pattern of hemicellulose in crisp fruit was oriented in the same direction, but was more irregular in soft fruits. Length of branch chain was unrelated to texture type, but crisp varieties had wider branches and a higher frequency of wide branched chains (Chen et al., 2009). Crisp texture in blueberry may also be a result of structural variations in the substitution and branching patterns of the pectic and hemicellulosic components.

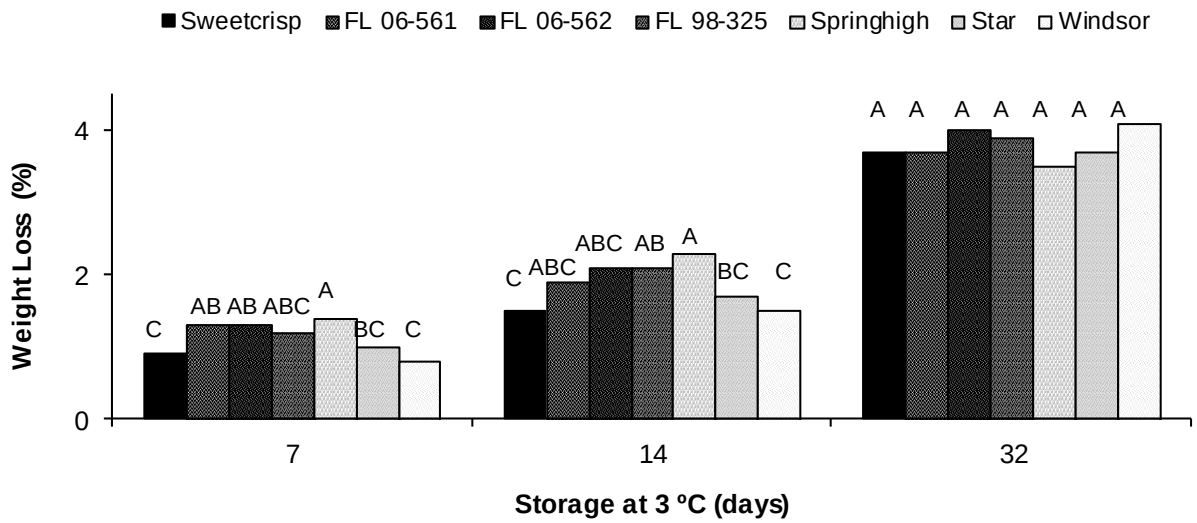

Figure 5-1. Weight loss (%) of four crisp (black) and three soft (gray) textured southern highbush blueberry genotypes after 7, 14, and 32 days postharvest storage at 3ºC. Different letters above graph bars indicate significant difference ($P < 0.05$) determined by Tukey's HSD test for genotypes within a developmental stage.

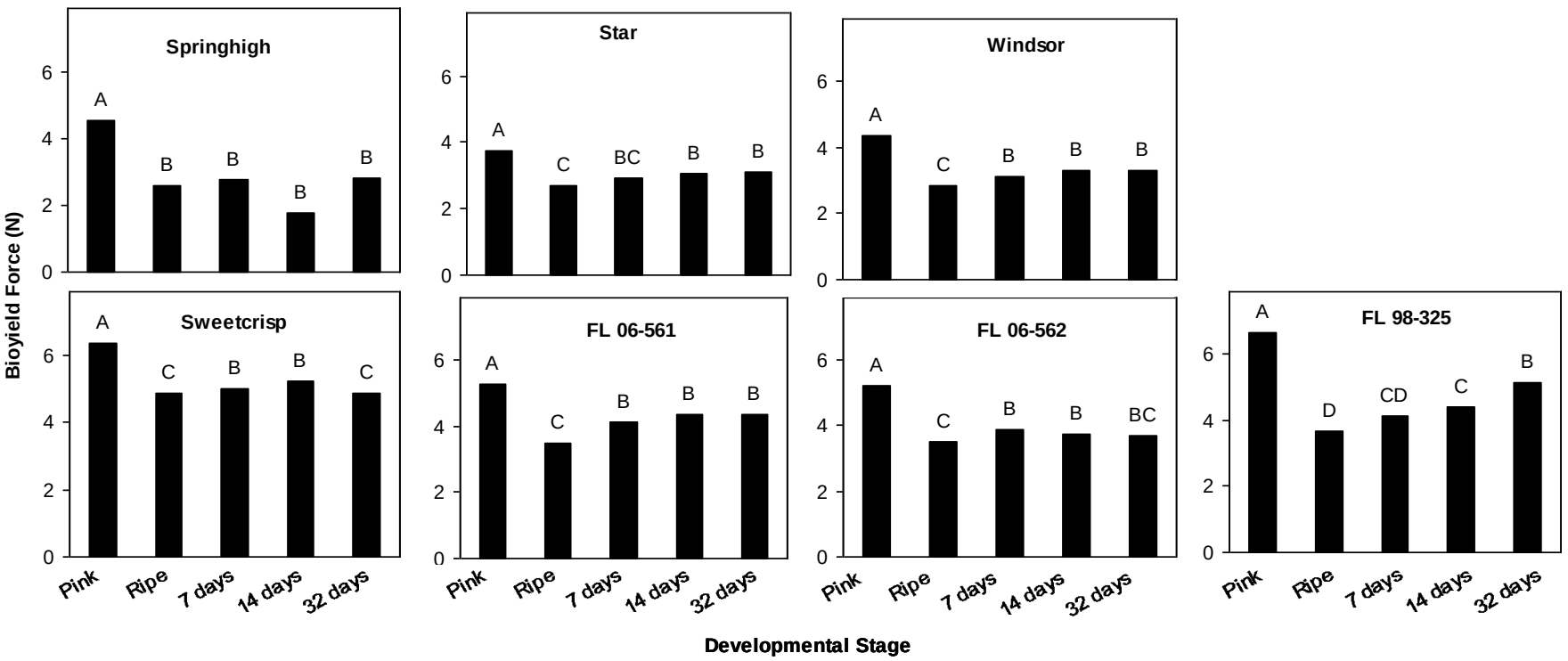

Figure 5-2. Bioyield force measurements (N) of fruit at pink, ripe, 7, 14, and 32 days storage at 3 ºC fruits from three soft (top) and four crisp (bottom) southern highbush blueberry genotypes. Different letters above graph bars indicate significant difference ($P < 0.05$) determined by Tukey's HSD test.

