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Calcium foliar fertilization and its effect on quality and shelf life in andean blackberry fruits (*Rubus glaucus* Benth.)

Fertilización foliar con calcio y su efecto sobre calidad y vida útil en frutos de mora (*Rubus glaucus* Benth.)

William Andrés Cardona[1]*; María Cristina García-Muñoz[2]; Blanca Lucía Botina-Azain[3]; Clara Viviana Franco-Flórez[4]; Pablo Edgar Jiménez-Ortega[5]

[1]Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA, Centro de investigación Tibaitatá. Mosquera - Cundinamarca, Colombia; e-mail: wcardona@agrosavia.co; mcgarcia@agrosavia.co; bbotina@agrosavia.co; cfranco@agrosavia.co; pjimenez@agrosavia.co
*corresponding author: wcardona@agrosavia.co

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ABSTRACT

Calcium is the third most crucial nutrient for andean blackberry; however, its absorption is limited in acid soils, and its low mobility in the plant decreases its effects on fruits. Therefore, the effect of foliar fertilizers with calcium on andean blackberry fruits was estimated. In three locations, experiments were established with five calcium sources (Ca chelate, CaB nitrate, Ca oxide, CaB and CaBZn gluconate) and a control without calcium, applied in three phenological stages and recording: weight, diameters, firmness, color, juice, pulp, pH, soluble solids, acidity, dehydration, and damage. Soil and tissue analysis was performed to identify the relationship between the plant's nutritional conditions and its fertilization response. For the statistical analysis were used mixed models, tests of means, and principal components. Foliar fertilization with chelate, nitrate, and calcium oxide in andean blackberry crops with thorns, in production, with pruning management, and in the phenological stages of flower bud, fruit set, and red fruit, is a viable alternative to improve firmness, weight, and diameter of the fruits. In contrast, the chemical and color parameters in andean blackberry fruits depend on the edaphoclimatic conditions of each zone. This fertilization should be considered as a complement in soils without acidity problems and with balanced cationic saturations.

Keywords: Dehydration; Fruit firmness; Fruit quality; Plant nutrition; Storage.

RESUMEN

El calcio es el tercer nutriente más importante para la mora; sin embargo, su absorción se ve limitada en suelos ácidos y su baja movilidad en planta disminuye su efecto en frutos. Por tanto, se estimó el efecto de fertilizantes foliares con calcio en frutos de mora. En tres localidades, se establecieron experimentos con cinco recursos de Ca (quelato de Ca, nitrato de CaB, óxido de Ca, KCaB y gluconato de CaBZn) y un control sin calcio, aplicados en tres etapas fenológicas y registrándose: peso, diámetros, firmeza, color, jugo, pulpa, pH, sólidos solubles, acidez, deshidratación y daños. Se realizó análisis de suelo y tejido, para identificar la relación entre las condiciones nutricionales de la planta y su respuesta a la fertilización. Para el análisis estadístico, se utilizaron modelos mixtos, pruebas de medias y componentes principales. La fertilización foliar con quelato, nitrato y óxido de calcio en cultivos de mora andina con espinas, en producción, con manejo de podas y en las etapas fenológicas de botón floral, cuajado y fruto rojo, es una alternativa viable para mejorar la firmeza, peso y diámetro de los frutos. En contraste, los parámetros químicos y de color en frutos de mora andina dependen de las condiciones edafoclimáticas propias de cada zona. Esta fertilización, se debe considerar como complemento en suelos sin problemas de acidez y con saturaciones catiónicas equilibradas.

Palabras clave: Deshidratación; Firmeza del fruto; Calidad del fruto; Nutrición vegetal; Almacenamiento.

INTRODUCTION

Andean blackberry is one of the most economically valuable fruits in Colombia, Ecuador, and Costa Rica. During 2019, Colombia's area harvested, and production was 16,669 ha and 169,751 t (Agronet, 2021). The departments of Cundinamarca and Santander cover 45% of the area planted with andean blackberry, with Cundinamarca being the department with the largest area and national production with 26% and 34%, respectively. However, andean blackberry fruits are highly perishable in postharvest. The foliar application of low mobility nutrients such as calcium could improve the cell wall structure and the tissue resistance to avoid their damage in postharvest and enhance their physicochemical properties (Paniagua *et al*. 2013; Aghdam *et al*. 2012).

Calcium is the third most crucial macronutrient in the andean blackberry crop, after nitrogen and potassium. Its insufficient levels are related to high aluminum saturation in the soil displacing potassium, calcium, and magnesium and reducing their availability (Cardona & Bolaños-Benavides, 2019). Adequate plant's nutrition is reflected in the fruit's productivity and quality, then, inadequate management of the edaphic fertilization causes antagonism between nutrients, for example, NH_4^+, K^+, and Mg^{+2} affect the plant entrance of Ca^{+2}, which as a secondary nutrient is the most influential in fruit yield and quality (Cardona & Bolaños-Benavides, 2019). The calcium application by the edaphic route in the andean blackberry crop in Colombia is made through different types of limes incorporated into the soil. Its absorption in the plant is done in an early stage of development.

