

Stomatal Functioning and Its Influence on Fruit Calcium Accumulation in Northern Highbush Blueberry

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Abstract. Accumulation of calcium (Ca) in fruit is largely caused by transpiration and varies depending on the concentration of Ca in the xylem fluid. The objective of the 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.). Stomata were scarce on the berries and were concentrated primarily on the distal end near the calyx. The density of the stomata was greatest at petal fall, averaging 5 to 108 stomata/mm² from the proximal end (pedicel end) to the distal end of the berries. Stomata were wide-open at the early green stage of berry development and 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; by the late green stage, almost none was found in the middle and proximal segments. The majority of these stomata were completely covered with wax when the berries began to change color and ripen (stage II and stage III). Stomatal conductance (g_s) 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 less than 15 mmol·m⁻²·s⁻¹ throughout the ripening period. In four cultivars, including Duke, Bluecrop, Aurora, and Elliott, Ca uptake in the berries increased rapidly during the early green stage; however, it slowed considerably between the late green and fruit coloring stages and stopped completely during fruit ripening. The results of this study strongly suggested that practices used to increase the Ca content of blueberries, such as the application of foliar fertilizers, should be performed early in the season during the first few weeks after flowering.

Calcium (Ca) is considered 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 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). Calcium deficiency also leads to blossom-end rot in tomato (*Solanum lycopersicum* L.) (Ho and White, 2005) and fruit cracking in litchi (*Litchi chinensis* Sonn.) (Huang et al., 2008).

Fruit firmness is commonly associated with the level of Ca in the fruit. Studies have shown that 60% to 70% of Ca is located in the cell

walls of the fruit, and that it mainly acts as a stabilizing agent that binds with pectin (Demarty et al., 1984). Spraying Ca on the fruit surface effectively maintains 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), 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 western United States and Canada, foliar Ca sprays are commonly used in an attempt to increase berry firmness in northern highbush blueberry. Gerbrandt et al. (2019) reported that high concentrations of foliar Ca applied frequently, starting immediately after

bloom, increased fruit Ca concentration and decreased fruit drop in ‘Draper’ blueberry. However, Arrington and DeVetter (2017) found that spraying various Ca products had no effect on firmness or fruit Ca content in two cultivars of blueberries grown at different locations in northwest Washington. Vance et al. (2017) reported similar results in Oregon and suggested that further research of fruit stomatal functionality was needed to identify the proper timing for Ca applications.

Studies of a number of crops, including grape, kiwifruit [*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson var. *deliciosa*], and tomato, have suggested that Ca primarily accumulates during early stages of fruit development and decreases at later stages due to changes in xylem flow (Ho and White, 2005; Montanaro et al., 2015; Rogiers et al., 2006). Reduced xylem flow at later stages appears to be caused by the restriction of the xylem vessels during cellular expansion (Ho and White, 2005) and by reduced transpiration due to more wax on the fruit (Blanke and Leyhe, 1987, 1988; Rogiers et al., 2004). In blueberry, we found that wax thickens on the berries during development (Yang et al., 2019); however, whether it affects stomatal functionality and subsequent Ca accumulation is still unknown.

The objective of the present study was to evaluate the relationship between fruit stomatal functioning and Ca accumulation during different stages of development in northern highbush blueberry. This information is necessary 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 (fine, mixed, superactive, mesic Pachic Ultic Argixerolls) 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 regarding establishment of the planting].

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 (9N–0P–0K) 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) 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

Table 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
1. Petal fall	The corolla was falling off the flower, revealing the small green berry	1 May	120–145
2. Early green	Pea-sized berry expanded by cell division and varied from small to large	7 May	55–75
3. Late green	Berries slowed in growth and turned pale green; some exposed berries developed a slight red blush	30 June	15–25
4. Fruit coloring	The largest berry on the cluster began to soften and turn pink; growth occurred through cell expansion	8 July	15–25
5. 10% blue	≈10% of the berries, on a bush or in the field, were ripe and nearly ready for harvest	15 July	15–25
6. 25% blue	≈25% of the berries in the field were ripe, coinciding with the first harvest	20 July	15–25
7. 75% blue	≈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).

