Acta Scientiarum

http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v42i1.42449

CROP PRODUCTION

Physical and chemical characterization and bioactive compounds from blackberry under calcium chloride application

Joyce Helena Modesto[1], Jackson Mirellys Azevedo Souza[1], Rafael Augusto Ferraz[1], Marcelo de Souza Silva[1], Magali Leonel[2] and Sarita Leonel[1*]

[1]Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho", Cx. Postal 237, 18610-307, Botucatu, São Paulo, Brazil.
[2]Centro de Raízes e Amidos Tropicais, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, São Paulo, Brazil. *Author for correspondence.
E-mail: sarinel@fca.unesp.br

ABSTRACT. Calcium chloride application on fruits maintains the firmness of fruits, decreases storage breakdown and rot, extends shelf-life, and increases vitamin C and calcium content. Blackberries have low post-harvest durability due to the intrinsic characteristics of their fruits, mainly the high respiratory rate and low firmness, which causes problems in the production chain of fresh fruits. The current study aimed to evaluate the effects of pre-harvest application of calcium chloride on the fruits of 'Tupy' blackberry (*Rubus* spp.). A randomized block design for a factorial scheme was used, i.e. calcium chloride concentrations (0, 1.5, 3, and 4.5%) and number of applications (1, 2, and 3). Multiple applications occurred between 5-day intervals. The results showed that the calcium content in the fruits increased with a single application of 2.2%, but a decrease in mass loss was observed with a 4.5% application. The results also indicated great firmness and ascorbic acid content of fruits of 'Tupy' blackberry, in addition to the significant increase in fruit size and mass. However, in general, the content of soluble solids, sugars, antioxidant activity and total polyphenols decreased.

Keywords: *Rubus* spp.; fruit texture; nutritional value; Ca^{+2}; phenolic compounds; antioxidant activity.

Received on April 19, 2018.
Accepted on August 13, 2018.

Introduction

The blackberry is a species within the *Rubus* genus, referred to as berries. Additionally, consumers have increased their demand for such fruit because of its flavour, colour, health benefits, and high antioxidant activity (Ferreira, Rosso, & Mercadante, 2010).

Blackberries have high antioxidant activity, as they are rich in polyphenols, which are bioactive compounds of interest due to their possible benefits to human health (Segantini et al., 2015). In Brazil, Tupy is the most cultivated blackberry due to its rusticity, upright growth, great vigour and productive performance, as well as a good balance between acidity and sugar content in fruits (Campagnolo & Pio, 2012). Among other traits, high perishability is an aggravating factor that generally impedes commercialization, i.e. high sensitivity to mechanical damage directly affects its useful life and requires most producers to send their fruits to domestic markets.

Blackberries adapt in temperate and subtropical climates, and in both cold and hot conditions, the Tupy cultivar has high productive potential (Curi et al., 2015). However, blackberries have little post-harvest durability due to the intrinsic characteristics of their fruits, especially the high respiratory rate and low firmness, which causes problems in the productive chain of fresh fruits (Souza et al., 2014).

Calcium is a nutrient associated with fruit quality and receives considerable attention due to its effect on reducing the senescence rate of fruits. Studies indicate that senescence rate during ripening often depends on tissue calcium levels (Aghdam, Hassanpouraghdam, Paliyath, & Farmani, 2012). Moreover, calcium bridges between pectic acids or between pectic acids and other polysaccharides; therefore, reducing the action of pectolytic enzymes that are either produced by fruits and cause soft tissues or produced by fungi and bacteria and cause deterioration (Conway, Sams, McGuire, & Kelman, 1992). Calcium applications result in positive effects on preserving cell wall integrity and functionality, thus maintaining the firmness of fruits (Ortiz, Graell, & Lara, 2011) and delaying physicochemical modifications of fruits (Chen et al., 2011).

Regarding the application method, there are also positive results that support the use of calcium in fruit quality, even when applied onto the soil during pre-harvest, or after harvesting by immersing fruits in solution containing this mineral (Angeletti et al., 2010). However, calcium is a poorly transferred nutrient in the plant system, due to its low mobility in phloem (Danner, Citadin, Sasso, Zarth, & Mazaro, 2009). Therefore, Ca is not translocated to fruits when applied on leaves. Thus, spraying applications directed at fruits becomes crucial, which is also the most adopted application method. Given all the above, the current study aimed to evaluate the effect of a pre-harvest spraying application of calcium chloride on the fruit quality of the 'Tupy' blackberry.

Matherial and methods

Location and characterization of the experimental area

The experiment was performed at the Experimental Farm for the School of Agriculture (FCA/UNESP), located in the city of São Manuel, São Paulo, Brazil (22°44'28" S, 48°34'37" W, at an altitude of 740 m). According to the Köppen climate classification, the area is classified as Cwa, that is, temperate hot (mesothermic). Rainfall is concentrated from November to April, and the average annual rainfall is 1,370 mm. The hottest month in São Manuel has an average temperature of 22°C (Cunha & Martins, 2009). The soil of the area is classified as a typical Dystrophic Red Latosol (Embrapa, 2006).

In June 2009, an orchard was planted (4.0 m between rows and 0.6 m between plants). Plants were cultivated with six more vigorous stems, under a T-flat system with two wires approximately 1.2m tall. Furthermore, soil fertilizations were carried out based on soil analyses (Table 1) for the experimental area and crop recommendations (Freire, 2007). Plants were grown in a rain-fed system and received all suggested cultural practices for blackberry.

Table 1. Soil analyses for the experimental area in the crop cycles of 2014 and 2015. São Manuel, São Paulo State, Brazil.