Due to the structural conformation of the andean blackberry fruit, calcium plays a fundamental role oriented to the strengthening and improvement of disorders in the morphological, physical, and chemical characteristics since imbalance causes physiological alterations and shortens the shelf life (Gao *et al.* 2019). In this sense, firmness is one of the essential quality properties when determining a visually striking and healthy fruit. Using calcium in fruits allows strengthening the integrity of the tissues during the conservation time. It influences membrane permeability by activating specific enzymes since it is accumulated in the middle lamella and interacts with pectic acid to form calcium pectate, redistributing the cells in a more uniform conformation, increasing firmness, and giving resistance to the fruit against pathogens (Spann & Schumann, 2010). The high calcium absorption reduces the respiration rate, ethylene production, and the incidence of physiological disorders in fruits (Tyagi *et al.* 2017). Singh *et al*. (2007) found that the application of $CaCl_2$ and boron reduced albinism, gray mold, and firmness in strawberries.

The most relevant phenological stages of the andean blackberry crop for the application of calcium foliar, considering the accumulation of dry matter of the fruit, which reaches its maximum peak in the state of red fruit (Franco & Giraldo C., 2001), are:

<u>Flower bud:</u> a propitious moment for the association of calcium with the structures.

<u>Fruit set:</u> increases sugars, antioxidant capacity, and acquires color.
<u>Development or red fruit:</u> It acquires physiological maturity.

The national market for andean blackberry has different demand segments, namely: the industry of drinks and pulps; hotels, restaurants, coffee shops; and markets fresh. Likewise, the short shelf life of the andean blackberry due to its sugary nature, as well as the rapid dehydration of the fruit cause it ferments quickly, adding urgency to the andean blackberry sales by farmers; therefore, the improvement and study of the physical and chemical properties of andean blackberry fruits take on relevant importance (SIOC, 2021).

Based on the high demand for calcium presented by the andean blackberry plant and its low availability in soils, the purpose of this research was to evaluate the effect of calcium foliar applications in pre-harvest in three localities on physicochemical parameters and the shelf life for andean blackberry fruits.

MATERIALS AND METHODS

Selection of study sites. This research was carried out in the department of Cundinamarca, which represents 26 % of the andean blackberry production registered in Colombia during 2021. San Bernardo, Silvania, and El Colegio's municipalities concentrate 8.1 % of production within this department. According to the General Study of Soils and Land Zoning of the department of Cundinamarca, soils are located within a mapping unit with Inceptisols and Andisols developed from mantles of ash volcanic on gravigenic clastic deposits. They are found in mountainous landscapes, of moderately broken relief, with slopes between 12 and 25% (IGAC, 2000). The farms had slopes greater than 15 %.

Crop selection. Andean blackberry crops with thorns (*Rubus glaucus* Benth.) were selected in the productive stage, previously doing pruning to promote flowering and have a uniform size of the plants.

Crop management. It was carried out mechanical weed management, sanitary pruning, and applications with NPK fertilizers in doses between 150 to 250 g/plant every two to three months. The only calcium application was with dolomite or agricultural lime at the transplantation time.

In this study, three factors were considered:

<u>Moments of calcium foliar application</u>. The applications were made when more than 50 % of the plants were in the respective phenological state according to each application (flower bud, fruit set, and red fruit for the first, second, and third calcium application, respectively).

<u>Localities – Municipalities</u>. L aurel alto – San Bernardo crops, located at 4°08'59" N and 74°23'22" W, at an altitude of 2,023 m, with 36 months, espalier system in vine system, and plant spacing of 2.5 m x 1.5 m. Agua Bonita – Silvania crops, located at 4°25'47" N and 74°20'07" W, at an altitude of 2,172 m, with 24 months,

espalier system in single wire and plant spacing of 3 m x 3 m. El Carmelo – El Colegio crops, located at 4°32'08" N and 74°23'28" W, at an altitude of 2,106 m, with 24 months, espalier system in vine system, and plant spacing of 3 m x 2 m.

Applied products. Selection of five source and a untreated control: Treatment 1 (3 g l^{-1} Chelate-Ca composed of 7 % N + 12 % CaO + chelate EDTA); Treatment 2 (5 g l^{-1} Nitrate-CaB composed of 15.45 % N + 25.5 % CaO + 0.3 % B); Treatment 3 (5 cm^3 Oxide-Ca composed of 21.4 % CaO); Treatment 4 (2 cm^3 KCaB composed of 3.55 % K_2O + 5.6 % CaO + 1 % B); Treatment 5 (2 cm^3 Gluconate-CaBZn composed of 14 % CaO + 3 % B + 3 % Zn + 7 % OC + chelate gluconate); and Treatment 6 (without-Ca). In all treatments, 0.5 cm^3 l^{-1} of a pH and hardness regulator was used with a concentration of 25 % organic carbon (OC) + 1 cm^3 l^{-1} of adherent with 1.5 % N + 3 % P_2O_5 + 19 % OC. The treatments were applied with a manual sprayer on both sides of the plant.

