

AN ABSTRACT OF THE DISSERTATION OF

Fan-Hsuan Yang for the degree of Doctor of Philosophy in Horticulture presented on February 28, 2018.

Title: Predictions and Practices for Reducing Heat Damage in Northern Highbush Blueberry (*Vaccinium corymbosum* L.).

Abstract approved:

David R. Bryla

Heat-related fruit damage is a common problem in the northern highbush blueberry (*Vaccinium corymbosum* L). This is particularly true in regions such as the northwestern United States, where summers are warm and dry, and daytime temperature regularly exceeds 32 °C. Millions of dollars of fruit damage are reported in blueberries grown in Oregon and Washington each year. To reduce heat damage, growers advance their picking schedules or use irrigation systems to cool their fields. While over-canopy sprinklers have been used traditionally to irrigate and cool blueberry during heat events, most new fields are irrigated by drip. Some growers are installing dual irrigation systems with micro-sprinklers for cooling. Currently, there is little information available on whether these systems can improve fruit quality in blueberries, and if so, how to operate cooling systems effectively. To address these

problems, four studies were conducted from 2014 to 2016 in western Oregon. In the first study, berry temperature patterns and ultrastructure of the berry cuticle were examined at various canopy positions. A chamber-free convective heater was also used to determine the critical temperatures and heating times for fruit damage of two popular blueberry cultivars, 'Aurora' and 'Elliott.' Results showed that berries exposed to full sun were warmer and had thicker cuticle and wax layers than those in the shade. Mature fruit tolerated higher temperatures for a longer duration than immature fruit, and 'Aurora' was less heat-tolerant than 'Elliott'. In the second study, cooling sprinklers and micro-sprinklers were analyzed for their ability to reduce heat damage and to improve fruit quality. Effects of different cooling frequencies on reducing fruit temperature were also evaluated. Results showed that both sprinklers and micro-sprinklers were effective tools for reducing fruit temperature and improving fruit quality. Using micro-sprinklers with short cycles may be the best practice because these use significantly less water than sprinklers and keep fruit from getting too wet. In the third study, local weather data were collected to construct an energy balance model to predict blueberry fruit temperature. This model was later incorporated with different operational specifications to simulate fruit temperature changes under various cooling practices. The result successfully simulated diurnal fruit temperature patterns and identified the impact of major environmental factors on fruit temperature. Additionally, the model accurately simulated the outcome of different cooling practices. The last study investigated the relationship between blueberry fruit transpiration and Ca uptake in fruit. In this study, the density and functionality of fruit stomata were compared with fruit Ca uptake throughout

developmental stages. Although this study did not directly relate to heat damage, cooling practices may potentially improve fruit transpiration during heat events. The data of this study indicated that blueberry fruit stomata density was low and mainly concentrated near the calyx. Fruit transpiration may mediate Ca uptake since the fruit stoma activity was concurrent with Ca accumulation patterns. Overall, the results of this work provide a better understanding of blueberry heat damage formation and lead to possible methods for problem resolution. By including more cultivar characteristics and farm specifications, this model can be applied as a forecasting tool to predict heat damage incidence and suggest feasible cooling practices. These outcomes may provide growers greater flexibility with adapting to changing climate conditions.

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Predictions and Practices for Reducing Heat Damage in Northern Highbush
Blueberry (*Vaccinium corymbosum* L.)

by

Fan-Hsuan Yang

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Fan-Hsuan Yang, Author

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Completing a Ph.D. has been quite a journey for me! I feel like it would be harder than hiking the Pacific Crest Trail. It is all about persistence! But I finally achieved my goal and I did not travel alone.

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CONTRIBUTION OF AUTHORS

Dr. David Bryla was involved in the experimental design, statistical interpretation, and writing of all the chapters of this document. Dr. Bernadine Strik assisted with experimental design and data interpretation in chapters 2, 3 and 5. Dr. Yanyun Zhao assisted with fruit quality analysis and data interpretation in chapter 3. Dr. Troy Peters assisted with the model concept and data interpretation in chapter 4.

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DEDICATION

This dissertation is dedicated to my father and mother, to my grandparents in heaven, to my sisters, and to my husband.

PREDICTIONS AND PRACTICES FOR REDUCING HEAT DAMAGE
IN NORTHERN Highbush Blueberry (*Vaccinium corymbosum* L.).

Chapter 1 – General Introduction

Northern highbush production

Northern highbush blueberry (*Vaccinium corymbosum* L.) is native to North American. Over the past few decades, it has become the second most important berry crop grown worldwide, following strawberry. From 2004 to 2015, annual global production increased from 23,000 to 550,000 t of blueberries (Brazelton, 2016). In 2005, most of the production was located in North America, which accounted for 67% of the fresh market crop and 94% of total global production (Strik, 2007).

Production of blueberry continues to increase in the United States and elsewhere. Many new fields are being planted in warmer growing regions, including in eastern parts of Oregon and Washington. The Pacific Northwest is now the leading producing region in the country, accounting for 23% of the production area and 36% of the volume (NASS, 2016).

Annual growing cycle

Northern highbush blueberry is a temperate deciduous crop. In the Pacific Northwest, blueberry plants usually stay dormant during the winter, break bud in early spring (March), and set fruit in late spring (late April through early May). After fruit set, the berries undergo three primary stages of growth. The first stage (Stage I) is rapid cell division, where the berries increase in size. This stage usually occurs from petal fall to what is called the late green stage of berry development. The second stage (Stage II) is embryo development. This is often referred to as the “lag stage”. Seeds develop in the berries at this stage, but there little increase in berry size. This usually occurs from the late green stage to fruit coloring. The third and final stage (Stage III) is cell expansion,

when the berries increase in size and weight, the skin turns blue, and the fruit accumulate sugars. The harvest season usually covers the entire summer, beginning with early season cultivars in mid- to late June and ending with late-season cultivars in early to mid-September. The harvest window for a cultivar usually lasts 2–5 weeks. In late summer to early fall, flower buds start developing on shoots from the current year. These flower buds will bloom the following spring.

Summer weather in the Pacific Northwest

Summer weather in the Pacific Northwest is usually sunny with little precipitation. The climate is favorable for growth and fruit development in northern highbush blueberry. Long days and cool nights promote accumulation of carbohydrates in the shoots and fruit, and the dry weather keeps disease pressure low. However, weather has become more extreme in the region over the past decade. During this span, there have been a record number of heat waves and less precipitation than usual in the summer (Abatzoglou, 2014; Abatzoglou et al., 2014). Such occurrences are not isolated to the Pacific Northwest, nor are they likely to subside in the near future (IPCC, 2014). With these extreme weather changes, blueberry fruit development during the summer is threatened, particularly in late-season cultivars. Over the past 5 years, reports of heat-related fruit damage have increased. The most severe case was in 2015, wherein fruit loss reported in Oregon and Washington exceeded several million dollars. In some cases, fruit became so damaged that it no longer was suitable for the fresh market and could only be used for the lower priced processing market.

Blueberry fruit quality

Like other berry crops, blueberries are recognized as having a fruity aroma, sweet taste, and beneficial health effects. Berry crops are generally high in dietary fiber and low in calories, fat, and protein. Most health benefits are derived from their micronutrients, vitamins, and polyphenolics (Talcott, 2007; Viskelis et al., 2012). Common criteria for berry quality are berry size, berry shape, soluble solids, titratable acidity, and firmness. Consumers prefer berries with good appearance, larger fruit size, and higher sugar content. Acids are important in processing the fruit, as well as for balancing the sugar flavor. Fruit firmness significantly contributes to a longer postharvest shelf life. Beyond taste-related parameters, there are many consumers interested in the health-promoting compounds in blueberries. Particularly important are phenolics and anthocyanins, which are commonly associated with antioxidant activity and health benefits.

Impact of high temperature on fruit crops

High temperature is a ubiquitous problem in many crops. It can cause visible physiological disorders in different plant parts and lead to metabolism changes. In fruit crops, the most prevalent visible disorder is sunburn or sunscald, which has been reported in apples, pomegranates, grapes, persimmons, peaches, and citrus, to name a few. Among these fruit crops, apple sunburn is the most-studied. Two types of apple sunburn have been classified, according to Schrader et al. (2011). The first type is “sunburn necrosis”, which is caused by short periods (10 min) of very high heat ($> 52\text{ }^{\circ}\text{C}$), and the second type is “sunburn browning.” The latter is sub-lethal and occurs at lower temperatures ($46\text{--}49\text{ }^{\circ}\text{C}$) and takes longer to develop ($> 1\text{ h}$). Sunburn is caused as high temperatures

disrupt the thermal stability of the membranes and accelerate programmed death of fruit cells (Bonada et al., 2013; Inaba and Crandall, 1988; Schrader et al., 2011). Besides sunburn damage, high temperatures also affect many physiological processes. It has been found that high temperatures can reduce plant photosynthesis by lowering enzyme activity and thylakoid membrane integrity. This reduction can potentially lead to lower sugar levels in the fruit and less carbohydrate storage in the plants. High temperature can also affect the synthesis of fruit pigments, which has been noted as an ABA-related process. It was found in grape that under high temperature, more ABA was oxidized, thus the ABA-mediated anthocyanins pathway was inhibited, which may result in bad coloration in fruit (Björkman et al., 1980; Mori et al., 2007; Yamane et al., 2006).

Northern highbush blueberry is native to the northeastern United States and is not heat-tolerant. In the Pacific Northwest, growers are usually concerned when ambient air temperature exceeds 32 °C. Following a heat event, the primary types of heat-related damage reported in blueberry are sunburn and fruit softening. Fruit with these symptoms have defects in their appearance and shorter shelf-life, thus rendering them unsuitable for the fresh market. Similar to other horticultural crops, high temperature also affects physiological processes of blueberries, such as inducing oxidative damage and causing cell leakage (Yu et al., 2016). Additionally, when blueberry leaves are exposed to high temperature, photosystem II efficiency and carbohydrate assimilation decline (Chen et al., 2012; Hancock et al., 1992; Moon et al., 1987).

Strategies to prevent heat damage

In general, fruit exposed to full sunlight receive more radiative heat than fruit in the shade and, therefore, suffer a higher rate of heat damage. To reduce this problem, two strategies are commonly used. The first is to exclude or reduce radiative heat on the fruit by using kaolin-based particle film and shade nets. Kaolin particle film is made of inert clay with reflective properties, which can reduce the heat load on fruit. When used on apples, it has been effective at reducing fruit temperature and sunburn. Unfortunately, blueberry fruit is soft, perishable, and difficult to wash, which can make it very difficult to remove the residue. An alternate method involves the placement of shade netting, which is a fabric that can control the amount of sunlight that penetrates to the blueberry plant. It was been successfully used to reduce fruit surface temperature on both apple and blueberry (Gindaba and Wand, 2005; Lobos et al., 2013). However, its main drawback is the restriction of air movement, which may lead to higher relative humidity and subsequently impede the fruit's ability to cool. Additionally, shade net is costly to install, can delay fruit ripening, and may reduce flower bud initiation and, therefore, yield. Accordingly, growers tend to cover and remove shade nets based on heat waves, resulting in higher labor costs.

The second strategy is to remove heat from the fruit by cooling. There are three types of mechanisms to cool the fruit: hydro-cooling, convective cooling, and evaporative cooling. Hydro-cooling uses continuous application of water to contact the fruit and remove sensible heat. This method is very effective in reducing fruit temperature, but it usually results in too much water being applied and waterlogging issues. Convective cooling uses fine water droplets to cool the air and create a temperature gradient for circulation, and relies on air circulation to cool the fruit. This method is relatively

ineffective in reducing fruit temperature (Evans, 1999). Evaporative cooling involves the application of water on the fruit surface and removes heat through water evaporation. Often, cooling is applied in cycles to encourage water evaporation. This method is typically considered the most efficient process of the three, since it effectively cools the fruit and minimizes water use.

Evaporative cooling has been found to be effective in mitigating heat stress in apples and grapes. Moreover, by maintaining an optimal canopy temperature, both photosynthesis and vegetative growth can be improved by evaporative cooling (Aljibury et al., 1975; Caravia et al., 2017; Gindaba and Wand, 2007; Kliewer and Schultz, 1973; Pelletier et al., 2016). Effective evaporative cooling relies on environmental factors and system design. In general, cooling is more effective on drier days with higher vapor pressure deficits, particularly when it is coupled with optimal wind speeds. However, extra water may need to be applied on very windy days to ensure that the water reaches the fruit. In terms of system design, Evans et al. (1995) suggested that water should be applied to apples at a minimum rate of $6.25 \text{ L} \cdot \text{s}^{-1} \cdot \text{ha}^{-1}$ in order to keep the fruit core temperature at the optimal level. Reduction in application frequency or water droplet size requires a high water flow rate.

The use of an energy balance model for predicting fruit temperature

Fruit temperature correlates with air temperature and is usually higher on hot days than cool days. However, air temperature is not the only factor affecting the temperature of the fruit. Environmental factors such as light intensity and wind also affect fruit temperature. Therefore, more accurate, meteorologically derived, fruit temperature

predictions are necessary to prevent the occurrence of heat-related damage on fruit.

Utilizing a mathematic model based on the energy balance concept may be a potential option for blueberry, since it is widely used in apples, peaches, grapes, and figs (Cola et al., 2009; Evans, 2004; Li et al., 2014; Patiño et al., 1994; Pitacco et al., 2000; Saudreau et al., 2007; Smart and Sinclair, 1976). Conceptually, energy balance is based on the principal that net gain/loss of an object is zero. For example, on a sunny day, the fruit gain energy from direct sunlight, diffuse light from the leaves, longwave radiation from the air, longwave radiation from the plants, soil, ground cover, etc., and lose energy through emission, heat exchange with the air, vaporization, etc. These components of the model will vary according to the object's geometry, surface characteristics, and environmental conditions.

Energy balances can also be used for predicting cooling efficiency. Evans (2004) simulated temperature changes during cooling in apple by calculating the amount of heat removal caused by water evaporation (Evans, 2004). Caravia et al. (2017) also recently estimated the water requirement for effective cooling practices in wine grape. By using an energy balance model, the evaporative cooling outcome can be predicted. These types of models can potentially provide growers with useful data to help them justify or modify their cooling practices.

Calcium uptake in fruit

Calcium (Ca) is an important micronutrient that is commonly associated with fruit firmness and overall quality. It can act as a chelating agent, binding with pectin to strengthen cell walls. Without adequate Ca levels, several fruit quality defects may occur

during fruit development. These defects include bitter pit disorder in apples, blossom-end rot in tomatoes, and fruit cracking in litchi (De Freitas et al., 2015; Ferguson and Watkins, 1989; Ho and White, 2005; Huang et al., 2008). Since Ca is mobile in xylem and immobile in phloem, when Ca is insufficient in fruit, it is difficult to replenish through roots. A potential alternative is to apply Ca directly to the leaves and fruit. Spraying Ca has been shown to prevent fruit cracking in sweet cherries and reduce postharvest fruit decay in strawberries (Brown et al., 1995; Lara et al., 2004). In the Pacific Northwest, blueberry growers may apply Ca to leaves and fruit with a goal of increasing fruit Ca concentration and fruit firmness. However, Vance et al. (2017) found no effect of foliar application of various commercial Ca products on leaf or fruit Ca.

A number studies on fruit crops (kiwifruits, grapes, tomatoes, etc.) have suggested that there is a relationship between fruit transpiration and Ca uptake. Fruit Ca accumulation primarily occurs during early stages of fruit development (Ho and White, 2005; Montanaro et al., 2015; Rogiers et al., 2006). Reduced Ca uptake may result from narrowing of xylem vessels or non-functional stomata. Blueberry fruit was found to have photosynthetic capabilities during the early green stage of fruit development, and a small number of stomata have been identified near the calyx (Aschan and Pfanz, 2003; Cipollini and Levey, 1991; Konarska, 2015). It is still unknown, however, whether stomatal functionality declines during fruit development and, thus, affects Ca accumulation.

Study objectives

The aim of the work in this dissertation was to develop useful practices for helping the blueberry industry to be more resilient to the changing climate, and also to contribute new knowledge to our fundamental understanding of blueberry physiology. The objectives were to 1) determine the critical temperatures and heating times for fruit damage in blueberry, 2) evaluate the efficacy of over-canopy sprinkler and micro-sprinkler systems at reducing fruit temperature and improving fruit quality, 3) develop a simple climatological model that could be used with local weather data and farm specifications to predict blueberry fruit temperature and the efficiency of cooling practices, and 4) evaluate the relationship between stomatal function and Ca accumulation in the berries during different stages of blueberry fruit development.

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**Chapter 2 – Critical Temperatures and Heating Times for Fruit Damage in
Northern Highbush Blueberry**

Abstract

Over-canopy sprinkler systems are often used to cool blueberry fields in the Pacific Northwest, but more information is needed to determine exactly when cooling is needed. The objective of this study was to identify the critical temperatures for heat damage in northern highbush blueberry (*Vaccinium corymbosum* L.). An initial study conducted in western Oregon in a mature planting of late-season ‘Elliott’ blueberry revealed that heat damage was typically observed within 1–3 d after an extreme heat event. Damage occurred primarily on sun-exposed berries—which, on hot days (> 35 °C), were 7–11 °C warmer than air temperature—and it happened at both green and blue stages of fruit development. A subsequent study was conducted to determine whether the critical temperature for heat damage differs between cultivars and green and blue fruit. In this case, a second cultivar, ‘Aurora’, was added to the study and was compared to ‘Elliott’. Berries from both cultivars were heated using a chamber-free convective unit and were exposed for up to 4 h to berry temperatures of 42, 44, 46, and 48 °C. ‘Aurora’ was more susceptible to heat damage than ‘Elliott’, particularly when the berries were green. Green fruit of ‘Aurora’ were damaged within 1.5 h at 42–46 °C and 1 h at 48 °C. After 4 h, ‘Aurora’ had damage to 17% of the green berries on the cluster at 42 °C and as much as 59% damage at 48 °C. In contrast, green fruit of ‘Elliott’ had much less damage than in ‘Aurora’ and remained mostly undamaged for at least 3 h at 42–46 °C. After 4 h, ‘Elliott’ had damage to 14% of the green berries at 46 °C and 23% at 48 °C. Neither cultivar had any damage on blue fruit at 42 °C, and both had < 4% damage after 4 h at 44 °C. ‘Elliott’ also sustained minimal damage to blue fruit at 46 °C but had ≈ 20% damage after 4 h at 48 °C. In ‘Aurora’, damage occurred to blue fruit

within 3.5 h at 46 °C and 2 h at 48 °C, which increased to 19% and 30% after 4 h at these respective temperatures. Wax and cutin layers thickened on the berries as they progressed from green to blue, which perhaps increased their tolerance to heat at later stages of development. Based on these results, northern highbush blueberry fields should be cooled at air temperatures > 32 °C during early stages of fruit development and > 35 °C during the later stages.

Introduction

Heat damage is becoming a prevalent problem for many blueberry growers in the northwestern United States. The region, which includes Oregon and Washington, is the leading producer of blueberries in the country. In 2015, these two states produced a combined total of 91,104 t of blueberries (36% of the total U.S. production) (USDA, 2016). However, the Washington blueberry industry lost an estimated \$10 million of fruit that year due to heat and inadequate water for cooling and irrigation (Schreiber, 2016). Similar losses were reported in Oregon (Ore. Blueberry Commission, personal communication). Extreme weather events such as this have become more common in the region over the last two decades (Abatzoglou et al., 2014).

Northern highbush blueberry (*Vaccinium corymbosum* L.) is the type of blueberry primarily grown in cooler regions such as Oregon and Washington. Unlike southern highbush (a complex hybrid based largely on *V. corymbosum* and *V. darrowii* Camp.) and rabbiteye blueberry (*V. virgatum* Ait.) types, which are typically grown in warmer climates, northern highbush cultivars tend to be poorly adapted to high temperatures. During hot weather, net photosynthesis in many of these northern cultivars declines considerably, and high leaf temperatures result in large increases in plant water use (Hancock et al., 1992; Bryla, 2011). When high temperatures coincide with fruiting, water and carbohydrates are diverted from the fruit to supply the plant, resulting in small or shriveled berries, hastened fruit ripening, and a reduction in fruit quality and storage (Lobos and Hancock, 2015). Berries exposed to direct sunlight appear to be the most susceptible to heat damage. Unlike leaves, which cool via transpiration, the berries have very few stomata on the surface and, therefore, do not have an effective means of cooling

(Konarska, 2015). At the cellular level, high temperatures disrupt the thermal stability of membranes and proteins, causing ion leakage and inhibition of physiological processes associated with fruit development (Inaba and Crandall, 1988; Schrader et al., 2001; Yu et al., 2016). Like many fruit, blueberries possess inherent qualities such as a waxy cuticle that provide natural protection against heat damage. The cuticle consists of a polyester matrix or cutin layer and an epicuticular wax layer. The latter, often referred to as the “bloom”, is deposited on and in the cutin matrix and contains long-chain alkanes, acids, alcohols and esters (Gülz, 1994). Without the wax, blueberries are prone to infections by bacteria and fungi, physical damage, and water loss (Jenks and Ashworth, 1999; Riederer and Schreiber, 2001). Knowledge of the ultrastructure and thickness of the cuticle may help us to understand how to select and manage cultivars for increased resistance to heat damage. Apart from transpiration, the cuticle protects the berries against sunburn, resulting from exposure to UV radiation and excess heat absorption.

Overhead sprinklers are sometimes used to cool blueberry fields during hot temperatures. However, most new blueberry fields in northwestern United States are irrigated by drip (Strik and Yarborough, 2005). Options for reducing heat damage with drip are currently limited. To contend with this problem, some growers are installing dual irrigation systems and use micro-sprinklers to cool the berries and drip tubing to irrigate the plants. These dual systems are similar to those used in apple [*Malus × sylvestris* (L.) Mill. Var. *domestica* (Borkh.) Mansf.], where cooling not only prevents heat damage but also improves fruit size and color (Gindaba and Wand, 2005; Iglesias et al., 2002). The micro-sprinklers are located above the canopy and produce fine droplets of water that evaporate quickly.

Currently, there are many questions regarding the use of micro-sprinklers to reduce heat damage in northern highbush blueberry, including the temperature at which cooling is needed. Most growers focus their efforts on later stages of berry development and initiate cooling whenever air temperature is expected to exceed 30–32 °C (F-H. Yang, personal observation). However, there is no scientific basis for this decision, nor is there any information on how frequently the system should be run for cooling. Some growers run their micro-sprinklers continuously in hot weather, while others cycle them on for 15–30 minutes every hour.

The objective of the present study was to characterize and determine the critical temperatures and heating times for fruit damage in northern highbush blueberry. We also examined the ultrastructure of the berry cuticle to determine whether resistance to heat damage may be a heritable function of the amount of wax on the fruit. Damage was assessed in late-season cultivars during green and blue stages of berry development. Late-season cultivars ripen in late summer when temperatures are warmer and, therefore, are often very vulnerable to heat damage.

Materials and Methods

Weather conditions. Precipitation and maximum daily temperature readings were downloaded from AgriMet weather stations (<https://www.usbr.gov/pn/agrimet>) in Oregon and AgWeatherNet stations (<https://weather.wsu.edu>) in Washington. Stations were selected from four primary regions for blueberry production in the Pacific Northwest, including Aurora and Corvallis, which are located in northern and southern parts of the Willamette Valley in western Oregon, respectively, and Lynden and Prosser, which are

located in northwest and eastern Washington, respectively. Each station has been operational for > 20 years.

Characterization of heat damage. Heat damage and diurnal changes in berry temperature were monitored during 2013–2015 in a mature, 0.6-ha planting of ‘Duke’ and ‘Elliott’ blueberry. There was no evidence of heat damage in ‘Duke’, and therefore, only ‘Elliott’ was used in the present study. ‘Elliott’ is a late-season cultivar that ripens in August in western Oregon. The planting was established in Apr. 2004 at the Oregon State University Lewis-Brown Horticulture Research Farm in Corvallis, OR (lat. 44°33′ N, long. 123°13′ W, 68 m elevation). The experimental plots used to assess heat damage comprised three adjacent rows of 24 plants and were replicated three times; experimental plants were selected randomly from six plants located in the center of the middle row of each plot. Plants were spaced 0.8-m apart on raised beds centered 3.0-m apart. The beds were mulched every 2–3 years with a 5-cm-layer of douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust. Irrigation was applied by drip. See Bryla et al. (2011) for complete details on the establishment of the planting.