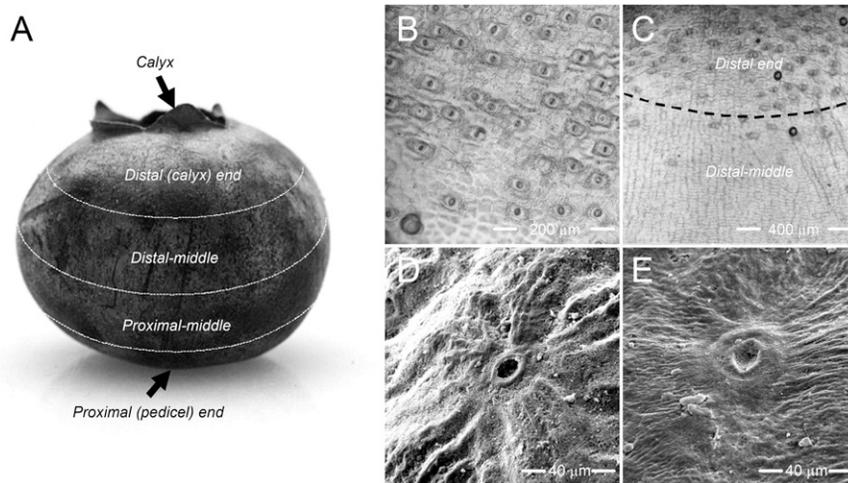


Fig. 1. (A) Illustration of the berry segments examined for stomata at different stages of development in ‘Elliott’ blueberry. Light microscopy: (B, C) stomata concentrated near the calyx at petal fall (1800× and 1200×). Scanning electron microscopy: stomata on the surface of the berries during the (D) early green and (E) fruit coloring (pink) stages of berry development (7200×).

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 never applied to the planting. Leaf tissue analysis (15 Aug. 2016) indicated that nutrients, including Ca, were sufficient at the site and within the ranges recommended

for northern highbush blueberry (Hart et al., 2006). The leaves were sampled and analyzed following the standard procedures for blueberry (Strik and Vance, 2015).

Berry dry weight and calcium content. Berry samples were collected at seven stages of fruit development: petal fall, early green, late green, fruit coloring, 10% blue, 25% blue, and 75% blue (Table 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 numbers of berries sampled at each stage of berry development are listed in Table 1. Samples were collected randomly from upper and lower parts of the canopy on both sides of the row. During 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 (ICP) optical emission spectrometer (model Optima 3000DV; Perkin Elmer, Wellesley, MA). Other nutrients, including potassium (K) and magnesium (Mg), were also measured in the berries during the ICP analysis; nitrogen (N) was measured using a combustion

analyzer (model TruSpec CN; Leco Corp., St. Paul, MN).

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 stage 6 (petal fall to 25% blue). Leaves were also measured on 10 plants at the late green stage (stage 3). 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 before each measurement, and the null point was set near ambient relative humidity. Each berry was clamped to the head of the porometer on the calyx side (i.e., facing the sensors). The aperture of the clamp was adjusted with different size openings on the foam pads (0.35–0.90 cm²), as needed, to accommodate different size berries. A complete seal and good contact between the instrument’s thermocouple and the berry were ensured before starting the measurements. Each measurement required 5 to 10 min to obtain a stable reading.

Stomatal density and distribution. The number of stomata on the berries was counted using a light microscope (model DM 2500; Leica, Wetzlar, Germany). To prepare the samples, six berries each from stage 1 through stage 6 (Table 1) 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: the distal (calyx) end, distal-middle end, proximal-middle end, and proximal (pedicel) end (Fig. 1A). Under the microscope, six photographs with an area of 1.65 × 1.30 mm 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 (ANOVA) using R version 3.4.2 (R Core Team, 2017). 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)

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

^aBerries in Corvallis and Aurora were selected based on the average stage of development in the field, whereas 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.

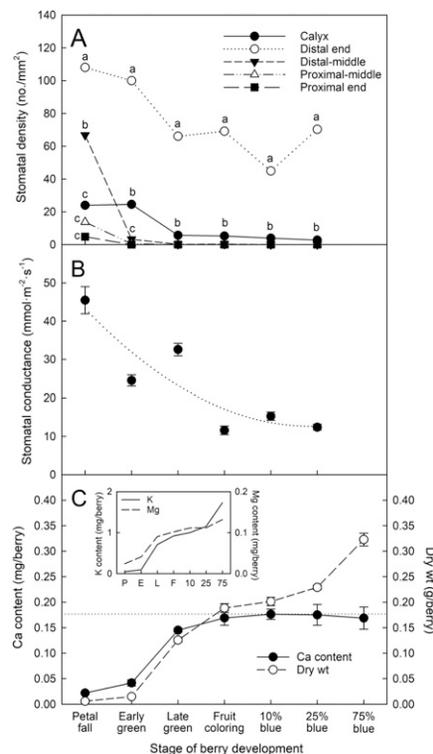


Fig. 2. (A) Stomatal density, (B) conductance, (C) Ca content, and dry weight of 'Elliott' blueberries from petal fall to the 75% blue stage of berry development (inset: K and Mg content in the berries). To assess stomatal density, berries were divided into the calyx and four equal spherical segments, including the distal (calyx) end, distal-middle end, proximal-middle end, and proximal (pedicel) end (see Fig. 1A). Means of stomatal density with different letters at a given stage were significantly different at $P \leq 0.05$. Error bars represent ± 1 SE.