These factors were evaluated under a design of three completely randomized blocks, for a total of 18 experimental units (E.U.) in each location and ten plants / E.U.

Harvest. It was carried out two weeks after each application in every E.U. Two samples of 500 g of fruits were collected in a maturity stage four (MS4) under NTC 4106 (Icontec, 1997) and in PET (Polyethylene terephthalate) packaging for the individual evaluation of physicochemical parameters and shelf life.

Record of climatic variables. Information was downloaded from IDEAM pluviometric stations, installed at a linear distance <6 km from each location. The registration period was 2019, emphasizing the monthly accumulated precipitation between July and November (installation of experiments). Likewise, were recorded maximum and minimum temperatures. Between July and November, the lowest precipitation records were presented with 33.2, 32.9, and 28 % of the total annual precipitation for the municipalities of Silvania with 796 mm, San Bernardo with 1,007 mm, and El Colegio with 1,350 mm, respectively. The low precipitation made it possible to reduce the loss of foliar fertilizers by "washing". Considering the isothermal condition of Colombia, average values of the maximum temperature of 25 °C and minimum of 15 °C were recorded, which are considered adequate for the andean blackberry cultivation (Castro-Retana & Cerdas-Araya, 2005).

The following parameters were recorded as response variables in each location:

Sample size. In each sampling and measuring physical parameters, five fruits/E.U. were harvested, and for chemicals, the juice was collected from a sample composed of 15 fruits/E.U.

Weight (g), using a Mettler PE 300 digital Scale (Ohio, USA) analytical balance **Polar and equatorial diameter** (mm) with a Mitutoyo digital 8 in Vernier caliper, reference 3416 (Mitutoyo, São Paulo, Brazil).

Color parameters were determined L, a *, and b * with a Konica Minolta CR-400 colorimeter (Minolta Camera Company, Osaka, Japan).

Fruit firmness (kg). It was determined by Chatillon TCD 200 texturometer (John Chatillon & Sons, Inc., NY, USA) applying compression tests at a point in the equatorial zone of the fruit with a 15 mm diameter plate plunger, and the force applied in a 20 kg x 0.01 kg cell at a constant speed of 60 mm/min until the fruit breaks.

Juice and pulp content (%). By manually macerating the samples of the fruits of each E.U., the juice was extracted, filtered (pulp content), and weighed (juice and pulp) on a Mettler PE 300 digital Scale analytical balance (Ohio, USA). Using this juice were determined pH, total soluble solids, and titratable acidity.

Total soluble solids (TSS), pH, and titratable acidity (TA). pH was determined with a Hanna Edge pH meter (Hanna instruments HI11310 scale 0-13 (Nușfalău, Romania). TSS was determined with a Hanna HI96801 precision digital refractometer, 0-85 % (Hanna Instruments, Woonsocket, RI, USA). Titratable acidity was determined by potentiometric titration with sodium hydroxide (0.1 N) until reaching pH 8.2 (Monroy-Cárdenas *et al*. 2019).

Shelf life. The fruits were distributed in 500 g PET packages, weighed, and stored between 5 and 7 °C. For nine days, they were inspected and weighed twice per week to determine the weight loss due to dehydration, microbiological damage and/or other damages. If contaminated fruits were found, they were removed, and the remaining fruits were recorded and weighed.

Soil and plant tissue analysis. 1 kg of soil was taken in each location before establishing the experiments to determine nutrient concentrations, cation exchange capacity, Al, Na, pH, and electrical conductivity. To analyze the nutrients in plant tissue, a month after the last application 50 leaves/treatment samples were taken in each location, the analysis procedures were done under ISO/IEC 17025.

Statistical analysis. Generalized linear mixed models were used to estimate fixed effects and interactions and compare factor levels by Student's t statistics test. A p-value of 5 % was used concerning the control to select a reasonable model for each variable. Verification of the fit of each model and normality was evaluated using Pearson's residual distribution. The statistical analysis of the concentration of nutrients in plant tissue was performed by Dunnett's test ($P \leq 0.05$) and principal component analysis (PCA). Analyzes were performed using SAS 9.4.

RESULTS AND DISCUSSION

Firmness. Triple interaction was found between evaluated factors, where the 1st application of chelate (T1) in El Carmelo and the 2nd application in Agua Bonita reduced fruits firmness. In contrast, the 3rd application in Laurel Alto produced an increase (Table 1). The use of compounds such as chelates can improve fruits' Ca^{+2} fixation (Gárate & Bonilla, 2000). Di Miro *et al.* (2005) did not find variations in the fruit firmness of peach trees through calcium chelate foliar applications.

Table 1. Effect calcium foliar fertilization on firmness, weight, and diameters in andean blackberry fruits.