Fruit temperature was measured on sun-exposed and shaded berries using 0.13-mm, copper-constantan wire thermocouples (Omega Engineering Inc., Stamford, CT). The junction of the thermocouples was inserted beneath the skin near the equator of the berries and secured at the insertion point with a small drop of silicone glue (General Electric, Boston, MA). Damage to the berries was limited to a few epidermal cells surrounding the thermocouple wires, and the thermocouples did not appear to interfere with berry development or ripening. Berries were selected at green (immature) and blue (mature) stages of development and were located in upper and lower portions of the

canopy on both sides of the row (east and west). Each temperature measurement was estimated from the average of four berries distributed vertically along the exterior face of a cluster and was replicated on three plants at each stage and location. An additional three set of clusters were measured to compare the difference in temperature between the surface and center of the berries. To do so, in each measured cluster, four thermocouples were inserted beneath the berry skin of four berries, and for each of these berries, another thermocouple was inserted in the center of the berry. Ambient air temperature was also measured using a temperature-humidity probe (HMP60; Vaisala, Woburn, WA). The probes were covered with a six-plate radiation shield (41303-5A; RM Young, Traverse City, MI) and mounted 1.8-m high. All thermocouples and air temperature data were collected every 5 min using data loggers (CR-1000; Campbell Scientific, Logan, UT).

Visual symptoms of heat damage were characterized by monitoring changes in the berries following warm weather events. Berries were also monitored for damage at commercial farms in Oregon and Washington. Heat damage usually occurred when air temperature exceeded 35 °C and was proceeded by cooler weather conditions (< 25 °C the previous day).

Ultrastructure of the berry cuticle. Thickness of the cutin and epicuticular wax layers on the berries were examined using a scanning electron microscope (SEM) (Quanta 600F; FEI Company, Hillsboro, OR) with an acceleration voltage of 10 kV. Sun-exposed and shaded berries of ‘Elliott’ blueberry were collected randomly at green and blue stages of development in 2014. Samples were taken from eight plants at the site described above and included a total of eight clusters. To avoid removing the wax during sampling, each berry was held by inserting a dissection needle through the calyx. Skin

was carefully removed from the equator of the berries using a razor blade. The skin was immediately submerged into a fixation solution (2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium cacodylate buffer) and stored overnight at 5 °C. The following day, skin samples were gradually washed with a series of 30%, 50%, 70%, 90%, and 100% acetone and, to maintain cellular structure, were dehydrated using a CO₂ critical point dryer (EMS 850, Electron Microscopy Sciences, Hatfield, PA). Once dried, the samples were frozen with liquid N and shattered into small specimens. The specimens were mounted onto aluminum stubs with double-sided carbon tape and coated with a 15-nm-thick layer of 60% gold and 40% palladium (Cressington 108 Auto Sputter Coater; Cressington, Watford, UK). Images of the specimens were captured at a magnification of 20645×. Thickness of the cutin and epicuticular layers were measured at three random locations in each SEM image and averaged.

Data were analyzed within locations (sun-exposed or shaded) by one-way analysis of variance using R v. 3.1.2 (Oregon State University, Corvallis, OR). Normality of the data was validated using the Shapiro–Wilk test, and homogeneity of variance was checked using Lavine’s test. Means of berries collected at green and blue stages of development were separated using Tukey’s honestly significant difference test ($\alpha = 0.05$). Means of sun-exposed and shaded berries were compared by paired t-test ($\alpha = 0.05$).

Critical temperature and heating times for damage to the berries. Critical temperatures for heat damage were evaluated during 2016 in a 0.3-ha planting of northern highbush blueberry located at the Lewis-Brown Horticulture Research Farm. The planting was established in Oct. 2008 with six cultivars. Two late-season cultivars, ‘Aurora’ and ‘Elliott’, were used in the present study. As in the previous experiment,

plants were spaced 0.8- × 3.0-m apart on raised beds mulched with douglas fir sawdust and were irrigated by drip. See Vargas et al. (2015) for complete details on establishment of the planting.

Berry clusters were heated *in situ* for 4 h to a constant berry temperature of 42, 44, 46, or 48 °C. These temperatures were chosen based on berry temperatures observed in the field in 2015 (see below) and preliminary observations of heat damage in the laboratory (data not shown). Each treatment was replicated on three plants and repeated on 3 d in August. All clusters were located on the upper, west side of the canopy and included at least 20 berries each at green and blue stages of development. Berries at the green stage were \approx 50% to 90 % smaller in diameter than those at the blue stage. Heating was achieved by forced convection using a 120-V heater (20055K111; McMaster-Carr, Los Angeles, CA) mounted to an axial fan (3110KL-04W-B40; NMB Technologies, Chatsworth, CA) (Fig. 2.1). Air flow was directed vertically to the clusters via insulated flexible duct (8.2-cm inside diameter) supported on the ends by a 0.3-m-long polyvinyl chloride pipe. Measurements of temperature in the cluster provided feedback at 5-s intervals to control delivery of the heated air [see Tarara et al. (2000) for details]. Average berry temperature of the clusters was measured with thermocouples and a data logger, as described previously, and the heaters were controlled by a relay power controller (SDM-16AC; Campbell Scientific, Logan, UT). The percentage of berries with heat damage (discoloration) in a cluster were recorded every 30 min during heating.

Percent heat damage was arcsine transformed and analyzed at each time interval by one-way analysis of variance using R v. 3.1.2. Means were separated using Tukey's honestly significant difference test ($\alpha = 0.05$). A t-test was used to determine whether

means were different than zero and to identify the time at which heat damage occurred within a given treatment.

Results and Discussion

Weather conditions. Heat damage was a prevalent problem for many blueberry growers in Oregon and Washington in 2014–2016 (F-H. Yang, personal observation).

Temperature was cooler than normal in spring and early summer in 2014. In June of that year, there was only one site in which temperature exceeded 30 °C (Fig. 2.2). However, by July, temperature was higher than normal and was > 35 °C on 1 or 2 d in Aurora and Corvallis, OR, respectively, and on 13 d in Prosser, WA. Many fields in these three regions had soft and heat damaged berries as a result of the warm weather (Peerbolt Crop Management, 2014). Fields in Lynden, WA, on the other hand, remained cool and had no heat damage.

The following year was warmer than normal. In 2015, temperature exceeded 35 °C by June in Aurora, Corvallis, and Prosser and by July in Lynden (Fig. 2.2). Heat damage was widespread and occurred in numerous cultivars throughout the Pacific Northwest, including in Lynden. Damage to the berries was exacerbated by drought and water restrictions at a number of locations. Five extreme heat events were documented in Corvallis in 2015, including three in July (Fig. 2.3A). In each case, temperature of sun-exposed berries was 7–11 °C warmer than the air temperature (Fig. 2.3B). The hottest day was 30 July, which reached 38.9 °C. Berry temperatures peaked at 49 °C on that date and were > 42 °C for nearly 4 h. These temperatures are close to the thresholds for heat

damage in other fruit crops, including apple (Schrader et al., 2011), pear (McClymont et al., 2016), and pomegranate (Yazici and Kaynak, 2009).

The weather was also warmer than normal in 2016. In this case, temperature exceeded 35 °C by June in Aurora and Prosser and by July in Corvallis (data not shown). Heat damage was less extensive than the previous year but remained a considerable concern in the region.

Characterization of heat damage. Heat damage on the berries was typically observed within 1–3 d after an extreme heat event. The most prevalent symptom was *necrosis* or necrotic spots on the fruit (Fig. 2.4A–C). Necrosis occurred in both green and blue-colored berries and usually happened on the upper portion of the fruit surface exposed to full sunlight. Browning and reddish spots often appeared prior to necrosis, and the skin then collapsed a few days later. Similar symptoms occur on sun-exposed apples (Schrader et al., 2011). A second type of heat damage was *spotting* on the berries (Fig. 2.4D). Spotting occurred during the green stage of fruit development and manifested as purple spots on the surface of the fruit. In many cases, spotted berries continued to ripen but formed crevices within the spots. A third type of heat damage was *shriveling* (Fig. 2.4E–F) or *wrinkling* (Fig. 2.4G). Like necrosis, shriveling occurred in both green and blue-colored berries. The berries in this case began to dry, shrink, and appear raisin-like. Shriveling occurred as a result of severe damage at the pedicel or on the entire berry. Krasnow et al. (2010) described the symptom as severe desiccation due to sun exposure in wine grapes. Wrinkling was similar to shriveling but had less effect on fruit size. It was observed only at the blue stage of fruit development and usually began with darkening on the fruit surface, followed by turgor loss and wrinkling a few days later. Darkening of the

berries has also been reported in grapes and appears to be due to degradation of surface crystalline wax (Bondada and Keller, 2012). The final type of heat damage observed was *poor coloration* during ripening (Fig. 2.4H). Berries with this type of damage tended to be smaller and less blue at maturity than other berries on the same cluster. Blueberries may also soften excessively during high temperature events. Soft berries are difficult to identify visually but are typically sorted out on the packing line.

In most cases, heat damage occurred in berries that were exposed to sunlight. Even on a relatively cool day ($< 25^{\circ}\text{C}$), the temperature of berries in full sun was $\approx 11\text{--}12^{\circ}\text{C}$ warmer than the ambient air temperature, while the temperature of those in the shade was never more than $1\text{--}3^{\circ}\text{C}$ above ambient (Fig. 2.5). Others have demonstrated that fruit surface temperature is function of advective and solar heating and, therefore, is correlated to both air temperature and solar radiation (Schrader et al., 2003; Yazici and Kaynak, 2009).

The number of berries with heat damage on the plants was similar on both sides of the row but was typically greater in the upper than in the lower portion of the canopy (F-H. Yang, personal observation). With rows running from north to south (most common row arrangement), berries located on the east side of the row heated up sooner in the day than those on the west side, but they also cooled down earlier as the sun moved across the sky (Fig. 2.6). As a result, total heat load was similar between the berries on both sides of the row. However, heat load was not similar between the upper and lower portions of the canopy. Berry temperature was cooler, on average, in the lower canopy and fluctuated more so than it did in the upper canopy due to periodic shading from the overhanging branches (Fig. 2.6). Peak temperatures reached in the lower canopy were

sufficient to occasionally cause heat damage in the fruit, particularly on berries located near the soil surface. Northern highbush blueberries tend to have thinner cuticle and wax layers in the lower canopy than in the upper canopy and, therefore, may be more susceptible to heat damage and sunburn (F-H. Yang, personal observation).

Fruit color had little effect on the temperature of the berries (Fig. 2.7). Basically, blue fruit were only 1–2 °C warmer than green fruit, which could partly explain why heat damage occurred at both green and blue stages of berry development (Fig. 2.4). Furthermore, the skin and core temperature of the berries also differed by < 1 °C over the course of the day, indicating the berries had little capacity to buffer changes in temperature (Fig. 2.8). Evans (2004) suggested that development of red skin color may reduce albedo and increase heat load during ripening in apples. However, apples are much larger than blueberries and, therefore, have greater thermal capacity and much lower rates of heat dissipation.

Ultrastructure of the berry cuticle. The berries were covered with a well-defined layer of epicuticular wax and cutin (Fig. 2.9A). The outer surface of the wax layer was primarily crystalline during the early stages of development (Fig. 2.9B) and later became amorphous as the berries turned pink and blue (Fig. 2.9C). Both the wax and cutin layers thickened as the berries ripened (Fig. 2.9D and E). On average, these layers increased by 84% and 70%, respectively, as the berries changed from green to blue (Fig. 2.9F and G). Konarska (2015) observed similar morphological changes on the fruit surface of ‘Bluecrop’ blueberries. Based on light microscopy, she estimated that the thickness of the wax layer increased by 45%, and the cutin layer increased by 47%, between 35 (early green stage) and 70 d after anthesis (maturity).

The wax and cuticle layers were also thicker on sun-exposed berries than on shaded berries (Fig. 2.9F and G). Similar results have been found in grape (Rosenquist and Morrison, 1989). Wax accumulation may protect sun-exposed berries against heat and light damage. Epicuticular wax is well known to increase reflection of visible and UV light in plants (Holmes and Keiller, 2002; Shepherd and Wynne Griffiths, 2006). Due to its strong UV-absorbing capabilities, phenolic fatty-acid esters in the wax appear to play an important role in sunscald resistance in apple (Whitaker, 1998). Perhaps cultivars with thicker wax layers on the fruit are less susceptible to heat damage and could be selected for better adaptation to warmer climates. Further research is warranted to determine whether the wax and cutin layers could be manipulated, with either breeding or management practices, to increase resistance to heat damage in northern highbush blueberry.

Critical temperatures for heat damage. The time and temperature required to cause heat damage differed by cultivar and the stage of berry development. Overall, ‘Aurora’ was more susceptible to heat damage than ‘Elliott’, particularly when the berries were green. Damage was clearly evident in green ‘Aurora’ berries within 1.5 h at berry surface temperatures of 42–46 °C and 1 h at 48 °C (Fig. 2.10A). A substantial percentage of these berries were damaged on the cluster after 4 h of heating, ranging from 17% at 42 °C to 59% at 48 °C. Green ‘Elliott’ berries, on the other hand, had much less damage than ‘Aurora’. In this case, the berries remained largely undamaged for at least 3 h at 42–46 °C, and after 4 h, damage was limited to < 10% at 42–44 °C, 14% at 46 °C, and 23% at 48 °C (Fig. 2.10B). The damage appeared initially as orange spots on the berries in

both cultivars. The spots eventually became necrotic and were similar to those observed previously as a result of natural heat damage (Fig. 2.4A).

Heat damage was also evident when the berries were blue (Fig. 2.10C and 10D). The damage at this stage began with darkening, which like in grape, appeared to be due to the wax layer melting on the surface of the berries (Bondada and Keller, 2012). These berries then either shriveled or developed reddish, necrotic spots. Again, symptoms were similar to those observed previously (Fig. 2.4B, 4C, and 4F). Generally, damage was less extensive and occurred later and at higher temperatures when the berries were blue than when they were green. Both cultivars had no damage on the blue fruit at 42 °C and < 4% damage after 4 h at 44 °C. Blue ‘Elliott’ berries also sustained minimal damage at 46 °C but had nearly 20% damage after 4 h at 48 °C (Fig. 2.10D). Damage at the latter temperature appeared within 2 h of heating. Blue ‘Aurora’ berries were also damaged within 2 h at 48 °C, as well as 3.5 h at 46 °C (Fig. 2.10C). In this case, damage increased to 19% and 30% after 4 h at 46 and 48 °C, respectively.

Conclusion

Based on our results, tolerance to heat appears to be primarily a function of cuticle thickness and less related to fruit color in northern highbush blueberry. Clearly, the berries were more tolerant to heat as the cuticle thickened during ripening. Thus, while temperatures are often warmer at later stages of berry development, the use of cooling practices is potentially more important when the berries are green. Depending on the cultivar, damage occurred in green berries within 1.5–3 h at berry surface temperatures of 42–46 °C and 1–1.5 h at 48 °C. Berries at the blue stage, on the other

hand, had little to no damage within 3–4 h at 42–46 °C. Heat damage occurred primarily on sun-exposed berries, which were 7–11 °C warmer than air temperature on hot days (> 35 °C).

Heat tolerance also differed between the two cultivars tested in the present study. We are uncertain why ‘Aurora’ was more susceptible to heat damage than ‘Elliott’, but the results suggest that tolerance to heat may be heritable and potentially selected for by breeding. If so, new cultivars could be developed not only to reduce heat damage at existing sites but also to extend the range of northern highbush blueberry to warmer and drier climates.

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Tables and Figures



Fig. 2.1. A forced-convection unit for heating blueberry clusters in the field.

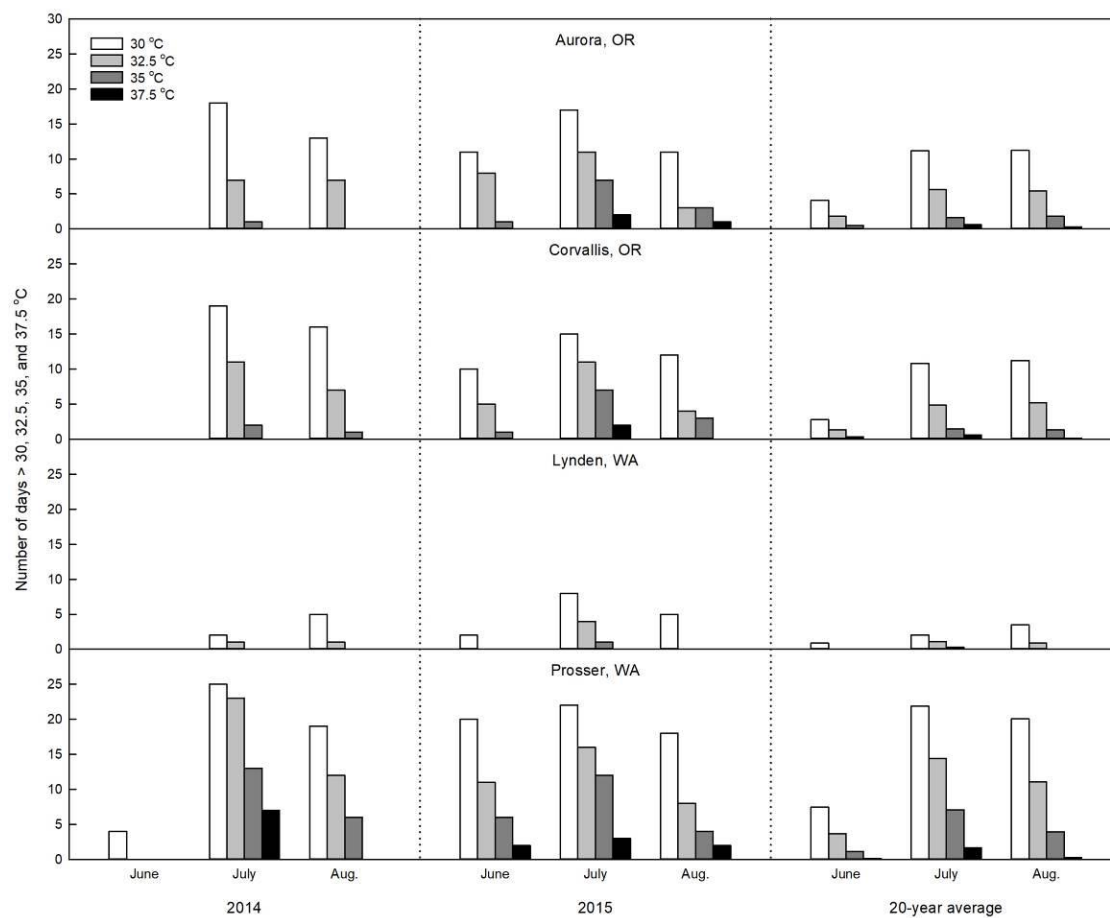


Fig. 2.2. Total number of days per month in which ambient temperature exceeded 30, 32.5, 35, and 37.5 °C at four locations in Oregon and Washington.

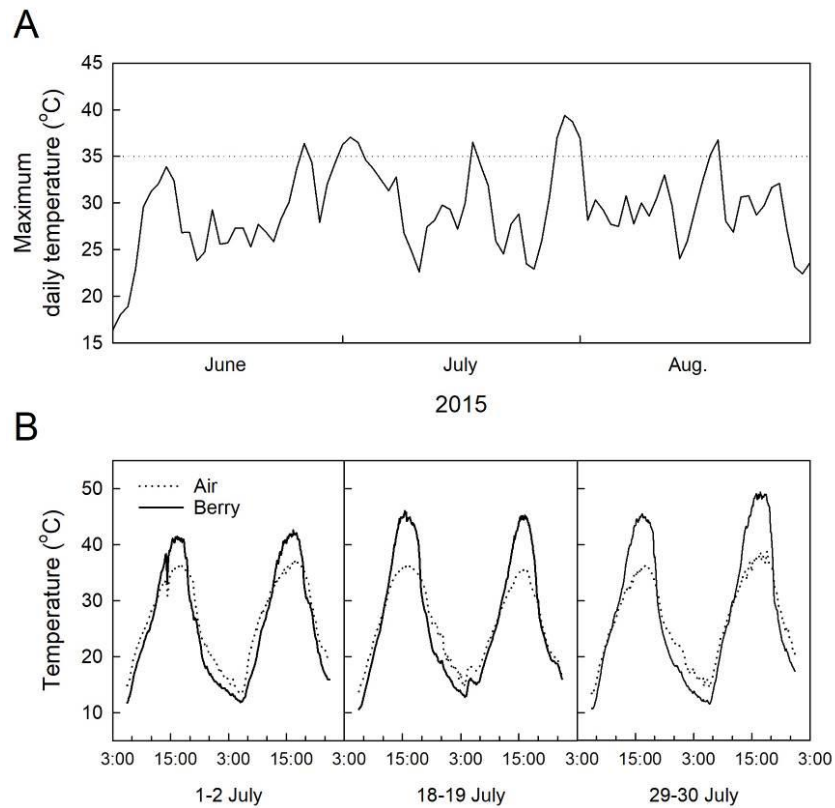


Fig. 2.3. (A) Maximum daily temperatures during June–Aug. 2015 in Corvallis, OR. (B) Diurnal changes in air and berry surface temperature in a field of ‘Elliott’ blueberry during three extreme heat events (air temperature > 35 °C) in July 2015 in Corvallis.

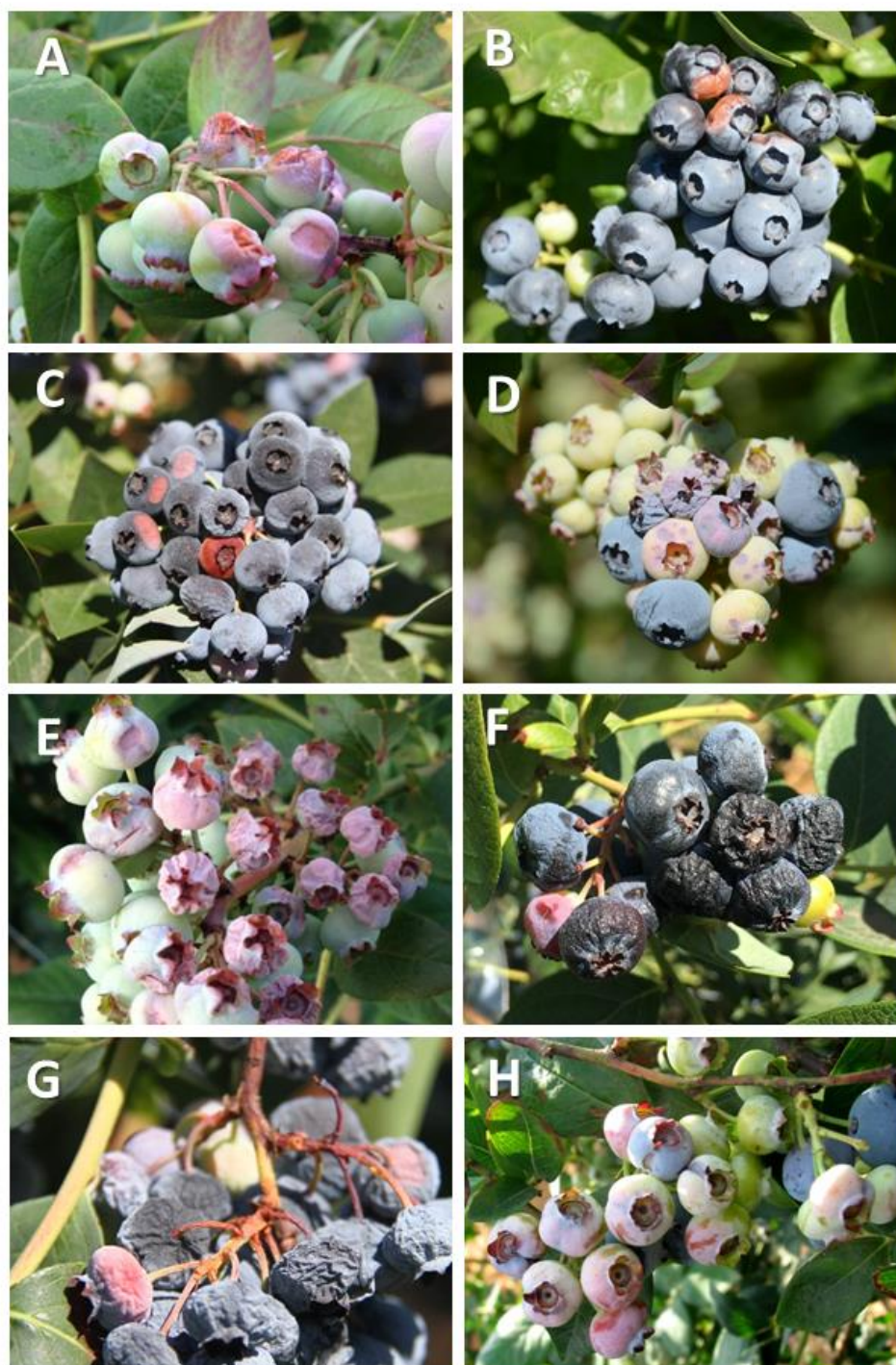


Fig. 2.4. Examples of heat damage at green and blue stages of fruit development in northern highbush blueberry. Typical symptoms include (A–C) necrosis, (D) spotting, (E, F) shriveling, (G) wrinkling, and (H) poor coloration on the berries.