2014												
Layer (cm)	pH $CaCl_2$	O.M. g dm^{-3}	P_{resin} mg dm^{-3}	Al^{3+}	H+Al	K	Ca	Mg	SB	CEC	V%	S mg dm^{-3}
				mmolc dm^{-3}								
0-20	4.7	6	15	1	21	3.7	19	6	29	50	58	4
20-40	4.9	5	8	0	16	4.1	22	6	32	48	66	4

	B	Cu	Fe	Mn	Zn
	mg dm^{-3}				
0-20	0.21	1,1	28	24.7	1.3
20-40	0.14	0,9	18	12.8	0.7

2015												
Layer (cm)	pH $CaCl_2$	O.M. g dm^{-3}	P_{resin} mg dm^{-3}	Al^{3+}	H+Al	K	Ca	Mg	SB	CEC	V%	S mg dm^{-3}
				mmolc dm^{-3}								
0-20	5.2	8	15	0	13	1.3	13	5	19	33	59	5
20-40	5.3	8	10	0	13	1.2	14	5	21	33	62	6

	B	Cu	Fe	Mn	Zn
	mg dm^{-3}				
0-20	0.24	1.,3	16	15.9	1.4
20-40	0.26	1.3	14	13.3	1.2

Source: Soil Fertility Laboratory. Department of Soils and Environmental Resources, UNESP/FCA.

Treatments and experimental design

A 4 x 3 (calcium chloride x application number) factorial experiment was carried out in a randomized block design with four replications (4 plants per plot), three of them useful and one along the border, totalling 144 useful plants. The first factor related to the calcium chloride concentrations tested (0, 1.5, 3, and 4.5%) and the second to the number of applications (1, 2, and 3). The experiment was evaluated in two agricultural cycles during 2014 and 2015.

Furthermore, fruits were harvested at the shiny-black stage, without any red drupes, which is the ideal blackberry harvesting point.

Conducting the experiment and harvesting the fruits

The application of calcium chloride took place at the beginning of October each year, with a costal spray. The product was diluted in water with a 1% adhesive spreader (Assist®) for all treatments. The applications were performed on sunny days, and the spray volume used per plant was 250 mL. The first application was performed when fruits were in the flower bud swelling phenological stage. Furthermore, multiple applications were conducted at 5-day intervals. Then, fruits were harvested for evaluation eleven days after the last application.

Variables evaluated

Ca^{+2} content in leaves and fruits: measured in leaves before and after $CaCl_2$ applications, by means of foliar analysis, and soon after harvesting in fruits. Leaf sampling was performed by collecting the sixth fully expanded leaf with pedicel, counted from the apex. Each treatment sample consisted of 80 leaves. To evaluate calcium content in fruits, as well as physical, physicochemical and biochemical (bioactive compounds) components, 20 mature fruits (shiny-black colour) were collected per replicate, totalling 80 fruits per treatment.

Average mass: obtained by weighing fruits in a semi-analytical balance and expressed in grams (g).

Length: obtained with a digital caliper (Starret 799) and expressed in millimetres (mm).

Diameter: obtained with a digital caliper (Starret 799) and expressed in millimetres (mm).

Firmness: determined in fruits with a texturometer (TA.XT Plus Texture Analyzer). Texture was analysed by penetration test with a SMS P/2N probe and 5.0 mm s^{-1} velocity. The reading was carried out at two different centres of the fruit, expressed in Newtons (N).

Mass loss: fruits were packed in trays of expanded polystyrene and wrapped in polyvinyl chloride film (PVC), stored in a cold room at 5 ± 1°C and RU 85 ± 5%, and weighted daily over six days. The fruit mass difference (plot) between intervals was calculated, expressed as percentage, by the equation: % fresh weight loss = ((MI - MA / MI) x 100), where MI is the initial mass (day zero) and MA is the mass on the evaluation day.

Ascorbic acid: performed by means of titration with a DCFI reagent until persistent pink staining, according to MAPA methodology (1986).

Titratable acidity (TA): expressed as a percentage of citric acid, titrated with 1.0 mol L^{-1} sodium hydroxide (NaOH), in 1 ml juice solution, 50 mL distilled water and 0.3 mL phenolphthalein (Instituto Adolfo Lutz, 2005).

Soluble solids (SS): expressed in °Brix, measured using a digital refractometer.

Maturity index: obtained by means of the relationship between soluble solids and titratable acidity contents.

Flavonoids: determined according to the method described by Popova et al. (2004) and adapted by Silva et al. (2016). The extraction was carried out in acidified acetone (70%) and absorbance was measured at 425 nm in a spectrophotometer (BEL Photonics®, SP 2000 UV/VIS). The results were expressed in mg equivalent of rutin per 100 g fresh weight.

Total anthocyanins: performed according to methodology proposed by Sims and Gamon (2002), with extraction in acetone solution (80% buffered TRIS pH 7.2). Readings were performed at 537 nm. All steps were performed in a dark environment, and the results were expressed in mg 100 g^{-1} of sample.

Total polyphenols: determined according to Swain and Hills (1959) methodology by means of the sample reaction with a Folin-Ciocalteau reagent. Samples were extracted in pure acetone, and readings were performed at 725 nm using a spectrophotometer. The results were expressed as mg of gallic acid per 100 g fresh weight.

Antioxidant activity: performed according to the methodology of Brand-Williams, Cuvelier, and Berset (1995), based on the use of the DPPH reagent (2,2-Diphenyl-1-picrylhydrazyl) (Sigma Aldrich, Brazil). Samples were extracted into pure acetone, and readings were carried out at 517 nm. The calibration curve was prepared with standard ascorbic acid (Merk®), and the results were expressed in μmol of ascorbic acid per g of sample.

Reducing and total soluble sugars: used the methodology described by Somogy and adapted by Nelson (1944). Readings were performed in a spectrophotometer at 535 nm and results were expressed as a percentage.

