

The Effect of Foliar Calcium Treatments on Fruit Weight and Firmness of Rabbiteye Blueberry (*Vaccinium virgatum* Aiton)

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Abstract

Foliar calcium applications are used in many fruiting crops to minimize disease and physiological disorders. In blueberry (*Vaccinium spp*), it is used to improve fruit firmness with varying success. Two applications of foliar calcium applied to rabbiteye blueberry (*V. virgatum* Aiton) cvs. Alapaha and Powderblue as calcium nitrate [$\text{Ca}(\text{NO}_3)_2$], neutralized calcium carbonate (CaCO_3), and chelated calcium (calcium glucoheptonate, $\text{C}_{14}\text{H}_{26}\text{CaO}_{16}$) were made at the label rate of 2.3 L ha^{-1} applied in a volume of 935.3 L ha^{-1} (697 ppm, 108 ppm, and 604 ppm Ca per application, respectively). The applications were made at 30 and 15 days preharvest in 2013 and 2014. Fruit were hand harvested at 40% ripe and evaluated for berry weight, color, firmness, soluble solids, and acidity. In 2013, fruit were stored at 1°C with 85% relative humidity and evaluated again at 7 and 15 days. In 2014, fruit and tissue samples were evaluated for Ca concentration. In 2013, 'Powderblue' had a 5% increase in firmness from the CaCO_3 treatment when compared to control fruit. The chelated calcium treatment significantly increased fruit weight by 12% compared to the control for 'Alapaha'. Fruit firmness increased 5% and fruit weight decreased 10% for the $\text{Ca}(\text{NO}_3)_2$ treatment compared to control for 'Alapaha' fruit sampled after 2 weeks of storage. In 2014, none of the treatments significantly increased fruit firmness or berry calcium concentration. For 'Powderblue' in 2014, all treatments significantly decreased firmness. Leaf Ca concentration was increased by 18% for 'Alapaha' and decreased by 26% for 'Powderblue' when comparing the chelated calcium treatment to non-treated leaves.

Rabbiteye blueberry harvest in South Georgia begins in late May and extends into July where high humidity, daily high temperatures that reach 35°C or above, and heavy rains can negatively affect fruit quality (Schermer and Krewer, 2003). Soluble solids, acidity, pH, berry size, firmness and color are all attributes used to characterize maturity. However, color is used to indicate harvest maturity by growers and the sorting line to evaluate firmness, color, and size, which determine if the fruit is subsequently fresh packed, processed/frozen, or culled. In Georgia, price per pound between fresh market and processed blueberry can have a disparity of 70%. For example, fresh packed blueberry price was $\$0.91 \text{ kg}^{-1}$ and processed $\$0.28 \text{ kg}^{-1}$ in 2011 (USDA, 2012). To reduce the risk of down-grading to processed or culled fruit, some growers use foliar calcium with

the intention of increasing firmness. Previous work with highbush blueberry (*V. corymbosum* L.) demonstrated very limited impact on fruit firmness (Hanson, 1995; Koron et al., 2009; Ochmian, 2012; Stückrath et al., 2008). However, the effect of foliar calcium treatments on rabbiteye blueberry fruit firmness has not been well studied.

In some years, mid- and late-season rabbiteye blueberry fruit lost firmness at harvest, even when field-heat was quickly removed (J. Cornelius, pers. comm.). To mitigate this effect, foliar calcium applications have been added as a management practice without consistent results. Identifying the effect of foliar applied calcium on fruit firmness will provide growers with information related to the cost effectiveness of this management practice. The objective of this study was to identify the effect of foliar applied calcium

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on fruit firmness of two rabbiteye blueberry cultivars.

Materials and Methods

Study site. Rabbiteye blueberry cvs. Alapaha and Powderblue, planted in 2008, were selected to study the effects of foliar applied calcium at the University of Georgia's Alapaha Blueberry Research Farm (31°20'46.91" N, 83°14'23.91" W) in Berrien County, GA. Both cultivars are suitable pollinators, 'Alapaha' ripens early to mid-June, whereas, 'Powderblue' ripens late June to early July (Krewer and NeSmith, 2006).