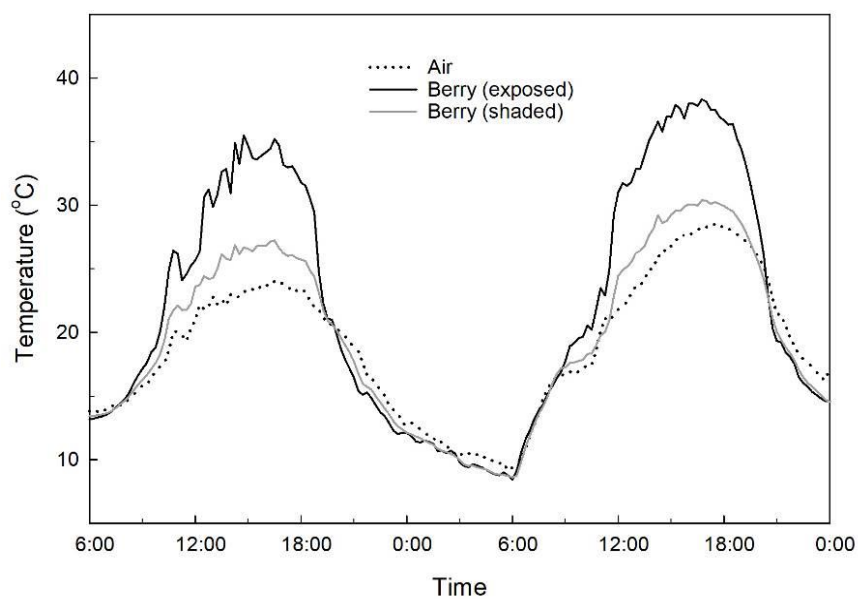


Fig. 2.5. Diurnal changes in surface temperature of sun-exposed and shaded berries of 'Elliott' blueberry. The measurements were taken on 2–3 Aug. 2013 in Corvallis, OR.

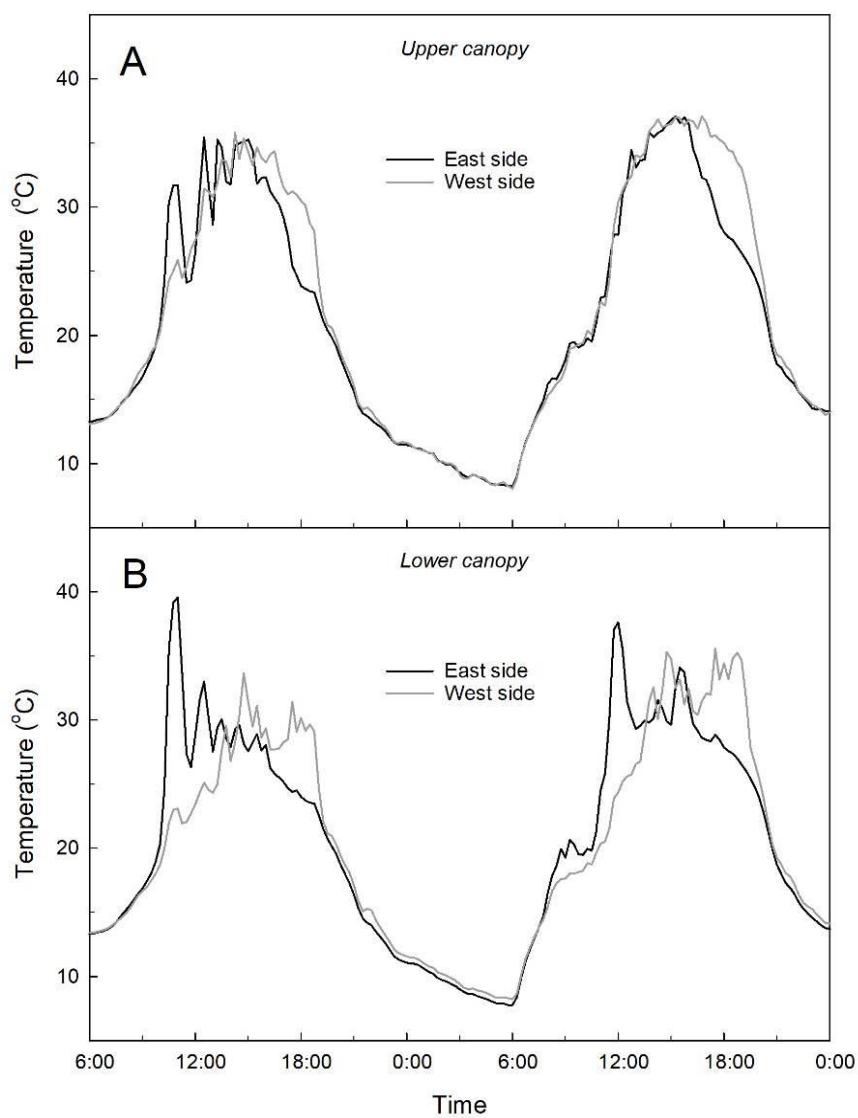


Fig. 2.6. Diurnal changes in surface temperature of berries located on the east and west side of the row in (A) upper and (B) lower parts of the canopy of 'Elliott' blueberry. The measurements were taken on 2–3 Aug. 2013 in Corvallis, OR.

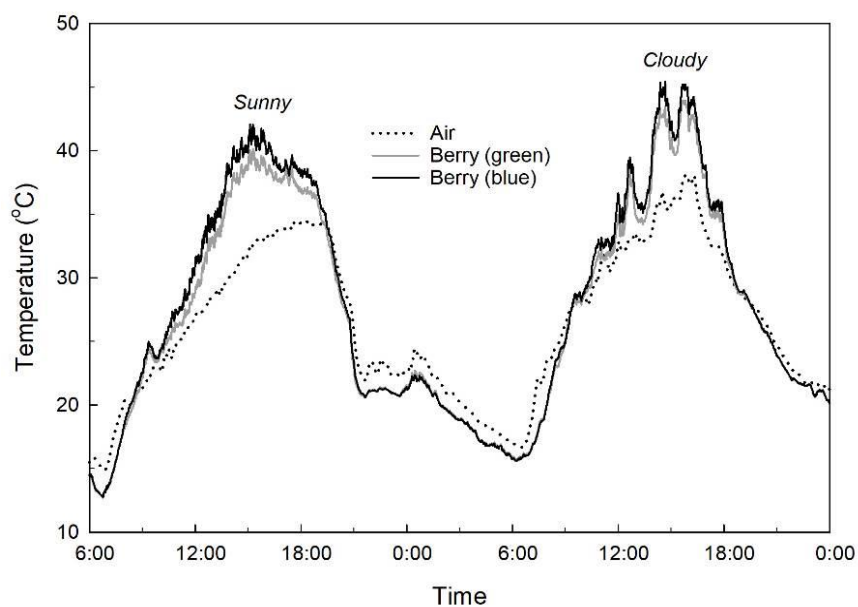


Fig. 2.7. Diurnal changes in berry surface temperature of green and blue fruit of 'Elliott' blueberry. The measurements were taken on 10–11 Aug. 2014 in Corvallis, OR.

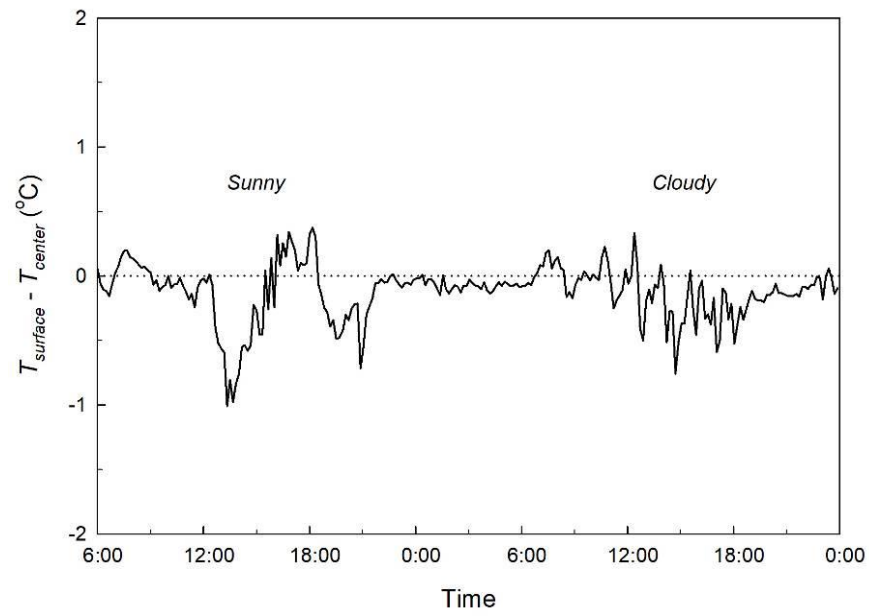


Fig. 2.8. Diurnal differences between the surface temperature (T_{surface}) and the temperature at the center of the berry (T_{center}) in 'Elliott' blueberry. The measurements were taken on 10–11 Aug. 2014 in Corvallis, OR.

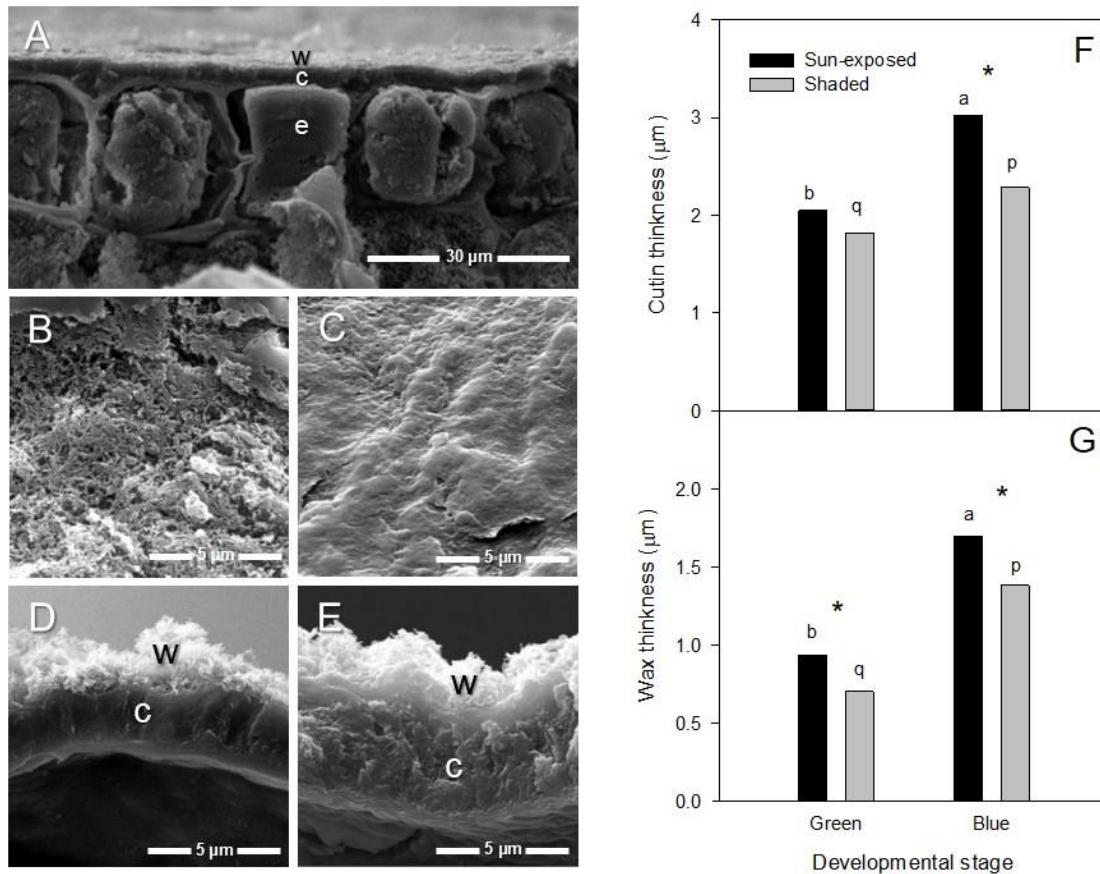


Fig. 2.9. SEM microscopy of 'Elliott' blueberry fruit: (A) cross-section of a berry at the early green stage; close-up of epicuticular wax on the surface of a berry at the (B) late green and (C) pink stage; and a close-up of the cutin layers at the (D) late green and (E) 10% blue stage. w – epicuticular wax; c – cuticle; and e – epidermal cell. Mean thickness of the (F) cuticle and (G) epicuticular wax layers on sun-exposed and shaded berries; the measurements were made at late green and 10% blue stages. Each bar represents the mean of eight replicates, and different letters above the bars indicate that means at green and blue stages of development were significantly different at $P \leq 0.05$, according to Tukey's honestly significant difference test. Asterisks above the bars indicate that the means of sun-exposed and shaded berries at each stage were significantly different $P \leq 0.05$ using paired *t*-test.

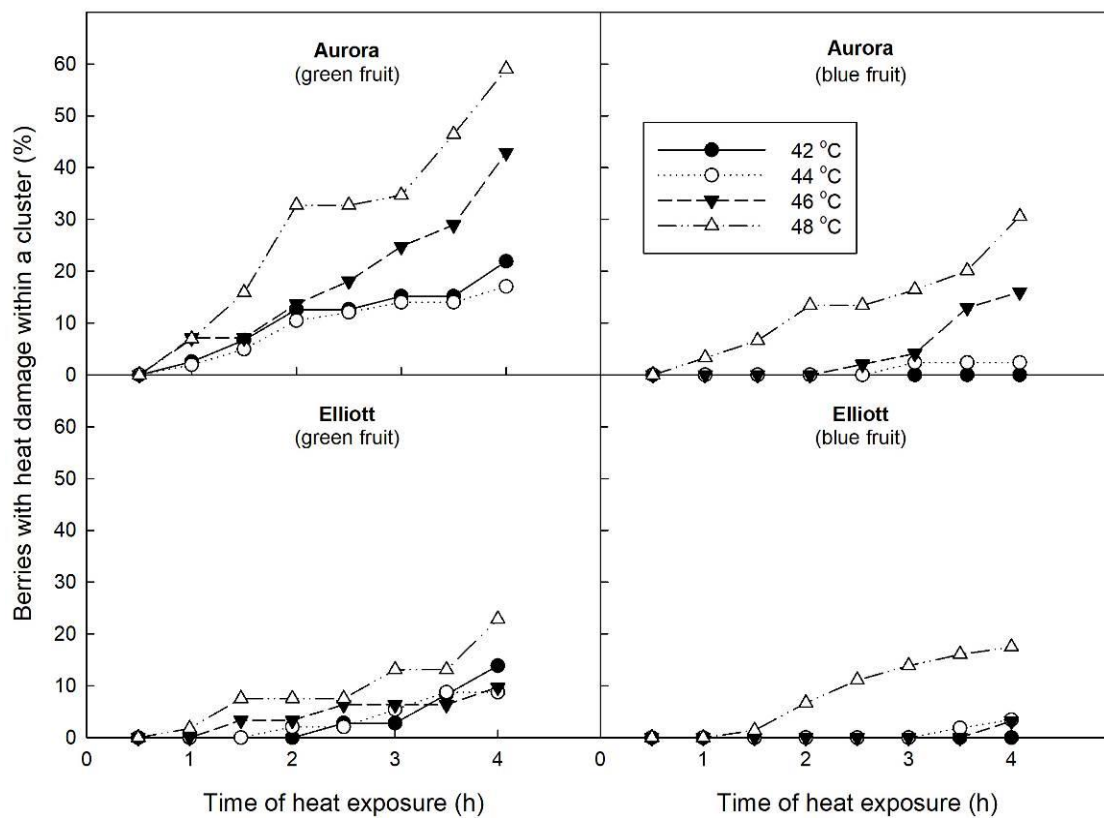


Fig. 2.10. Development of heat damage in green and blue fruit of 'Aurora' and 'Elliott' blueberry in Corvallis, OR in 2016. Clusters were heated for up to 4 h to average berry surface temperatures of 42, 44, 46, or 48 °C.

Chapter 3 – Thermal Cooling with Over-Canopy Irrigation Systems Reduces Heat Damage and Improves Fruit Quality in Northern Highbush Blueberry

Abstract

Hot and sunny weather causes a considerable amount of fruit damage in northern highbush blueberry (*Vaccinium corymbosum* L.) and can result in millions of dollars of crop loss each year. To combat this issue, many growers use over-canopy sprinkler or micro-sprinkler systems to cool the fruit, but questions remain on the amount of time and frequency these systems should be run and whether they have any effect on fruit quality. Therefore, two series of studies were conducted to evaluate the efficacy of using sprinklers or micro-sprinklers for reducing blueberry fruit temperature and improving fruit quality in western Oregon. With sprinklers, treatments were applied in 2014 and 2015 and included irrigation (night) and cooling (day) with sprinklers, sprinkler irrigation (at night only) with no cooling, and drip irrigation with no cooling in ‘Elliott’ blueberry. For cooling, sprinklers were pulsed for 15 min every hour when air temperature was ≥ 32 or $35\text{ }^{\circ}\text{C}$. Berry temperatures dropped rapidly within the first 15 min of cooling and never exceeded ambient air temperature during the cooling cycles. While the percentage of fruit with heat damage was low even without cooling ($< 2\%$), cooling reduced the damage to nearly 0% in one out of 2 years and, when run at $\geq 32\text{ }^{\circ}\text{C}$, increased berry weight relative to no cooling in both years. However, cooling also reduced the concentration of soluble solids (sugars) in the berries in 2014 and had no effect on yield, fruit firmness, titratable acidity, harvest date, or anthocyanin content and total phenolics in the berries in either year. With micro-sprinklers, cooling was tested at both a commercial farm and an experimental site. At the farm, 5-ha blocks of ‘Aurora’ blueberry either had no cooling or were cooled using micro-sprinklers run continuously or pulsed in 1-h or 20-min cycles during three extreme heat events ($\geq 35\text{ }^{\circ}\text{C}$) in 2015. Continuous cooling was the most

effective treatment for reducing temperature of the berries, but it resulted in wetter conditions afterwards, which could impede fruit harvest and increase slugs and fungal diseases. Pulsed cooling, especially with short cycles, effectively maintained fruit temperatures near that of ambient air and reduced water use by 50%. At the experimental site, cooling with micro-sprinklers reduced heat damage in ‘Aurora’ and ‘Elliott’ in 2016. Cooling also increased the weight and firmness of the berries in ‘Aurora’ but slightly reduced total soluble solids. Overall, cooling with either sprinklers or micro-sprinklers was very effective for reducing berry temperature and improving fruit quality during warm weather events in northern highbush blueberry.

Introduction

Blueberry (*Vaccinium* sp.) is the second most valuable berry crop following strawberry (*Fragaria × ananassa* Duch.), and world production volume has increased 24-fold in the past two decades (Brazelton, 2016). In the United States, the Pacific Northwest is the major blueberry producing region, accounting for 23% of the production acreage and 36% of the volume in 2016 (NASS, 2017). The majority of the blueberry cultivars in this region are northern highbush (*V. corymbosum* L.) and ripen during the summer months (June to August). However, summer weather in the Pacific Northwest has become more extreme in the past decade – more days with high temperature and less precipitation (Abatzoglou et al., 2014). Hot and sunny weather during the summer is a persistent threat to blueberry production.

High temperatures can reduce carbohydrate assimilation and trigger oxidative stress in highbush blueberry plants (Hancock et al., 1992; Moon et al., 1987; Yu et al., 2016) and diminish fruit quality and shelf life (Lobos and Hancock, 2015). In chapter 2, we found that necrosis and softening in the berries were the two most common fruit quality disorders that occurred after extreme temperature events. We also determined that visible signs of heat damage can develop in < 2 h when berry surface temperature exceeds 42–48 °C. Necrosis occurs in both immature and ripe berries, and usually starts with distinct browning or reddish spots that eventually collapse within a few days. When necrosis occurs, those berries are unmarketable, particularly in the fresh market. Fruit softening also reduces the marketability of the berries and shortens the shelf life (Ballinger et al., 1973).

Common strategies to prevent heat damage in fruit crops include the use of kaolin-based particle film, shade nets, and evaporative cooling. Kaolin particle film is not an option for blueberry since the surface residue is difficult to remove (Spiers et al., 2003). Shade net successfully reduces fruit surface temperature in apple [*Malus × sylvestris* (L.) Mill. Var. *domestica* (Borkh.) Mansf.] (Gindaba and Wand, 2005) and blueberry (Lobos et al., 2013); however, a study in plum (*Prunus salicina* Lindl.) revealed that the netting may not always cool the fruit due to slower air movement (Murray et al., 2005). Shade netting is also costly to install and can delay harvest and impede flower bud initiation, thus reducing yield (Lobos et al., 2013). Evaporative cooling is a process that uses water to remove heat from an object through evaporation. It is relatively easy to use in blueberry fields since growers can use their existing irrigation systems, and it tends to be more efficient than hydro-cooling and convective cooling since it requires less water and directly cools the fruit (Evans, 2004).

A previous study demonstrated that using over-tree irrigation for evaporative cooling can reduce apple surface temperatures and solar injury (Parchomchuk and Meheriuk, 1996). Historically, growers in the Pacific Northwest irrigated blueberry using sprinklers (Strik and Yarborough, 2005). When the weather was hot, they usually adapted apple practices using sprinklers to cool the berries. Most new blueberry plantings, however, are irrigated by drip (Bryla and Strik, 2015), so growers are seeking alternative systems to protect the fruit from summer heat. Adding overhead micro-sprinklers to existing drip-irrigated fields is one option. Using micro-sprinklers to mitigate high temperatures has been found to be effective in many horticultural crops, including apples and grapes (*Vitis* sp.) (Aljibury et al., 1975; Caravia et al., 2017a; Gindaba and Wand,

2005; Kliewer and Schultz, 1973; Iglesias et al., 2002). By lowering canopy temperature, evaporative cooling can improve C assimilation, as well as encourage shoot growth (Aljibury et al., 1975; Gindaba and Wand, 2007; Pelletier et al., 2016).

No prior research exists that has examined whether evaporative cooling can improve fruit quality or alter harvest date in northern highbush blueberry. Since micro-sprinklers use relatively less water than sprinklers, the amount of time and frequency that the micro-sprinklers should be run on any given day and whether cooling causes any problems, such as flooding or plant diseases, are important research questions. Therefore, the objective of the present study was to evaluate the efficacy of using over-canopy sprinklers and micro-sprinkler systems to reduce berry temperature, prevent heat damage, and improve fruit quality in northern highbush blueberry.

Materials and Methods

Sprinkler study. A 2-year study was conducted in 2014 and 2015 to evaluate the efficacy of using over-canopy sprinklers for cooling in northern highbush blueberry. The study was set up in an existing planting of ‘Elliott’ blueberry established in Apr. 2004 at Oregon State University Lewis-Brown Horticultural Research Farm in Corvallis, OR (lat. 44°33′ N, long. 123°13′ W, 68 m elevation). Soil at the site was a Malabon silty clay loam (fine, mixed, superactive, mesic Pachic Ultic Argixerolls). The plants were spaced 0.8 × 3.0 m apart within and between rows, respectively, and grown on 0.4-m high × 0.9-m wide raised beds. The beds were mulched every 2–3 years with a 5-cm-deep layer of douglas fir [*Pseudotsuga menziesii* Mirb. (Franco)] sawdust. Grass alleyways were

planted between the beds and mowed as needed. See Bryla et al. (2011) for complete details on establishment and irrigation of the planting.