Statistical analysis

Statistical analyses were performed on the arithmetic average of each characteristic evaluated in two agricultural cycles (2014 and 2015), excluding the effect of cycles, to determine the effects of $CaCl_2$ applications. A normality test (Kolmogorov-Smirnov) on the means was applied, and then, the data were submitted to analysis of variance at 1 and 5% levels of significance. When significant, a regression analysis was performed for data corresponding to the concentration factor of calcium chloride. For the factor number of applications, a Tukey's test was performed at the 5% level of significance to compare the means.

For the mass loss evaluation, in addition to the mentioned factors, a factor was added for days after harvesting; therefore, a triple factorial (concentrations x applications x days after harvesting) was carried out on this variable. When the variance was significant, a regression analysis was performed.

It should be noted that because of non-normal data for calcium in leaves and fruits, mass loss, ascorbic acid and reducing sugar, a square root of x + 0.5 transformation was applied.

Results and discussion

There was a significant interaction between $CaCl_2$ concentration and the number of application amounts on the Ca in the leaves (Table 2). A quadratic effect was observed between $CaCl_2$ concentrations and 2 or 3 applications in the leaves. The results indicated that two applications promoted a decrease in Ca^{+2} content in the leaves (9.9 g kg^{-1}) at the minimum estimated point of 0.7% $CaCl_2$, whereas at three applications, there was a quadratic decrease until the estimated concentration of 0.5% $CaCl_2$ to give 10.2 g kg^{-1} of Ca^{+2}. However, from these estimated concentrations, there was a quadratic increase in the averages up to 4.5% of $CaCl_2$, obtaining 12.5 (2 applications) and 13.0 g kg^{-1} (3 applications) of Ca^{2+} in the leaves; these averages were higher than that obtained with an application of the same concentration (Figure 1a). In the city of São Mateus do Sul (state of Paraná), 'Tupy' presented 0.96% calcium concentration in plants, leaves and pruned stems (Ferreira et al., 2013).

Table 2. Values of the F test, coefficients of variation and P-value of the normality test for the amount of Ca in leaves and fruits, fruit diameter, fruit length, fruit mass and firmness of blackberries, as a function of $CaCl_2$ concentrations and number of applications.

SV	Amount of Ca in leaves	Amount of Ca in fruits	Fruit diameter	Fruit length	Fruit mass	Firmness
Blocks	0.4^{ns}	0.5^{ns}	4.5**	4.1*	5.1**	2.4^{ns}
Concentrations (A)	73.6**	1.9^{ns}	10.1**	3.7*	2.0*	1.6**
Applications (B)	21.7**	1.9^{ns}	1.8^{ns}	0.1^{ns}	0.1^{ns}	1.1^{ns}
A x B	10.5**	3.4**	2.6*	0.2^{NS}	0.5^{ns}	0.5^{ns}
VC (%)	1.7	3.9	2.5	2.9	7.3	19.9
P-value[1]	<0.01	<0.01	>0.15	0.05	0.14	>0.15

^{ns} Not significant, * Significant at 5% probability, ** Significant at 1% probability. [1]P-value of the Kolmogorov-Smirnov normality test: values higher than 0.05 indicate normality of the data; data with values smaller than 0.05 were transformed (square root of x + 0.5).

When a single application was performed, no effect was observed on the contents of Ca^{+2} in the leaves (Figure 1a). This result can be related to the fact that at the concentrations used, the amount of calcium applied in a single application was not enough to affect the already existing content. This result was different from when 2 or 3 applications were carried out, as observed, which promoted an increase in the calcium content in the leaves.

Similar to the content of Ca^{2+} in the leaves, an interaction between the concentrations and the number of applications for Ca^{2+} content in the fruits was also verified (Table 2). However, there was a significant effect only when a single application was used. There was an increase in the content up to the maximum estimated concentration of 2.2%, which resulted in 2.9 g kg^{-1} of Ca^{2+} in the fruits.

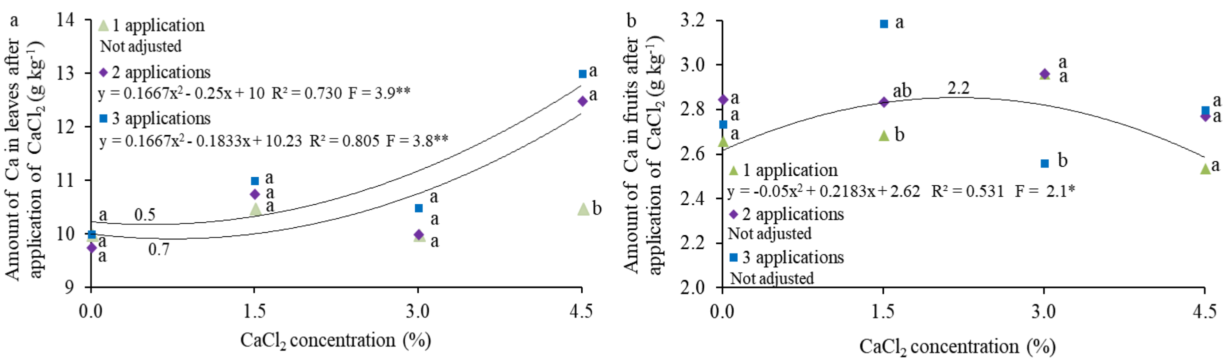

Figure 1. Calcium content in leaves (a) and fruits (b) of 'Tupy' blackberry after the $CaCl_2$ application during pre-harvest, as a function of $CaCl_2$ concentrations and number of applications. São Manuel, state of São Paulo, Brazil. 2014 and 2015.