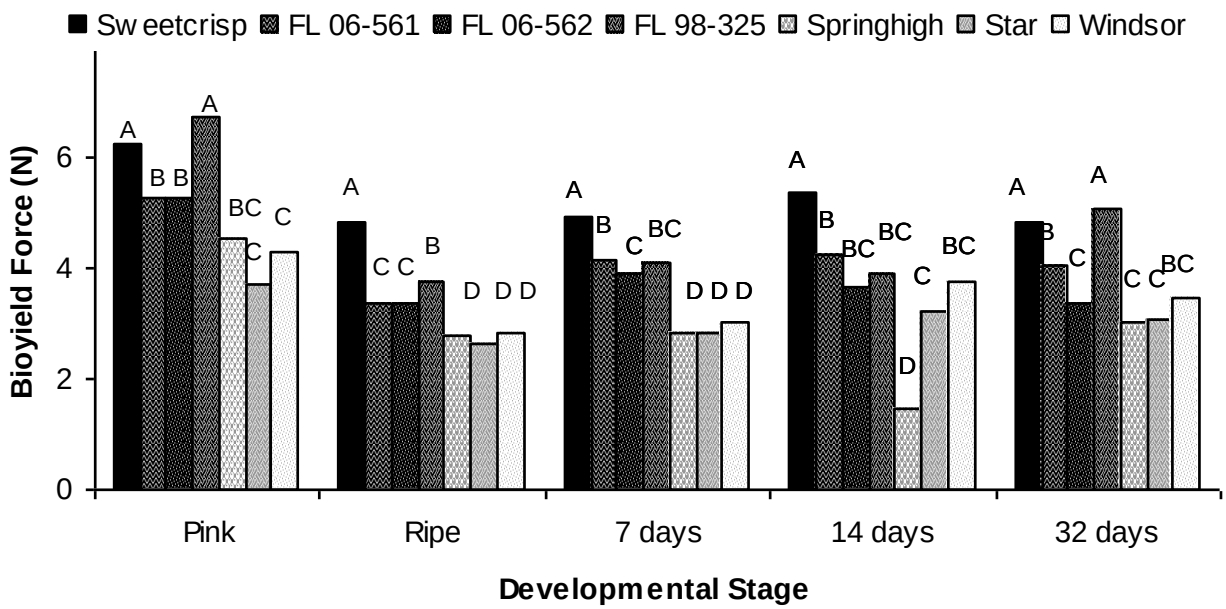

Figure 5-3. Bioyield force measurements (N) of four crisp (black) and three soft (gray) textured southern highbush blueberry genotypes at the pink and ripe stage, and after 7, 14, and 32 days postharvest storage at 3ºC. Different letters above graph bars indicate significant difference ($P < 0.05$) determined by Tukey's HSD test for genotypes within a developmental stage.

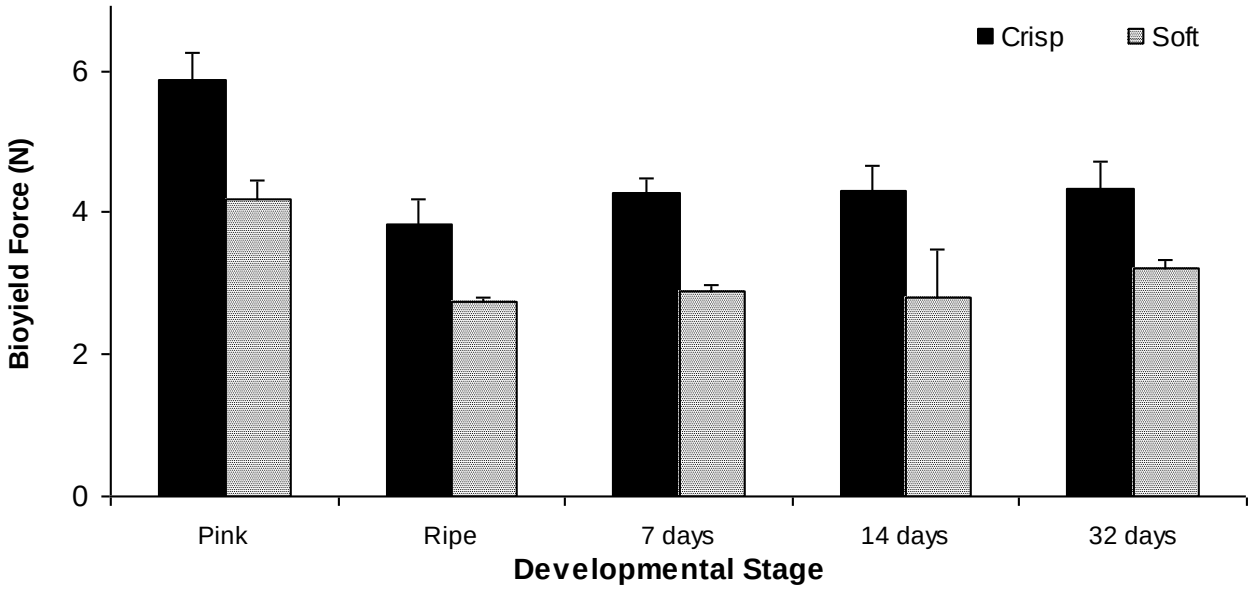

Figure 5-4. Bioyield force measurements (N) of combined crisp and soft-textured southern highbush blueberry fruits at five maturity and postharvest stages. Error bars denote SE.