Four treatments were assigned to existing irrigation plots in the field, including two controls with no cooling that were irrigated by drip or sprinklers (at night only), and two cooling treatments that were both irrigated (night) and cooled (day) by the sprinklers. The cooling treatments were run for 15 min every hour when air temperature was ≥ 32 or $35\text{ }^{\circ}\text{C}$. Any water applied for cooling was subtracted to offset the weekly irrigation requirements. The treatments were arranged in a randomized complete block design and replicated four times. Each block included three rows of plants and was divided into treatment plots with eight plants per row. All measurements were made on the center six plants in the middle row of each plot; the remaining plants served as buffers between treatments.

Sprinkler treatments were cooled and irrigated using $2.8\text{ L}\cdot\text{min}^{-1}$ pop-up spray heads with pre-installed dual spray quarter-pattern (90°) nozzles (model 1802QDS; Rain Bird Corp., Glendora, CA). The spray heads were mounted on steel posts on the corners of the plots at $\approx 1\text{ m}$ above the top of the canopy. Each sprinkler covered a radius of $\approx 6\text{ m}$ when operated at a pressure of 170–210 kPa. The drip treatment was irrigated using lines of drip tubing (UniRam 570; Netafim, Fresno, CA) positioned on each side of the row at a distance of $\approx 0.2\text{ m}$ from the base of the plants. The tubing had $2\text{ L}\cdot\text{h}^{-1}$ pressure-compensating emitters integrated every 0.45 m. Irrigation was scheduled based on weather and daily estimates of crop evapotranspiration (Bryla, 2011) and run once or twice per week, as needed, with sprinklers, and three to seven times per week with drip. Cooling was scheduled based on air temperatures measured in a drip-irrigated plot and

was run automatically using a 16-channel AC/DC relay controller and data logger (model SDM-CD16AC and CR-1000, respectively; Campbell Scientific, Logan, UT).

Ripe fruit were picked by hand on 1 Aug., 15 Aug., and 28 Aug. 28 in 2014 and 5 Aug., 20 Aug., and 2 Sept. in 2015. Fertilizers were applied following standard commercial practices for northern highbush blueberry (Bryla and Strik, 2015). Weeds were controlled, as needed, by hand-weeding on the top of beds and by applying glyphosate herbicide at the base of beds. No insecticides or fungicides were applied to the field during the study.

Micro-sprinkler studies. Two studies were performed to evaluate the use of micro-sprinklers for cooling blueberries. The first was conducted in 2015 in a 6-ha field of ‘Aurora’ blueberry located at a commercial farm (Pan-American Berry Growers) near Salem, OR (lat. 45°00' N, long. 123°56' W). The second study was conducted in 2016 at the Lewis-Brown Farm and included ‘Aurora’ and ‘Elliott’ blueberry. In both cases, plants were grown on raised beds and spaced 0.8 × 3.0 m apart; grass alleyways were maintained between the beds; and irrigation was applied by drip (two lines per row).

The field in Salem was established in Sept. 2004 and was managed using conventional pesticides and fertilizers. Soil at the site was a Woodburn silt loam (fine-silty, mixed, superactive, mesic Aquultic Argixerolls). The beds were unmulched, and irrigation was scheduled by the farm manager. Micro-sprinklers (Model SuperNet; Netafim, Fresno, CA) were used for cooling and chemigation. The micro-sprinklers were spaced 3.6-m apart in every other row (triangular pattern) and installed ≈ 2 m in height. The system was operated at a pressure of 240 kPa and applied 58 L·h⁻¹ of water per micro-sprinkler. Treatments were applied in 2015 and included no cooling, continuous

cooling, and pulsed cooling in 1-h or 20-min cycles. Each of these treatments was applied to 11 rows of plants (1.3-ha). Cooling (continuous and pulsed) was initiated on days when air temperature was ≥ 32 °C and was stopped at 19:00 HR. The field was hand-harvested by commercial crews in late July and early August. Nearly 30 t·ha⁻¹ of marketable fruit was produced in the field in 2015.

The planting at Lewis-Brown was established in Oct. 2008 with 12 rows of 84 plants each (0.23 ha). Mulching and irrigation were done as described above in the sprinkler study. See Vargas et al. (2015) for complete details on establishment of the planting. The present study was set up as a 2×2 factorial experiment, arranged in a randomized complete block design, and included no cooling or cooling in both cultivars. Each treatment plot consisted of one row of eight plants and was replicated four times. Only the middle six plants in each plot were used for measurements. Hanging fan-jet micro-sprinklers (DC series; Bowsmith, Exeter, CA) were suspended ≈ 0.6 m above the canopy between every other plant in the cooling treatments. The micro-sprinklers had a flow rate of 20 L·h⁻¹ and were operated at a pressure of 100 kPa. Cooling was controlled automatically, as described above, and was pulsed in 20-min cycles when air temperature was ≥ 32 °C. The plants were harvested by hand on 1 Aug., 15 Aug., and 24 Aug. 2016.

Measurements. Berry temperature was measured in each study using 0.13-mm, copper-constantan wire thermocouples (Omega Engineering Inc.; Stamford, CT). The thermocouples were inserted beneath the epidermal layer of four berries per cluster. The clusters were randomly selected from the upper west side of the canopy (1.2- to 1.8-m high) on three or four plants in each treatment plot. Ambient air temperature and relative humidity were measured in one plot per treatment using a temperature/humidity probe

(model HMP60; Vaisala, Woburn, WA). The probes were covered with a six-plate radiation shield (model 41303-5A; RM Young, Traverse City, MI) and mounted 1.8-m high. Each measurement was recorded every 5 min using data loggers (model CR-800 or CR-1000). In Salem, an hourly average of wind speed was obtained from a weather station located at the farm (Puresense Environmental Inc., Fresno, CA).

Yield and fruit quality were measured on each harvest date at Lewis-Brown; however, they were not recorded in Salem due to the logistics. The berries were first weighed to determine yield. Then, a random sample of 100 berries was weighed to calculate the average berry weight and to estimate the total number of berries in each treatment plot. Another 25 berries were randomly sampled to determine firmness using a firmness tester (FirmTech2; BioWorks Inc., Wamego, KS). Each berry was placed on its side on the instrument turntable, with the calyx facing inward. The compression force threshold procedure with a fixed range of compression forces (selected by the operator) was used to measure the firmness, which is reported as the mean gram force (N) of compression per millimeter. Heat damage was assessed visually (chapter 2) and calculated by dividing the number of damaged berries on the plants (counted prior to harvest) by the total number of berries from each harvest.

About 150 g of berries were frozen from each replicate on each harvest date and later analyzed for soluble solids ($^{\circ}\text{Brix}$), titratable acidity, and the total content of phenolics and monomeric anthocyanins. The frozen samples were thawed and pureed in a blender and measured for soluble solids using a temperature-compensated refractometer (PAL-1; Atago USA Inc., Bellevue, WA). A 10-g sample of the puree was then mixed with 100 mL of distilled water and titrated with $0.1 \text{ mol}\cdot\text{L}^{-1}$ NaOH to an endpoint pH of

8.1. Titratable acidity was calculated as a percentage of citric acid. Finally, to determine phenolics and anthocyanins in the berries, samples were homogenized with liquid N and extracted with acidified 60% methanol solution (Wang et al., 2016). Due to time and expense, this last set of procedures was only done in the sprinkler study. Phenolics were measured by colorimetry, using Folin–Ciocalteu (FC) reagent and gallic acid (Sigma, St. Louis, MO) as a standard for measuring the changes in absorbance at 765 nm (Duan et al., 2011). Anthocyanins were measured using a pH differential method, which is based on the different structure and color absorbance of anthocyanins at 520 and 700 nm and pH of 1.0 and 4.5, respectively (Wrolstad et al., 2005; Cavender et al., 2014). All colorimetric measurements were made using the same spectrometer (model UV160U; Shimadzu Co., Kyoto, Japan).

Statistical analysis. Data from the sprinkler study were analyzed by one-way analysis of variance using R v. 3.1.2 (R Development Core Team, 2014). Each measurement, including heat damage, yield, berry weight, firmness, and other quality characteristics of the berries, differed between 2014 and 2015 ($P \leq 0.05$) and, consequently, were analyzed independently by year. However, none of the measurements were significantly different within a given year among harvest dates. Therefore, each measurement was totaled or average and weighted, as appropriate, and reanalyzed across harvest dates each year. Planned comparisons between the control(s) and cooling treatments were separated at the 5% level using Fisher's protected least significant difference (LSD) test.

Data from the micro-sprinkler study were analyzed by two-way analysis of variance with R and assessed for the effects of cultivar and cooling and any interactions

between them. Heat damage, yield, and berry weight did not differ among harvest dates and, therefore, were totaled or averaged and weighted, as appropriate, and reanalyzed for the year. Firmness and other quality characteristics of the berries, on the other hand, were affected by harvest date. Combined effects of cultivar and cooling were separated using Tukey's honestly significant difference test ($P \leq 0.05$).

Results and Discussion

Cooling with sprinklers

Berry temperatures. Pulsed cooling with sprinklers was a very effective means of reducing berry temperatures in the field on warm days (Fig. 3.1). Berry temperature dropped quickly with each irrigation pulse and remained well below the temperature of the non-cooled berries throughout the cooling period. Interestingly, berry temperatures were also a few degrees lower in cooled than non-cooled plots during mornings prior to cooling (e.g., see the beginning of day 2 in Fig. 3.1). Iglesias et al. (2002) observed a similar pattern in apple and suggested this was due to higher humidity and more evaporation and convection in the orchard during the morning on days after cooling.

When the sprinklers were set to run at air temperatures ≥ 32 °C, cooling occurred on 20 d in 2014 and 21 d in 2015. Delaying the process until 35 °C resulted in only slightly higher berry temperatures (Fig. 3.1B); however, it reduced irrigation water use considerably. At the higher setting, cooling only occurred on 3 and 10 d per year, respectively, and was initiated a few hours later on each of these days. As a result, we reduced water use during cooling by ≈ 3.4 million L·ha⁻¹ during the first year and 3.0

million L·ha⁻¹ the following year. When using sprinklers, this water could be subtracted from the irrigation schedule; however, this would not be the case when a field is irrigated by drip and cooled by micro-sprinklers (see below).

In 2016, we evaluated two alternative strategies for reducing water usage when cooling with sprinklers (Fig. 3.2). The first was to shorten the cooling time from 15 min to 5 min every hour. By doing so, the temperature of the berries did not decline as much during cooling. Consequently, the berries were warmer between cycles and approached the same temperatures as the non-cooled berries. The second strategy was to add an additional hour of “off” time between each cooling cycle. In this case, berry temperatures were well above air temperature for 60–70 min between each cooling cycle and, again, were very similar to the temperatures of the non-cooled berries. Thus, while these alternative strategies only used 33% and 50% as much water as cooling for 15 min every hour, respectively, both were less effective at reducing berry temperature. In one instance, berry temperature exceeded 40 °C for nearly 90 min (Fig. 3.2C). We previously found that ‘Aurora’ blueberries can be damaged within 1–1.5 h at 42 °C (chapter 2). Heat stress might also accumulate in the berries during repeated exposure to high temperature and, over the course of a day, could lead to as much damage as continuous exposure to the same temperature. For example, Horowitz and Taylorson (1983) found that multiple short-term heat events were more lethal than a single long-term event in certain weed seeds.

Heat damage, yield, and fruit quality. Cooling with sprinklers reduced heat damage in the berries in 2014 (Table 3.1). However, this was not the case the following year. In 2015, a 3-d power outage occurred on 17–19 July and prevented the sprinklers

from running during a heat wave. At that point, many of the berries on the clusters were still green. Blueberries are more sensitive to heat damage at the green stage of development than during ripening (chapter 2). Consequently, many berries were damaged during the heat wave, including those in the cooling treatment.

Cooling with sprinklers had no effect on yield, firmness, titratable acidity, or the sugar:acid ratio of the berries, but when run at air temperatures ≥ 32 °C, it increased berry weight relative to no cooling in 2014 and 2015 (Table 3.2). Running sprinklers at temperatures ≥ 35 °C also increased berry weight relative to using sprinklers for irrigation only in 2014. However, cooling also reduced the concentration of soluble solids (sugars) in the berries in 2014, which potentially could affect their flavor and processing quality (Nindo et al., 2005). Reduced soluble solids was likely a dilution effect and a result of larger berries in the treatment (Greer and Weedon, 2014). The effects of cooling on fruit soluble solids are inconsistent in other crops, such as apple (Iglesias et al., 2002; Parchomchuk and Meheriuk, 1996) and grape (Aljibury et al., 1975; Caravia et al., 2017b; Greer and Weedon, 2014; Kliewer and Schultz, 1973), and often varies depending on fruit size.

We found no evidence that cooling delayed fruit ripening or had any effect on anthocyanin content or total phenolics in the berries (data not shown). However, it is noteworthy that each treatment had higher levels of anthocyanins and phenolics during the second year of the study, which was warmer and drier than the previous year (chapter 2).

Cooling with micro-sprinklers

Berry temperatures. The use of micro-sprinklers was very effective at reducing berry temperature at the commercial site in Salem, OR. Within 15 min of continuous operation, temperature of the berries dropped to $< 30\text{ }^{\circ}\text{C}$ and remained below air temperature, even after the system was turned off at sunset (Fig. 3.3A). Pulsed cooling with micro-sprinklers was also effective and reduced berry temperature by an average rate of $0.17\text{ }^{\circ}\text{C}/\text{min}$ during each 1-h cycle (Fig. 3.3B) and $0.31\text{ }^{\circ}\text{C}/\text{min}$ during each 20-min cycle (Fig. 3.3C). The berries likely cooled faster in the latter case due to less heat accumulation during the “off” portion of the cycle. At the warmest time of the day, cooling at either cycling frequency reduced berry temperature by $\approx 3\text{ }^{\circ}\text{C}$ below ambient and by $\approx 12\text{ }^{\circ}\text{C}$ below the temperature of the non-cooled berries; however, berry temperature increased to as high as $41\text{ }^{\circ}\text{C}$ when the system was “off” between 1-h cycles and up to $38\text{ }^{\circ}\text{C}$ between 20-min cycles. In total, berry temperature was $> 37.5\text{ }^{\circ}\text{C}$ during 39% of the time with 1-h cycling and 15% of the time with 20-min cycling. Therefore, with shorter breaks between cycles, berries were less likely to exceed the temperature thresholds for heat damage (chapter 2).

In general, both continuous and cyclic cooling with micro-sprinklers worked better on windy days. For example, in Salem, wind speed averaged 4.7 (gentle breeze) and 2.1 (calm) $\text{m}\cdot\text{s}^{-1}$ on July 29 and 30, respectively. Consequently, cooling was less effective on the latter date, where berry temperature with continuous cooling was similar the air temperature (Fig. 3.3). Wind reduces the boundary layer on the surface of the berries allowing more water vapor to evaporate into the air and creates temperature gradients within the canopy that increase convective cooling (Evans, 2004). Cooling also appeared to be more effective on sunny days than on cloudy days (data not shown).

Relative humidity is typically higher on cloudy days, reducing the vapor pressure deficit and potential for evaporative cooling (Iglesias et al., 2002; Lakatos and Żyromski, 2012). Less efficient evaporative cooling under higher relative humidity may be explained by considering wet bulb temperature. Wet bulb temperature is used to calculate the lowest temperature a wet object can reach by evaporation. A greater difference between wet bulb and dry bulb temperature (ambient air) indicates a higher evaporation efficiency. Commonly, on days with high relative humidity (>40 %), wet-bulb temperature is much closer to ambient air temperature, and, thus, cooling efficiency is lower. It is expected under a more humid climate such as Florida, where relative humidity is often > 80% during the summer, cooling efficiency would be much lower.

Pulsed cooling (at either 1-h or 20-min cycles) reduced water use by 50% relative to continuous cooling and resulted in lower relative humidity, not only during cooling, but also in the night afterwards (data not shown). Excessive moisture created by continuous cooling may be detrimental to berry production. For example, we observed more slugs in plots with continuous cooling than with no cooling at the site in Salem in 2014 (data not shown). The field was also much wetter the day after the system was run continuously than when it was pulsed for cooling, which could affect the harvest schedule and increase the potential for fruit rot (Miles et al., 2013).

Heat damage, yield, and fruit quality. Cooling with micro-sprinklers significantly reduced the percentage of berries with heat damage at the experimental site ($P < 0.05$). However, damage was only reduced by 38% in ‘Aurora’ and 21% in ‘Elliott’ (Fig. 3.4). The micro-sprinklers employed in these plots were smaller and provided less coverage

than those typically used in commercial operations, including at the site in Salem.

Therefore, cooling may have been less effective than normal at preventing heat damage.

Between cultivars, ‘Elliott’ was less susceptible to heat damage than ‘Aurora’ ($P < 0.001$). Even without cooling, the cultivar had $< 2\%$ total fruit loss in both this and the previous study with sprinklers (Table 3.1 and Fig. 3.4). Differences in tolerance to high temperatures may occur among cultivars due to variations in surface wax on the berries (Chen et al., 2012; chapter 2). The wax develops naturally during fruit ripening and acts both as a water barrier and as a reflectant for solar radiation (Jenks and Ashworth, 1999). Chu et al. (2017) found that composition of the wax varies among blueberry cultivars and suggested that different forms of β -diketones in the wax may be associated with drought or heat tolerance. Differences in thermo-tolerance may also be the result of different lipid compositions in the membrane of the fruit cells. High levels of saturated fatty acids in the phospholipid bilayer is generally associated with greater membrane stability under high temperature conditions in different plant organelles (Molina-Bravo and Zamora-Melendez, 2016), including berries (Wang and Lin, 2006).

Cooling with the micro-sprinklers had no effect on yield, titratable acidity, or the sugar:acid ratio in either ‘Aurora’ or ‘Elliott’, but it increased berry weight (size) in both cultivars (Table 3.3). A cooler microclimate created by cooling practices could increase photosynthesis and minimize the loss of carbohydrates (Aljibury et al., 1975; Caravia et al., 2017a; Gindaba and Wand, 2005; Iglesias et al., 2002; Kliewer and Schultz, 1973). In blueberry, since carbohydrate assimilation declines when air temperature is $> 30\text{ }^{\circ}\text{C}$ (Hancock et al., 1992; Moon et al., 1987), reducing leaf or berry temperature by evaporative cooling may also improve assimilation of carbohydrates, and, thus, increase

the fruit mass. Micro-sprinklers also maintained higher berry firmness in ‘Aurora’ on the first harvest date (Table 3.3). Preliminary measurements likewise suggested that cooling (continuous or pulsed) increased firmness of the berries ($190\text{--}200\text{ g}\cdot\text{mm}^{-1}$ per treatment) relative to no cooling ($175\text{ g}\cdot\text{mm}^{-1}$) at the commercial site. Both berry weight and firmness are important traits for fresh market production of northern highbush blueberries. Generally, heavier berries are larger and cost less to hand-pick than smaller berries, while firmer berries ship better and have a longer shelf life than softer berries (Ballinger et al., 1973). However, as in the sprinkler study, cooling also reduced the concentration of soluble solids in the berries (Table 3.3). Again, this was likely a dilution effect due to larger berries in the treatment.

Fruit firmness typically declines as fruit begin to change color. This softening is associated with cell wall hydrolysis, which commonly involves depolymerization of pectin, cellulose, and hemicellulose (Fischer and Bennett, 1991). In ‘Collins’ blueberry, pectin hydrolysis enzymes increase as fruit begin to turn pink (Proctor and Miesle, 1991). In ‘Duke’, fruit soften during fruit development mainly due to depolymerization of hemicellulose (Vicente et al., 2007). The transcription level of these enzyme may be affected by heat stress. It was reported in grape that under heat stress, genes related to cell wall hemicellulose modification were enhanced, which may result in the expansion or loosening of cell walls (Rienth et al., 2014; Lecourieux et al., 2017). It is possible that blueberry fruit undergo cell wall modifications under high temperatures, which may explain why the berries were firmer after cooling. Further research is needed to investigate changes in the cell wall components of blueberry during heat stress.

Neither berry weight nor firmness were affected by cooling on the subsequent two harvest dates (data not shown). Berries picked later in the season are often shaded within the canopy or cluster and, therefore, tend to have less heat damage than sun-exposed berries, which usually ripen the earliest (chapter 2). However, the percentage of berries with heat damage was similar between the first and third harvest in ‘Aurora’ (Fig. 3.4). In this case, temperature reached 38 °C just prior to the third harvest, causing heat damage in many of the remaining berries exposed to sunlight. Most of these berries were only exposed to sunlight after other berries in the cluster were removed.

Conclusion

Cooling with over-canopy sprinklers or micro-sprinklers was very effective for reducing berry temperature during warm weather events in northern highbush blueberry. In addition to protecting the berries from heat damage, in some cases, cooling increased berry weight and berry firmness. Cyclic cooling was generally as effective as continuous cooling, but it used 50% less water and resulted in lower relative humidity during and after cooling. Less humidity after cooling could improve fruit harvest (drier berries in the morning) and reduce the magnitude of slugs and fungal diseases.

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Tables and Figures

Table 3.1. Effects of cooling with sprinklers on heat damage in ‘Elliott’ blueberries during 2014 and 2015 in Corvallis, OR.

| Treatment ^z | Heat damage (%) ^y | |
|--|------------------------------|------|
| | 2014 | 2015 |
| Drip (no cooling) | 1.7 a ^x | 1.5 |
| Sprinklers (no cooling) | 0.8 b | 1.9 |
| Sprinklers (cooling at T _{air} ≥ 32 °C) | 0.1 c | 1.0 |
| Sprinklers (cooling at T _{air} ≥ 35 °C) | 0.1 c | 1.6 |
| Significance | ** | NS |

^zTreatments included irrigation by drip and no cooling, irrigation by sprinklers (night only) and no cooling, and irrigation (night) and cooling (day) by sprinklers. During cooling, sprinklers were run for 15 min every hour when air temperature (T_{air}) was ≥ 32 or 35 °C.

^yCalculated by dividing the number of damaged berries by the total number of berries harvested from a plant.

^xMeans followed by the same letter within a column are not significantly different ($P > 0.05$), according to Fisher’s protected LSD test ($P > 0.05$).

NS, **Non-significant or significant at $P \leq 0.01$, respectively.

Table 3.2. Effects of cooling with sprinklers on yield and fruit quality of ‘Elliott’ blueberry during 2014 and 2015 in Corvallis, OR.

| Treatment ^z | Yield (kg/plant) | | Berry wt (g) | | Firmness (g·mm ⁻¹) | |
|--|------------------|------|----------------------|---------|--------------------------------|------|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| Drip | 4.8 | 5.5 | 1.42 bc ^y | 1.15 b | 161 | 203 |
| Sprinklers (irrigation only) | 4.2 | 5.9 | 1.25 c | 1.29 ab | 176 | 189 |
| Sprinklers (cooling at T _{air} ≥ 32 °C) | 3.9 | 5.6 | 1.69 a | 1.35 a | 156 | 193 |
| Sprinklers (cooling at T _{air} ≥ 35 °C) | 5.1 | 5.3 | 1.45 ab | 1.19 b | 148 | 201 |
| Significance | NS | NS | ** | * | NS | NS |

| | Soluble solids (%) | | Titratable acid (%) | | Sugar:acid ratio ^x | |
|--|--------------------|------|---------------------|------|-------------------------------|------|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| Drip | 13.3 ab | 14.3 | 1.25 | 0.77 | 10.9 | 19.0 |
| Sprinklers (irrigation only) | 13.4 a | 14.2 | 1.27 | 0.93 | 11.3 | 16.0 |
| Sprinklers (cooling at T _{air} ≥ 32 °C) | 11.8 bc | 14.6 | 1.27 | 0.97 | 9.5 | 15.4 |
| Sprinklers (cooling at T _{air} ≥ 35 °C) | 11.7 c | 14.5 | 1.20 | 1.03 | 9.9 | 14.3 |
| Significance | ** | NS | NS | NS | NS | NS |

^zTreatments included irrigation by drip and no cooling, irrigation by sprinklers (night only) and no cooling, and irrigation (night) and cooling (day) by sprinklers. During cooling, sprinklers were run for 15 min every hour when air temperature (T_{air}) was ≥ 32 or 35 °C.