Location	Parameter	Application	Treatment (source)	Estimation*	Mean values/ Application**
El Colegio (El Carmelo)	Firmness (kgf)	1st (flower bud)	T1 (Chelate-Ca)	- 0.30	
		2nd (fruit set)	T5 (Gluconate-CaBZn)	+ 0.25	
	Weight (g)	1st and 2nd	T2 (Nitrate-CaB)	+ 22% and + 20%	**1st**: 6.3 – 7.7 **2nd**: 5.7 – 7.9 **3rd**: 4.8 – 8.5
		3rd (red fruit)	T2 (Nitrate-CaB)	+ 77%	
		3rd (red fruit)	T1 and T3	+ 43% and + 45%	
		3rd (red fruit)	T4 (KCaB)	+ 28%	
	Equatorial diameter (mm)	1st and 2nd	T2 (Nitrate-CaB)	+ 7%	**1st** and **2nd**: 20.0 – 22.4 **3rd**: 19.7 – 23.0
		3rd (red fruit)	T2 (Nitrate-CaB)	+ 16.5%	
		1st (flower bud)	T5 (Gluconate-CaBZn)	+ 6%	
		3rd (red fruit)	T1, T3 and T4	+ 8% and +11%	
	Polar diameter (mm)	2nd and 3rd	T2 (Nitrate-CaB)	+ 15% and +26%	**1st**: 26.4 – 28.4 **2nd**: 25.0 – 29.0 **3rd**: 22.5 – 28.5
		3rd (red fruit)	T1 (Chelate-Ca)	+ 16%	
		3rd (red fruit)	T3 (Oxide-Ca)	+ 21%	
Silvania (Agua Bonita)	Firmness (kgf)	1st (flower bud)	T5 (Gluconate-CaBZn)	+ 0.21	
		2nd (fruit set)	T1 and T5	- 0.26	
		2nd and 3rd	T2 (Nitrate-CaB)	- 0.32 and -0.19	
		2nd (fruit set)	T3 (Oxide-Ca)	- 0.19	
	Weight (g)	1st (flower bud)	T1 and T3	- 33% and -41%	**1st**: 3.9 – 6.5 **2nd**: 5.3 – 6.8 **3rd**: 6.6 – 7.9
		2nd (fruit set)	T3 (Oxide-Ca)	+ 28%	
		2nd (fruit set)	T5 (Gluconate-CaBZn)	+ 24%	
	Equatorial diameter (mm)	1st (flower bud)	T1 and T3	- 7% and -13%	**1st**: 17.7 – 20.4 **2nd**: 19.9 – 21.5 **3rd**: 21.2 – 23.1
		2nd and 3rd	T3 (Oxide-Ca)	+ 8% and + 7%	
		2nd (fruit set)	T5 (Gluconate-CaBZn)	+ 7%	
	Polar diameter (mm)	1st (flower bud)	T1 (Chelate-Ca)	- 19%	**1st**: 21 – 27 **2nd** and **3rd**: 25 – 29
		1st (flower bud)	T3 (Oxide-Ca)	- 23%	
San Bernardo (Laurel alto)	Firmness (kgf)	3rd (red fruit)	T1 (Chelate-Ca)	+ 0.22	
	Weight (g)	1st and 2nd	T2 (Nitrate-CaB)	- 22% and - 28%	**1st**: 4.7 – 6.1 **2nd**: 5.2 – 7.3 **3rd**: 6.1 – 6.7
		2nd (fruit set)	T1 (Chelate-Ca)	- 25%	
		2nd (fruit set)	T3 (Oxide-Ca)	- 20%	
	Equatorial diameter (mm)	1st (flower bud)	T2 (Nitrate-CaB)	- 6%	**1st**, **2nd**, and **3rd**: 18.7 – 21.1
		2nd (fruit set)	T2 (Nitrate-CaB)	- 6%	
	Polar diameter (mm)	1st (flower bud)	T1 (Chelate-Ca)	- 12%	**1st**: 21.9 – 26.6 **2nd**: 23.8 – 29.0 **3rd**: 26.3 – 27.7
		1st (flower bud)	T2 (Nitrate-CaB)	- 18%	

*: increase or decrease with respect to the control fruits that did not receive fertilization (T6). **: Mean values obtained in each application.

Regarding the effect of gluconate (T5), the 1st application in Agua Bonita and the 2nd application in El Carmelo increased firmness (Figure 1). The firmness values recorded in this research are like those found by Aghdam *et al*. (2012), who determined a linear relationship between calcium applications and fruit firmness. The greater firmness can be explained by the accumulation of Ca between the cell wall and the middle lamella, which contributes to the restructuring of the integrity of both structures (Romero-Gomezcaña *et al*. 2006).