Table 5-1. Changes in dry weight and alcohol insoluble residue (AIR) of flesh and skin tissue from crisp and soft-textured southern highbush blueberry genotypes during ripening. Different letters in a column of the same tissue origin indicate significant difference ($P < 0.05$) determined by Tukey's HSD.

		Dry Weight (% Fresh Weight)										AIR (µg 100 mg^{-1})									
Tissue	Genotype	Pink		Ripe		7 days		14 days		32 days		Pink		Ripe		7 days		14 days		32 days	
Flesh	Sweetcrisp	13.3	AB	17.1	A	16.8	A	17.5	A	16.5	A	1.45	ABC	1.31	AB	1.32	A	1.28	A	1.36	AB
	FL 06-561	13.4	AB	14.3	BC	13.2	CD	13.7	CD	13.9	C	1.34	BC	1.33	AB	1.23	A	1.18	A	1.20	AB
	FL 06-562	13.2	AB	12.8	C	12.7	D	13.1	CD	13.1	C	1.37	BC	1.38	AB	1.28	A	1.16	A	1.37	AB
	FL 98-325	14.0	A	15.2	AB	15.3	AB	13.7	CD	15.9	AB	1.57	AB	1.43	A	1.44	A	1.23	A	1.48	A
	Springhigh	11.4	B	14.1	BC	14.2	BCD	11.2	D	13.8	C	1.25	C	1.31	B	1.28	A	1.11	A	1.08	B
	Star	12.6	AB	15.2	AB	14.7	BC	15.0	BC	14.2	BC	1.49	ABC	1.31	AB	1.33	A	1.25	A	1.32	AB
	Windsor	12.7	AB	15.1	ABC	15.4	AB	16.9	AB	15.3	ABC	1.72	A	1.56	A	1.49	A	1.33	A	1.54	A
Skin	Sweetcrisp	21.4	A	27.5	A	24.3	A	21.2	B	23.0	AB	7.55	A	8.12	AB	7.54	ABC	6.84	AB	6.47	ABC
	FL 06-561	20.4	A	24.2	A	21.8	A	21.9	B	20.9	AB	7.60	A	8.24	AB	6.78	BC	7.77	AB	5.97	BC
	FL 06-562	19.0	A	26.5	A	21.5	A	20.9	B	19.9	B	6.89	A	9.57	AB	7.60	ABC	7.91	A	6.23	ABC
	FL 98-325	20.3	A	24.4	A	21.6	A	30.4	A	23.4	AB	7.71	A	6.15	B	6.46	BC	5.50	B	6.45	ABC
	Springhigh	21.0	A	26.6	A	25.8	A	31.5	A	23.3	AB	8.08	A	10.13	A	9.37	A	8.39	A	7.51	AB
	Star	20.7	A	21.9	A	21.9	A	19.9	B	20.8	B	7.70	A	5.83	B	5.82	C	5.93	B	5.45	C
	Windsor	21.9	A	23.4	A	23.0	A	22.9	B	24.9	A	8.31	A	7.69	AB	8.52	AB	8.15	A	7.87	A

Table 5-2. Differences between southern highbush blueberry genotypes for uronic acids and neutral sugars in the flesh and skin tissue of berries at two stages of ripening (pink and blue) and 3 stages during postharvest storage (7, 14, and 32 days). Different letters in a column of the same tissue origin indicate significant difference ($P < 0.05$) determined by Tukey's HSD.

		Uronic Acids (µg mg^{-1})					Neutral Sugars (µg mg^{-1})				
Tissue	Genotype	Pink	Ripe	7 days	14 days	32 days	Pink	Ripe	7 days	14 days	32 days
Flesh	Sweetcrisp	316 A	187 A	225 A	192 BC	206 A	674 A	577 A	588 A	560 A	524 A
	FL 06-561	334 A	248 A	261 A	225 ABC	251 A	542 A	464 A	492 A	482 A	483 A
	FL 06-562	327 A	214 A	161 A	112 C	158 A	505 A	540 A	455 A	515 A	479 A
	FL 98-325	253 A	251 A	222 A	436 A	238 A	531 A	591 A	569 A	496 A	565 A
	Springhigh	225 A	194 A	170 A	361 AB	192 A	533 A	464 A	592 A	508 A	563 A
	Star	272 A	241 A	249 A	194 BC	269 A	522 A	579 A	539 A	452 A	530 A
	Windsor	207 A	228 A	147 A	103 C	208 A	517 A	620 A	558 A	529 A	563 A
Skin	Sweetcrisp	319 A	239 A	258 A	214 A	285 AB	428 A	418 A	429 A	414 A	444 A
	FL 06-561	265 A	192 A	266 A	222 A	300 AB	420 A	462 A	403 A	409 A	363 A
	FL 06-562	293 A	242 A	226 A	196 A	279 AB	393 A	424 A	381 A	436 A	372 A
	FL 98-325	323 A	284 A	252 A	362 A	169 B	419 A	408 A	396 A	413 A	456 A
	Springhigh	183 A	360 A	268 A	270 A	183 B	443 A	437 A	467 A	361 A	399 A
	Star	258 A	256 A	259 A	267 A	317 A	397 A	395 A	441 A	394 A	368 A
	Windsor	347 A	263 A	186 A	249 A	284 AB	568 A	488 A	458 A	404 A	336 A

CHAPTER 6
SENSORY AND INSTRUMENTAL MEASUREMENTS OF CRISP TEXTURED BLUEBERRIES IN AN F_1 POPULATION

Literature Review

Southern highbush blueberry (SHB, *Vaccinium corymbosum* L. hybrids) belongs to the family Ericaceae, genus *Vaccinium*, and section *Cyanococcus,* which includes 16 species (Uttal, 1987). Blueberries sold commercially for the fresh market typically come from two species: *V. corymbosum* and *V. virgatum,* commonly known as "northern highbush" and "rabbiteye" respectively. More recently, SHB resulting from hybrids between northern highbush and several section *Cyanococcus* species native to the southeastern U.S. (e.g., *V. darrowii, V. virgatum, V. myrsinites*), have been developed for production in low-chill climates (Moore, 1965; Sharpe, 1953; Lyrene, 1997).