^yMeans followed by the same letter within a column are not significantly different, according to Fisher’s protected LSD test ($P > 0.05$).

^xCalculated by dividing soluble solids content by titratable acidity.

NS, *, ** Non-significant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3.3. Effects of cooling with micro-sprinklers on yield and fruit quality of ‘Aurora’ and ‘Elliott’ blueberry during 2016 in Corvallis, OR.

| Treatment | Yield (kg/plant) | Berry wt (g) | Firmness (g·mm ⁻¹) ^z | Soluble solids (%) ^z | Titrateable acidity (%) ^z | Sugar-acid ratio ^{z,y} |
|--------------------|---------------------|-----------------|--|------------------------------------|---|------------------------------------|
| Aurora | | | | | | |
| No cooling | 6.1 | 1.74 | 170 c ^x | 15.6 | 1.16 | 13.5 |
| Cooling | 6.1 | 2.04 | 224 a | 14.6 | 1.11 | 13.2 |
| Elliott | | | | | | |
| No cooling | 6.5 | 1.46 | 200 a | 14.7 | 1.02 | 14.6 |
| Cooling | 8.5 | 1.49 | 192 a | 14.1 | 1.01 | 14.1 |
| Significance | | | | | | |
| Cultivar | 0.0153 | <0.0001 | NS | NS | 0.0087 | NS |
| Cooling | NS | 0.0282 | 0.0155 | 0.02479 | NS | NS |
| Cultivar × cooling | NS | NS | 0.0032 | NS | NS | NS |

^zData on fruit firmness, soluble solids, titrateable, and sugar-acid ratio are from the first of three harvest dates in 2016, which accounts for 42% and 38% of the total yield with and without cooling, respectively, in ‘Aurora’, and 65% and 70% of the total yield with and without cooling, respectively, in ‘Elliott’. None of these measurements were affected by cooling on the latter two harvest dates.

^yCalculated by dividing soluble solids content by titrateable acidity.

^xMeans followed by the same letter within a column are not significantly different ($P > 0.05$), according to Tukey’s honestly significant difference test.

^{NS}Non-significant.

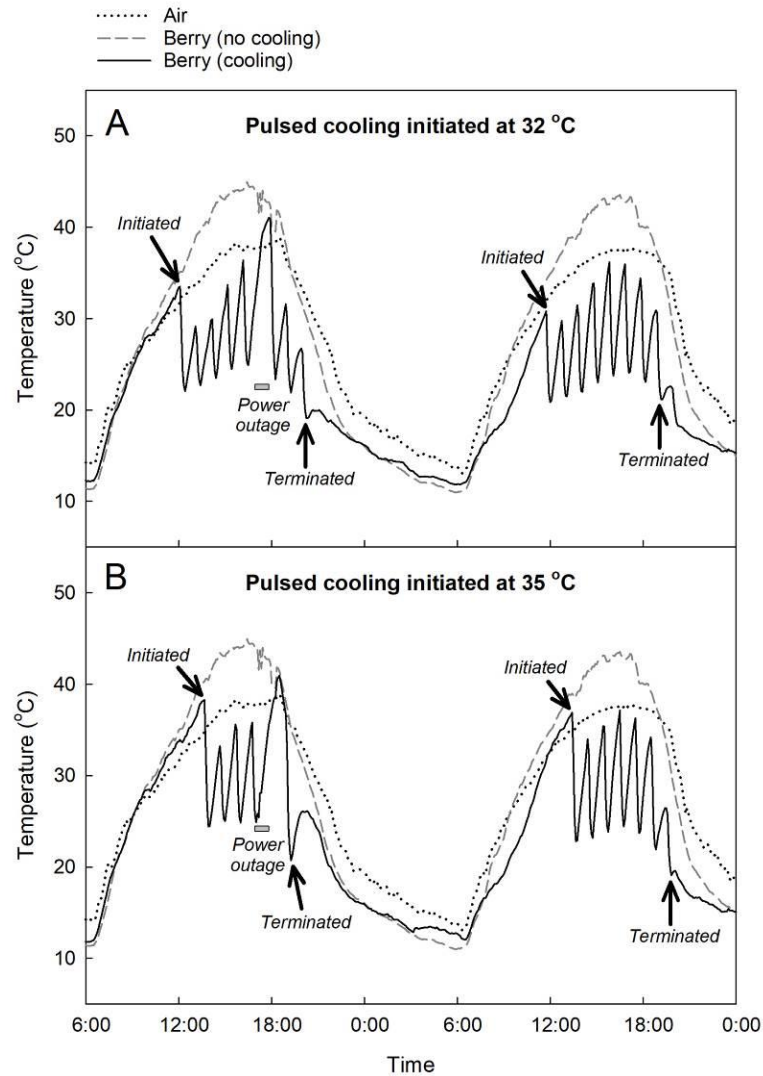


Fig. 3.1. Ambient air and berry surface temperatures measured in experimental plots of 'Elliott' blueberry in Corvallis, OR. The plots either had no cooling or were cooled using over-canopy sprinklers. The sprinklers were run (pulsed) for 15 min every hour when air temperature was \geq (A) 32 °C or (B) 35 °C. The measurements shown here were taken on 30-31 July 2015 (hottest days of the year at the site); note that a cooling cycle was missed on the first date due to a power outage.

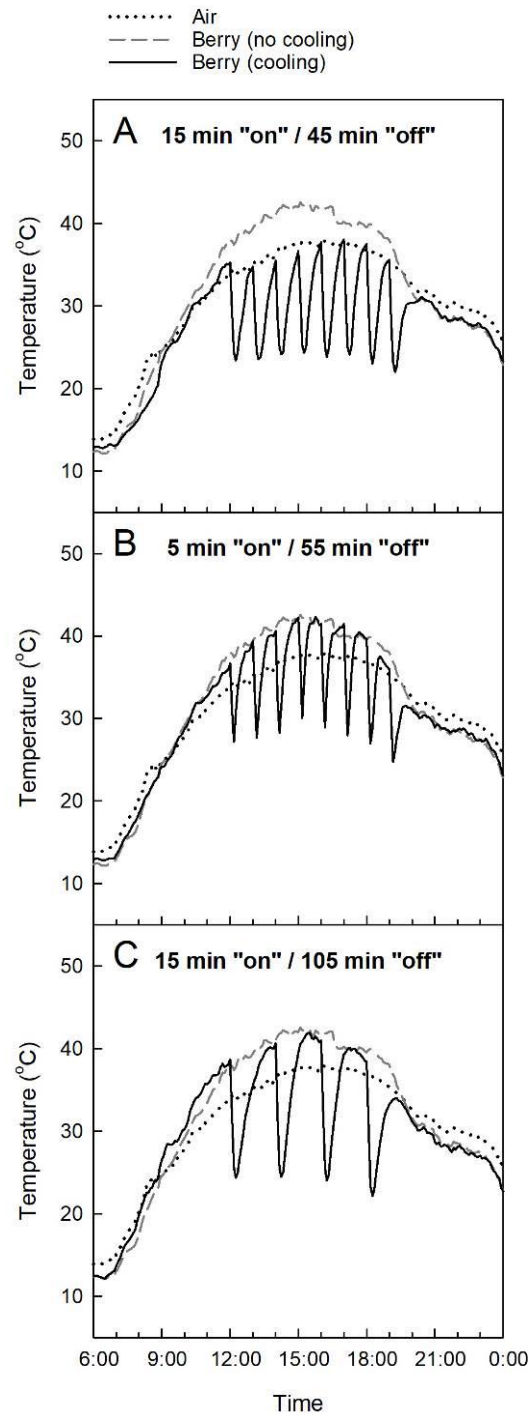


Fig. 3.2. Ambient air and berry surface temperature measured in experimental plots of 'Elliott' blueberry. The plots either had no cooling or were cooled using over-canopy sprinklers that were run (pulsed) for (A) 15 min every hour, (B) 5 min every hour, or (C) 15 min every 2 h when air temperature was ≥ 32 °C. The measurements shown here were taken on 19 Aug. 2016.

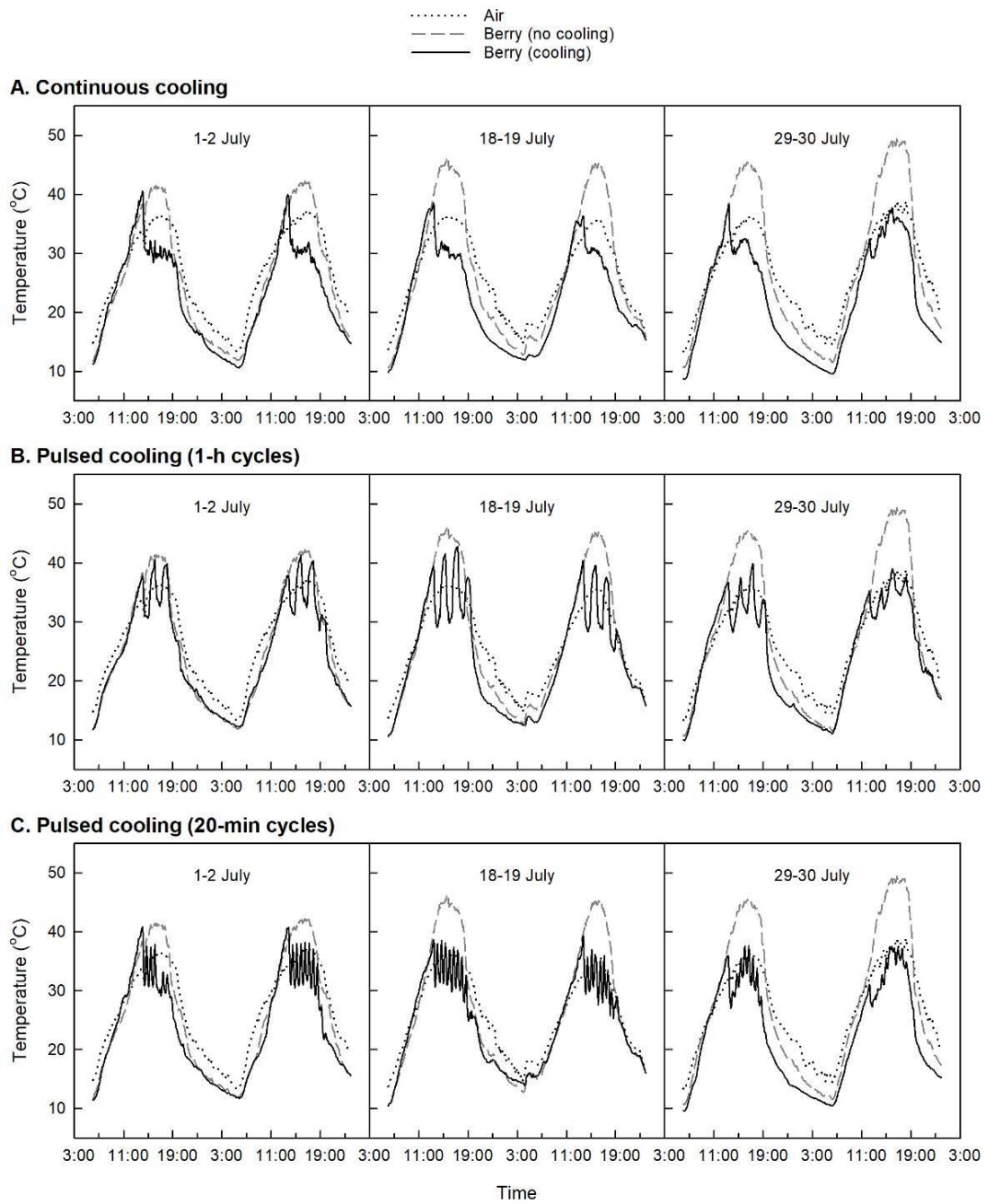


Fig. 3.3. Ambient air and berry surface temperatures measured in a commercial field of 'Aurora' blueberry in Salem, OR. Five-hectare blocks at the site either had no cooling or were cooled using over-canopy micro-sprinklers that were (A) run continuously or pulsed in (B) 1-h or (C) 20-min cycles during three extreme heat events ($\geq 35^\circ\text{C}$) in 2015. On each date, cooling was initiated between 12:00 and 14:00 HR and was terminated at approximately 19:00 HR.

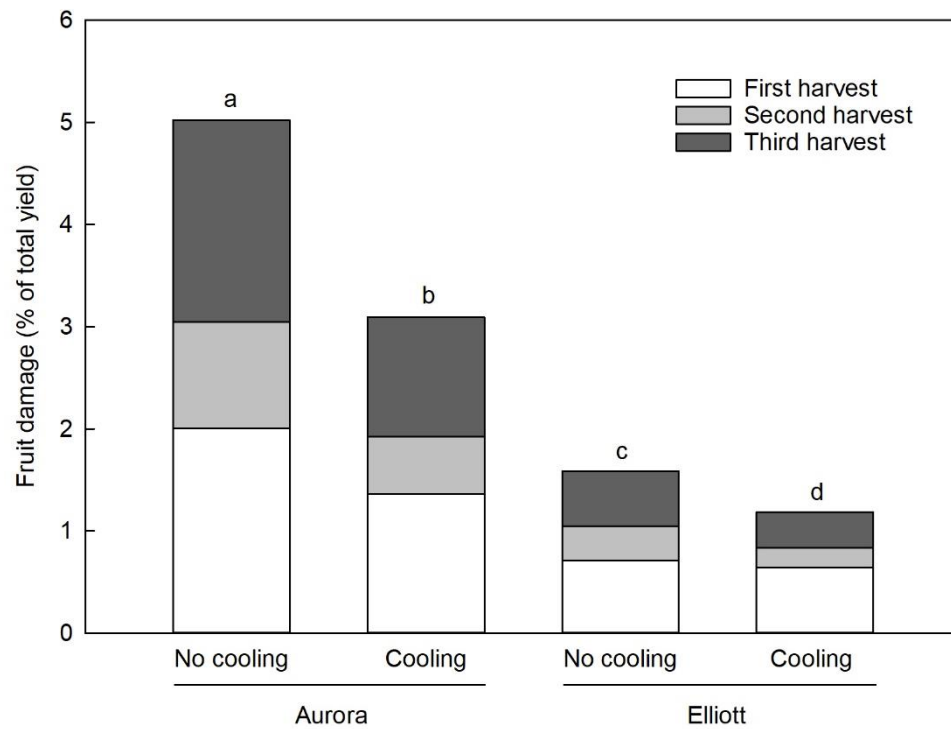


Fig. 3.4. Percentage of berries with heat damage in experimental plots of 'Aurora' and 'Elliott' blueberry in Corvallis, OR in 2016. The plots either had no cooling or were cooled using over-canopy micro-sprinklers that were pulsed in 20 min cycles when air temperature was ≥ 32 °C.

Chapter 4 – An Energy Balance Model for Predicting Berry Temperature and Scheduling Evaporative Cooling with Sprinklers in Northern Highbush Blueberry

Abstract

Heat related-fruit damage is a prevalent issue in northern highbush blueberries (*Vaccinium corymbosum* L.) in the Pacific Northwest. In the current study, a simple climatological model was developed to predict blueberry fruit temperatures based on local weather data and to simulate the effects of using over-canopy sprinklers for cooling the fruit. During sunny days, the predicted fruit temperature was strongly correlated with the actual value ($R^2 = 0.91$) and had a root-mean-square error of ≈ 2 °C. Among the model parameters tested, ambient air temperature and light intensity were found to have the greatest impact on fruit temperature, while wind speed and fruit size had less impact, and relative humidity had no impact. During cooling with sprinklers, water temperature and nozzle flow rate affected the extent to which fruit temperature was reduced. Prolonging the runtime of the sprinklers did not guarantee greater temperature reduction, since cooling efficiency declined as fruit temperature approached the temperature of the irrigation water. Cooling efficiency under different cooling intervals was successfully estimated by incorporating a water application factor. The outcome of this study provides increased understanding of how fruit temperature in blueberry changes under various conditions. This model can potentially be used as a tool for evaluating feasible cooling practices and for making cooling decisions according to the local weather conditions.

Introduction

Northern highbush blueberry is sensitive to high temperatures, particularly during fruit ripening (Lobos and Hancock, 2015). After major heat events, reduction in fruit quality and physical disorders such as sunburn, fruit softening, and bad coloration are commonly reported (chapter 2). To reduce the impact of high temperature on fruit, some blueberry growers in the Pacific Northwest (Oregon and Washington) either advance their harvest schedules to escape the heat, or they use overhead irrigation systems to cool the berries (Houston et al., 2017). As more growers begin to adopt these practices, some key questions are arising, such as when is the risk of heat damage economically critical in blueberries, and how can cooling practices be optimized to efficiently prevent the damage?

Previously in chapter 2, we found that visual heat damage occurs when the surface temperature of berries reached 42–48 °C for multiple hours. We also determined that fruit temperature was up to 7–11 °C warmer than daytime air temperatures during an extreme heat event. Unfortunately, predictions of heat damage based simply on air-temperature measurements are not always accurate, because there are other environmental factors, such as light intensity and wind, that affect the temperature of the plant (Cellier et al., 1993; Monteith and Unsworth, 2008; Saudreau et al., 2009). To better estimate blueberry temperatures according to local environmental conditions, mathematic models based on energy balance are an option. In an energy balance model, the overall gain and loss of energy on an object is the same (Monteith and Unsworth, 2008). The energy flux of a fruit is estimated according to its geometry and surface characteristics, and the environmental conditions. Energy models have been widely used for predicting

fruit temperature in a number of crops, including apple [*Malus × sylvestris* (L.) Mill. Var. *domestica* (Borkh.) Mansf.], peach [*Prunus persica* (L.) Batsch], wine grape (*Vitis vinifera* L.), and fig (*Ficus carica* L.) (Cola et al., 2009; Evans, 2004; Li et al., 2014; Patiño et al., 1994; Pitacco et al., 2000; Saudreau et al., 2007; Smart and Sinclair, 1976).

Energy balances can be used for predicting the efficiency of cooling systems to reduce fruit heat damage. Running overhead sprinklers or micro-sprinklers during heat events is an effective means of reducing temperature in blueberry (chapter 3) and other fruit crops (Caravia et al., 2017; Greer and Weedon, 2014; Iglesias et al., 2002; Kliwer and Schultz, 1973; Parchomchuk and Meheriuk, 1996; Pelletier et al., 2016). Evans (2004) provided equations for estimating skin and core temperatures in apple and was able to accurately simulate changes in fruit temperature during cooling using an irrigation system with small spray nozzles. He calculated the amount of heat removed from the apples based on estimates of water interception, sprinkler spacing, and temperature differences between the fruit and irrigation water. A simple energy balance model was also used to predict potential water use during evaporative cooling in wine grape (Caravia et al., 2016). Prior to the present study, no similar research had been done in blueberry.

In this study, we developed an energy balance model specifically for northern highbush blueberry. Our objectives included: 1) developing a model for predicting blueberry fruit temperature based on the weather conditions; 2) evaluating the impact of different weather parameters on fruit temperature; and 3) predicting the efficacy of cooling on fruit temperature based on sprinkler specifications and cooling frequency. This information is needed to design effective management practices and strategies for preventing heat damage prior to harvest in blueberries.

Materials and Methods

Study site

Data for the model were collected in 2015 in a mature, test planting of ‘Elliott’ blueberry established in Apr. 2004 at Oregon State University Lewis-Brown Horticultural Research Farm in Corvallis, OR (lat. 44°33′ N, long. 123°13′ W, 68 m elevation). The plants were grown 0.8-m apart on rows of raised beds (0.4-m high × 0.9-m wide). The beds were spaced 3.0-m apart, in an east-west direction, and were mulched every 2–3 years with a 5-cm-deep layer of douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] sawdust. Blueberry plants were hand-pruned annually in late winter. The field was arranged in a randomized block design with drip and sprinkler irrigation treatments. Each treatment plot consisted of three rows of eight plants (all measurements were made in the middle row on the center six plants in the plot). Drip plots were irrigated using pressure-compensating drip tubing (model UniRam 570; Netafim, Fresno, CA) with integrated emitters (2.0 L·h⁻¹) every 0.45 m. Sprinkler plots were irrigated using 2.8 L·min⁻¹ pop-up spray heads with pre-installed dual spray quarter-pattern (90 °) nozzles (model 1802QDS; Rain Bird Corp., Glendora, CA). In this study, four replicates of plots with drip and sprinklers were used as “no cooling” and “cooling” treatments. Sprinklers were used for both irrigation (night) and cooling (day) as needed. Cooling was run for 15 min every hour when air temperature was ≥ 35 °C. Any water used for cooling was subtracted from the irrigation schedule. See chapter 3 for more information on the study site.

Measurements

Fruit surface temperatures were measured at the site using 0.13-mm, copper-constantan wire thermocouples (Omega Engineering Inc., Stamford, CT). The thermocouples were inserted beneath the epidermal layer of four berries per cluster. Four clusters exposed to full sun on the west side of the plants were randomly selected from the upper part of the canopy (1.2- to 1.5-m high) in each plot.

Ambient air temperature and relative humidity were measured using a temperature-humidity probe (model HMP60; Vaisala, Woburn, WA). The probe was covered by a six-plate radiation shield (model 41303-5A; RM Young, Traverse City, MI) and mounted 1.8-m high. Wind speed was measured at a height 2.4 m above the ground using a high-performance wind sensor (model 05103; RM Young, Traverse City, MI). This sensor has a sensitivity threshold of $1 \text{ m} \cdot \text{s}^{-1}$, and therefore, readings that were $< 1 \text{ m} \cdot \text{s}^{-1}$ were rounded up to $1 \text{ m} \cdot \text{s}^{-1}$. Total solar irradiance, diffuse irradiance, and net radiation were measured using a pyranometer (model SP-110; Apogee, Logan, UT), a sunshine sensor (model BF-5; Delta-T, Cambridge, UK), and a net radiometer (model Q*7.1; Radiation and Energy Balance Systems, Seattle, WA), respectively. Each radiation sensor was mounted 3-m high.

Each measurement was recorded every 5 min using data loggers (models CR-800, CR-1000, and CR-3000; Campbell Scientific, Logan, UT).

Energy balance model

According to the energy conservation law, energy cannot be created or destroyed, but can be modified in forms. In a given object, the input of energy will be equivalent to

storage and loss of the energy. According to Evans (2004), the energy balance of fruit can be categorized into total incoming radiation (R_{abs}), emitted radiation (R_e), sensible heat loss (H), latent heat loss (λE), and sensible heat flux within the fruit (E_f) (Fig. 4.1A). Sensible heat removal by water (E_w) will also occur when sprinklers are run for cooling in the field.

Total incoming and emitted radiation. Net radiation (R_n) is the difference between R_{abs} , which includes both short- and longwave radiation, and R_e . Shortwave radiation absorbed by the fruit (R_s) is calculated by multiplying total shortwave radiation (S_T) by the fraction of reflectance ($1 - \alpha_f$), where S_T is the sum of direct irradiance (S_b) and diffuse irradiance (S_d) and α_f is albedo of the fruit (Cole et al., 2009). Smart and Sinclair (1976) determined that α_f was equal to 0.22 in red wine grapes, which we assumed here was similar to blueberries. Direct light reaching the fruit was corrected (S_b') by dividing S_b by sin of the solar elevation angle (β) (Smart and Sinclair, 1976). In this study, we simulated the hottest scenario, which is when the solar beam is normal (perpendicular) to the surface of the fruit. Incoming long-wave radiation (R_l) includes irradiance from both the sky (L_u) and the ground (L_d). The combination of the two were derived from net radiometer output (R_n') ($\text{W} \cdot \text{m}^{-2}$). Since the net radiometer was installed 3 m above the soil surface, the albedo was equivalent to an orchard canopy (α_{canopy}), which has a value of about 0.2 (Monteith and Unsworth, 2008). Therefore, to calculate R_l , we used the following formula:

$$R_l = R_n' - (1 - \alpha_{canopy}) S_T \quad [\text{Eq. 1}]$$

Energy emission from the fruit, R_e , was calculated based on Stephan-Boltzmann's law. Assuming the fruit act as a gray body, R_e was calculated as:

$$R_e = \varepsilon \sigma T_f^4 \quad [\text{Eq. 2}]$$

where ε is emissivity of the atmosphere (0.97; Monteith and Unsworth, 2008), σ is the Stephan-Boltzman constant ($5.67\text{E}-8 \text{ W}\cdot\text{m}^{-2}$), and T_f is the surface temperature of the fruit ($^{\circ}\text{C}$).