(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 distal (calyx) end. 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 and 1% paraformaldehyde in 0.1 M sodium cacodylate buffer) and stored overnight at 5 °C. Very little fruit pulp was retained on the skin during

removal, and most of it separated from the skin after fixation. Approximately 20 h later, skin samples were gradually washed with a series of 30%, 50%, 70%, 90%, and 100% acetone; to maintain cellular structure, they 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 on 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 \times .

Study 2

Additional berry samples were collected from three cultivars of northern highbush blueberry at three sites in northwestern United States in 2016. The cultivars included: an early season cultivar, Duke, which ripens in late June and July; a midseason 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 more than 70% of the total planted area in the region. The sites included the field at Lewis-Brown in Corvallis (see study 1), a certified organic field 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 drip-irrigated (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 sodium nitrate blend (4N-0.9P-0.4K; Converted Organics of California, Gonzales, CA), 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. (2017b) for more information regarding the organic site]. A standard analysis of the leaves (15-19 Aug. 2016) indicated that most nutrients, including Ca, were sufficient at each site and within the range recommended for northern highbush blueberry; however, boron (B) was below normal in 'Aurora' blueberry at the site in Corvallis,

OR (21 ppm B) (Hart et al., 2006; Strik and Vance, 2015).

Berries from each cultivar were 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 2). A set of berries was sampled from three individual plant replicates at each site. The berries were collected from the same plants at each developmental stage at the two sites in Oregon, and from different plants (within three replicated plots of six plants each) for each stage of development at the site in Washington. Each sample was washed, weighed, and analyzed for Ca and other nutrients, as described (study 1). An additional 15 berries were collected at the 75% blue stage to assess the distribution of dry matter and Ca in the skin, pulp, and seeds. The data were analyzed using an ANOVA and R software v. 3.1.2.

Results

Stomatal density and distribution. Stomata were scarce on the berries and concentrated primarily on the distal end near the calyx (Fig. 1B and C). Density of the stomata was greatest at petal fall, averaging 5 to 108 stomata/mm² from the distal end to the proximal end of the berries (Fig. 2A). In comparison, the mean density on the abaxial surface of the leaves was, on average, 496 stomata/mm². Stomata were wide-open at the early green stage of berry development and had only a slight deposit of wax along the guard cells (Fig. 1D).

As the berries expanded during the initial period of rapid growth (stage I), most of the stomata remained near the distal end of the berries, and by the late green stage, almost none was found in the middle and proximal segments (Fig. 2A). The majority of these stomata were completely covered with wax when the berries began to change color and ripen (stages II and III; Fig. 1E). By the time the berries were blue, it was nearly impossible to locate the stomata with the 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. 2B). By the fruit coloring stage, conductance was low and remained less than 15 mmol·m⁻²·s⁻¹ throughout the ripening period. Leaf g_s measured at the green stage averaged ≈ 100 mmol·m⁻²·s⁻¹.