The plants were grown on Lee field loamy sand (loamy, siliceous, subactive, thermic Arenic Plinthic Paleudults). The bushes were planted in pine bark culture at a spacing of 1.5 m (in-row) x 3.7 m (between-row) on 1.2 m (width) x 0.5 m (height) beds (Williamson et al., 2006). Irrigation was provided through a single line of drip tape (John Deere Ro-Drip, Moline, Ill; 1.57 cm dia., 5 mm wall thickness, 30.5 cm emitter spacing, emitter flow rate 0.9 L·hr⁻¹) positioned down the center of the bed and over the crown of each plant. Water was applied based on a total of 2.54 cm of water per week including precipitation during the growing season. Plants were managed according to standard agricultural practices for the southeastern region of the U.S. (Krewer and NeSmith, 2001; Burack et al., 2013). A composite soil sample was taken in March 2014 before fertilization. From under the drip line of the same plants used in this study, each sample was taken at 0 to 15 cm depth with the surface 2.5 cm removed: 'Alapaha' soil analyses, pH 4.6, and P, K, Ca, Mg levels of 99, 88, 421, 72 kg·ha⁻¹, respectively; 'Powderblue' soil analyses, pH 4.3, and P, K, Ca, Mg levels of 138, 67, 425, 81 kg·ha⁻¹, respectively (tested by University of Georgia's Soil, Plant, and Water Laboratory, Athens, GA). Soil nutrients were extracted using a Mehlich I procedure and pH was measured using a 0.01 molar calcium chloride solution in a 1:1 soil to CaCl₂ mixture and reported as an adjusted pH value of

+ 0.6 units (Kissel and Sonon, 2008). The study was conducted over the 2013 and 2014 seasons.

Treatments. A non-treated control and three calcium salts, calcium nitrate [Ca(NO₃)₂] (Cell Force with N= 6%, Ca= 10%, B= 0.2%; Miller Chemical & Fertilizer Corp. Hanover, PA), neutralized calcium carbonate (CaCO₃) (Calexin with Ca = 2% Miller Chemical & Fertilizer Corp. Hanover, PA), and chelated calcium (calcium gluconate, C₁₄H₂₆CaO₁₆) (KeyPlex Calcium Plus with Ca = 9%; KeyPlex, Winter Park, FL) were applied via backpack sprayer (Solo 3.79 L 473-P, Newport News, VA). Each application of the calcium solution was applied to runoff at the product label rate of 2.3 L·ha⁻¹ in a volume of 935.3 L·ha⁻¹ regardless of concentration (697 ppm, 108 ppm, and 604 ppm Ca per application, respectively) and water was applied to the control. Treatments were a completely randomized design with five plots of five plants. In each plot, only the middle three plants were treated and harvested separately for triplicate analyses of fruit quality measurements. The plants at the end of the plots were non-treated guard plants, which left a minimum of two guard plants between each treatment. The plots were within rows of contiguous cultivars with three plants as a border at the end of each row and a border row on either side of the treatment row. Applications were made at 30 and 15 days prior to harvest for each cultivar for a total of two applications. 'Alapaha' applications were made 8 and 22 May in 2013 and 15 and 29 May in 2014. 'Powderblue' applications were made 22 May and 5 June in 2013 and 29 May and 12 June in 2014. No surfactants were used and no phytotoxicity was observed.

Harvest and Fruit Quality. All fruit were hand harvested when the control bushes had ~40% ripe fruit. The calcium treated fruit were also at ~40% maturity. Harvest dates were 6 June 2013 and 13 June 2014 for 'Alapaha' and 19 June 2013 and 26 June 2014 for 'Powderblue'. A random sample of 1.5 L of