Sensible heat loss. Sensible heat flux ($\text{W}\cdot\text{m}^{-2}$), H , arises as a result of the temperature difference between the surface of an object and the air above. This heat must transfer through a boundary layer above the object before exchanging with the air. The process is defined as:

$$H = C_p \times \rho_a \times g_H \times (T_f - T_a) \quad [\text{Eq. 3}]$$

where C_p is the specific heat of air ($\text{J}\cdot\text{kg}^{-1}\cdot\text{K}^{-1}$), ρ_a is air density ($\text{kg}\cdot\text{m}^{-3}$), g_H is heat transfer conductance ($\text{m}\cdot\text{s}^{-1}$) (Monteith and Unsworth, 2008), and T_a is ambient air temperature ($^{\circ}\text{C}$). Heat transfer conductance, g_H , can be calculated as:

$$g_H = 1.5 \times \frac{\kappa \text{Nu}}{d} \quad [\text{Eq. 4}]$$

where 1.5 is a ratio to adjust a sphere to a plate based on the average Reynolds number (Re ; Campbell and Norman, 1998), κ is the thermal diffusivity of air ($\text{m}^2\cdot\text{s}^{-1}$), d is the

average diameter of a berry cluster (m), and Nu is the Nusselt number, which, in the present study, was equal to $0.34 \times Re^{0.6}$ (based on the geometry of the fruit and the range of Re) (Monteith and Unsworth, 2008).

Latent heat loss. Latent heat flux ($W \cdot m^{-2}$), λE , is associated with the phase change of water without changing its temperature. Commonly, latent heat exchange occurs as evaporation and transpiration (Campbell and Norman, 1998; Li et al., 2014) and can be calculated as:

$$\lambda E = \lambda g_v \rho_a \frac{\Delta (T_f - T_a)}{P} + \lambda g_v \rho_a \frac{e_s(T_a) - e_a}{P} \quad [\text{Eq. 5}]$$

where λ is the latent heat of vaporization ($kJ \cdot kg^{-1}$), g_v is water vapor conductance ($m \cdot s^{-1}$), Δ is the slope of the saturation vapor pressure curve ($kPa/^\circ C$), P is atmospheric pressure (kPa), $e_s(T_a)$ is the saturation vapor pressure at T_a (kPa), and e_a is ambient vapor pressure (kPa). For water vapor to transfer from intracellular to outer air space, it must pass through the cuticle (g_c) and boundary layer (g_{bl}) on the surface of the fruit. Therefore, g_v can be written as:

$$g_v = \frac{1}{\frac{1}{g_c} + \frac{1}{g_{bl}}} \quad [\text{Eq. 6}]$$

We assumed that g_c of blueberries was equivalent to mature wine grapes ($\approx 4 \text{ mmol} \cdot m^{-2} \cdot s^{-1}$; Zhang and Keller, 2015) and defined g_{bl} as:

$$g_{bl} = \frac{\kappa_v}{\delta} \quad [\text{Eq. 7}]$$

where κ_v is the thermal diffusivity of water vapor ($\text{m}^2 \cdot \text{s}^{-1}$) and δ is the boundary layer thickness (mm). Based on Nobel (1975), δ was calculated as:

$$\delta = 2.8 \sqrt{\frac{d}{u} + \frac{0.25}{u}} \quad [\text{Eq. 8}]$$

where u is wind speed ($\text{m} \cdot \text{s}^{-1}$).

Sensible heat flux within the fruit. Sensible heat flux, or in other words the change of heat storage in the fruit, can be calculated as:

$$E_f = C_f \times \frac{d}{6} \times \frac{dT}{dt} \quad [\text{Eq. 9}]$$

where C_f is specific heat of fruit ($4172 \text{ J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$; Mercali et al., 2011) and dT/dt is the change in temperature over time in the fruit cluster.

An example of outputs from the model are illustrated in Fig. 4.1B. In this case, we input weather data from 31 July 2015, which was the hottest day of the year. The total amount of incoming radiation absorbed by the fruit increased from 497 to 887 $\text{W} \cdot \text{m}^{-2}$ between 10:00 and 18:00 HR (typically the warmest period of the day). Most of the radiation absorbed by the fruit was shortwave radiation. Heat loss was dominated by emittance, which accounted for 63% to 92% of the total energy loss. Based on Stephan-Boltzmann's law [Eq. 2], heat emittance depends on the temperature of the fruit and is

independent of the external environment. Sensible heat loss, in contrast, depends on wind speed and the temperature difference between the fruit and surrounding ambient air. In our model, sensible heat accounted for 7% to 35% of the total heat loss from the berries. Latent heat accounted for only 1% to 3% of the total energy loss and change of heat storage accounted for < 0.5% loss throughout this study. Since the latter term was trivial, it was excluded from the energy balance calculation. Similar changes in heat flux were observed during other days in which the model was run.

Sensible heat removal by water. In apple (Evans, 2004), heat removal by water was calculated as:

$$E_w = I \frac{dMa}{dt} \times (T_w - T_f) \times \frac{C}{S} \quad [\text{Eq. 10}]$$

where I is the water interception ratio by plants, M_a is the mass of water applied by sprinklers over time ($\text{kg} \cdot \text{s}^{-1}$), T_w is the temperature of the water at the fruit surface ($^{\circ}\text{C}$), C is a conversion factor to change $\text{kg} \cdot \text{s}^{-1}$ to $\text{W} \cdot \text{K}^{-1}$ [which is calculated by multiplying the specific heat of water ($4190 \text{ J} \cdot \text{kg}^{-1} \cdot \text{K}^{-1}$) by the density of water ($1 \text{ kg} \cdot \text{L}^{-1}$)], and S is the spacing of the sprinklers (m^2). However, rather than calculating water interception from sprinkler flow rate and spacing, we directly collected water applied near the fruit during cooling using containers with known volume and surface area. Based on the amount of water applied in a given amount of time and area, we obtained a water application factor, A ($\text{L} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Therefore, E_w was redefined as:

$$E_w' = A \times f \times (T_w - T_f) \quad [\text{Eq. 11}]$$

where f is a unit for cooling time. In general, fruit temperature declines over time during cooling. However, since fruit temperature was calculated using weather data measured in drip-irrigated plots, the result could not reflect the accumulation of heat removal over time. To solve this issue, f was added to estimate changes in fruit temperature over consecutive time series. During each cooling cycle (“on” time), heat removal from the previous cooling cycles was added by 1 unit of f . On the other hand, between cycles (“off” time), f decreased over time as result of evaporation of water from the fruit surface. The water application factor during “off” times (A_{off}) was calculated based on the amount of water applied during “on” times and the amount of time required for all water to evaporate from the fruit surface. Time required for evaporation was based on empirical observations. During cooling, it took ≈ 55 min for the fruit to dry following 15 min of water application. Therefore, $A_{off} = A_{on} \times (\text{on time}) \div (\text{time required for evaporation}) = 0.0102 \text{ L} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \times 15 \text{ min} \div 55 \text{ min} = 0.0028 \text{ L} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Weather data collected from the field were entered into the equations. Fruit temperature was then calculated at 5-min time intervals using Excel Solver (Microsoft, Seattle, WA). Solver is an add-in program used for what-if analysis. Energy parameters (R_{abs} , R_e , H , λE , E_f , and E_w) were initially calculated at a fruit temperature of 25 °C. The sum of these parameters was then set to zero, and fruit temperature was set up as a changing variable. Solver automatically found the best-fitting fruit temperature by optimizing the result based on the minimum sum of square. Sensitivity analysis was performed to evaluate the impact of different variables on the model output, including air

temperature, light intensity, wind speed, relative humidity, and diameter of the berry cluster (cluster size). To test the weight of each variable, one factor was changed at a time, and the output was monitored. Based on data from the hottest day of year, the parameters were initially set at 38 °C, 826 W·m⁻², 4.2 m·s⁻¹ (classified as ‘gentle breeze’, according to the Beaufort scale), 16%, and 0.1 m, respectively.

Results

Validation of the model. The model was validated using data collected on 13 sunny days in July and Aug. 2015. Over the course of each day, berry temperatures predicted by the model tended to fluctuate frequently relative to the actual measurements but, in general, remained within a few degrees of the temperatures measured in the field (Fig. 4.2). Fluctuations in the simulated values were due to minor changes in wind speed, which is a key parameter for estimating heat transfer conductance (Eq. 4) and boundary layer thickness (Eq. 8) on the berry clusters. The relationship between the predicted and actual temperature was significantly linear ($P < 0.0001$) and had a coefficient of determination (R^2) of 0.91 and root-mean-square error of 2.1 °C (Fig. 4.2, inset). Tests for normality and constant variance, however, failed ($P < 0.0001$). Most of the variability occurred at temperatures > 30 °C and was likely due to periodic shading of the berries, produced by nearby leaves or by adjacent berries on the cluster, as the sun moved across the sky.

Sensitivity analysis indicated that estimates of berry temperature were largely affected by air temperature, light intensity, and wind speed, and were only slightly influenced by relative humidity or size of the berry cluster (Fig. 4.3). For example,

adjusting air temperature in the model from 34 to 38 °C (+11% gain) increased our estimate of berry temperature by 3.5 °C. Likewise, a 10% gain in light intensity increased berry temperature by 1.4 °C. In both cases, the relationship between variation in these parameters and changes in fruit temperature was linear. Wind speed was the only factor that was negatively correlated with fruit temperature. In this case, the relationship was sigmoidal and dominated by the boundary layer on the fruit cluster. Consequently, berry temperature was affected much more as a result of changes in wind speed under light breeze conditions ($1.6\text{--}3.3\text{ m}\cdot\text{s}^{-1}$) than under a moderate breeze ($5.5\text{--}7.9\text{ m}\cdot\text{s}^{-1}$). For example, reducing wind speed from 4.2 to $2.1\text{ m}\cdot\text{s}^{-1}$ increased berry temperature by 1.5 °C, whereas increasing it to $8.5\text{ m}\cdot\text{s}^{-1}$ reduced berry temperature by only 1.1 °C. Berry temperature was barely affected by changes in relative humidity ($< 0.1^\circ\text{C}$) and, with the exception of very small berry clusters (e.g., 5-cm diameter), was only slightly affected by differences in cluster size ($< 1^\circ\text{C}$).

Predicting fruit temperatures during cooling with sprinklers. Pulsed cooling with sprinklers reduced irrigation water use and allowed time for water to evaporate and cool the fruit surface (Fig. 4.4). Typically, fruit temperature dropped well below ambient air temperature during each 15-min cooling cycle and gradually increased between cycles. Simulated fruit temperatures calculated at the outset of cooling were initially quite similar to the actual berry temperatures measured in the field. However, unlike the predicted values, actual berry temperatures continued to drop by 2–3 °C each time irrigation was turned off during a cooling cycle, and often peaked at somewhat higher temperatures between cycles. Although fruit temperature during cooling was somewhat more variable than predicted by the model, mean differences between measured and simulated values

were $< 1.4^{\circ}\text{C}$ within each irrigation cycle. Note that, due to the nature of the model, fruit temperature could not be predicted after sunset (20:40 HR).

Based on Eq. 11, predicting the efficiency of cooling was primarily dependent on the temperature of the irrigation water and the rate and frequency in which the water was applied. To illustrate, we ran the model under four different operating scenarios (Fig. 4.5). The default was set to run sprinklers for 15 min every hour using water with a temperature of 20°C (scenario 1). When temperature of the water was increased to 25°C (scenario 2), fruit temperature was $\approx 1\text{--}3^{\circ}\text{C}$ warmer during cooling than in the previous scenario, but it was still well below the temperature of the uncooled fruit. Not surprisingly, running sprinklers continuously (scenario 3) reduced fruit temperature to nearly the same temperature as the irrigation water (20°C). The final scenario was an extreme case where water applications were reduced by 33% (scenario 4). In this case, fruit temperature declined as expected during cooling but increased between cycles to the same temperature as the uncooled fruit.

Discussion

An energy balance model was developed in the present study to predict changes in fruit temperature on warm, sunny days in northern highbush blueberry. It was not surprising that most of the energy gained from incoming solar radiation was lost in the form of emittance and sensible heat. Latent heat only accounted for a small portion of the energy lost, which was likewise found in apple and grape (Li et al., 2014; Saudreau et al., 2007; Smart and Sinclair, 1976). During cooling with sprinklers, additional sensible heat

was removed through contact between the fruit and the irrigation water applied. Using local weather data, estimates of fruit temperature during cooling were fairly accurate.

Ambient air temperature was the most important variable affecting our estimates of berry temperature. Light intensity was also important, followed by wind speed and size of the berry cluster. Relative humidity had very little impact. Light intensity and air temperature are confounding factors, as incremental increases in light results in higher air temperature. Wind speed, on the other hand, was negatively correlated with fruit temperature. Faster wind speeds increase sensible heat loss and thereby reduce fruit temperature (Smart and Sinclair, 1976). Cluster size was of minor importance in our estimates of fruit temperature ($< 1\text{ }^{\circ}\text{C}$). Fruit temperature increase with larger fruit (or clusters in this study) due to a thicker boundary layer (Patiño et al., 1994). While relative humidity was also of minor importance ($< 0.1\text{ }^{\circ}\text{C}$), it would be interesting to further investigate the response of fruit temperature in a hot and humid climate such as Florida.

To simulate energy reception, estimates of fruit temperature were based on the assumption that the shape of a blueberry cluster is a sphere. For a spherical object, the direct beam of radiation received from sunlight varies with solar altitude. To simulate the hottest scenarios, we assumed that the beam was normal to the fruit surface (“hot spot”) (Smart and Sinclair, 1976). While this correction proved to result in reasonable estimates of berry temperature during most of the day, it led to overestimates of incoming radiation in the early evening due to the low solar elevation angle near sunset (Smart and Sinclair, 1976). Based on our observations, we suggest that the user does not correct the total direct beam when solar elevation angle is $< 20^{\circ}$. Instead, this angle should be calculated

using the arctangent function and adjusted according to the row direction, row spacing, and the height of the plants in the field.

In the present study, we assumed that a berry cluster is a thermal isotropic object. Previous results indicated that there was $< 1^{\circ}\text{C}$ difference between the surface and the center of a blueberry fruit on a sunny day (chapter 2). Therefore, temperature was only measured and simulated on the surface of the cluster. In apple, fruit temperatures were monitored separately at the skin and core, and heat conduction flux within the fruit was included in the energy balance model (Evans, 2004). In our case, heat storage accounted for $< 0.5\%$ total energy flux within the fruit and, therefore, was not included in the model. However, given the fact that temperature gradients normally occur within a blueberry cluster, more precise fruit temperature predictions could be achieved potentially by either estimating the light interception ratio of different clusters using a thermal camera (Li et al., 2014) or by modeling the upper and lower hemisphere of a fruit cluster separately (Saudreau et al., 2007; Smart and Sinclair, 1976).

We noted that actual fruit temperatures continued to decline by $2\text{--}3^{\circ}\text{C}$ after the sprinklers were turned off during each cooling cycle. This effect was likely due to evaporation of water from the fruit surface, which removes a large amount of heat ($2.43\text{ MJ}\cdot\text{kg}^{-1}$ of water at 30°C). In the current model, the time required for water to evaporate from the fruit was based on visual observations, which may be why the simulations failed to account for the additional heat loss. Perhaps a surface wetness sensor could be used to improve the estimates. Hewett and Young (1980) reported that leaf wetness sensors could be used to control evaporative cooling systems for delaying blooming in fruit trees in the spring time. Leaf wetness sensor can also be connected to dehumidifying devices to

reduce high relative humidity inside a greenhouse (Seginer and Zlochins, 1997). However, a preliminary study revealed that leaf wetness sensors tended to dry faster than a typical berry cluster (F.-H. Yang, unpublished data). A correlation between leaf wetness and fruit surface water retention would need to be developed in order to use this type of sensor for estimating fruit temperatures and scheduling cooling in a blueberry field.

In blueberry, most heat damage occurs on berries exposed to full sunlight (chapter 2), which is why the current model focused on predicting temperatures of sun-exposed fruit. However, unlike in wine grape vineyards and high-density apple orchards, which rely on training practices to maximize fruit light exposure, berry clusters in blueberry are located throughout the canopy. Clusters located at lower or shaded positions generally have much lower fruit temperatures during the day than those located in the sun (chapter 2). Thus, scheduling cooling practices based on estimates of fully-exposed clusters could result in wetter fruit in the shaded areas of the canopy, and thereby increase potential for infection by fungal pathogens. Further work is needed to adjust for variations in cluster temperature.

Conclusion

Two significant outcomes were achieved in this study. First, a simple energy balance model was used successfully to predict fruit temperature based on local weather measurements. Potentially, this model can be incorporated into a weather forecast program to predict the incidence of heat damage on any given day. Second, the model also successfully predicted the fruit temperature patterns during evaporative cooling

practices. The model can be possibly used as a tool for making cooling decisions and evaluating the best cooling practices for northern highbush blueberry.

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Tables and Figures

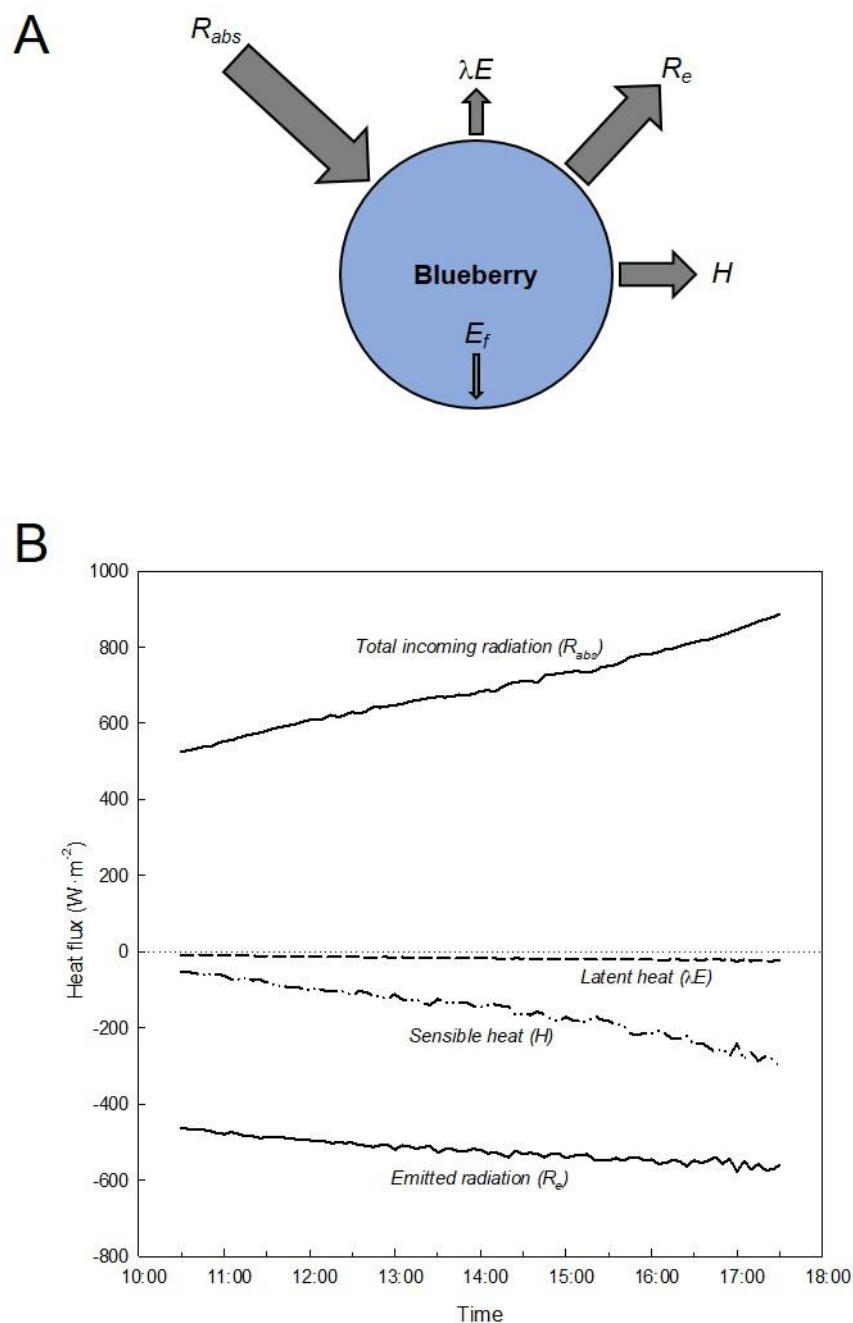


Fig. 4.1. (A) Illustration of the components of the heat energy balance model and (B) simulated heat fluxes at the surface of a sun-exposed blueberry. Fluxes were calculated using weather data collected in a field of ‘Elliott’ blueberry on 31 July 2015 (hottest day of the year). Sensible heat flux (E_f) within the fruit accounted for < 0.5% heat loss.

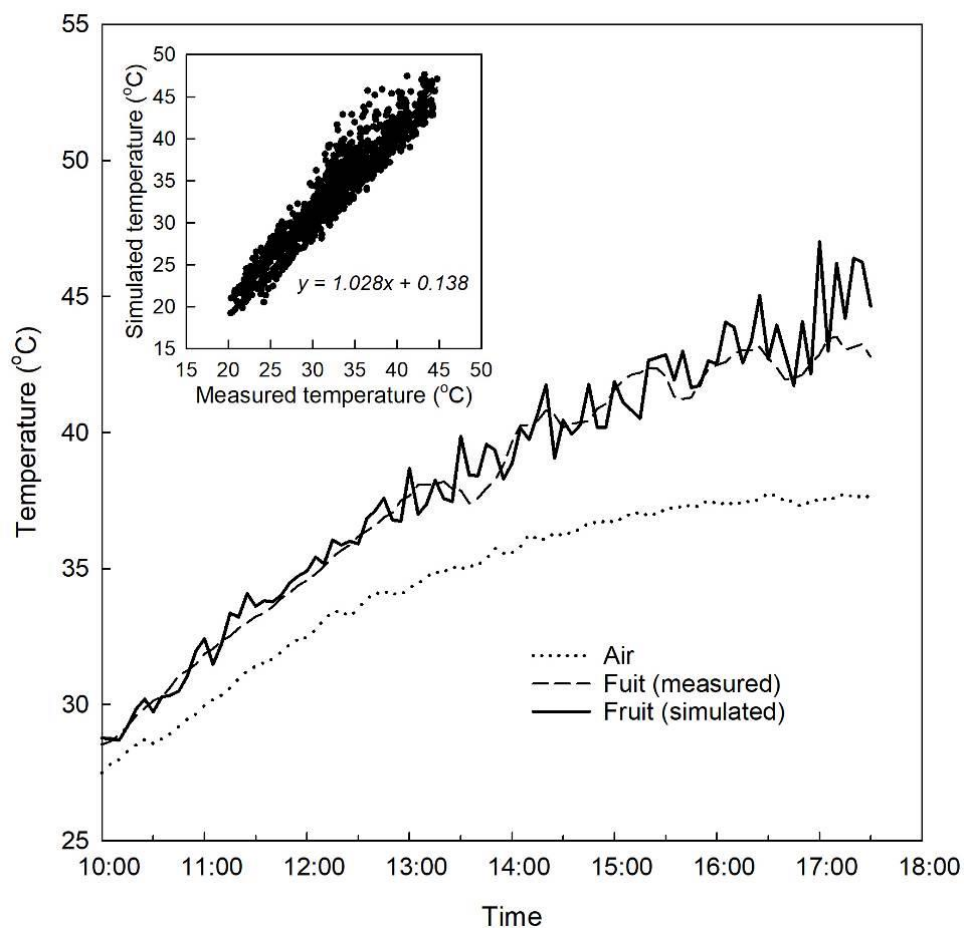


Fig. 4.2. Comparison of model results to actual surface temperature of sun-exposed blueberries measured in a field of ‘Elliott’ blueberry on 31 July 2015 (hottest day of the year). Inset: The relationship between simulated and measured fruit temperatures on 13 sunny days in 2015.