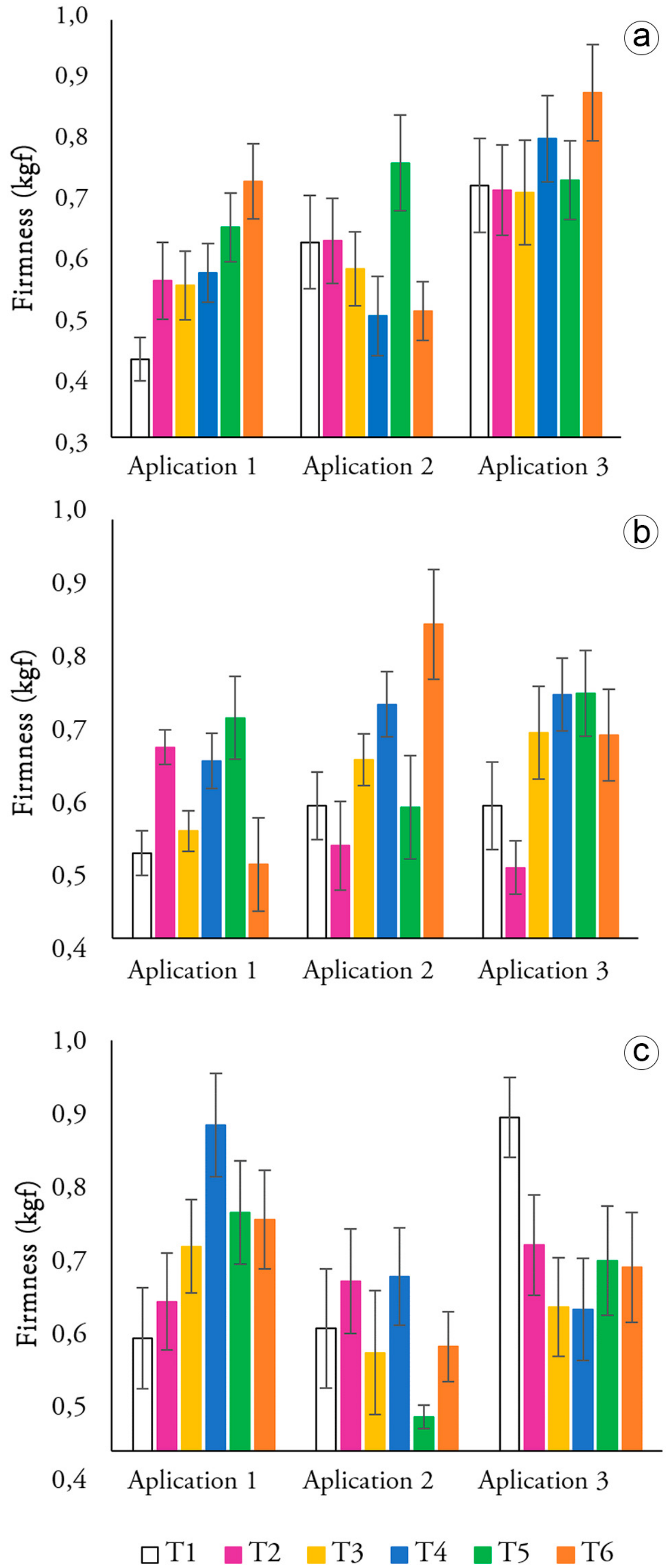

Figure 1. Effect of foliar applications of calcium on the firmness of andean blackberry fruits in the localities: a) El Carmelo; b) Agua bonita; c) Laurel alto.
Values are averages (n = 15) ± standard error. T1: Chelate-Ca; T2: Nitrate-CaB; T3: Oxide-Ca; T4: KCaB; T5: Gluconate-CaBZn and T6: Without-Ca.

In general, the applications of chelate (T1) and nitrate (T2) in El Carmelo and Laurel Alto increased the firmness over time, while the oxide (T3) allowed an increase in El Carmelo and Agua Bonita (Figure 1). These three calcium salts would be fulfilling the function of firming agents, increasing the cell wall strength, as occurs in fruits and vegetables (Rincón-Pérez & Martínez-Quintero, 2015). The calcium contributions through the application periodically of fertilizer sources produce fruits with high firmness, allowing a greater manipulation and enduring longer transportation to new markets (Monroy-Cárdenas *et al.* 2019).

Fruit weight. The nitrate (T2) applications in El Carmelo allowed increases (Table 1). Likewise, the 3rd application of chelate (T1), oxide (T3), and KCaB (T4) produced increases (P ≤0.05) (Table 1). In contrast, Di Miro *et al.* (2005) found that calcium chelate foliar applications did not produce variations in the fresh weight of peach fruits. The 1st application of T1 and T3 in Agua Bonita produced decreases (P ≤0.05) (Table 1). Contrary to the 2nd application of oxide (T3) and gluconate (T5) that allowed increases (P ≤0.05) (Table 1). Concerning Laurel alto, the 1st application with T2 showed a decrease (P ≤0.05) (Table 1). Likewise, the 2nd application of T1, T2, and T3 caused reductions (P ≤0.05) (Table 1). Yfran *et al.* (2017) did not find significant differences between the different foliar fertilization treatments with potassium, calcium, and boron (K+Ca+B) on the weight of 'Nova' mandarin fruits. In contrast, Cooman *et al.* (2005) mention that fruits gain greater weight by adding calcium.