ripe fruit was collected from each bush within each plot at harvest. In this study, the objective was to determine foliar applied calcium's effect on firmness and no yield (total, partial, or marketable) was measured. On each harvest date the fruit were transported to the lab, allowed to equilibrate to room temperature, and sorted. Only marketable fruit were evaluated and fruit that was green, red, or damaged were discarded. From each subsample, 100 ripe fruit were placed into its respective 0.5 L clear clamshell (APET Clamshell 480, Pactiv LLC, Lake Forest, IL). The marketable fruit was analyzed for weight, firmness, color, soluble solids (SS), and acidity. The weight of 100 berries was measured in grams (g) and fifty fruit per subsample were measured at the equator for firmness (g mm^{-1}) (FirmTech2, Bioworks, Inc. Wamego, KS). Ten fruit from each subsample were measured for color at the stem end as $L^* a^* b^*$ CIELAB (Konica-Minolta CR400, Osaka, Japan). Soluble solids were measured with a digital hand-held refractometer ($^{\circ}$ Brix) (BrixStix, Cole Palmer, Vernon Hills, IL), and 0.1 N sodium hydroxide (NaOH) was used to determine the titratable acidity (TA) of the fruit (Mettler Toledo DL15 Titrator, Columbus, OH). For SS and TA analyses, 25 fruit per subsample were pulped (PowerGen 500, Fisher Scientific, Waltham, MA) and centrifuged (Allegra 25R Centrifuge, Beckman Coulter, Brea, CA) at $4100g_n$ in 50 ml high-speed plastic centrifuge tubes (Fisher Scientific, Waltham, MA). The liquid portion was collected and evaluated for SS and TA. In this study, sugar and acidity were reported as a ratio (sugar/acid) as an indicator for fruit quality to simplify the interpretation of these two fruit quality characteristics. Beaudry (1992) suggested that the SS and acidity for marketable blueberry fruit be $>10\%$ w/w and 0.3–1.3% w/w, respectively, which estimates the sugar/acid ratio from 10 to 33 for marketable fruit. Included in Beaudry's (1992) assessment of fruit quality was color as blue for blueberry. Hue angle was calculated from the arctangent (\tan^{-1}) of a^*/b^* and corrected

for negative values (McGuire, 1992). The calculation represents a 360° color wheel where red/purple ($0/360^{\circ}$), yellow (90°), bluish green (180°), and blue (270°) are given angles to simplify color interpretation. Shade is expressed as L^* where 0 = black and 100 = white. All CIELAB fruit quality data were reported as L^* and hue.

Postharvest. In 2013, at harvest, two 100-fruit samples per subsample per treatment were placed in 0.5 L plastic clamshell containers (APET Clamshell 480, Pactiv LLC, Lake Forest, Ill, USA) and stored for either 7 or 14 days at 1°C with 85% relative humidity (Dade Service Corporation, Daytona Beach, FL, USA). At the end of each storage period, one clamshell of fruit per subsample was allowed to stabilize to room temperature as determined by infrared thermometry (Fluke IR Thermometer, Everett, WA) before being analyzed for fruit quality as reported above.

Tissue Analysis. In 2014, leaf and fruit tissue samples were collected at the same time per plot per treatment on 16 May before calcium applications and at harvest for each cultivar. Sample dates were selected to determine if a similar trend of decreasing berry Ca existed in rabbiteye blueberry as reported by Strückrath et al. (2008) in highbush blueberry 'Elliott' and increasing leaf Ca (Spillers, 1982). In addition, leaf and berry tissue was collected in the control plants on 10 Apr when the first leaves were fully expanded. Leaf tissue collected were fully expanded leaves from non-fruiting shoots that were exposed to full sun with 75 leaves collected from each treatment bush. Berries were collected from racemes on vigorously growing shoots that were part of the plant growing from the crown. The number of berries depended on maturity and size: 50, 25, and 25 fruit sampled on 10 Apr, 16 May, and at harvest, respectively. Tissue collection from suckering shoots was avoided. All tissue collected was washed in a dilute phosphate-free detergent solution (0.1% detergent) followed by rinsing with distilled water. The tissue

samples were then dried to a constant weight at 80 °C (Grieve model 13-261-28A, Round Lake, IL). The samples were analyzed for Ca (Waters Agricultural Laboratories, Inc., Camilla, GA), where the dried leaves were ground to pass a 20-mesh screen, the samples were reduced to ash in a muffle furnace, acid digested, and measured by inductive coupled plasma spectrophotometer (ICP) coupled to a Digiblock 3000 (SCP Science, Baie D'Urfé, Quebec, Canada).

Statistics. The experiment was analyzed within cultivar and within the year to avoid additional interactions using SAS's 9.3 Proc GLM (SAS Institute Inc., Cary, NC, U.S.).

Means were separated at $P < 0.05$ level using Fisher's least significant difference (LSD) test.

Results and Discussion

'Alapaha'. In 2013, the Ca applications did not significantly affect fruit firmness or weight except that chelated calcium increased fruit weight by 12% at harvest, but not after storage (Table 1). In 2014 the trend was similar; where the chelated calcium treated fruit weight was 10% greater than the control, though not significantly different. The control fruit sugar/acid ratio was greater than the calcium treated fruit (Table 2) in 2014 only. The sugar/acid ratio was 20,

Table 1. Foliar applied calcium effects on berry firmness and weight of 'Alapaha' and 'Powderblue' rabbiteye blueberry at harvest and after harvest in 2013 and 2014.