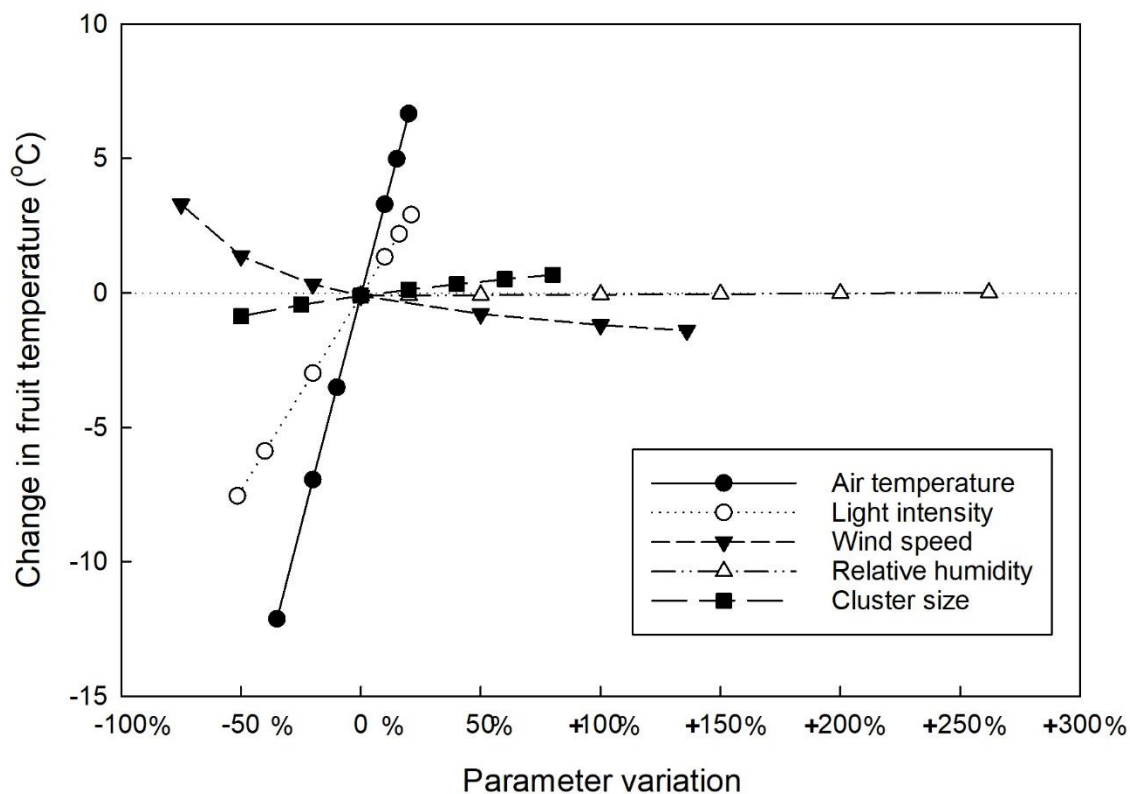


Fig. 4.3. Sensitivity analysis of various physical parameters in the energy balance model on estimates of fruit surface temperature. Range of each parameter: Air temperature (25–46 °C), light intensity (400–1000 W·m⁻²), wind speed (1–10 m·s⁻¹), relative humidity (16% to 60%), and cluster size (5–18 cm).

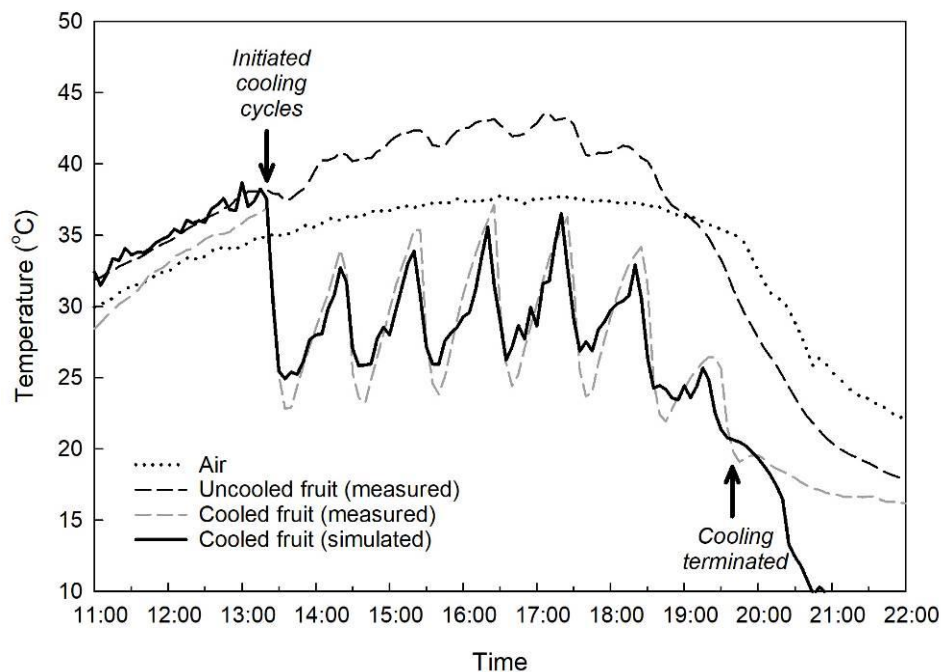


Fig. 4.4. Comparison of measured and simulated fruit surface temperatures during cooling with overhead sprinklers in a field of 'Elliott' blueberry on 31 July 2015 (hottest day of the year). Cooling was initiated when air temperature reached 35 °C (1:20 HR) and run for 15 min every hour until air temperature was once again < 35 °C (19:40 HR).

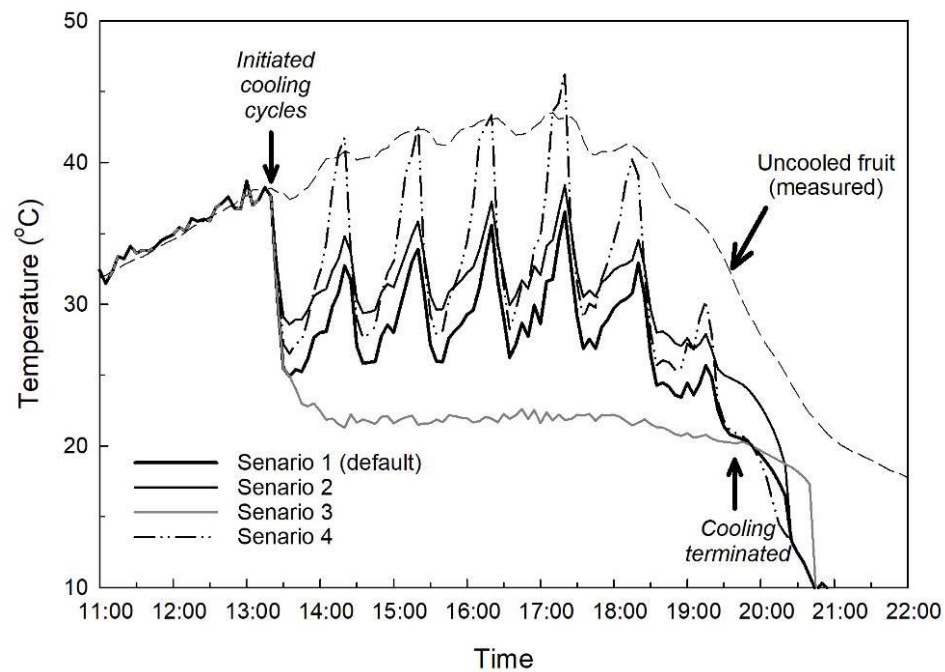


Fig. 4.5. Simulated fruit surface temperatures under different cooling scenarios with over-canopy sprinklers in northern highbush blueberry. In scenario 1, sprinklers were run for 15 min every hour, and temperature of the irrigation water was set at 20 °C. Sprinklers were also run for 15 min every hour in scenario 2, but water temperature was increased to 25 °C. In scenario 3, sprinklers were run continuously at a water temperature of 20 °C. Scenario 4 was similar to scenario 1, except in this case, the rate of water application was reduced by 33%. The first scenario was used as the default setting and was based on the parameters measured on 31 July 2015 (see Fig. 4.4). Measured temperatures of uncooled fruit was included as a reference.

**Chapter 5 –Stomatal Functioning and Its Influence on Calcium Accumulation
During Fruit Development in Northern Highbush Blueberry**

Abstract

Accumulation of Ca in fruit is largely driven by transpiration and varies depending on the concentration of Ca in the xylem fluid. The objective of present study was to evaluate the relationship between fruit stomatal functioning and Ca accumulation during different stages of development in northern highbush blueberry (*Vaccinium corymbosum* L.). The information is needed to develop feasible practices for increasing Ca levels in the fruit. Stomata were scarce on the berries and were concentrated primarily on the distal end near the calyx. Density of the stomata was greatest at petal fall, averaging 5–108 stomata/mm² from the proximal (pedicel end) to the distal end of the berries. In comparison, mean density on the abaxial surface of the leaves averaged 496 stomata/mm². Stomata were wide open at the early green stage of berry development and only had a slight deposit of wax along the guard cells. As the berries expanded during the initial period of rapid growth (stage I), most of the stomata remained near the distal segment of the berries, and by the late green stage, almost none were found in the middle and proximal segments. The majority of these stomata were completely covered with wax once the berries began to change color and ripen. Stomatal conductance of the berries averaged 45 mmol·m⁻²·s⁻¹ at petal fall and rapidly declined as the fruit developed. By the fruit coloring stage, conductance was low and remained < 15 mmol·m⁻²·s⁻¹ throughout the ripening period. Leaf stomatal conductance measured at the late green stage averaged ≈ 100 mmol·m⁻²·s⁻¹. Dry matter accumulated in the berries in a typical double-sigmoid pattern, with an initial period of rapid growth (Stage I) from petal fall to fruit coloring, followed by a short lag period of growth (Stage II) during fruit coloring, and finally a second period of rapid growth (Stage III) during fruit ripening and prior to harvest.

Calcium likewise accumulated rapidly during the initial stage of berry development, but in this case, accumulation slowed considerably between the late green and fruit coloring stages and stopped completely during fruit ripening. In general, Ca accumulation appeared to end sooner in early and mid-season cultivars (Duke and Bluecrop) than in late-season cultivars (Aurora and Elliott). Although stomatal conductance is low in developing blueberries, it appears to be an important mechanism by which Ca is delivered to the berries.

Introduction

Calcium is considered to be important for plant health and fruit quality. Without a sufficient level of Ca, several fruit quality defects may occur during fruit development (de Freitas and Mitcham, 2012). For example, multiple studies have concluded that insufficient Ca or an imbalance ratio of Ca with other nutrients (P, Mg, K, etc) can cause bitter pit disorder in apple [*Malus × sylvestris* (L.) Mill. var. domestica (Borkh.) Mansf.] (De Freitas et al., 2015; Ferguson and Watkins, 1989). In tomato (*Lycopersicum esculentum* Mill.), Ca deficiency in the distal part of the fruit leads to blossom-end rot (Ho and White, 2005). In litchi (*Litchi chinensis* Sonn.), fruit with cracking symptoms usually had lower Ca concentrations (Huang et al., 2008).

Fruit firmness is also commonly associated with Ca. Studies show that 60% to 70% of Ca is located in the cell walls of the fruit, which mainly acts as a stabilizing agent binding with pectin (Demarty et al., 1984). Spraying Ca on the fruit surface effectively maintained fruit firmness in grape (*Vitis vinifera* L.) (Ciccarese et al., 2013; Marzouk and Kassem, 2011) and apple (Dris and Niskanen, 1999; Siddiqui and Bangerth, 1995). Spraying Ca also prevents fruit cracking in sweet cherry (*Prunus avium* L.) (Brown et al., 1995) and reduces postharvest fruit decay as a result of increased Ca-bound pectins in strawberry (*Fragaria × ananassa* Duch.) (Lara et al., 2004). In the Pacific Northwest, foliar Ca sprays are commonly used in an attempt to increase berry firmness in northern highbush blueberry. However, Vance et al. (2017) recently found that spraying various Ca products has no effect on firmness or fruit Ca content in blueberries and suggested that further research on fruit stomatal functionality is needed to identify the proper timing for Ca applications.

Compared with vegetative leafy organs, fruit is commonly considered a C sink. While many fruit crops set flowers before leaf emergence, many young green fruits actually have the ability for photosynthesis (Aschan and Pfanz, 2003; Cipollini and Levey, 1991). However, very few stomata occur on blueberries, and most are concentrated near the calyx (Konarska, 2015). An early study in rabbiteye blueberry (*V. virgatum* Aiton) indicated that fruit photosynthesis was active from petal fall to fruit coloring, and C fixed by the berries supplied 15% of the total fruit C requirements (Birkhold et al., 1992). These findings suggest that blueberry fruit stomata may function actively at early stages of fruit development.

Accumulation of Ca in fruit is largely driven by transpiration and varies depending on the concentration of Ca in the xylem fluid (Marschner, 2012). Studies in a number of crops, including grape, kiwifruit [*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson var. *deliciosa*], and tomato, suggest that Ca primarily accumulates during early stages of fruit development and decrease in later stages due to changes in the flow of xylem (Ho and White, 2005; Montanaro et al., 2015; Rogiers et al., 2006). Reduced xylem flow to the fruit appears to be the result of cell expansion and restriction of the xylem vessels (Ho and White, 2005) or reduced transpiration due to more wax on the fruit (Blanke and Leyhe, 1987; Rogiers et al., 2004). In blueberry, we found that wax thickens on the berries during development (chapter 2), but whether it affects stomatal functionality and, thus, Ca accumulation is still unknown.

The objective of present study was to evaluate the relationship between fruit stomatal functioning and Ca accumulation during different stages of development in

northern highbush blueberry. The information is needed to develop feasible practices for increasing Ca levels in the fruit.

Materials and Methods

Study 1

Study site. The study was conducted using mature plants of ‘Elliott’ blueberry located in a field with five other cultivars at the Oregon State University Lewis–Brown Horticultural Research Farm in Corvallis, OR (lat. 44°33′ N, long. 123°13′ W). ‘Elliott’ is a late-season cultivar that typically ripens in August and late September in western Oregon. The field was planted in Oct. 2008. Soil at the site was a Malabon silty clay loam with a pH of 5.5. The plants were spaced 0.8 × 3.0 m apart on 0.4-m high raised beds and were mulched every other year with a 5-cm-deep layer of douglas fir [*Pseudotsuga menziesii* Mirb. (Franco)] sawdust. Grass alleyways were planted between the beds and mowed as needed. See Vargas et al. (2015) for complete details on establishment of the planting.

The plants were irrigated using two lines of drip tubing (Netafim, Fresno, CA) per row and were fertigated weekly (from mid-April through July) with ammonium sulfate (9–0–0) at a total rate of 168 kg·ha⁻¹ N per year. Urea, monoammonium phosphate, and boric acid were also applied in previous years (Vargas et al., 2015). The drip lines had integrated pressure-compensating emitters (2 L·h⁻¹) located every 0.45 m and were located ≈ 0.45 m from the base of the plants on each side of the row. Weeds were controlled, as needed, by hand-weeding on the top of beds and by applying

glyphosate herbicide at the base of beds. No insecticides or fungicides were applied to the field during the study. Calcium fertilizer was also never applied to the planting.

Berry dry weight and Ca content. Berry samples were collected at seven stages of fruit development, including petal fall, early green, late green, fruit coloring, 10% blue, 25% blue, and 75% blue (Table 5.1). The percentage of blue was approximated and represented the proportion of berries that turned blue in the entire field on a given day. At each stage, berries were sampled from three individual plant replicates. The number of berries sampled at each stage of berry development are listed in Table 5.1 Samples were collected randomly from both sides of the row and in the upper and lower parts of the canopy. For the last stage (75% blue), additional samples (15 berries/replicate) were collected and separated into skin, pulp, and seeds to determine the distribution of dry matter and Ca in the berries. The berries and their components were digested using 70% (v/v) nitric acid, as described by Gavlak et al. (2005), and then analyzed for Ca using an inductively coupled plasma optical emission spectrometer (model Optima 3000DV; Perkin Elmer, Wellesley, MA).

Stomatal conductance. Stomatal conductance of the berries was measured on sunny days using a steady-state porometer (model LI-1600; LI-COR, Lincoln, NE). Ten berries each were randomly measured from stage 1 through 6. Leaves were also measured on 10 plants at the late green stage. For each measurement, a single berry was selected from a cluster and the rest of the berries were removed before turning on the porometer. The porometer was equilibrated to site conditions for 30 min prior to each measurement, and the null point was set near ambient relative humidity. Each berry was clamped into the head of the porometer on the calyx side (i.e., facing the sensors). The aperture of the

clamp was adjusted with different size foam pads, as needed, to adjust for different size berries (0.35–0.90 cm²). A complete seal and good contact between the instrument's thermocouple and the berry were ensured before starting the measurements. Each measurement required 5–10 min to get a stable reading.

Stomatal density and distribution. The number of stomata on the berries was counted using a light microscopy (model DM 2500; Leica, Wetzlar, Germany). To prepare the samples, six berries from each of stage 1 through 6 were randomly collected and coated with clear lacquer (nail polish). Leaf samples were also collected from plants at the late green stage and coated on the abaxial side with the lacquer. After drying, the lacquer was carefully peeled from the berries and leaves and placed on glass slides. For each berry sample, the berry was divided into the calyx and four equal spherical segments, including the top (pedicel end), upper middle, lower middle, and bottom (calyx end). Under the microscope, six photographs with an area of 1.65×1.30 mm² per image were randomly taken from each position at 1800× magnification. The stomata were then counted in the photographs and divided by the area to calculate the stomatal density on each segment of the berries. A total of 1080 photographs were processed. The results were analyzed by one-way analysis of variance using R software v. 3.1.2 (R Development Core Team, 2014). Means were separated using Tukey's honestly significant difference test ($\alpha = 0.05$).

Stomata on the berries were also examined under a scanning electron microscope (SEM) (Quanta 600F; FEI Company, Hillsboro, OR) with an acceleration voltage of 10 kV. Eight berries from the sun-exposed part of the canopy were collected at three stages, including early green, fruit coloring, and 25% blue. To avoid removing the wax during

sampling, each berry was held by inserting a dissection needle through the calyx end of the berry. Skin (epidermis) was carefully removed from the equator of each berry using a razor blade. The skin was immediately submerged into a fixation solution (2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M sodium cacodylate buffer) and stored overnight at 5 °C. There was little fruit pulp retained on the skin during removal and most of it separated from the skin after fixation. The following day, skin samples were gradually washed with a series of 30%, 50%, 70%, 90%, and 100% acetone and, to maintain cellular structure, were dehydrated using a CO₂ critical point dryer (EMS 850, Electron Microscopy Sciences, Hatfield, PA). Once dried, the samples were frozen with liquid N and shattered into small specimens that were mounted onto aluminum stubs with double-sided carbon tape and coated with a 15-nm-thick layer of 60% gold and 40% palladium (Cressington 108 Auto Sputter Coater; Cressington, Watford, UK). Images of the specimens were captured at a magnification of 7200×.

Study 2

Additional berry samples were collected from three cultivars of northern highbush blueberry at three sites in the Pacific Northwest in 2016. The cultivars included an early season cultivar, ‘Duke’, which ripens in late June and July, a mid-season cultivar, ‘Bluecrop’, which ripens in July and early August, and a late-season cultivar, ‘Aurora’, which ripens in August and early September. These cultivars represent > 70% of the planted area in the region. The sites included the field at Lewis-Brown in Corvallis (see study 1), a certified organic planting located at the North Willamette Research and Extension Center in Aurora, OR (lat. 45°28′ N, long. 122°76′ W), and three conventional

fields located at a commercial farm (Sakuma Bros.) in Burlington, WA (lat. 48°30' N, long. 122°22' W). Plants at each location were irrigated by drip (one or two lines per row) and fertigated or fertilized with ammonium sulfate, urea, or organic fertilizers (organic planting), including a soluble grain fermentation and nitrate of soda blend (4N–0.9P–0.4K; Converted Organics of California, Gonzales, CA) and a fish hydrolysate and fish emulsion blend combined with molasses (5N–0.4P–1.6K; True Organic Products, Spreckels, CA), and a granular soybean meal (7N–0.4P–1.6K; California Organic Fertilizers, Fresno, CA). Calcium sulfate (gypsum) was also applied to the organic planting at a rate of 258 kg·ha⁻¹ Ca in 2013. See Strik et al. (2017a) for more information on the organic site.

Each cultivar was sampled at the same seven stages of development as ‘Elliott’ (study 1), but the actual date on which the berries were collected varied among the locations and cultivars (Table 5.2). A set of berries were sampled from three individual plant replicates at each site. We collected the berries from the same plants at each developmental stage at the sites in Oregon and used different plants for each stage in the commercial field at the Washington site. Each sample was washed and weighed and analyzed for Ca, as described above (study 1). An additional 15 berries were also collected at the 75% blue stage to assess the distribution of dry matter and Ca in the skin, pulp, and seeds.

Data were analyzed by analysis of variance using R software v. 3.1.2. Results of a three-way analysis (location × cultivar × developmental stage) indicated that dry weight and Ca content of the berries were significantly affected by each main effect, as well as two-way interactions between cultivar and stage and location and stage ($P \leq 0.05$). A

two-way analysis revealed that allocation of dry matter and Ca to the skin, pulp, and seeds of the berries was also affected by cultivar at the final stage of development ($P \leq 0.01$) but, in this case, was not affected by location or the interaction between cultivar and location in each tissue.

Results

Stomatal density and distribution. Stomata were scarce on the berries and were concentrated primarily on the bottom near the calyx (Fig. 5.1A and B). Density of the stomata was greatest at petal fall, averaging 5–108 stomata/mm² from the top (pedicel end) to the bottom (calyx end) of the berries (Fig. 5.2A). In comparison, mean density on the abaxial surface of the leaves averaged 496 stomata/mm². Stomata were wide open at the early green stage of berry development and only had a slight deposit of wax along the guard cells (Fig. 5.1C).

As the berries expanded during the initial period of rapid growth (stage I), most of the stomata remained near the bottom segment of the berries, and by the late green stage, almost none were found in the middle and top segments (Fig. 5.2A). The majority of these stomata were completely covered with wax once the berries began to change color and ripen (Fig. 5.1D). By the time the berries were blue, it was nearly impossible to locate the stomata with SEM.

Stomatal conductance. Stomatal conductance of the berries averaged 45 mmol·m⁻²·s⁻¹ at petal fall and rapidly declined as the fruit developed (Fig. 5.2B). By the fruit coloring stage, conductance was low and remained < 15 mmol·m⁻²·s⁻¹ throughout the

ripening period. Leaf stomatal conductance measured at the green stage averaged $\approx 100 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Accumulation and partitioning of dry matter and Ca. In ‘Elliott’, dry matter accumulated in the berries in a typical double-sigmoid pattern, with an initial period of rapid growth (Stage I) from petal fall to fruit coloring, followed by a short lag period of growth (Stage II) during fruit coloring, and finally a second period of rapid growth (Stage III) during fruit ripening and prior to harvest (Fig. 5.2C). Calcium likewise accumulated rapidly during the initial stage of berry development, but in this case, accumulation slowed considerably between the late green and fruit coloring stages, and stopped completely during fruit ripening (Fig. 5.2C).

Calcium accumulation also typically slowed or stopped by the late green stage in ‘Duke’, ‘Bluecrop’, and ‘Aurora’ (Fig. 5.3). In general, Ca accumulation appeared to end sooner in the early and mid-season cultivars (Duke and Bluecrop) than in the late-season cultivar [Aurora; also see Elliott (Fig. 5.2C)]. However, there were two exceptions. In Burlington, WA, ‘Duke’ continued to accumulate Ca until the berries were 25% blue (Fig. 5.3A), while ‘Aurora’ appeared to stop accumulating Ca by the late green stage and then resumed uptake once the berries began turning blue (Fig. 5.3C).

By harvest, Ca content was $> 0.15 \text{ mg/berry}$, on average, in each of the cultivars examined (Fig. 5.3). However, levels were well below average in two cultivars in Burlington, WA, averaging only 0.12 mg of Ca each in ‘Bluecrop’ and ‘Aurora’ berries. ‘Duke’ also had less Ca in the berries at Burlington than at the organic site in Aurora, OR. Despite the large amount of dry matter in the berries (0.33 mg/berry), ‘Duke’ only

had 0.08 mg/berry of Ca at the Burlington site, which was about half the Ca content found in the berries of ‘Duke’ in Corvallis.