Accumulation and partitioning of dry matter and calcium. With 'Elliott', dry

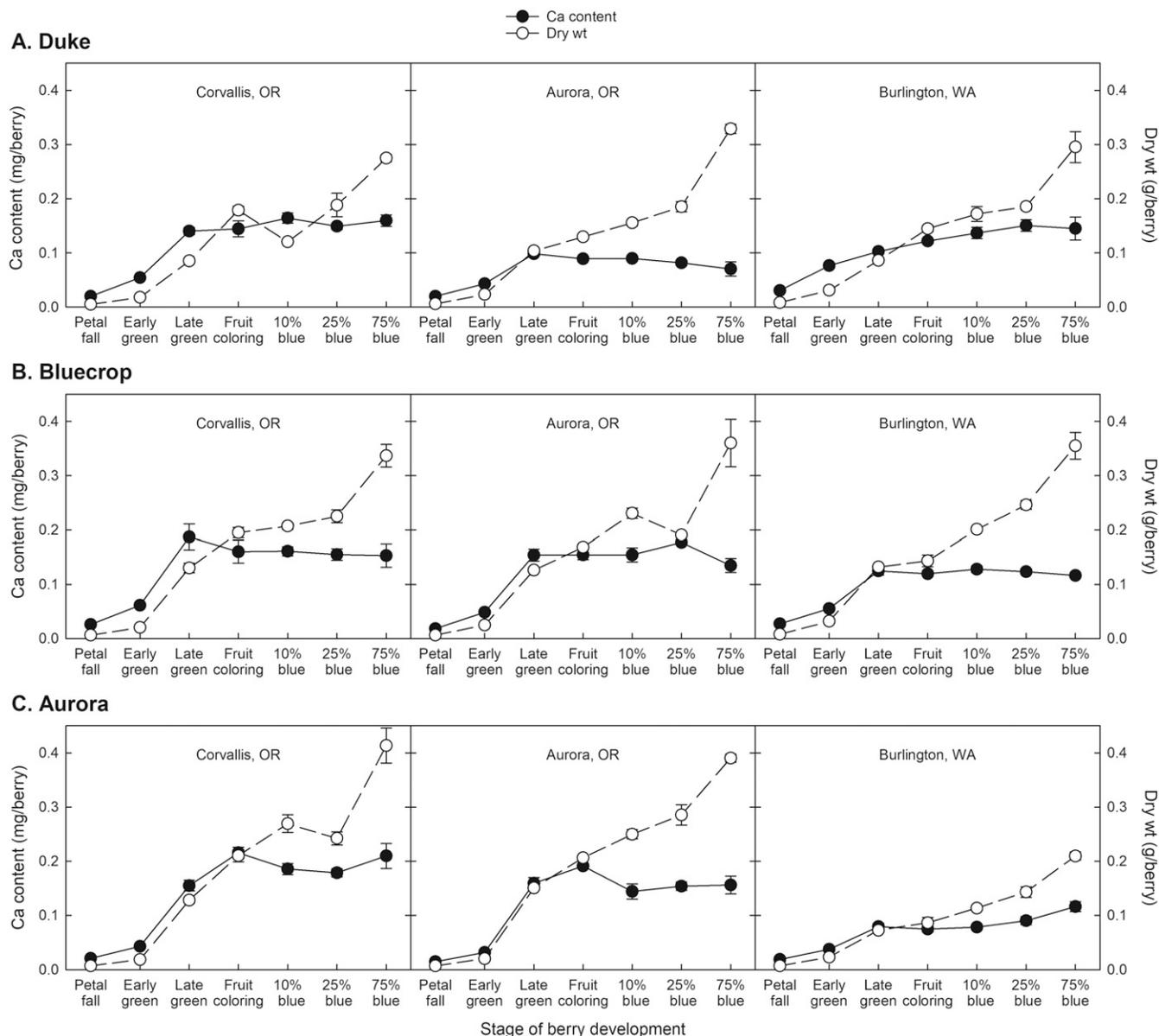


Fig. 3. Calcium content and dry weight of (A) ‘Duke’, (B) ‘Bluecrop’, and (C) ‘Aurora’ blueberries from petal fall to the 75% blue stage of berry development. Berries were collected in 2016 from sites located (from south to north) in Corvallis, OR, Aurora, OR, and Burlington, WA. The sites in Corvallis and Burlington were managed conventionally, whereas the site in Aurora was certified organic. Error bars represent ± 1 SE.

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 before harvest (Fig. 2C). Calcium also accumulated rapidly during the initial stage of berry development; however, in this case, accumulation slowed considerably between the late green and fruit coloring stages, and it stopped completely during fruit ripening (Fig. 2C). In contrast, K and Mg continued to accumulate throughout development of the berries (Fig. 2C, inset).

Calcium accumulation also typically slowed or stopped by the late green stage in ‘Duke’, ‘Bluecrop’, and ‘Aurora’ (Fig. 3), whereas uptake of K, Mg, and other nutri-

ents such as N, phosphorus (P), and sulfur (S) continued throughout berry development in each cultivar (data not shown). Results of a three-way analysis indicated that the dry weight and Ca content of the berries were significantly affected by location, cultivar, and stage of development, as well as by two-way interactions of cultivar \times stage and location \times stage ($P \leq 0.05$). In general, Ca accumulation appeared to end sooner in the early and midseason cultivars (Duke and Bluecrop) than in the late-season cultivar (Aurora; also see Elliott) (Fig. 2C). However, there were two exceptions. In Burlington, WA, ‘Duke’ continued to accumulate Ca until the berries were 25% blue (Fig. 3A); however, ‘Aurora’ appeared to stop accumulating Ca by the late green stage but then resumed uptake once the berries were 25% blue (Fig. 3C).

By harvest (25% and 75% blue stage of the field), the Ca content was less than 0.15 mg/berry, on average, in each of the cultivars examined. However, levels were low in two cultivars in Burlington, WA, averaging only 0.12 and 0.10 mg/berry of Ca each in ‘Bluecrop’ and ‘Aurora’, respectively (Fig. 3B and C). ‘Duke’ also had low Ca in the berries at the organic site in Aurora, OR. Despite a large amount of dry matter (0.33 mg/berry), ‘Duke’ berries from the organic site accumulated only 0.07 mg/berry of Ca by the 75% blue stage, which was less than half the amount measured at the same stage in Corvallis and Burlington (Fig. 3A).