Equatorial diameter. Applications of nitrate (T2) in El Carmelo allowed increases (P ≤0.05) (Table 1). The 3rd application of chelate (T1), oxide (T3), and KCaB (T4) caused increases (P ≤0.05) (Table 1). The increase in fruit length because of Ca foliar applications may be due to their functions in cell division, elongation, and permeability of cell membranes (Carpita & McCann, 2000). The 1st application of T3 in Agua Bonita caused reductions (P ≤0.05), while the 2nd and 3rd application of T3 allowed increases (P ≤0.05) (Table 1). The 1st and 2nd applications of T2 in Laurel alto caused decreases (P ≤0.05) (Table 1). Yfran *et al.* (2017) did not find significant differences between different foliar fertilization treatments with K+Ca+B on the equatorial diameter of 'Nova' mandarin fruits.

Polar diameter. The 2nd and 3rd applications of nitrate (T2) in El Carmelo increased (P ≤0.05) polar diameter (Table 1). After the 3rd application of chelate (T1) and oxide (T3), an increase was found (P ≤0.05); in contrast to Agua Bonita, the 1st application of T1 y T3 produced decreases (Table 1). Carra *et al.* (2017) did not find a relationship between applied calcium and the diameter of pear fruits. For Laurel alto, the 1st application of T1 and T2 produced decreases (P ≤0.05) (Table 1). The 2nd application of calcium sources decreased (P ≤0.05) this parameter between 11 % and 18 %. Ayala Sánchez *et al.* (2013) recorded polar and equatorial diameter values of 27.29 ± 2.87 and 18.81 ± 1.63 mm; respectively, for andean blackberry fruits in MS4 and from crops located in Ibagué (Tolima, Colombia).

Parameter L. The 1st and 3rd applications of oxide (T3) and gluconate (T5) in El Carmelo decreased (P ≤0.05) the luminosity (Table 2). The 2nd application of T5 increased it, while in Agua Bonita decreased it (P ≤0.05) (Table 2). The 2nd and 3rd applications of nitrate (T2) in Agua Bonita and El Carmelo decreased (P ≤0.05) this parameter (Table 2). In this regard, Romero-Gomezcaña *et al.* (2006) found that the application of $Ca(NO_3)_2$ produced mango fruits with a lower value of L. Likewise, the last two applications of chelate (T1) and KCaB (T4) reduced (P ≤0.05) the luminosity (Table 2). In contrast, Di Miro *et al.* (2005) found that calcium chelate applications allowed higher L values in peach. Regarding Agua Bonita, fruits that received applications of T3 registered reductions (Table 2). About Laurel alto, the 1st and 2nd applications of T1 and T3 decreased (P ≤0.05) the luminosity (Table 2).

Parameter a*. El Carmelo fruits that received the 2nd and 3rd applications of chelate (T1), nitrate (T2), and KCaB (T4) showed reductions (Table 2). Likewise, the 1st application of gluconate (T5) produced reductions, while in Agua Bonita, there was an increase (Table 2). However, with the 2nd application of T5 in Agua Bonita, this parameter was reduced (P ≤0.05) (Table 2). On the other hand, the applications of T1 in Agua Bonita produced decreases (P ≤0.05) (Table 2). The 1st and 3rd applications of T2 reduced this parameter (P ≤0.05), and through the 2nd and 3rd applications of oxide (T3) and KCaB (T4), there were decreases (P ≤0.05) (Table 2). In Laurel alto, the 2nd application of T1 and the 3rd of T2 caused decreases (P ≤0.05) (Table 2).

Parameter b*. In the locality of El Carmelo, the 2nd application of gluconate (T5) allowed an increase (P ≤0.05) (Table 2). In contrast, fruits that received the 2nd and 3rd applications of nitrate (T2) in El Carmelo and Agua Bonita showed decreases (P ≤0.05) (Table 2). Likewise, with the 3rd application of oxide (T3), there were reductions (P ≤0.05) in both localities (Table 2). The 2nd application of chelate (T1) and KCaB (T4) in Agua Bonita and El Carmelo decreased this parameter (P ≤0.05) (Table 2). The 1st and 3rd applications of T3 in Laurel alto caused a reduction (P ≤0.05) (Table 2). Calcium oxide applications allowed obtaining fruits with reddish to purple colorations of greater intensity, with more significant accumulation of pigments and anthocyanins, as stated by Fischer *et al.* (2018).

Juice. The 1st application of chelate (T1) in El Carmelo caused an increase (P ≤0.05) (Table 2), in contrast to the first two applications of nitrate (T2) and oxide (T3) that led to reductions (P ≤0.05) (Table 2). The 2nd application of KCaB (T4) also caused a decrease (P ≤0.05) (Table 2). Concerning Agua Bonita, there was an increase (P ≤0.05) through the 2nd application of T1 and T3 (Table 2). In Laurel alto, the 2nd application of nitrate (T2), oxide (T3), and KCaB (T4) caused increases (P ≤0.05) (Table 2). Yfran *et al.* (2017) did not find significant differences by fertilization with K+Ca+B on mandarin juice; results like those found in this research.

Pulp. The first two applications of nitrate (T2) in El Carmelo increased it (P ≤0.05) (Table 2). In contrast, the 1st application of chelate (T1) produced a decrease (P ≤0.05) (Table 2). Regarding

Table 2. Effect calcium foliar fertilization on color parameters, juice, pulp, and chemical parameters in andean blackberry fruits.