Once the berries matured, 18% of the total dry matter in ‘Elliott’ berries was allocated to the skin, 75% was in the pulp, and < 7% was in the seeds (Table 5.3). Calcium, on the other hand, was about three times more concentrated in the skin and seeds than in the pulp, and consequently, nearly half of the Ca in the berries was in the skin (35%) and seeds (13%) (Table 5.3). Proportionally less dry matter and more Ca were also allocated to the skin and seeds of ‘Duke’, ‘Bluecrop’, and ‘Aurora’ berries (Fig. 5.4). In these cultivars, partitioning of dry matter and Ca were similar at each site, but ‘Aurora’ allocated more dry matter and Ca to the skin and less to the seeds than either ‘Duke’ or ‘Bluecrop’ ($P \leq 0.05$).

Discussion

The results of this study indicate that Ca accumulation mainly occurs during the early stages of fruit development in northern highbush blueberry and appears to be limited at later stages by reduced stomatal functioning and development of wax on the berry surface. In the four cultivars studied, most Ca uptake occurred while the berries were small and green and actively transpiring during stage I. Calcium also accumulated primarily during early fruit development in other crops, including apple, apricot (*Prunus armeniaca* L.), grape, kiwifruit, and nectarine (Creasy et al., 1993; Dichio et al., 2003; Drazeta et al., 2004; Montanaro et al., 2010; 2014). Unlike most nutrients in plants, the movement of Ca relies on xylem fluid driven by transpiration. Thus, as water translocation switches from xylem to phloem during fruit development, Ca movement

becomes limited (Hanger, 1979). Once the xylem is no longer functional, any additional accumulation of Ca in the fruit is strongly reduced (Ho and White, 2005; Montanaro et al., 2015; Rogiers et al., 2006). In contrast, we found that uptake of other nutrients, including K and Mg, continued to increase throughout each stage of development of the berries, including fruit coloring and ripening (F.-H Yang, unpublished data). Translocation of these nutrients to various organelles primarily occurs via the phloem (Marschner, 2012).

Stomatal density on the berries declined as the fruit matured. Grusak and Pomper (1999) observed similar results in snap bean (*Phaseolus vulgaris* L.) pods. As in the present study, the decline in stomatal density was related to the expansive growth of the fruiting structures. It would appear that blueberries begin with a certain number of stomata, and these become more diffuse as the berries expand. During expansion, most of the stomata remained near the calyx end of the berry. Eventually, these stomates became covered with wax and were probably no longer functional during the later stages of berry development. Thus, by the time fruit coloring begins, water movement through the surface of the berries would have to occur via minute cracks in the cuticle (Konarska, 2015). Significant reduction in transpiration is also associated with thickening of epicuticular waxes after véraison in wine grape (Blanke and Leyhe, 1987; Rogiers et al., 2004).

The rate of stomatal conductance measured in the berries after petal fall was roughly half of what was measured in actively transpiring leaves on the plants. Fruit stomata are well known to be essential for gas exchange and C assimilation during early fruit development in many crops (Aschan and Pfan, 2003; Cipollini and Levey, 1991; Palliotti and Cartechini, 2001). However, rates measured at later stages of development

were comparable to leaves with closed stomata (Bryla and Strik, 2016). Cuticle water loss apparently predominates in the berries during later stages of development.

Among the cultivars examined in this study, Ca content was below average in ‘Bluecrop’ and ‘Aurora’ blueberries in Burlington, WA. The Burlington site was the most northern of the sites sampled in the study, and the climate there is cooler and wetter than at the other two sites sampled in Oregon (chapter 2). Low temperatures and high humidity reduces vapor pressure deficit and, therefore, might have resulted in a reduction in the cumulative influx of Ca in the xylem sap at this site (Zhang and Keller, 2015). Seeds in the fruit are also involved in fruit Ca accumulation (Buccheri and Di Vaio, 2005). Cultivars that regularly produce low numbers of seeds or are grown in seasons with poor environmental conditions at pollination are expected to have lower fruit Ca levels (Arrington and DeVetter, 2016). Weather conditions were poor during pollination in Washington in 2016 (L. DeVetter, personal observation), which probably reduced the number of seeds per berry and the associated accumulation of Ca in the fruit.

Calcium content was also below average in organic ‘Duke’ blueberries. Many organic fertilizers, including those used in this study, tend to be high in K and low in Ca. Yard debris compost, which is also high in K, was also incorporated into the soil and used as mulch on the beds in the planting (Strik et al., 2017a). Excessive K can inhibit Ca uptake in plants and, therefore, interfere with Ca movement to the leaves and fruit (Marshner, 2012). For whatever reason, ‘Duke’ appears to be particularly sensitive to high K levels and any negative effects it might have on fruit and leaf Ca (Strik and Vance, 2015; Strik et al., 2017b).

Although stomatal conductance is low in developing blueberries, it appears to be an important mechanism by which Ca is delivered to the berries. Therefore, to increase Ca and enhance the quality of the fruit, cultivars could be developed with more and/or larger stomata on the berry surface. Cultural practices such as heavier pruning to increase light exposure or running fans to increase air circulation (e.g., for containerized production of blueberries in tunnels and greenhouses) could also be employed as a more immediate means to improve xylem influx and movement of Ca into the berries (Montanaro et al., 2006; Mazzeo et al., 2011; 2013; Rebucci et al., 1997). Finally, based on the result of present study, blueberry fruit can potentially uptake exogenous Ca through their stomata early in the season; however, the efficiency of uptake may be limited due to the low fruit stomata number.

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Tables and Figures

Table 5.1. Berry sampling dates of ‘Elliott’ blueberry in Corvallis, OR in 2016 (study 1).

| Stage of berry development ² | Description | Date | No. of berries per replicate |
|---|---|---------|------------------------------|
| Petal fall | The corolla was falling off the flower, revealing the small green berry. | 1 May | 120–145 |
| Early green | Pea-sized berry expanding by cell division and varying from small to large. | 7 May | 55–75 |
| Late green | Berry growth slowed, becoming pale green; exposed berries may have developed a slight red blush. | 30 June | 15–25 |
| Fruit coloring | The largest berry on the cluster began to soften and turn pink. Cell division stopped and growth occurred through cell expansion. | 8 July | 15–25 |
| 10% blue | About 10% of the berries, on a bush or in the field, were ripe and nearly ready for harvest. | 15 July | 15–25 |
| 25% blue | About 25% of the berries in the field were ripe; coinciding with the first hand harvest. | 20 July | 15–25 |
| 75% blue | About 75% of the berries in the field were ripe; coinciding with the second harvest. | 27 July | 4–6 |

²From Michigan State University Extension (http://www.canr.msu.edu/blueberries/growing_blueberries/growth-stages).

Table 5.2. Berry sampling dates of ‘Duke’, ‘Bluecrop’, and ‘Aurora’ blueberry from fields located in Oregon and Washington in 2016 (study 2).

| Stage of berry development ^z | Corvallis, OR | | | Aurora, OR | | | Burlington, WA | | |
|---|---------------|----------|---------|------------|----------|---------|----------------|----------|---------|
| | Duke | Bluecrop | Aurora | Duke | Bluecrop | Aurora | Duke | Bluecrop | Aurora |
| Petal fall | 23 Apr. | 25 Apr. | 27 Apr. | 26 Apr. | 26 Apr. | 26 Apr. | 2 May | 2 May | 3 May |
| Early green | 1 May | 1 May | 3 May | 2 May | 2 May | 2 May | 9 May | 9 May | 9 May |
| Late green | 19 May | 7 June | 30 June | 18 May | 31 May | 29 June | 24 June | 29 June | 26 June |
| Fruit coloring | 27 May | 10 June | 8 July | 26 May | 6 June | 5 July | 29 June | 29 June | 2 Aug. |
| 10% blue | 7 June | 17 June | 15 July | 31 May | 15 June | 13 July | 5 July | 5 July | 2 Aug. |
| 25% blue | 10 June | 24 June | 20 July | 6 June | 24 June | 19 July | 5 July | 5 July | 19 Aug. |
| 75% blue | 16 June | 30 June | 27 July | 15 June | 29 June | 26 July | 5 July | 18 July | 19 Aug. |

^zBerries in Corvallis and Aurora were selected based on the average stage of development in the field, while those in Burlington were selected based on development of individual plants. See Table 1 for a full description of the stages and the number of berries sampled at each stage.

Table 5.3. Mean (\pm SE) dry weight and concentration and content of Ca in the skin, pulp, and seeds of ‘Elliott’ blueberries.^z

| Berry component | Dry wt (g/berry) | Ca concn (%) | Ca content (mg/berry) |
|-----------------|-------------------|-------------------|-----------------------|
| Skin | 0.055 ± 0.005 | 0.099 ± 0.002 | 0.054 ± 0.004 |
| Pulp | 0.224 ± 0.019 | 0.036 ± 0.002 | 0.080 ± 0.003 |
| Seeds | 0.019 ± 0.001 | 0.112 ± 0.006 | 0.021 ± 0.003 |
| Total | 0.297 ± 0.021 | 0.052 ± 0.002 | 0.155 ± 0.003 |

^aMeasured at the 75% blue stage of berry development.

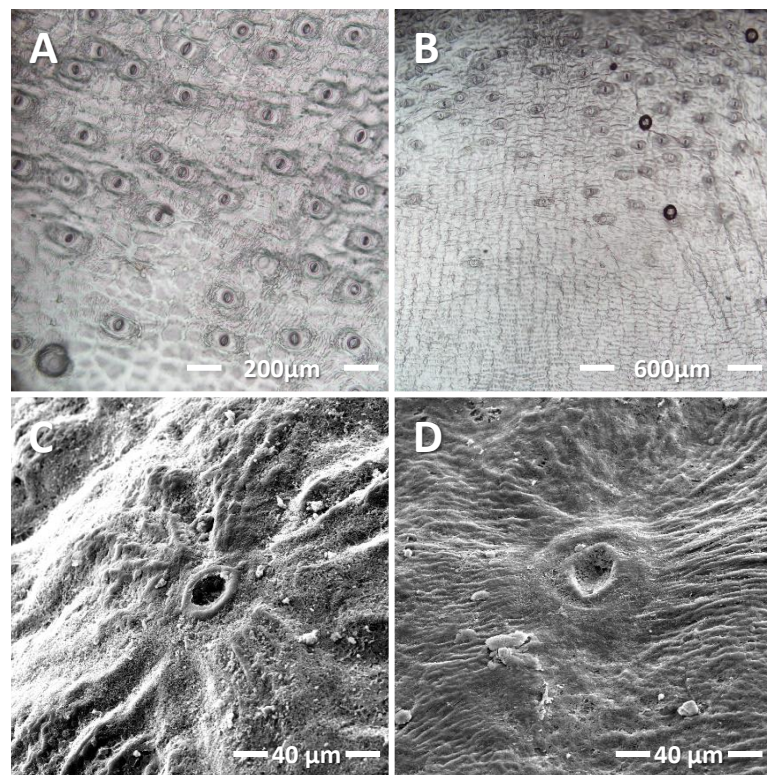


Fig. 5.1. Light microscopy: (A, B) berry stomata concentrated near the calyx of an 'Elliott' blueberry at petal fall (1800 \times and 1200 \times). Scanning electron microscopy: stomata on the surface of an 'Elliott' blueberry during the (C) early green and (D) fruit coloring (pink) stages of berry development (7200 \times).

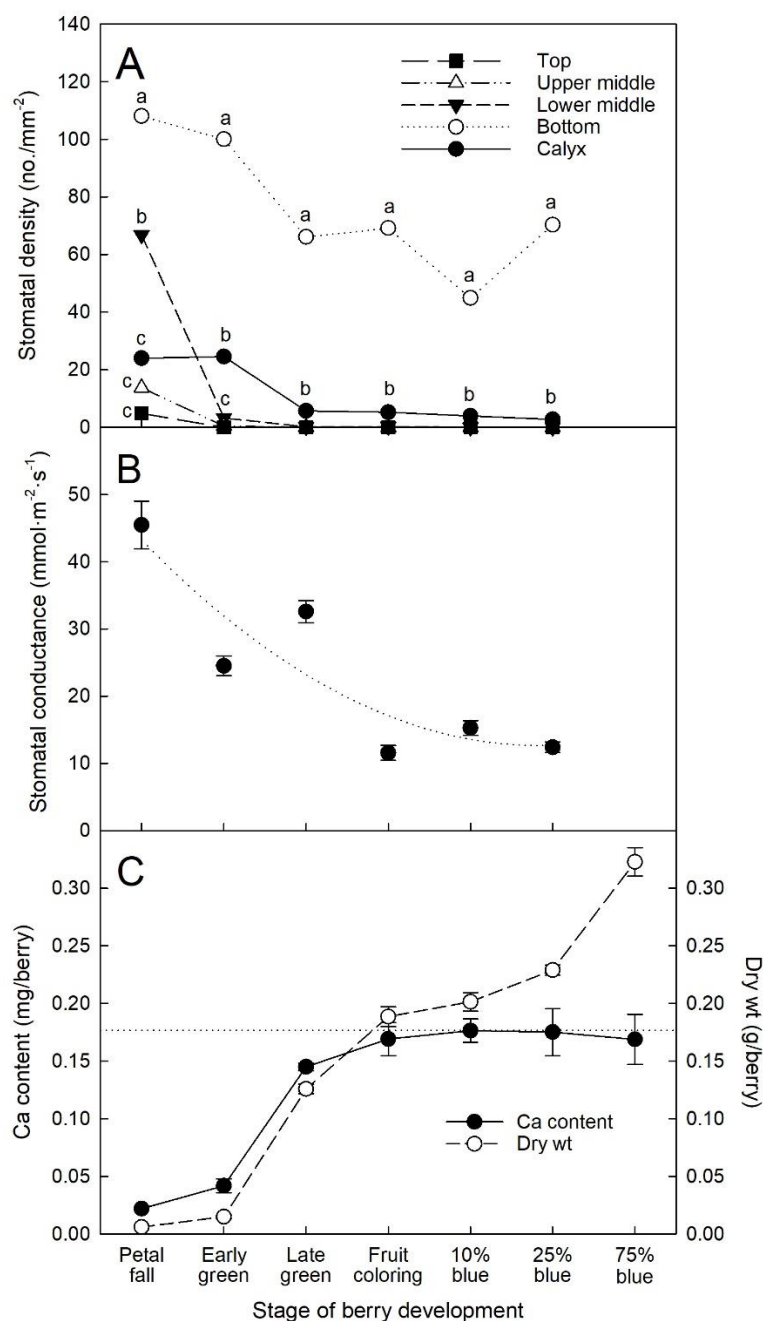


Fig. 5.2. (A) Stomatal density and (B) conductance, and (C) Ca content and dry weight of 'Elliott' blueberries from petal fall to the 75% blue stage of berry development. To assess stomatal density, berries were divided into the calyx and four equal spherical segments, including the top (pedicel end), upper middle, lower middle, and bottom (calyx end). Means of stomatal density with different letters at a given stage were significantly different at $P \leq 0.05$. Error bars represent ± 1 SE.

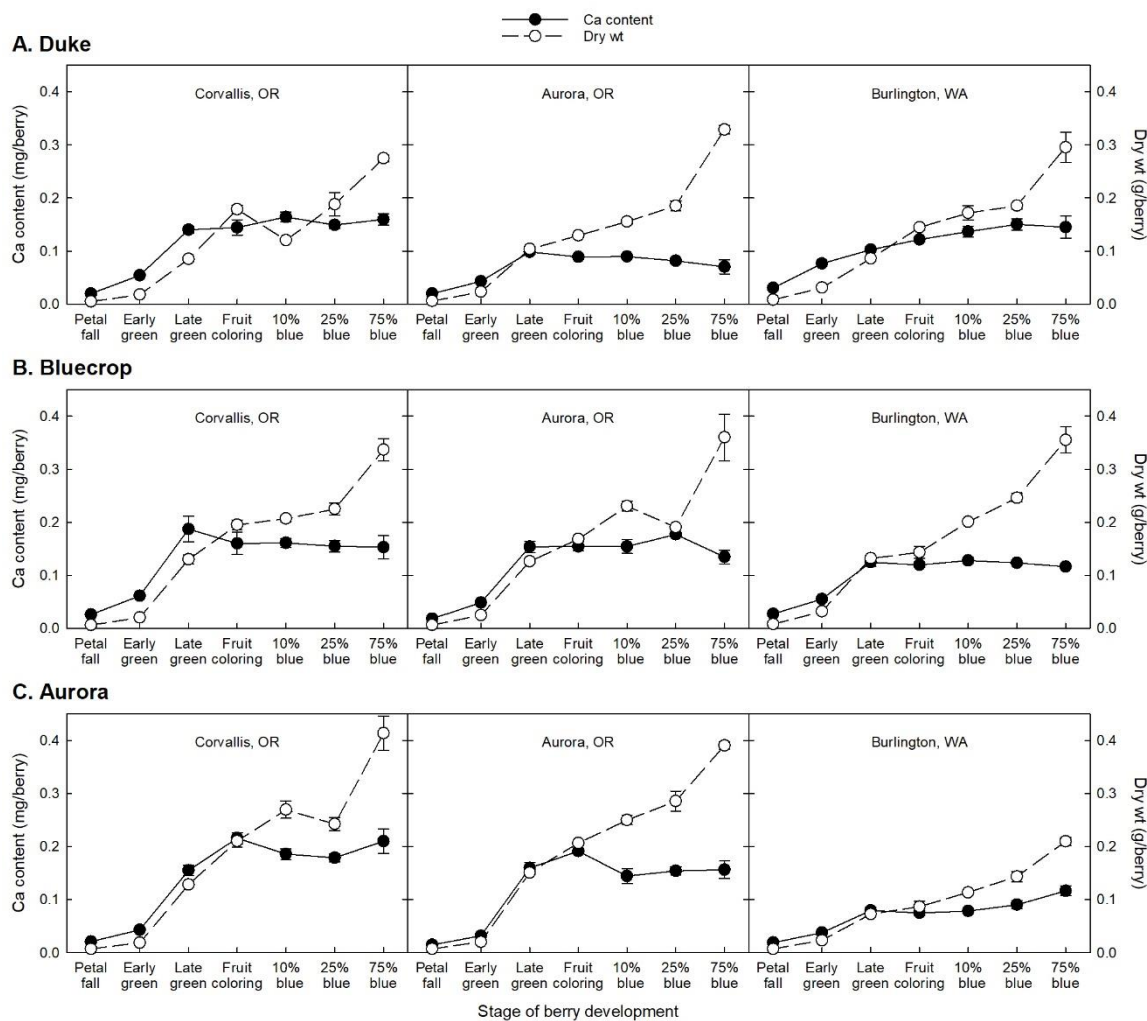


Fig. 5.3. Calcium content and dry weight of (A) 'Duke', (B) 'Bluecrop', and (C) 'Aurora' blueberries from petal fall to the 75% blue stage of berry development. Berries were collected in 2016 from fields located (from south to north) in Corvallis and Aurora, OR and Burlington, WA. The fields in Corvallis and Burlington were managed conventionally, while the site in Aurora was certified organic. Error bars represent ± 1 SE.

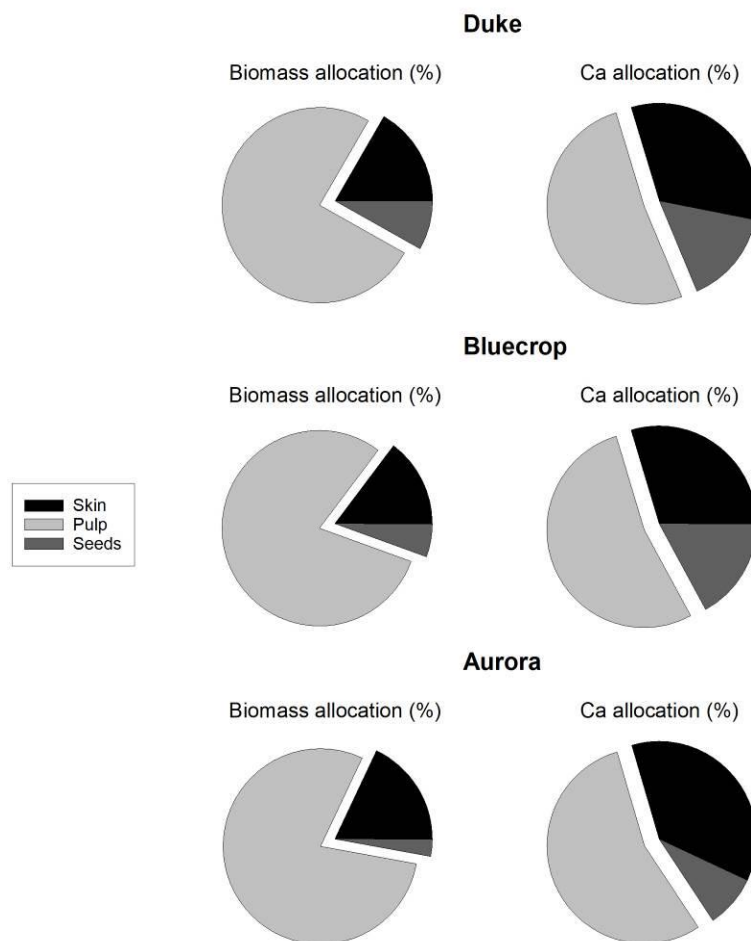


Fig. 5.4. Allocation of dry biomass and Ca among the skin, pulp, and seeds of 'Duke', 'Bluecrop', and 'Aurora' blueberries. Data are pooled from berries collected at the 75% blue stage of berry development in Corvallis and Aurora, OR and Burlington, WA.

Chapter 6 – General Conclusions

The work revealed that berries fully exposed to the sun were much warmer than those in the shade, which explains why heat damage usually occurs on the upper part of the canopy. It also showed that heat damage occurred on both green and blue-colored berries, with necrosis and softening being the two most prevalent types of heat damage. Critical temperatures and the required times for blueberries to develop heat damage were determined. Results indicated that mature fruit have higher critical temperatures and tolerate longer periods of heat exposure than immature fruit. This is possibly due to a thicker cuticle and wax layer on the mature berries. Heat tolerance differed between two cultivars tested. ‘Aurora’ was more heat sensitive and had a lower critical temperature than ‘Elliott’. These results provide information for developing cultural practices to reduce blueberry heat damage. Since there are differences between cultivars and developmental stages, any preventive practice should be designed according to the heat susceptibility of each specific cultivar.

Heat damage can be resolved by using evaporative cooling tools such as over-canopy sprinklers and micro-sprinklers. Our results demonstrated that cooling with sprinklers was very effective at reducing berry temperature during warm weather events. This temperature reduction can mitigate heat damage and possibly increase fruit size. Cooling applied at 32 and 35 °C had negligible effects in ‘Elliott’ blueberry, which is possibly due to the fact that its threshold temperature was > 48 °C. Compared with over-canopy sprinklers, micro-sprinklers used less water but were equally effective for keeping berries cool. Operating micro-sprinklers in short cycles yielded the effect of reducing berry temperature equal to that of continuous cooling but reduced water use and limited the amount of water retained on the fruit. Reducing water retention would decrease the

potential for slugs and fungal disease. Cooling with micro-sprinklers effectively reduced heat damage and increased fruit size and firmness in both ‘Aurora’ and ‘Elliott’ blueberry.

Maintaining an optimal canopy temperature with cooling has been shown to reduce heat damage and maintain high rates of leaf transpiration. This mechanism may apply to berries and result in other potential benefits. Results of this study showed that even though stomatal density was much lower on the fruit than on the leaves, fruit stomata were active during the early green stage of fruit development (but declined as the fruit ripened). This result implies that at the early green stage, blueberry fruit may be able to uptake Ca through their stomata, but uptake may be limited by the low number of stomata on the surface of the berries. Similar to other fruit crops, fruit transpiration mediates Ca uptake into the fruit in blueberry. Therefore, using cooling to maintain an optimal fruit temperature may increase transpiration and Ca in the fruit.

To better predict the incidence of heat damage and evaluate cooling practices, a simple energy balance model for simulating changes in fruit temperature was developed. This model could be very useful for blueberry growers, since it can be adjusted for a variety of parameters such as blueberry size, cooling system specifications, and local environmental conditions. It successfully predicted diurnal fruit temperature patterns during sunny days and accurately simulated changes in fruit temperature under various cooling scenarios. The model could be potentially incorporated into weather forecasting systems and used as a decision-making tool to determine the best cooling practice for blueberry.

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