Harvest Date	Alapaha 6/6/2013		Powder Blue 6/19/2013	
	Firmness (g/mm)	100 Fruit Wt (g)	Firmness (g/mm)	100 Fruit Wt (g)
Harvest 2013				
Control ^z	227 a ^x	121 b	215 b	146 ab
CaCO ₃	228 a	125 b	226 a	136 b
Ca(NO ₃) ₂	228 a	119 b	210 b	152 a
Chelated Ca	230 a	136 a	216 ab	149 a
1 Week of Storage				
Control	217 a	122 ab	201 a	130 b
CaCO ₃	214 a	119 ab	201 a	132 b
Ca(NO ₃) ₂	220 a	114 b	192 a	145 a
Chelated Ca	216 a	129 a	193 a	146 a
2 Weeks of Storage				
Control	222 bc	111 ab	179 a	128 a
CaCO ₃	217 c	107 b	179 a	126 a
Ca(NO ₃) ₂	234 a	100 c	172 a	135 a
Chelated Ca	225 b	114 a	175 a	135 a
Harvest 2014				
	6/13/2014		6/26/2014	
Control	120 a	123 a	113 a	150 a
CaCO ₃	114 b	130 a	96 c	144 ab
Ca(NO ₃) ₂	121 a	128 a	101 b	134 b
Chelated Ca	117 ab	135 a	107 b	139 ab

^z Control = non-treated fruit; CaCO₃ = calcium carbonate, Calcein; Ca(NO₃)₂ = calcium nitrate, Cell Force; Chelated Ca = calcium glucoheptonate, KeyPlex;

^x Means followed by a different letter within a column are significantly different at $P < 0.05$ according to Fisher's least significant difference (lsd) test.

Table 2. Foliar applied calcium effects on berry color (L^* = shade and *Hue* = color) and sugar/acid ratio of ‘Alapaha’ and ‘Powderblue’ rabbiteye blueberry at harvest in 2013 and 2014.

Harvest Date		‘Alapaha’ 6/6/2013		‘Powderblue’ 6/19/2013		
Treatment	L	Color Hue	Sugar/Acid Ratio °Brix/%Acid	L	Color Hue	Sugar/Acid Ratio °Brix/%Acid
Control ^x	22.6 a ^z	284.1 a	19.8 a	38.1 b	274.4 a	14.9 a
CaCO ₃	22.1 a	290.7 a	20.6 a	38.6 ab	273.5 a	15.4 a
Ca(NO ₃) ₂	22.8 a	287.1 a	17.8 a	37.9 b	275.1 a	15.7 a
Chelated Ca	22.1 a	287.7 a	20.6 a	39.4 a	273.7 a	15.9 a
6/13/2014 Harvest			6/26/2014 Harvest			
Control	28.8 b	282.4 a	23.8 a	38.3 a	271.3 a	15.4 a
CaCO ₃	31.0 a	278.5 a	18.1 b	35.0 b	274.6 a	12.8 bc
Ca(NO ₃) ₂	29.6 b	281.5 a	17.2 b	37.8 a	273.9 a	11.7 c
Chelated Ca	29.2 b	283.7 a	19.1 b	36.2 b	273.4 a	14.9 ab

^x Control = non-treated fruit; CaCO₃ = calcium carbonate, Calcein; Ca(NO₃)₂ = calcium nitrate, Cell Force; Chelated Ca = calcium glucoheptonate , KeyPlex;

^z Means followed by a different letter within a column are significantly different at $P < 0.05$ according to Fisher’s least significant difference (lsd) test.

24, and 28% lower than the control fruit for chelated calcium, calcium carbonate, and calcium nitrate, respectively (Table 2). The shade or L^* value for calcium carbonate was 7% lighter than the control; however, for only the harvest date in 2014. Ochmian (2012) reported that the L^* value for ‘Duke’ highbush blueberry can decrease or become darker by ~43% when the wax coating is removed, this suggests that comparing L^* values is a good indication of the level of handling through harvesting, sorting, and/or analytical manipulation. The hue was not statistically different within each harvest date for either ‘Alapaha’ or ‘Powderblue’. In addition, a sugar/acid ratio between 10-33 was suggested to be commercially acceptable (Beaudry, 1992) and though there was variability among the treatments, in all cases, the fruit was of marketable quality. Considering the hue and sugar/acid ratio, the fruit tested were of similar maturity (Table 2).