Location	Parameter	Application	Treatment (source)	Estimation*	Mean values/ application**
El Colegio (El Carmelo)	L (units)	2^{nd} (fruit set)	T5 (Gluconate-CaBZn)	+ 4.2	**1^{st}** and **2^{nd}**: 23 – 32 **3^{rd}**: 20 – 25
		1^{st} and 3^{rd}	T3 and T5	- 5 and – 6	
		2^{nd} and 3^{rd}	T1, T2, and T4	- 4 and – 5	
	a* (units)	2^{nd} and 3^{rd}	T1, T2, and T4	- 7 and – 9	**1^{st}**, **2^{nd}**, and **3^{rd}**: 16 – 29
		1^{st} and 3^{rd}	T5 (Gluconate-CaBZn)	- 5	
	b* (units)	2^{nd} (fruit set)	T5 (Gluconate-CaBZn)	+ 3	**1^{st}** and **3^{rd}**: 3 – 8 **2^{nd}**: 6 – 16
		2^{nd} and 3^{rd}	T1, T2, and T4	- 3 and – 6	
		3^{rd} (red fruit)	T3 (Oxide-Ca)	- 6	
	Juice (%)	1^{st} (flower bud)	T1 (Chelate-Ca)	+ 9%	**1^{st}**: 27 – 47 **2^{nd}**: 20 – 37 **3^{rd}**: 31 – 37
		1^{st} and 2^{nd}	T2 and T3	- 9% and - 18%	
		2^{nd} (fruit set)	T4 (KCaB)	- 7,4%	
	Pulp (%)	1^{st} and 2^{nd}	T2 (Nitrate-CaB)	- 11.3% and - 8%	**1^{st}**: 43 – 59 **2^{nd}** and **3^{rd}**: 53 – 65
		1^{st} (flower bud)	T1 (Chelate-Ca)	- 18.7%	
	TSS (%)	2^{nd} (fruit set)	T1 (Chelate-Ca)	- 1.2%	**1^{st}** and **2^{nd}**: 5 – 7 **3^{rd}**: 7.0 – 7.6
	Acidity (%)	1^{st} (flower bud)	T1 and T4	- 0,8% and - 0,5%	**1^{st}** and **2^{nd}**: 2.5 – 3.5 **3^{rd}**: 1.8 – 2.0
Silvania (Agua Bonita)	L (units)	1^{st}, 2^{nd} and 3^{rd}	T3 (Oxide-Ca)	- 2.9 and - 6.9	**1^{st}** and **3^{rd}**: 19 – 24 **2^{nd}**: 23 – 32
		2^{nd} (fruit set)	T1, T4, and T5	- 6 and – 8	
		2^{nd} and 3^{rd}	T2 (Nitrate-CaB)	- 3 and – 4	
	a* (units)	1^{st} , 2^{nd}, and 3^{rd}	T1 (Chelate-Ca)	- 4.5 and -5.8	**1^{st}**: 10 – 21 **2^{nd}**: 19 – 26 **3^{rd}**: 16 – 22
		1^{st} and 3^{rd}	T2 (Nitrate-CaB)	- 6	
		2^{nd} and 3^{rd}	T3 and T4	- 4 and – 8	
		1^{st} and 2^{nd}	T5 (Gluconate-CaBZn)	+ 5 and – 5	
	b* (units)	2^{nd} (fruit set)	T1, T4, and T5	- 7.2 and - 9.5	**1^{st}** and **3^{rd}**: 3 – 8 **2^{nd}**: 6 – 16
		2^{nd} and 3^{rd}	T2 (Nitrate-CaB)	- 4.9 and - 2.8	
		2^{nd} and 3^{rd}	T3 (Oxide-Ca)	- 7.2 and - 3.2	
	Juice (%)	2^{nd} (fruit set)	T1 and T3	+ 10.1 and + 9.9	**1^{st}**: 30 – 42 **2^{nd}** and **3^{rd}**: 25 – 35
	Pulp (%)	2^{nd} (fruit set)	T1 and T3	- 6.9 and - 9.5	**1^{st}**, **2^{nd}**, and **3^{rd}**: 52 – 68
	TSS (%)	2^{nd} (fruit set)	T4 (KCaB)	+ 1.2%	**1^{st}**, **2^{nd}**, and **3^{rd}**: 6.1 – 7.6
	Acidity (%)	2^{nd} (fruit set)	T2 and T4	+0.7% and +0.6%	**1^{st}** and **2^{nd}**: 3.0 – 4.1 **3^{rd}**: 1.7 – 2.0

Continuación tabla 2.