In 1997, the SHB cultivar 'Bluecrisp' was released by the University of Florida (UF) and was considered by many to have a uniquely crisp texture (Okie, 1999). Similar crisp texture was also noted in several other genotypes from UF breeding germplasm including FL 97-136, FL 98-325, FL 02-22, FL 03-161 and 'Sweetcrisp', which was released for commercial production in 2005 (Olmstead, 2011). The firmness of crisp berries makes them attractive for use in mechanical harvesting and as a means of extending postharvest shelf life (Mehra et al., 2013). The texture of these fruits is also considered to have increased consumer appeal (Padley, 2005; Olmstead, personal communication). Ehlenfeldt and Martin, (2002) reported that SHB cultivars were generally more firm than northern highbush cultivars when compression force measurements were made, and suggested it may be due to southern native *Vaccinium* species in their ancestry. However, the crisp texture trait has not been traced back to any one genetic source through pedigree analysis. Furthermore, it is unclear whether

the genetic basis of crisp texture is the result of monogenic or polygenic inheritance. Because SHB is considered to be autotetraploid (Lyrene et al., 2003), simply inherited traits are phenotypically much more complex than in diploids and can appear to be quantitative (Lyrene, 1993). The objective of this study was to evaluate segregation patterns of crisp texture in five F1 populations produced from parents expressing varying degrees of crisp texture. Phenotypic data was assembled both by sensory evaluation and instrumental measurements of bioyield using a texture analyzer.

Methods

Plant Material

Five crosses were made in spring 2009 ('Sweetcrisp' x FL 98-325; FL 03-161 x FL 98-325; FL 98-325 x FL 97-136; FL 98-325 x FL 02-22; FL 98-325 x 'Sweetcrisp') between parents considered to have a crisp texture as determined by firmness measurements and sensory evaluations made by breeders in the field (Padley, 2005). Hand-pollinated berries were picked at full maturity and seeds were extracted by blending the total number of berries from a single cross in a Waring blender (model no. 38BL54, Torrington, CT). Seed from each cross were stored at 4 °C for approximately six months before being planted in 3.75 L plastic pots containing 100% peat soil. After germination, approximately 1,000 seedlings from each cross were transplanted into trays of peat at 2.54 cm spacing and grown in the greenhouse to a height of approximately 30 cm. Seedlings were randomly planted by population in April 2010 at 0.76 m spacing in raised bed rows (3 m apart) consisting of pine bark mixed with native soil and covered with black woven weed barrier. Standard Florida blueberry management practices were applied uniformly to all seedlings (Williamson et al., 2004, 2013). High tunnels were constructed over these plants in October-November 2010 and

commercial bumblebee hives were placed centrally under each tunnel in January 2011 and 2012 to facilitate pollination. The first two hundred healthy plants in a row from each cross were selected and labeled for phenotypic evaluation.

Phenotypic Evaluation

In spring 2011, fruit from two hundred F_1 seedlings from each of the five populations were rated for crisp texture using a 9-point scale ranging from “not crisp” (1) to “most crisp” (9). To compensate for fruit-to-fruit variability, three to six berries from each seedling were tasted, and only undamaged berries were selected at the ripe (100% blue surface area) stage of development (Shutak et al., 1980). Following each evaluation period, plants were harvested for commercial production to ensure that overripe fruits would not be present at the subsequent harvest for sensory analysis. To account for climate and fruit quality differences throughout the approximate six week harvest season, each seedling was evaluated on three separate dates during the harvest period. Plants that could not be evaluated more than one time during the season due to low plant yield or excessive fruit damage were excluded from the population. The sensory scores of each seedling were averaged and any seedlings with a standard deviation of ≥3 were also excluded. The total number of seedlings that were included in the analysis of each population is given in Table 6-1.

In spring 2012, 25 undamaged ripe (100% blue surface area) fruits were picked from 200 F_1 seedlings of the FL 98-325 x ‘Sweetcrisp’ population. Berries were packed in 170 g plastic clamshells (Pactiv, Lake Forest, IL) , stored in coolers filled with ice and transported to the blueberry breeding lab at UF in Gainesville, FL for instrumental analysis on the same day. Because this particular population had variable yield and fruit had to be harvested from a single seedling, harvests were sometimes performed on

multiple days in order to reach the 25 berry goal. Seedlings from which 25 berries could not be acquired due to plant death, low fruit yield, or damaged fruit were excluded from the population. Bioyield (N) was measured using a TA.HD plus Texture Analyzer (Texture Technologies, Corp., Scarsdale, NY) fitted with a 4 mm probe to bioyield through the skin of a single berry oriented equatorially upright as described by Ehlenfeldt and Martin, (2002).

Data Analyses

A chi square goodness-of-fit test was used to determine whether the segregation pattern of each population fit expected segregation ratios for a Mendelian mode of inheritance.