The current study showed higher results of Ca content in fruits (Figure 1b) than those found by Souza, Rodrigues, Gomes, Gomes, and Vieites (2015) (1.44 g kg^{-1}) and Guedes et al. (2013) (1.16 g kg^{-1}) for the same blackberry cultivar. These differences likely occurred due to several factors, such as the maturation stage of the fruits. According to Souza et al. (2015), calcium content has a slight tendency to fall during the most advanced harvest stage of blackberries (i.e. 100% black). The higher Ca^{+2} content resulting from an application at the concentration of 2.2% is of great importance because according to Taiz, Zeiger, Møller, and Murphy (2017), this mineral provides great firmness, increases nutritional value, acts in cell wall synthesis, functions as a secondary messenger in response to biotic and abiotic signals, and is required as a cofactor by enzymes that participate in the hydrolysis of ATP.

Mass loss was significantly influenced by the interaction between $CaCl_2$ concentrations and number of applications (Table 3). For the number of applications, there was a linear decrease in the averages as a function of the concentration (Figure 2a). However, when one application was used, there was less mass loss at the concentration of 1.5%. At concentrations of 3.0 and 4.5%, one or three applications resulted in lower mass loss (Figure 2a). Therefore, it is advisable to use the highest concentration (4.5% $CaCl_2$) in a single application, consequently saving either time and hand labour.

Table 3. Values of the test F, coefficients of variation and P-value of the normality test for mass loss in blackberries, as a function of $CaCl_2$ concentrations and number of applications.

SV	Mass loss
Blocks	24.3**
Concentrations (A)	20.1**
Applications number (B)	22.7**
Storage period (C)	4330.6**
A x B	2.1*
A x C	4.1**
B x C	1.9*
A x B x C	0.8ns
VC	6.94
P-value[1]	<0.10

ns Not significant, * Significant at 5% probability, ** Significant at 1% probability. [1] P-value of the Kolmogorov-Smirnov normality test: values higher than 0.05 indicate normality of the data; data with values smaller than 0.05 were transformed (square root of x + 0.5).

In the current study, the sixth day became the storage time limit, so that fruits did not present a significant decrease in quality (absence of rotting and odour) under cold chamber conditions (at 5 ± 1°C and RU 85 ± 5%). The evaluation of mass loss ceased on the sixth day in fruits because fruits, in general, started to show signs of mould or deterioration on the seventh day, making fruits inappropriate for commercialization and consumption.

As observed, the use of $CaCl_2$, regardless of the concentration used and the number of applications, promoted reduction of mass loss, proving to be an important technique for the conservation of blackberry. According to Chitarra and Chitarra (2005), mass loss can be considered harmful, because it reduces fruit quality. An eficiência do uso de cálcio na redução da perda de massa já foi observada em outras frutas, i.e. Al-Eryani-Raqeeb et al. (2008) obtained a significant reduction in mass loss for 'Eksotica II' papaya in a post-harvest study with calcium chloride applied by vacuum infiltration, whereas Angeletti et al. (2010) observed reduced fruit mass loss in 'O'Neal' and 'Bluecrop' blueberries, when calcium was applied onto soil (fertilization with $CaSO^4$) during pre-harvest.

The reduction in the mass loss with the use of $CaCl_2$, observed in this study, is related to the fact that Ca2+ ions increased cell wall stability by binding non-esterified pectins, increasing resistance to water flow, and making it difficult for water to exit the cell (Angeletti et al., 2010).

Regardless of $CaCl_2$ concentration and number of applications, a quadratic increase was observed in mass loss, as a function of storage time (Figure 2b and c). After six days of storage, the concentrations of 3.0 and 4.5% allowed less mass loss (Figure 2b). Therefore, there was lower mass loss for a single application on the fifth and sixth days of storage (Figure 2c). The best results observed for a single application may be related to the fact that with 2 and 3 applications, there was excess calcium in the fruits, which promotes damage to the plant tissue.