‘Powderblue’. In 2013, fruit from the calcium carbonate treatment was 5% firmer than the control fruit at harvest; however, the fruit was 7% less in weight (Table 1). Across all other treatments, fruit firmness and weight

measurements were similar. In 2014, neither fruit firmness nor weights were improved by the application of calcium; the firmness measured 15%, 11%, and 5% less than the control fruit for calcium carbonate, calcium nitrate, and chelated calcium, respectively (Table 1). There was significant variation in L^* value or shade of the fruit but results differed depending on the year (Table 2). Because the hue angle measurements were similar, the variation in the shade of the fruit can be associated with the level of bloom or fragile epicuticular wax layer (Retamales and Hancock, 2012) being disturbed or removed. In addition, the treatments lacked consistency between the years, where chelated Ca was 3% lighter in 2013 and 5% darker in 2014 suggesting the bloom was being rubbed from the fruit through handling.

Postharvest. In 2013, ‘Alapaha’ fruit assessed after one week in storage showed no significant differences among treatments for firmness or weight when compared to the control (Table 1). However, calcium nitrate treated fruit was 5% firmer and weighed 10% less than the control fruit after the second week of storage. For ‘Powderblue’ fruit after

one week of storage, the weight of calcium nitrate, and chelated calcium treated fruit was 11% and 12% greater than the control fruit, respectively (Table 1). The second week of storage, the fruit firmness and weight were not significantly different. The differences at harvest did not continue through storage even though the normal processes of postharvest fruit softening and weight loss occurred (Ehlenfeldt, 2002).

Fruit harvested in 2014 were not assessed following storage due to low firmness values. Saftner et al. (2008) reported that for instrument-sensory analyses of compression firmness, a correlation exists between the crispness of the fruit and the amount of compression reported by the FirmTech 2. Measurements in the 150 g mm⁻¹ range are associated with an undesirable but not unacceptable crispness. 'Coastal' rabbiteye blueberry was too soft at 140 g mm⁻¹ (Saftner et al., 2008). None of the fruit harvested in 2014 for either 'Alapaha' or 'Powderblue' were above 121 g mm⁻¹ suggesting that this fruit would have been rejected for fresh market and further analysis was not warranted. In addition, 'Alapaha' and 'Powderblue' fruit measuring ≤ 120 g mm⁻¹ by the FirmTech 2 tended to split and expel the mesocarp and endocarp, losing fruit for further analyses.

Tissue Ca concentration. In 2014, berry and leaf tissue calcium analysis follow the same trend that Strückrath et al. (2008) describes in highbush blueberry 'Elliott' where the Ca concentration falls in the fruit and rises in the leaf over time (Table 3 and Fig 1). There was no effect from Ca treatments on

calcium concentrations in the fruit for either cultivar. However, chelated calcium significantly increased 'Alapaha' leaf Ca concentration by 15%, but reduced 'Powderblue' leaf Ca concentration by 26% compared to the control. The increasing level of calcium in the leaves is a function of mobility. In general, plants have low Ca mobility in the phloem and high mobility through the xylem (White, 2012) including highbush blueberry (Gough, 1994). The transpiration stream is a continuous column of water from the soil through the xylem to the leaves and into the atmosphere. Conduction of water through this stream is a factor of water availability in the soil, air temperature, relative humidity, stomata number and opening, and uninhibited movement through the xylem. Calcium is a plant nutrient that is primarily xylem mobile and accumulation of calcium in leaf tissue should increase as the season progresses as water moves through the plant (White, 2012). Leaf tissue calcium increased in highbush blueberry (Doughty et al., 1981) and a similar trend occurred in this study (Fig 1 & Table 3).

Conclusions

Earlier studies have shown that calcium, when sufficiently available to the plant, does not improve firmness without a reduction in weight (Hanson 1995; Strückrath et al. 2008; Ochmian, 2012). When a significant increase in firmness resulted from foliar Ca applications, fruit mass was reduced compared to control fruit (Ochmian, 2012). Fruit calcium was not significantly increased by the foliar

Table 3. Regression analysis of non-treated fruit leaf tissue Ca (y) sampled in 2014 for rabbiteye blueberry cvs. Alapaha and Powderblue regressed against sample dates (x): Apr 10 (100 Julian days [J]), May 16 (136 J) and at harvest [Jun 13 (164 J)] for 'Alapaha' and Jun 26 (177 J) for 'Powderblue'. Berry and leaf Ca percent for 'Alapaha' and 'Powderblue' are reported in Fig. 1.