When the berries matured, 18% of the total dry matter in ‘Elliott’ berries was in the skin, 75% was in the pulp, and less than 7% was in the seeds (Table 3). Calcium, however, was approximately three-times more

Table 3. Mean (\pm SE) dry weight and concentration and content of Ca in the skin, pulp, and seeds of 'Elliott' blueberries (n = 3).^z

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

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

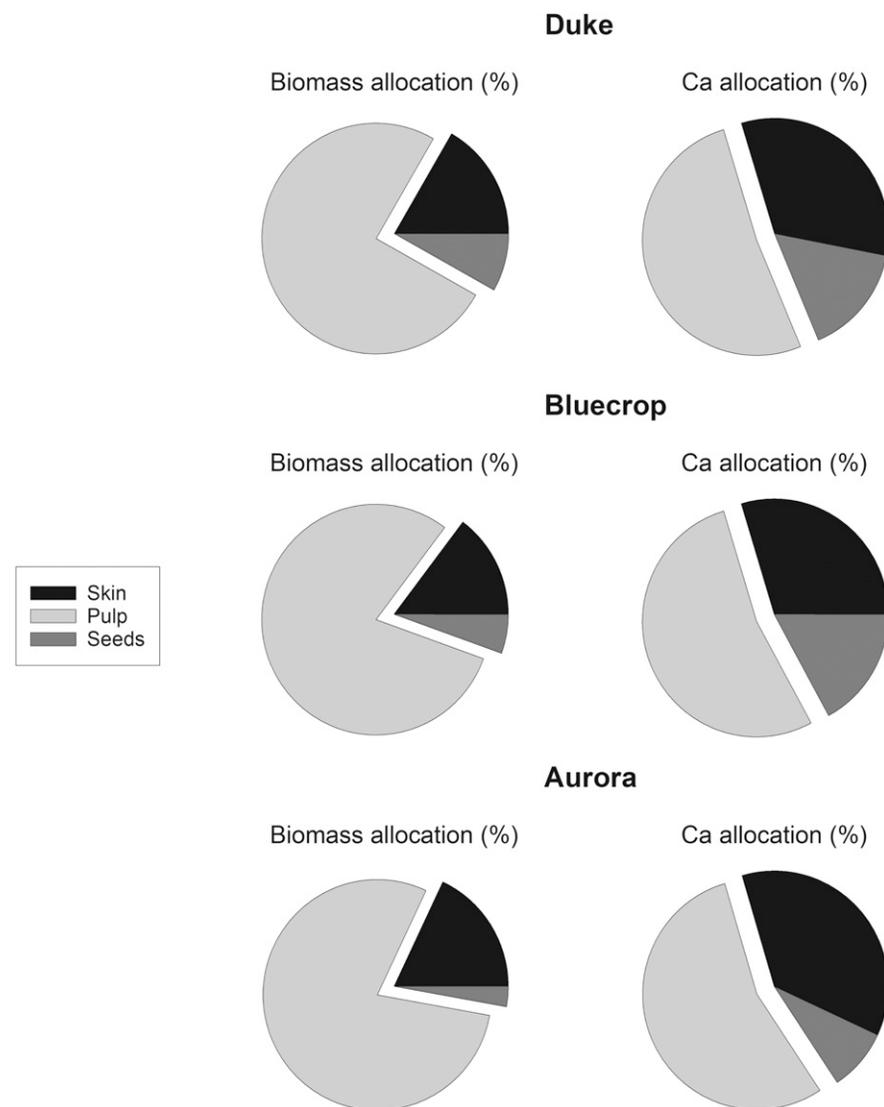


Fig. 4. Allocation of dry biomass and Ca among the skin, pulp, and seeds of 'Duke', 'Bluecrop', and 'Aurora' blueberries. Data are pooled from berries collected at the 75% blue stage of berry development in Corvallis, OR, Aurora, OR, and Burlington, WA (n = 3).

concentrated in the skin and seeds than in the pulp; consequently, nearly half of the Ca in the berries was in the skin (35%) and seeds (13%) (Table 3). Proportionally less dry matter and more Ca were also allocated to the skin and seeds of 'Duke', 'Bluecrop', and 'Aurora' berries (Fig. 4). A two-way analysis revealed that allocation of dry matter and Ca to each tissue was affected by cultivar ($P \leq 0.01$), but it was unaffected by the location in which the berries were collected or by the interaction of cultivar \times location. In these cultivars, partitioning of dry matter and Ca were similar at each site, although 'Aurora'

allocated significantly more dry matter and Ca to the skin and less to the seeds than either 'Duke' or 'Bluecrop'.