Results and Discussion

Sensory scores for crisp texture reported for 2011 were averaged from three evaluations per seedling and ranged from 1.3 to 8.3 on a nine-point scale across all five F_1 populations. Because population size was not the same for each family, the distribution of sensory scores was expressed as the percent of seedlings with mean sensory scores ranging from one to nine (Figure 6-1). In three out of five populations, no seedling received a sensory score greater than 7.6. The FL 98-325 x FL 02-22 population had the lowest mean sensory score of 3.8, and the FL 98-325 x 'Sweetcrisp' population had the highest mean sensory score of 6.0. The distribution of sensory scores for seedlings from the FL 98-325 x 'Sweetcrisp' population was skewed toward the higher texture rating compared to all other populations. In 2011 a trained sensory panel evaluated four of the five parents used to develop these populations (FL 02-22, FL 03-161, FL 98-325, and 'Sweetcrisp'). Several fruit textural qualities were determined including "bursting energy" which was rated on an eleven point scale (0 to 10) based on

the panelist's impression at the first bite, from mushy to crunchy (see Chapter 2). Sensory scores determined by the trained panel for FL 02-22, FL 03-161, FL 98-325, and 'Sweetcrisp' were 3.4, 4.5, 6.3, and 7.3 respectively. Populations having one parent (FL 02-22 or FL 03-161) that received lower sensory scores by the trained panel for bursting energy also had lower overall sensory scores for crisp texture in this study as compared to those populations having the two parents (FL 98-325 and 'Sweetcrisp') that received higher scores from the trained panel.

There were several limitations to the sensory score method of phenotyping used for these populations in 2011. The process of rating so many samples in the field could not be performed under the same conditions at each evaluation. Temperature is well known to have an effect on blueberry fruit firmness (NeSmith et al., 2002, 2005). Evaluation of these populations often required the entire day, such that fruits evaluated in the cool morning were not under the same conditions as those evaluated during the heat of the afternoon. For a more consistent and less subjective measure of texture, a single population was selected for evaluation by instrumental measures in 2012.

Bioyield (defined as the point at which deformation increases and force decreases or remains the same) was measured using a texture analyzer on 25 berries from 124 F_1 seedlings of the FL 98-325 x 'Sweetcrisp' population (Jackman and Stanley, 1995). A normal distribution, with bioyield forces for individual genotypes ranging from 3.27 to 6.05 N and a mean force of 4.41 N, was observed for this population (Figure 6-2). The parents of this population were not located in the same field, however, the mean bioyield force of FL 98-325 and 'Sweetcrisp' fruit collected from multiple nearby locations during the same harvest time period was 3.70 and 4.89 N respectively. In comparison, standard texture, non-crisp commercial cultivars such as 'Springhigh', 'Star', and

‘Windsor’ were also collected from multiple locations in 2012 and had a mean bioyield force of 2.57, 2.67, and 2.84 N respectively (data not shown). Each of these non-crisp cultivars had an average bioyield that was below the lowest bioyield force from any individual in the FL 98-325 x ‘Sweetcrisp’ population.

Chi square analysis was used to test the five populations that were phenotyped by sensory scores in 2011 to determine whether they could be explained by qualitative inheritance. Based on sensory and instrumental data previously obtained on four of the five parents used in these crosses, we hypothesized that crisp texture was the result of incomplete dominance and that degree of crisp texture was dependent on allelic dosage in autotetraploid southern highbush blueberry. ‘Sweetcrisp’ had received the highest sensory scores for texture by trained panelists in 2010 and 2011, and also had the highest bioyield force measured by a texture analyzer (see Chapter 2). However, because fruit from several seedlings from the FL 98-325 x ‘Sweetcrisp’ F_1 population received higher sensory and bioyield scores than ‘Sweetcrisp’, we hypothesized that this parent contributed a higher allelic dosage than any of the other parents but did not have the highest allelic dosage possible for this putative locus. Therefore, ‘Sweetcrisp’ was designated to contribute three out of four potential alleles to the crisp phenotype at a single locus. Based on previous sensory scores, FL 98-325 was designated to contribute two out of four alleles, and the remaining three parents (FL 97-136, FL 02-22, and FL 03-161) were designated as contributors of one allele to the crisp phenotype. According to this model, the FL 98-325 x ‘Sweetcrisp’ F_1 population was expected to demonstrate a 1:5:5:1 segregation ratio in which 8.3% of the population was homozygous for the crisp allele, 42% carried three crisp alleles, 42% carried two alleles, and 8.3% carried one allele. The ‘Sweetcrisp’ x FL 02-22 population was expected to

follow a 1:2:1 segregation ratio where 25% of the progeny carried three crisp alleles, 50% carried two crisp alleles, and 25% carried one crisp allele. The remaining three populations (FL 03-161 x FL 98-325, FL 98-325 x FL 97-136, and FL 98-325 x FL 02-22) were expected to follow 1:5:5:1 segregation ratios with classes representing three, two, one, or zero crisp alleles, respectively. Because sensory scores were subjective and did not correspond to the number of potential alleles at a single locus, the scores were divided into classes as follows: Sensory scores of 8.0-9.0 were designated to represent seedlings having four crisp alleles, scores of 6.5-7.9 represented seedlings with three crisp alleles, scores of 3.5-6.4 represented seedlings with two crisp alleles, scores of 2.5-3.4 represented seedlings with one crisp allele, and scores of 1.0-2.4 represented seedlings with no crisp allele. Because the distributions of four out of five populations were heavily skewed toward less crisp ratings and because of limited ability to differentiate between softer fruits at the low end of the sensory scale, seedlings having one or zero crisp alleles were combined into a single class and represented by sensory scores <3.4. These sensory score classes were applied uniformly to the varying expected segregation ratios in the chi square analysis of all five populations.

Four out of five populations fit expected segregation ratios based on the hypothesis that 'Sweetcrisp', FL 98-325, and the other three parents (FL 97-136, FL 02-22, and FL 03-161) contributed three, two, and one crisp alleles respectively ($P > 0.2$) (Table 6-1). Segregation of the 'Sweetcrisp' x FL 02-22 population was found significantly different from the expected 1:2:1 segregation pattern ($P < 0.02$). Fewer individuals were able to be evaluated from this population due to poor fruit quality and pest damage. This diminished sample and overall reduction in fruit firmness among

seedlings of this population may account for the deficit of individuals with higher sensory scores.