	'Alapaha'		'Powderblue'	
	Berry	Leaf	Berry	Leaf
Linear Regression				
Eq.	$y = -0.0017x + 0.411$	$y = 0.0042x - 0.0238$	$y = -0.0014x + 0.4011$	$y = 0.0066x - 0.2561$
R-square	0.993	0.997	0.916	0.937
Significance	0.0047	0.0016	0.0271	0.0015

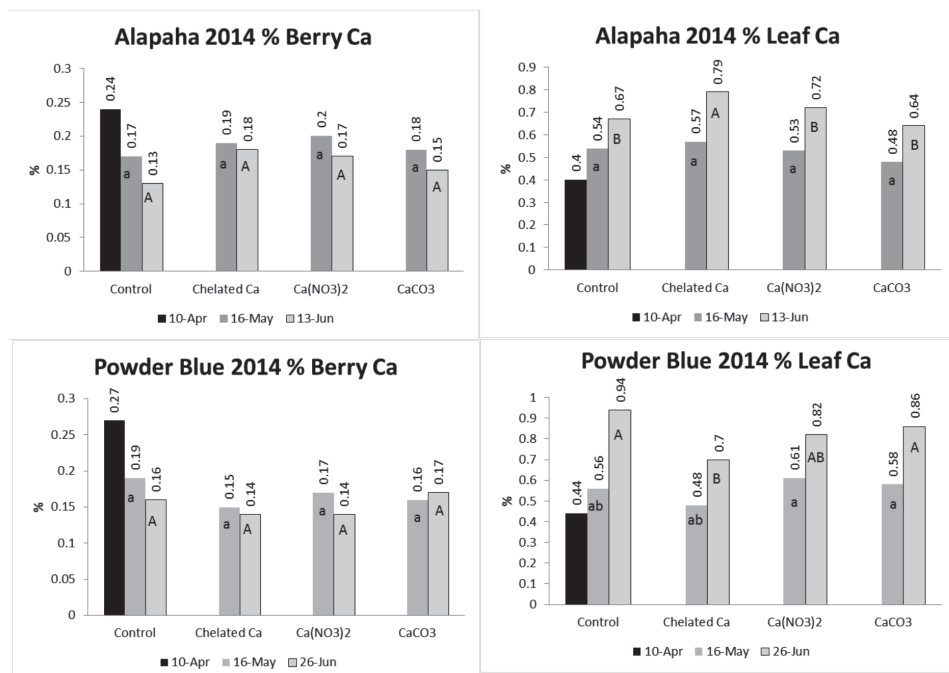


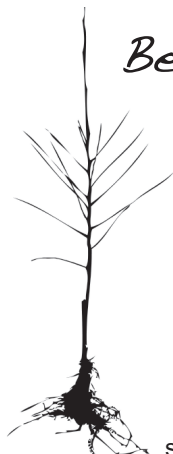
Figure 1. Berry and leaf calcium concentrations in 2014 for ‘Alapaha’ and ‘Powderblue’ rabbiteye blueberry as affected by foliar calcium treatment. Control = non-treated fruit; Chelated Ca = calcium glucoheptonate, KeyPlex; Ca(NO₃)₂ = calcium nitrate, Cell Force; CaCO₃ = calcium carbonate, Calixin. Control tissue sampling on 10 Apr and the comparisons are within the sample date, lower case letters are 16 May sampling and upper case letters are harvest date sampling (‘Alapaha’ 13 Jun and ‘Powderblue’ 26 Jun); means followed by a different letter within a bar are significantly different at $P < 0.05$ according to Fisher’s least significant difference (LSD) test.

application and the firmness in all treatments for 2014 was below the standard for fresh market quality. Considering the use of foliar calcium is to improve firmness, the chemistries used in this experiment did not perform to expectation, which suggests foliar calcium applications for rabbiteye blueberry will likely not sufficiently improve fruit firmness to warrant the cost of application.

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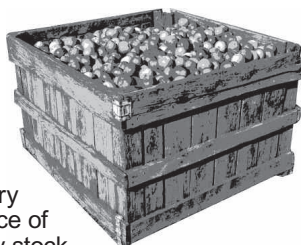
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