Discussion

The results of this study indicated that Ca accumulation occurs primarily 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. Most of the Ca uptake occurred while the berries were small and green and

actively transpiring during stage I. Calcium accumulates mainly during early fruit development in other crops, including apple, apricot (*P. armeniaca* L.), grape, kiwifruit, and nectarine [*P. persica* (L.) Batsch var. *nucipersica* (Suckow) C.K. Schneid.] (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. Therefore, as water translocation switches from xylem to phloem during fruit development, Ca movement becomes limited (Hanger, 1979). When 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. Translocation of these nutrients to various organs occurs primarily via the phloem (Marschner, 2012).

Stomatal density of the blueberries 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 that become more diffuse as the berries expand. During expansion, most of the stomata remained near the calyx or distal end of the berry. Eventually, these stomata became covered with wax and were probably no longer functional during the later stages of berry development. Therefore, by the time fruit coloring begins, water loss 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, 1988; Rogiers et al., 2004).

The rate of g_s measured in the berries after petal fall was approximately half of what was measured in actively transpiring leaves on the plants. Fruit stomata are well-known as essential for gas exchange and C assimilation during early fruit development in many crops (Aschan and Pfanz, 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, 2006). Cuticular 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. These anomalies may have been because the berries in Washington were collected from different plants at each stage of development, whereas those in Oregon were sampled from the same plants at each stage. However, 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 (Yang et al., 2019). Low temperatures and high humidity reduce vapor pressure deficit; therefore, they 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 exposed to poor environmental conditions during 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 could have reduced the number of seeds per berry and the associated accumulation of Ca in the fruit. In contrast, seed number was consistently high in Aurora, OR, due to the favorable climate and a high potential for cross-pollination among the cultivars at the site (Strik et al., 2017b; Strik and Vance, 2019).

The Ca 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 in the soil and used as a mulch in the planting (Strik et al., 2017b). Excessive K can inhibit Ca uptake in plants, thereby interfering with Ca movement to the leaves and fruit (Marschner, 2012). 'Duke', in particular, demonstrates a genetic tendency toward sensitivity to high K and any negative effects it might have on fruit and leaf Ca (Strik and Vance, 2015; Strik et al., 2017a, 2019).

Although g_s is low in developing blueberries, it appears to be an important mechanism by which Ca is delivered to the fruit. Therefore, to increase Ca and enhance the quality of the fruit, cultivars could be developed with more stomata on the berry surface. Less wax would also increase stomatal functioning, particularly at later stages of berry development, but it might lead to problems such as increased infection by fungi and bacteria, more physical damage, and higher rates of water loss after harvest (Jenks and Ashworth, 1999; Riederer and Schreiber, 2001). Alternatively, cultivars could be developed with a higher proportion of Ca in the skin of the berries, which, as mentioned, increases berry firmness and decreases cracking and postharvest decay of fruit in other crops. 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 used as a more immediate means to improve xylem influx and movement of Ca into the berries (Mazzeo et al., 2011, 2013; Montanaro et al., 2006; Rebucci et al., 1997). Finally, applying soil or foliar Ca fertilizers during early stages of berry development can increase Ca in the berries

(Gerbrandt et al., 2019; Hanson and Berkeimer, 2004), but the efficacy of uptake may be limited by the rate of application, cultivar, and weather conditions (Arrington and DeVetter, 2017; Vance et al., 2017).