Because bioyield was only measured in one F_1 population in 2012 a chi-square analysis was not performed, since division of measurements into classes corresponding to allelic dosage would be arbitrary and unable to be compared between multiple populations. However, based on the bioyield measurements of the parents (FL 98-325 and 'Sweetcrisp') in relation to the population distribution, the most plausible model assuming crisp is a monogenic trait is that in which 'Sweetcrisp' carries three crisp alleles and FL 98-325 carries two crisp alleles. The bioyield of thirteen individuals was less than the bioyield of FL 98-325 (3.70 N) and the bioyield of twenty-five individuals was greater than 'Sweetcrisp' (4.89 N), leaving 84 individuals with bioyield scores between these two parents. Regardless of how bioyield scores are grouped according to allelic dosage, the segregation ratio of AAaa x AAAa (1:5:5:1) is more descriptive of this population than any other alternative for a monogenic trait. Unfortunately, in an autotetraploid, the segregation ratio of Aaaa x AAaa (1:5:5:1) cannot be ruled out, as it is not distinguishable from the previous allelic combination. However, assigning an allele dosage of Aaaa to FL 98-325 and AAaa to 'Sweetcrisp' could not be expanded to fit the parents (FL 97-136, FL 02-22, and FL 03-161) used to develop the populations phenotyped by sensory score in 2011, and does not correlate with sensory panel and firmness measurements of the parents (FL 02-22, FL 03-161, FL 98-325, and 'Sweetcrisp') which demonstrate increasing levels of crispness.

While the normal distribution of bioyield force in the FL 98-325 x 'Sweetcrisp' population is descriptive of quantitative inheritance, it could also explain a monogenic trait that is disguised by polyploidy and environmental factors that make the crisp

phenotype difficult to classify in discrete categories. Thus, it remains unclear whether the genetic basis of crisp texture is regulated by one or several genes. Although the subjective rating scales used in 2011 can be made to fit a single-gene model, the polyploid nature of blueberry and the potential for environmental interactions make assessing texture difficult. Until a more precise method of determining the crisp phenotype is developed, ideally at the genome level, it may be difficult to conclusively determine whether crisp texture is a monogenic or polygenic trait.

Table 6-1. Segregation data for crisp texture in five F_1 southern highbush blueberry populations tested to fit expected single-gene segregation ratios for a trait of incomplete dominance in an autotetraploid species.

Cross	No. of Seedlings	Allelic Dosage[z]	Observed[y]	Expected	X^2	*P*-value
SC[x] x 02-22[v]	152	aaaa	44[w]	0 + 38		
		Aaaa				
		AAaa	86	76		
		AAAa	22	38		
		AAAA	0	0	9	<0.02
03-161[v] x 98-325[u]	190	aaaa	82	16 + 79		
		Aaaa				
		AAaa	86	79		
		AAAa	15	16		
		AAAA	0	0	2.5	>0.25
98-325 x 97-136[v]	188	aaaa	83	16 + 78		
		Aaaa				
		AAaa	85	78		
		AAAa	20	16		
		AAAA	0	0	2.9	>0.2
98-325 x 02-22	192	aaaa	91	17 + 83		
		Aaaa				
		AAaa	91	83		
		AAAa	17	17		
		AAAA	0	0	1.6	>0.25
98-325 x SC	184	aaaa	16	0 + 15		
		Aaaa				
		AAaa	83	75		
		AAAa	65	75		
		AAAA	17	15	2.5	>0.25

[z]Allelic dosage representing genotypes with sensory scores of AAAA = 8.0-9.0; AAAa = 6.5-7.9; AAaa = 3.5-6.4; Aaaa = 2.5-3.4, and aaaa = 1.0-2.4.

[y]Each seedling was evaluated by sensory score 2-3 times during the season. Sensory scores were averaged and any seedlings with a standard deviation of ≥3 were excluded.

[x]AAAa = genotype with 3 crisp alleles.

[w]Because subjective rating differences between the aaaa and Aaaa allelic classes were difficult to separate, these two classes were uniformly combined and the chi-square analysis was applied to the combined class.

[v]Aaaa = genotype with 1 crisp allele.

[u]AAaa = genotype with 2 crisp alleles.

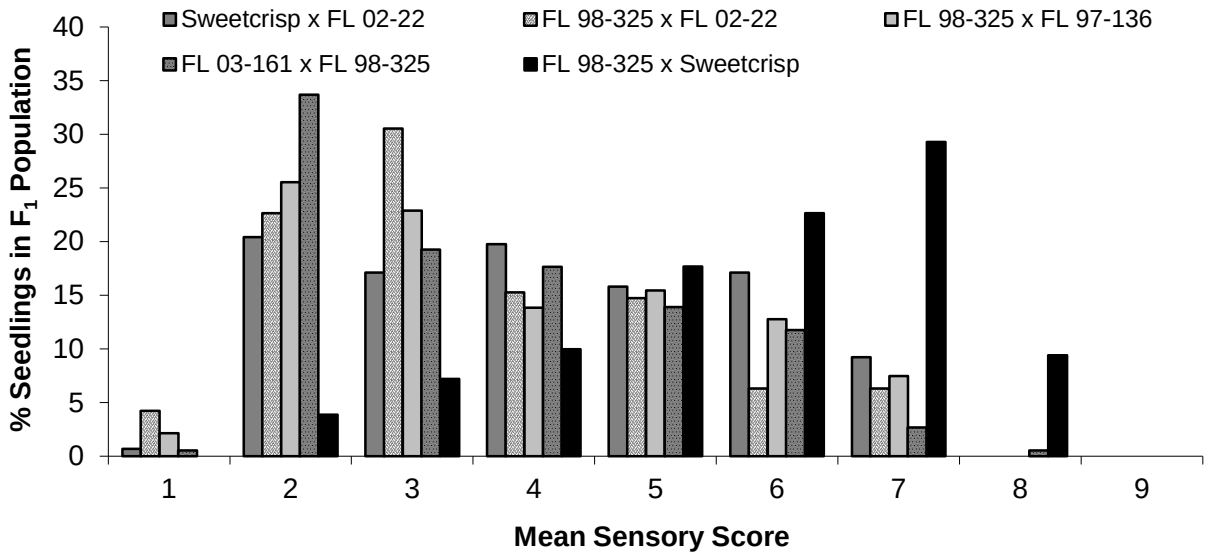

Figure 6-1. Distribution of mean sensory scores for seedlings from five F_1 southern highbush blueberry populations rated in April 2011. Sensory scores follow a 1-9 scale where 1 = not crisp and 9 = most crisp.