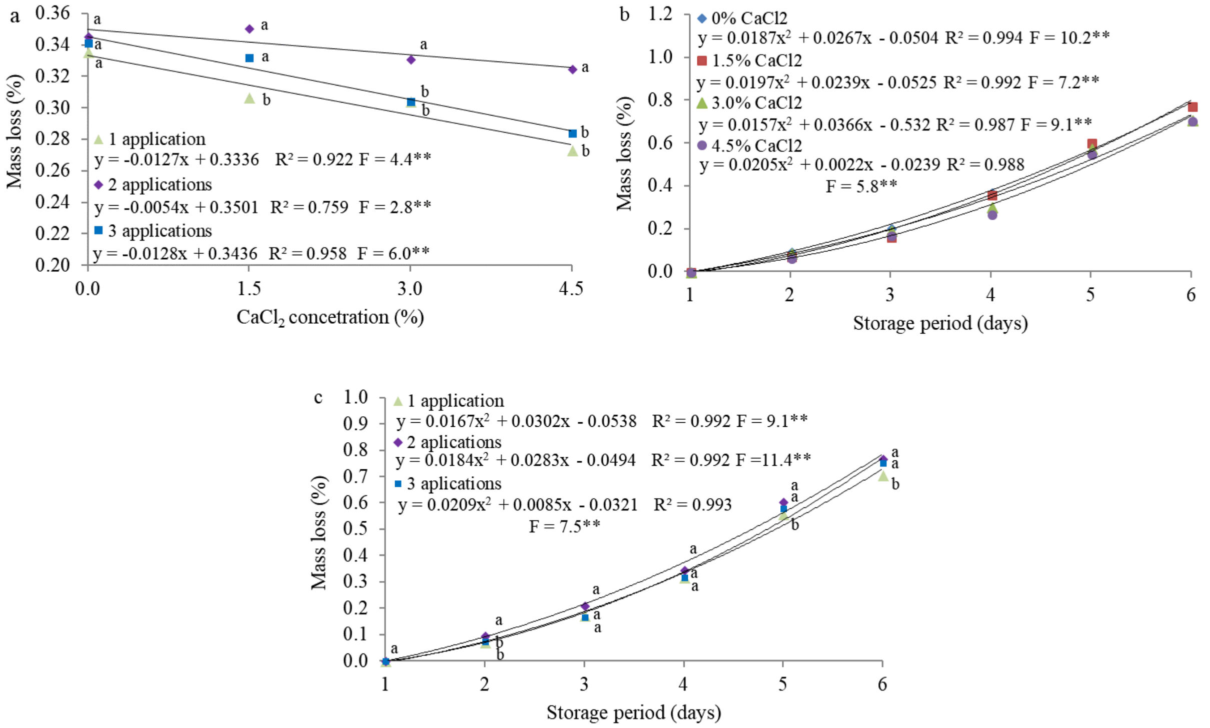

Figure 2. Mass loss of 'Tupy' blackberry fruits, as a function of the number of applications x $CaCl_2$ concentrations (a), $CaCl_2$ concentrations x storage period (b) and number of applications x storage period (c). São Manuel, state of São Paulo, Brazil. 2014 and 2015.

The only interaction for physical traits was observed in fruit diameter between concentrations and number of applications (Table 2). Moreover, the other traits only presented isolated effects of $CaCl_2$ concentrations (Table 2).

The diameter of the fruits linearly increased when three applications were performed, whereas when a single application was used, the means decreased slightly to the estimated concentration of 2.0% $CaCl_2$; they increased again from this point, with the maximum value observed with a concentration of 4.5%. However, when three applications with a concentration of 4.5% were carried out, blackberry diameter increased (Figure 3a). In São Manuel (state of São Paulo), Segantini et al. (2015) found diameters from 18 to 23.60 mm in non-irrigated plants without $CaCl_2$ application for 'Tupy' blackberry. Moreover, Campagnolo and Pio (2012) observed a mean diameter of 23.6 mm in Marechal Cândido Rondon (state of Paraná). These values are similar to those observed in the current study (21.27 to 23.10).

The increase in diameter obtained with the use of calcium may be the result of the action of this mineral on fruit growth because calcium acts in several metabolic processes, such as division, differentiation, polarity and cell elongation, according to White and Broadley (2003).

With regard to fruit length and fresh mass, the means of both traits increased in a quadratic form until the estimated concentrations of 2.4 and 1.9% $CaCl_2$, which allowed the fruit to obtain a length of 28.0 mm and a fresh mass of 7.1 g. (Figure 3b and c). Campagnolo and Pio (2012) found a lower value of fresh mass of 'Tupy' blackberry (5.3 g). The $CaCl_2$ concentration values cited above decreased fruit length and mass average (Figure 3b and c). These results can be explained by the increase of calcium in the cytosol from higher concentrations, causing physiological disorders in cells that negatively affected these traits.

In an experiment with the Florida King peach tree, Ali, Abbasi, and Hafiz (2014) observed higher fresh mass (148.3 g) and fruit diameter (65.9 mm) in plants that received 1% $CaCl_2$ via spraying, when compared with the control; however, higher $CaCl_2$ concentrations demonstrated toxic effects.

When fruit firmness was evaluated, a linear increase was observed in the means, as a function of enhancing $CaCl_2$ concentrations, reaching the maximum value at 4.5% $CaCl_2$ (Figure 3d). One characteristic of Ca is to maintain cell wall and middle lamella integrity because it fixes the bonds between pectin molecules. According to Poovaiah (1986), calcium maintains fruit pulp firmness by forming connections between cell wall pectins and the middle lamella, promoting greater stability. In addition, high levels of calcium in fruit apoplast may contribute to the persistent inhibition of pectin hydrolysis during ripening (Huang, Liu, Lu, & Xia, 2007). Therefore, the greater fruit firmness that occurred with the highest concentration of $CaCl_2$ can be explained.

Similar to this study, other authors have reported the effect of calcium on steady fruit in various fruit types. In an experiment with 'Tupy' blackberry, Ferreira et al. (2013) evaluated the number of calcium applications (0, 2, 4, and 8 applications) in the form of liquid foliar fertilizer that contained 19% calcium and 2% boron. They reported that there was no effect of the product on fruit firmness. However, Ortiz et al. (2011) evaluated pre-harvest calcium application on 'Fuji Kiku 8' apples and found greater fruit firmness with mineral treatments. Similarly, Angeletti et al. (2010) evaluated pre-harvest calcium application on blueberry and verified greater fruit firmness.