Literature Cited

- Arrington, M. and L.W. DeVetter. 2016. Fruit set improvement of highbush blueberry (*Vaccinium corymbosum*): A review. *J. Amer. Pomol. Soc.* 70:124–137.
- Arrington, M. and L.W. DeVetter. 2017. Foliar applications of calcium and boron do not increase fruit set or yield in northern highbush blueberry (*Vaccinium corymbosum*). *HortScience* 52:1259–1264.
- Aschan, G. and H. Pfanzer. 2003. Non-foliar photosynthesis – a strategy of additional carbon acquisition. *Flora - Morphology Distribution Functional Ecol. Plants* 198:81–97.
- Blanke, M.M. and A. Leyhe. 1987. Stomatal activity of the grape berry cv. Riesling, Müller-Thurgau and Ehrenfelser. *J. Plant Physiol.* 127:451–460.
- Blanke, M.M. and A. Leyhe. 1988. Stomatal and cuticular transpiration of the cap and berry of grape. *J. Plant Physiol.* 132:250–253.
- Brown, G., S. Wilson, W. Boucher, B. Graham, and B. McGlasson. 1995. Effects of copper-calcium sprays on fruit cracking in sweet cherry (*Prunus avium*). *Scientia Hort.* 62:75–80.
- Bryla, D.R. and B.C. Strik. 2006. Variation in plant and soil water relations among irrigated blueberry cultivars planted at two distinct in-row spacings. *Acta Hort.* 715:295–300.
- Buccheri, M. and C. Di Vaio. 2005. Relationship among seed number, quality, and calcium content in apple fruits. *J. Plant Nutr.* 27:1735–1746.
- Ciccarese, A., A.M. Stellacci, G. Gentilello, and P. Rubino. 2013. Effectiveness of pre- and post- veraison calcium applications to control decay and maintain table grape fruit quality during storage. *Postharvest Biol. Technol.* 75:135–141.
- Cipollini, M.L. and D.J. Levey. 1991. Why some fruits are green when they are ripe: Carbon balance in fleshy fruits. *Oecologia* 88:371–377.
- Creasy, G.L., S.F. Price, and P.B. Lombard. 1993. Evidence for xylem discontinuity in Pinot noir and Merlot grapes: Dye uptake and mineral composition during berry maturation. *Amer. J. Enol. Viticult.* 44:187–192.
- de Freitas, S.T., C.V.T. do Amarante, and E.J. Mitcham. 2015. Mechanisms regulating apple cultivar susceptibility to bitter pit. *Scientia Hort.* 186:54–60.
- de Freitas, S.T. and E.J. Mitcham. 2012. Factors involved in fruit calcium deficiency disorders. *Hort. Rev.* 40:107–146.
- Demarty, M., C. Morvan, and M. Thellier. 1984. Calcium and the cell wall. *Plant Cell Environ.* 7:441–448.
- Dichio, B., D. Remorini, and S. Lang. 2003. Developmental changes in xylem functionality in kiwifruit fruit: Implications for fruit calcium accumulation. *Acta Hort.* 610:191–195.
- Drazeta, L., A. Lang, A.J. Hall, R.K. Volz, and P.E. Jameson. 2004. Causes and effects of changes in xylem functionality in apple fruit. *Ann. Bot.* 93:275–282.
- Driscoll, R. and R. Niskanen. 1999. Calcium chloride sprays decrease physiological disorders following long-term cold storage of apple. *Plant Foods Hum. Nutr.* 54:159–171.
- Ferguson, I.B. and C.B. Watkins. 1989. Bitter pit in apple fruit. *Hort. Rev.* 11:289–355.
- Gavlak, R.G., D.A. Horneck, and R.O. Miller. 2005. The soil, plant and water reference methods for the western region. 3rd ed. West-ern Regional Ext. Publ. 125. Univ. Alaska, Fairbanks, AK.
- Gerbrandt, E.M., C. Mouritzen, and M. Sweeney. 2019. Foliar calcium corrects a deficiency causing green fruit drop in 'Draper' highbush blueberry (*Vaccinium corymbosum* L.). *Agriculture* 9:63. doi: 10.3390/agriculture9030063.
- Grusak, M.A. and K.W. Pomper. 1999. Influence of pod stomatal density and pod transpiration on the calcium concentration of snap bean pods. *J. Amer. Soc. Hort. Sci.* 124:194–198.
- Hanger, B.C. 1979. The movement of calcium in plants. *Commun. Soil Sci. Plant Anal.* 10:171–193.
- Hanson, E.J. and S.F. Berkeimer. 2004. Effect of soil calcium applications on blueberry yield and quality. *Small Fruits Rev.* 3:133–139.
- Hart, J., B. Strik, L. White, and W. Yang. 2006. Nutrient management for blueberries in Oregon. Oregon State University Extension Service. EM 8918. 2 Aug. 2019. <<http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/20444/em8918.pdf>>.
- Ho, L.C. and P.J. White. 2005. A cellular hypothesis for the induction of blossom-end rot in tomato fruit. *Ann. Bot.* 95:571–581.
- Huang, X.-M., H.-C. Wang, W.-L. Zhong, W.-Q. Yuan, J.-M. Lu, and J.-G. Li. 2008. Spraying calcium is not an effective way to increase structural calcium in litchi pericarp. *Scientia Hort.* 117:39–44.
- Jenks, M.A. and E.N. Ashworth. 1999. Plant epicuticular waxes: Function, production, and genetics. *Hort. Rev.* 23:1–68.
- Konarska, A. 2015. Morphological, anatomical, and ultrastructural changes in *Vaccinium corymbosum* fruits during ontogeny. *Botany* 93:589–602.
- Lara, I., P. Garcia, and M. Vendrell. 2004. Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria × ananassa* Duch.) fruit. *Postharvest Biol. Technol.* 34:331–339.
- Marschner, P. (ed.). 2012. Marschner's mineral nutrition of higher plants, 3rd ed. Academic Press, New York.
- Marzouk, H.A. and H.A. Kassem. 2011. Improving yield, quality, and shelf life of Thompson seedless grapevine by preharvest foliar applications. *Scientia Hort.* 130:425–430.
- Mazzeo, M., B. Dichio, M.J. Clearwater, G. Montanaro, and C. Xiloyannis. 2013. Hydraulic resistance of developing *Actinidia* fruit. *Ann. Bot.* 112:197–205.
- Mazzeo, M., B. Dichio, C. Xiloyannis, and A. Lang. 2011. Fruit transpiration increases with windspeed in *Actinidia deliciosa* 'Hayward'. *Acta Hort.* 913:385–388.
- Montanaro, G., B. Dichio, A. Lang, A.N. Mininni, V. Nuzzo, M.J. Clearwater, and C. Xiloyannis. 2014. Internal versus external control of calcium nutrition in kiwifruit. *J. Plant Nutr. Soil Sci.* 177:819–830.
- Montanaro, G., B. Dichio, A. Lang, A.N. Mininni, and C. Xiloyannis. 2015. Fruit calcium accumulation coupled and uncoupled from its transpiration in kiwifruit. *J. Plant Physiol.* 181:67–74.
- Montanaro, G., B. Dichio, and C. Xiloyannis. 2010. Significance of fruit transpiration on calcium nutrition in developing apricot fruit. *J. Plant Nutr. Soil Sci.* 173:618–622.
- Montanaro, G., B. Dichio, C. Xiloyannis, and G. Celano. 2006. Light influences transpiration and calcium accumulation in fruit of kiwifruit plants (*Actinidia deliciosa* var. *deliciosa*). *Plant Sci.* 170:520–527.
- Palliotti, A. and A. Cartechini. 2001. Developmental changes in gas exchange activity in flowers, berries, and tendrils of field-grown Cabernet Sauvignon. *Amer. J. Enol. Viticult.* 52:317–323.