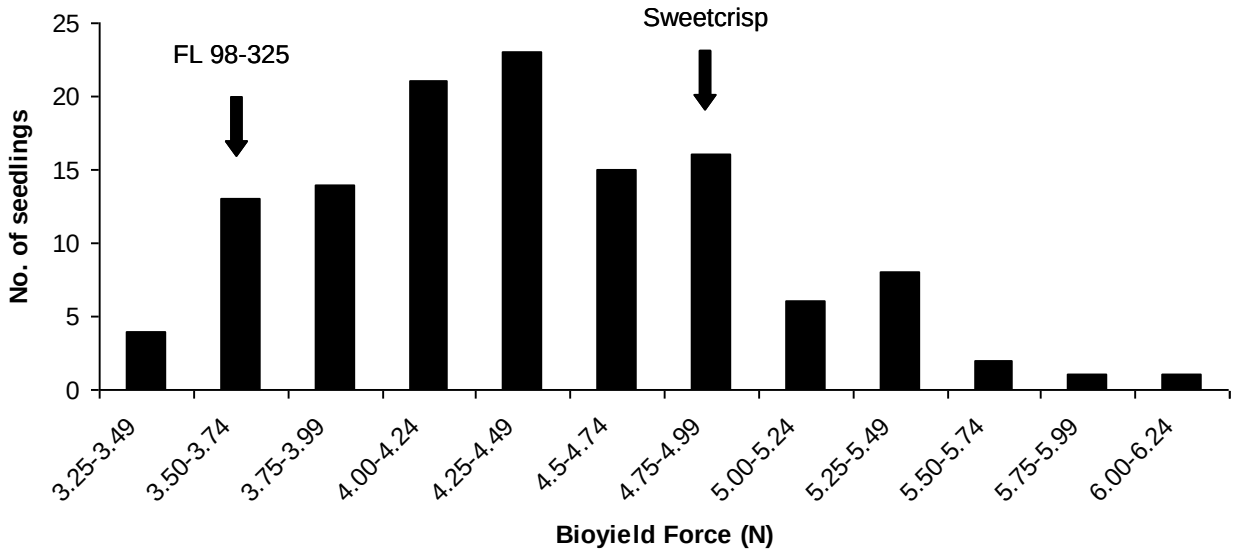

Figure 6-2. Distribution of bioyield force (N) of seedlings from the FL 98-325 x 'Sweetcrisp' F_1 southern highbush blueberry population.

CHAPTER 7
CONCLUSION

The objective of this research was to use compression and bioyield force measures to identify crisp and soft-textured southern highbush blueberry (SHB) genotypes determined by a trained sensory panel, to evaluate how genotypes of these identified texture classes respond to ethylene inhibition, to investigate differences in cellular structure and composition between genotypes, and to phenotype seedling populations from putative crisp parents in order to determine segregation patterns of crisp texture in blueberry.

Descriptors for textural traits in blueberry were devised using a trained sensory panel to survey a broad range of germplasm, including crisp and soft-textured SHB cultivars and selections developed at the University of Florida. Differences in sensory perception of berry texture and instrumental measurements of compression and bioyield force on the berry tissue were detected between genotypes, and correlations between trained panel ranking and instrumental measurements of blueberry texture were found. Instrumental measures of compression and bioyield forces were significantly different among genotypes and correlated with sensory scores for bursting energy, flesh firmness, and skin toughness. These results suggest that there is a distinction between crisp and soft-textured cultivars in blueberry, and that crispness is related to the sensory perception of bursting energy, flesh firmness, and skin toughness, as well as higher compression and bioyield force measurements.

In an effort to determine the physiological basis for the increased firmness observed in crisp-textured genotypes, we began by exploring the role of ethylene in blueberry ripening. Fruit species can be divided into two categories based on how they

ripen: climacteric and non-climacteric. Because blueberry had been characterized as a climacteric fruit, we decided to first investigate how genotypes of the previously identified texture classes would respond to ethylene inhibition. Two SHB cultivars with soft and crisp fruit texture were treated at five and nine days prior to berry coloration with a preharvest application of 1-methylcyclopropene (1-MCP), which is a known ethylene inhibitor. Compression firmness was measured on berries from each treatment and genotype, but none of the preharvest treatments with 1-MCP resulted in significantly greater firmness when compared to the untreated control. Ethylene sensitive (climacteric) fruits are expected to show negative ripening responses to ethylene inhibitors such as 1-MCP, and to our knowledge no climacteric fruit has yet been found to be unresponsive to 1-MCP. These results suggested that ethylene inhibition does not have an effect on ripening in blueberry and that blueberry has been inappropriately identified as a climacteric fruit. These results also demonstrate that differences in fruit texture were not the result of genotypic differences in ethylene sensitivity.

In conjunction with our findings that neither crisp nor soft-textured fruits demonstrated ethylene sensitivities during ripening, reports from an unpublished study between crisp and soft-textured SHB genotypes showed that crisp genotypes had consistently greater firmness throughout ripening and storage but were not different from soft-textured genotypes in the rate at which they softened. Together these findings led us to focus on differences in the cellular structure between these two texture types rather than changes over time. More specifically, we chose to narrow our studies by focusing on the structure and composition of the fruit peel.