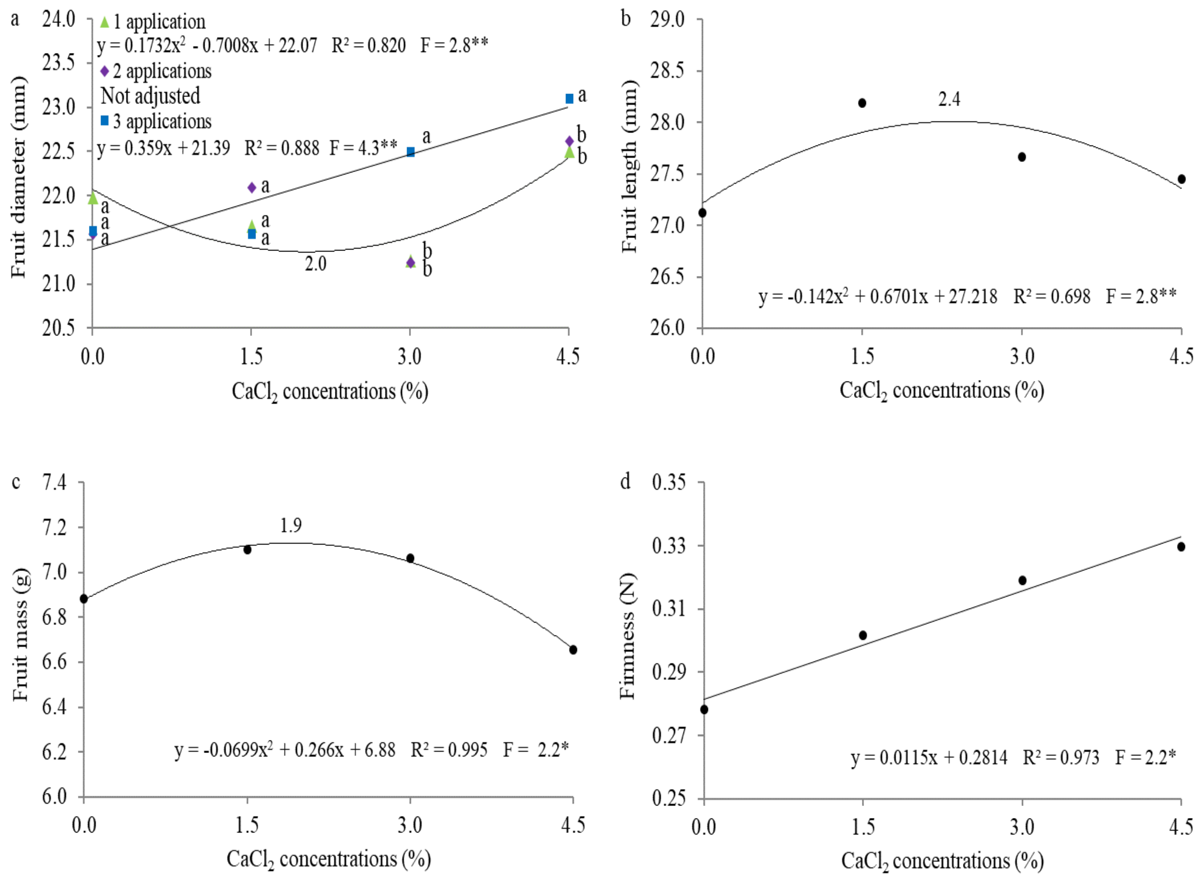

Figure 3. Fruit diameter as a function of the $CaCl_2$ concentrations and number of applications (a), length (b), fresh mass (c) and firmness (d) of 'Tupy' blackberry as a function of the different concentrations of $CaCl_2$ applied during pre-harvest. São Manuel, state of São Paulo, Brazil. 2014 and 2015.

Regarding physicochemical variables, there was no significant interaction between factors for any traits; the same occurred when application numbers were evaluated. However, the $CaCl_2$ concentration was significant for soluble solids, titratable acidity and reducing soluble sugar ($p < 0.05$), and ascorbic acid and total soluble sugar ($p < 0.01$) (Table 4).

Table 4. Values of the F test, coefficients of variation and P-value of the normality test for soluble solids (SS), titratable acidity (TA), maturity index (MI), ascorbic acid (AA), reducing soluble sugar (RSS), total soluble sugar (TSS), antioxidant activity (AAC), total polyphenols (POL), total flavonoids (FLAV), and total anthocyanins (ANT) in blackberries, as a function of $CaCl_2$ concentrations and number of applications.

SV	SS	TA	MI	AA	RSS	TSS	AAC	POL	FLAV	ANT
Blocks	0.8^{ns}	2.8*	2.9^{ns}	0.3^{ns}	0.7^{ns}	0.7^{ns}	2.5^{ns}	4.6**	0.3^{ns}	0.1^{ns}
Concentrations (A)	1.8*	1.5*	1.3^{ns}	22.6**	4.4*	3.3**	5.6**	20.9**	2.7^{ns}	0.6^{ns}
Applications (B)	0.5^{ns}	0.2^{ns}	1.3^{ns}	0.3^{ns}	3.4^{ns}	1.0^{ns}	3.7^{ns}	3.3^{ns}	1.7^{ns}	0.4^{ns}
A x B	0.7^{ns}	0.7^{ns}	0.8^{ns}	1.2^{ns}	0.3^{ns}	0.4^{ns}	0.7^{ns}	1.7^{ns}	1.6^{ns}	1.4^{ns}
VC (%)	7.9	11.2	16.7	10.0	6.4	18.7	21.1	10.3	24.8	36.0
P value[1]	>0.15	>0.15	0.09	<0.10	0.02	>0.15	>0.15	>0.15	>0.08	>0.15

^{ns} Not significant, * Significant at 5% probability, ** Significant at 1% probability. [1] P-value of the Kolmogorov-Smirnov normality test: values higher than 0.05 indicate normality of the data; data with values smaller than 0.05 were transformed (square root of x + 0.5).

Furthermore, when the application of $CaCl_2$ increased, the means of soluble solids and total and reducing sugars decreased, reaching the lowest value when applied at the highest concentration (Figure 4a, e, and f). The increase in soluble solids and reducing sugars as a function of increasing calcium concentrations is due, according to Angeletti et al. (2010), to the fact that calcium reduces pectin solubilization; consequently, this scenario slows down cell-wall disruption. The increase in sugar content in fruits comes from the natural disruption of the cell wall, which is promoted by pectolytic enzymes, so that there is an increase in the solubility of polysaccharides rich in neutral sugars (Vicente et al., 2007).