- R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2 Oct. 2017. <<https://www.R-project.org/>>.
- Rebucci, B., S. Poni, C. Intriери, E. Magnanini, and A.N. Lakso. 1997. Effects of manipulated grape berry transpiration on post-veraison sugar accumulation. *Austral. J. Grape Wine Res.* 3:57–65.
- Riederer, M. and L. Schreiber. 2001. Protecting against water loss: Analysis of the barrier properties of plant cuticles. *J. Expt. Bot.* 52:2023–2032.
- Rogiers, S.Y., D.H. Greer, J.M. Hatfield, B.A. Orchard, and M. Keller. 2006. Mineral sinks within ripening grape berries (*Vitis vinifera* L.). *Vitis* 45:115–123.
- Rogiers, S.Y., J.M. Hatfield, V.G. Jaudzems, R.G. White, and M. Keller. 2004. Grape berry cv. Shiraz epicuticular wax and transpiration during ripening and preharvest weight loss. *Amer. J. Enol. Viticult.* 55:121–127.
- Siddiqui, S. and F. Bangerth. 1995. Effect of preharvest application of calcium on flesh firmness and cell-wall composition of apples—influence of fruit size. *J. Hort. Sci.* 70:263–269.
- Strik, B.C. and A.J. Vance. 2015. Seasonal variation in leaf nutrient concentration of northern highbush blueberry cultivars grown in conventional and organic production systems. *HortScience* 50:1453–1466.
- Strik, B.C. and A.J. Vance. 2019. Highbush blueberry cultivars differ in the relationship between seed number and berry weight during the harvest season. *HortScience* 54:1728–1736.
- Strik, B.C., A. Vance, D.R. Bryla, and D.M. Sullivan. 2017a. Organic production systems in northern highbush blueberry. I. Impact of planting method, cultivar, fertilizer, and mulch on yield and fruit quality from planting through maturity. *HortScience* 52:1201–1213.
- Strik, B.C., A. Vance, D.R. Bryla, and D.M. Sullivan. 2019. Organic production systems in northern highbush blueberry. II. Impact of planting method, cultivar, fertilizer, and mulch on leaf and soil nutrient concentrations and relationships with yield from planting through maturity. *HortScience* 54:1777–1794.
- Strik, B.C., A. Vance, and C.E. Finn. 2017b. Northern highbush blueberry cultivars differed in yield and fruit quality in two organic production systems from planting to maturity. *HortScience* 52:844–851.
- Vance, A.J., P. Jones, and B.C. Strik. 2017. Foliar calcium applications do not improve quality or shelf life of strawberry, raspberry, blackberry, or blueberry fruit. *HortScience* 52:382–387.
- Vargas, O.L., D.R. Bryla, J.E. Weiland, B.C. Strik, and L. Sun. 2015. Irrigation and fertigation with drip and alternative micro irrigation systems in northern highbush blueberry. *HortScience* 50:897–903.
- Yang, F.-H., D.R. Bryla, and B.C. Strik. 2019. Critical temperatures and heating times for fruit damage in northern highbush blueberry. *HortScience* (In press).
- Zhang, Y. and M. Keller. 2015. Grape berry transpiration is determined by vapor pressure deficit, cuticular conductance, and berry size. *Amer. J. Enol. Viticult.* 66:454–462.