A survey of blueberry firmness (measured as compression force $g \cdot mm^{-1}$) by Ehlenfeldt and Martin (2002) found that SHB with southern native species (*V. virgatum*,

V. darrowii, and *V. tenellum*) in their pedigree, were firmer than SHB with introgression from northern species (*V. angustifolium*). Silva et al (2005) measured the bioyield force of three rabbiteye (*V. virgatum*) and two highbush (*V. corymbosum*) cultivars and similarly found that the rabbiteye genotypes originating in the south had a greater bioyield force. The texture of rabbiteye cultivars has also been described as having increased seediness and tougher skins Silva et al., 2005).

In addition, Gough (1983) reported sclereids beneath the skin tissue of three highbush blueberry cultivars and suggested that these highly lignified cells give fruit a gritty texture (which could easily be confused with “seediness”) and may increase firmness. It has also been observed that unlike standard soft blueberry fruits, which form a sauce when heated, the skins of crisp fruits remain intact (Olmstead, personal communication). These reports, in combination with our own findings (see chapter 2) in which bioyield force, bursting energy (“crispness”), and skin toughness were highly associated, led us to focus our research on the fruit peel. To examine the structural features of the fruit peel, a histological analysis was performed on the outermost cell layers of four soft, four crisp, and one intermediate-textured genotype at the mature green and ripe blue maturity stage. The results of this study found variability among genotypes for stone cell frequency, cell size, cell shape, and thickness of the epicarp, but no differences between classes of soft and crisp-textured genotypes were found, which led us to a more detailed study of cellular composition.

Similar to the crisp SHB genotypes that we evaluated (see chapter 2), rabbiteye blueberries have been shown to have greater firmness when measured by bioyield force than highbush types, and also were shown to contain more soluble and insoluble fiber (Silva et al., 2005). Fruit firmness is largely determined by cell wall strength and

thickness (Goulao and Oliveira, 2008; Li et al., 2010), therefore, the next logical step towards investigating the physiological differences between crisp and soft-textured blueberries, was to look in more detail at the cell wall. We continued to focus our research on the fruit peel and collected the alcohol insoluble residue (AIR), comprised of mostly cell wall material, from separated skins and flesh tissue from three crisp, three soft, and one intermediate-textured SHB genotype from two field locations. To confirm unpublished findings of the degree to which crisp and soft-textured genotypes vary in firmness over time, we evaluated fruits at two maturity stages (pink and ripe) and after three postharvest storage durations (7, 14, and 32 days at 3ºC). Dry weight, AIR, uronic acids, and neutral sugars were measured in the separated flesh and skin tissues of each genotype at each developmental stage, and were compared between crisp and soft-textured genotypes. There were differences in the dry weight and AIR of flesh and skin tissue between genotypes, and select genotypes varied in uronic acids, but these differences did not correspond to crisp and soft-texture classes. To further pursue an explanation of these differences, fractions of the AIR should be separated based on the solubility of cell wall polysaccharides to estimate polymer size and sugar composition of each fraction (Brummell, 2006). If textural differences cannot be detected based on evidence of the solubilization and depolymerization of pectin and hemicelluloses, then further analysis of polymer substitution and branching patterns would be warranted. For example, several monoclonal antibodies are available that bind specific pectin domains with the ability to differentiate between varying amounts and patterns of methyl-esterification (Willats et al., 2005) Several other techniques are available for determining the fine structure of cell wall polymers as well, and could also be applied if no differences are detected by fractionation between texture types (Willats et al., 2005).

Fruit texture is a complex trait and despite our inability to identify the physiological factors governing its unique expression of “crisp” in blueberry with the body of work we have contributed here, we were able to successfully eliminate several plausible explanations of what might have been responsible for the textural differences in blueberry, and we were able to develop a reliable set of tools for phenotyping crisp texture that will be informative and useful for future studies of texture in blueberry.

We used the tools developed through sensory descriptors and instrumental bioyield force measures to phenotype five F_1 populations segregating for crisp texture. These populations were evaluated by sensory score and tested by chi square analysis to determine whether segregation patterns could be explained by qualitative inheritance. One population was phenotyped by bioyield force and demonstrated a distribution that could support the qualitative model that we proposed in which the crisp trait is controlled by a single gene expressing incomplete dominance in an autotetraploid genetic system. In the populations that we evaluated and with the limited bioyield force measurements we were able to obtain, it was not possible to determine the genotypic nature of crisp, despite four out of five populations fitting expected ratios for a monogenic trait. Future segregation studies would be improved by selecting parents based on results from Chapter 2, such that parents with sensory and instrumental scores likely to represent each possible dosage class (0, 1, 2, 3, and 4 crisp alleles) for a putative single locus should be crossed in order to form F_1 populations representing every possible crisp allelic dosage combination. The phenotyping of F_1 populations would also improve with the use of bioyield force, which we found to be more consistent and objective than onsite sensory scores assigned to fruit from seedlings in the fluctuating weather conditions of the field.

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BIOGRAPHICAL SKETCH

Kendra was born to Donald and Susan Blaker in Bradenton, Florida. She is the third of four children: Bryan, Amber, and Courtney. She is sister-in-law to Mark Mattson and Melissa Blaker and the proud aunt of seven beautiful nieces: Annabelle, Grace, Selah, Aubrey, Georgia, Aniyah, and Tovah.

Kendra received her primary education in Manatee County public schools, and earned a Bachelor of Science degree in biochemistry from Berry College, Rome, Georgia, and a Master of Arts degree in theology from Gordon-Conwell Theological Seminary, South Hamilton, Massachusetts. In 2006, she moved to Gainesville, FL and taught in the secondary school at Cornerstone Academy. In 2007, she was admitted to the stone-fruit breeding program of Dr. José Chaparro at the University of Florida, and graduated with a Master of Science in Horticultural Sciences in May 2010. That same semester she began research as a doctoral student in the blueberry breeding program of Dr. James Olmstead at the University of Florida. Upon completing her Ph.D, she hopes to find work in horticultural plant breeding.