Moreover, the increase of applied $CaCl_2$ concentrations promoted a quadratic reduction in titratable acidity of 'Tupy' blackberry, with the minimum value (1.3 mg citric acid per 100 g) reached with 2.5% $CaCl_2$ (Figure 4b). However, means increased again from this point. The maintenance of fruit acidity is crucial because it guarantees product flavour and odour (Chitarra & Chitarra, 2005). Several current studies identified different results for the effects of calcium treatment on titratable acidity. For example, Omaima and Karima (2007) verified a decrease in titratable acidity under pre-harvest application of $CaCl_2$ (0.4%) in 'Anna' apples, whereas Ali et al. (2014) reported higher titratable acidity in peaches treated with 1% $CaCl_2$, but observed that results did not differ from untreated plants at 2 - 3% concentrations.

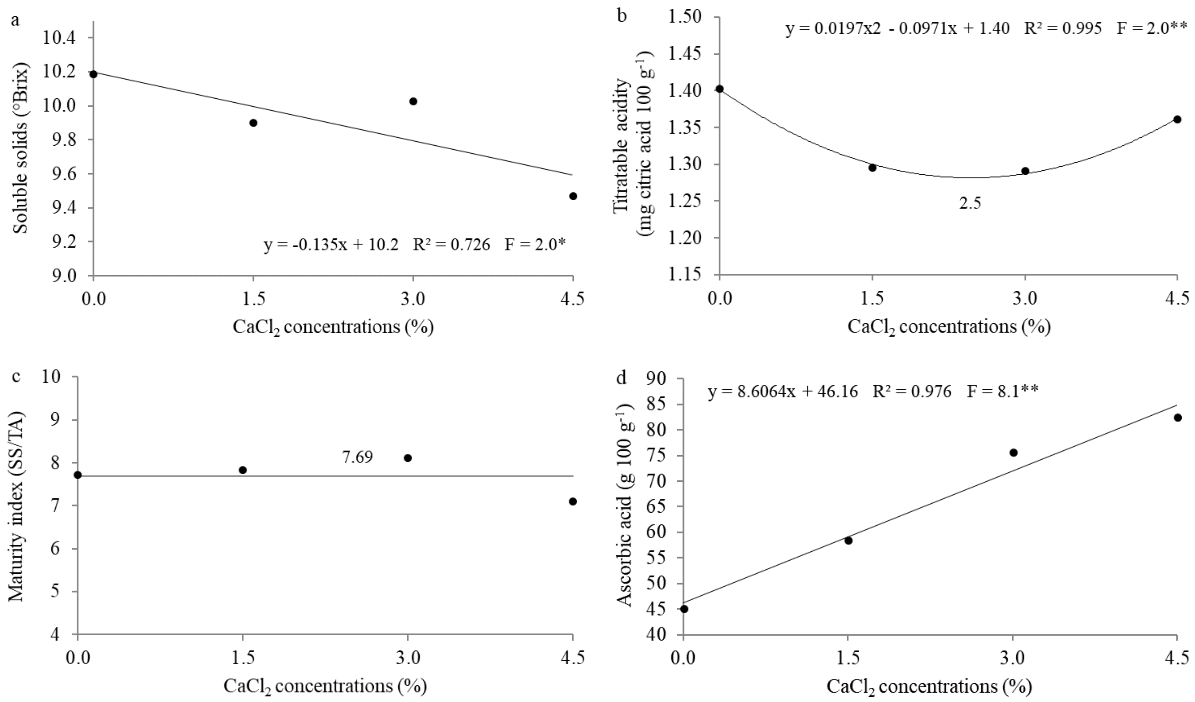

Figure 4. Soluble solids (a), titratable acidity (b), maturity index (c), ascorbic acid (d), reducing soluble sugar (e) and total soluble sugar (f) of 'Tupy' blackberry under different $CaCl_2$ concentrations applied during pre-harvest. São Manuel, state of São Paulo, Brazil. 2014 and 2015.

With regard to the maturity index, Ferreira et al. (2013) also stated that this variable was not affected by calcium application for different numbers of pre-harvest applications in the Tupy cultivar, similar to the current study (Figure 4c). Thus, the ripeness index was obtained between the soluble solids and titratable acidity relationship, which is expressed by the sweet taste of fruits. It was noted that even with the lowest sugar content because acidity was also affected, such traits remained the same, even in fruits with a higher amount of sugar, which indicates that $CaCl_2$ applications did not affect sweet taste expression.

Ascorbic acid content showed a linear increase in the means, as a function of increasing $CaCl_2$ concentrations, reaching a maximum value with 4.5% $CaCl_2$ (Figure 4d). This result occurred due to the increase in the calcium content in the intercellular spaces; this ion can be translocated to the cell cytoplasm to improve the ascorbate-glutathione cycle, consequently leading to great synthesis of ascorbic acid (Huang et al., 2007). Ascorbic acid content can be used as a food quality index. The application of the highest concentration of calcium chloride (4.5%) during pre-harvest in 'Tupy' blackberry generated fruits with high levels of ascorbic acid. Similar to this study, Ali et al. (2014) verified higher levels of ascorbic acid in peaches during pre-harvest application of 1% $CaCl_2$, and when 2 - 3% applications were used, means did not differ from control.

Regarding the bioactive compounds, there was no significant interaction between the concentrations and the number of $CaCl_2$ applications. Evaluating the isolated factors, there was a significant effect of the concentrations for antioxidant activity and total polyphenols (Table 4). When antioxidant activity and total polyphenols were evaluated, the increase in $CaCl_2$ concentrations promoted a mean reduction of these traits, reaching the minimum values (0,55 μmol g $^{-1}$ and 1269 mg 100 g^{-1}, respectively) by the time the highest $CaCl_2$ concentration (4.5%) was applied (Figure 5a and b). However, these values in antioxidant activity and total polyphenols can be considered high; therefore, the use of higher concentrations does not affect, in a pronounced way, the functional value of the fruits.

Evaluating the antioxidant activity of 'Tupy' blackberry (100% black), Souza, Vieites, Gomes, and Vieira (2018) found an average of 0.29 μmol g $^{-1}$. These authors stated that fruit antioxidant capacity has been reported in the literature by the direct relationship between total phenol content and anthocyanins. However, the use of $CaCl_2$ did not significantly affect total flavonoid and anthocyanin content in the current study (Figure 5c and d). Angeletti al. (2010) also reported an absence of calcium effect, when applied onto the soil, on total anthocyanin content in blueberries.

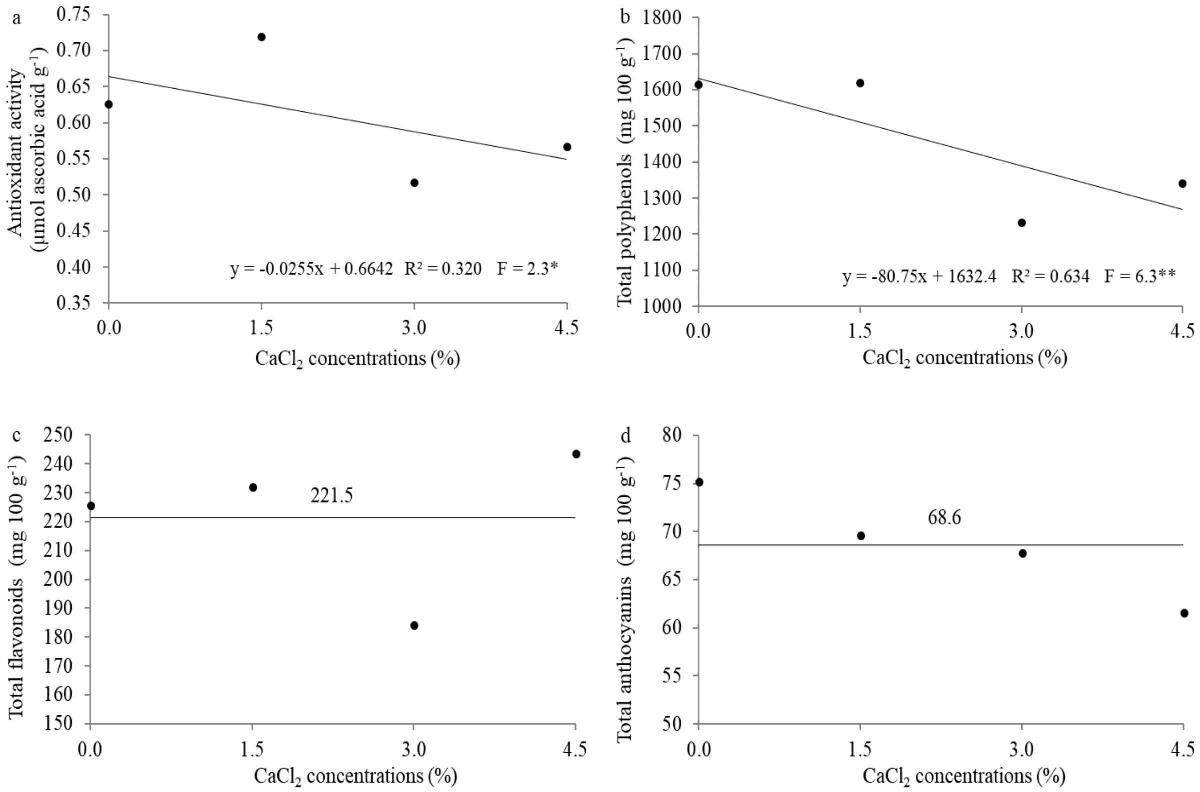

Figure 5. Antioxidant activity (a), total polyphenols (b), total flavonoids (c) and total anthocyanins (d) of 'Tupy' blackberry, as a function of different $CaCl_2$ concentrations, applied during pre-harvest. São Manuel, state of São Paulo, Brazil. 2014 and 2015.

Considering the results, it appears that the use of a single $CaCl_2$ application is more advantageous, since few characteristics were affected by the number of applications, the use of only one application also reduces expenses with hand labour.

Regarding the recommended concentration, it should be considered that for some characteristics there was a linear effect, while for others, the effect was quadratic. Mass loss decreased linearly to 4%, a positive result, however, sugars, antioxidant activity and phenolic compounds also decreased to this concentration, which is not interesting. Firmness and ascorbic acid content increased linearly to the highest dose, while the highest lengths and mass were observed between 1.5 and 3%, followed by lower acidity. Thus, the use of concentrations between 1.5 and 3% $CaCl_2$, with single application, is more interesting for blackberry, since it results in fruits with greater mass and length, as well as intermediate values of loss of mass, firmness, content. ascorbic acid, avoiding very low sugars, antioxidant activity and phenolic compounds.

Conclusion

In general, there were positive effects of calcium chloride applications on physical and chemical characterization and bioactive compounds for the fruit characteristics of 'Tupy' blackberry. A single 2.2% $CaCl_2$ application promoted a higher content of this nutrient in fruits; at 4.5%, there was decreased mass loss. The number of applications did not affect the physical, physicochemical and bioactive compound traits of the fruits. The use of $CaCl_2$ resulted in fruits with greater firmness and higher content of ascorbic acid, as well as greater size and fruit mass. However, it promoted a decrease in soluble solids, reducing and total sugars, antioxidant activity, and total polyphenols.

Acknowledgements

We acknowledge CNPq, process (304571/2013-0, 303373/2014-8, 406341/2017-6) and CAPES for financial support.

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