Location	Parameter	Application	Treatment (source)	Estimation*	Mean values/ application**
San Bernardo (Laurel alto)	L (units)	1st (flower bud)	T1, T2, and T3	- 4 and - 4.9	**1st**: 20 – 25 **2nd** and **3rd**: 22 – 28
		2nd (fruit set)	T1 and T3	- 2.9 and - 4.1	
	a* (units)	2nd (fruit set)	T1 (Chelate-Ca)	- 5.2	**1st**: 14 – 18 **2nd** and **3rd**: 21 – 30
		3rd (red fruit)	T2 (Nitrate-CaB)	- 4.0	
	b* (units)	1st (flower bud)	T3 (Oxide-Ca)	- 3.1	**1st**: 5 – 8 **2nd** and **3rd**: 8 – 13
		2nd (fruit set)	T1 and T3	- 3.6 and - 3.0	
	Juice (%)	2nd (fruit set)	T2, T3, and T4	+9.9% and +18.1	**1st**: 36 – 41 **2nd**: 29 – 47 **3rd**: 33 – 37
	Pulp (%)	2nd (fruit set)	T1, T2, T3, and T4	- 8% and -13%	**1st**: 48 – 53 **2nd**: 49 – 62 **3rd**: 61 – 63
	Acidity (%)	1st (flower bud)	T2 and T3	+1.4% and +1.2%	**1st**: 1.8 – 3.3 **2nd**: 3.0 – 3.5 **3rd**: 1.6 – 1.9

*: increase or decrease with respect to the control fruits that did not receive fertilization (T6). **: Mean values obtained in each application

Agua Bonita, the 2nd application of chelate (T1) and oxide (T3) produced reductions (P ≤0.05) (Table 2). In Laurel alto, the 2nd application of chelate (T1), nitrate (T2), oxide (T3), and KCaB (T4) produced reductions (P ≤0.05) (Table 2).

TSS. El Carmelo fruits showed a decrease (P ≤0.05) with the 2nd application of chelate (T1) (Table 2), which reported shallow values in the applications (6.1 to 6.7 °Brix). In Agua Bonita, there was an increase (P ≤0.05) with the 2nd application of KCaB (T4) (Table 2). In Laurel alto, there were no significant differences. During the 1st application, TSS values were presented between 6.7 and 7.7 °Brix, and values between 7.5 and 8.3 °Brix for the 2nd and 3rd applications. TSS variations obtained in other locations can be attributed to the edaphoclimatic characteristics of each production site (temperature, relative humidity, solar radiation, cultivation practices, degree of soil fertility, among others), which affect the ability of plants to synthesize and translocate photoassimilates to fruits (Ali *et al*. 2011; Monroy-Cárdenas *et al*. 2019). Romero-Gomezcaña *et al*. (2006) by $Ca(NO_3)_2$ foliar application found a significant increase of TSS mango. Yfran *et al*. (2017) found no differences between fertilization treatments with K+Ca+B in TSS mandarin. In the present investigation, the calcium chelate applications in El Carmelo produced the lowest TSS values in andean blackberry fruits, results like those reported by Di Miro *et al*. (2005) in peach fruits.

pH. Calcium applications in El Carmelo and Agua Bonita had no significant effect. In both localities, values between 2.4 and 3.0 units were presented. In the Laurel alto locality, the 1st application of nitrate (T2), oxide (T3), and gluconate (T5) produced reductions (P ≤0.05) of 2.33, 2.30, and 0.47; respectively. The fruits recorded pH between 2.7 – 5.4, 2.8 – 2.9, and 2.5 – 2.7 units for the 1st, 2nd, and 3rd application. Andean blackberry fruits in MS4 present average 2.7 units (Ayala Sánchez *et al*. 2013).

Acidity. In locality El Carmelo, there were decreases (P ≤0.05) with the 1st application of chelate (T1) and KCaB (T4) (Table 2). Calcium chelate applications decreased peach acidity (Di Miro *et al*. 2005). In locality Agua Bonita there were increases (P ≤0.05) with the 2nd application of nitrate (T2) and KCaB (T4) (Table 2). Through the 1st application of nitrate (T2) and oxide (T3) in Laurel alto, there were increases (P ≤0.05) (Table 2). Romero-Gomezcaña *et al*. (2006) by $Ca(NO_3)_2$ applying found an increase in mango acidity. In general, 2.8 % and 3.2 % values have been reported for andean blackberry fruits in MS4 (Ayala Sánchez *et al*. 2013).

Fruit dehydration. The 1st application of chelate (T1) in El Colegio reduced (P ≤0.05) dehydration between 4.3 % and 10.8 % on the 5th, 7th, and 9th storage days. This treatment allowed a decrease (P ≤0.05) on day 7 of 3.6 % compared to the control evaluated on day 5 and a reduction (P ≤0.05) on day 9 of 10.3 % compared to the control evaluated on day 7. The 1st application of nitrate (T2) reduced (P ≤0.05) dehydration by 9.2 % and 8.9 % on days 7 and 9. This treatment allowed a decrease (P ≤0.05) in dehydration on day 9 of 8.4 % compared to the control evaluated on day 7. The 1st application of oxide (T3) reduced (P ≤0.05) dehydration by 3.7 %, 10.0 %, and 9.5 % on days 5, 7, and 9. This treatment allowed a decrease (P ≤0.05) on day 9 of 8.9 % compared to the control on day 7. The 1st application of KCaB (T4) reduced (P ≤0.05) dehydration by 7.3 % on days 7 and 9. Furthermore, this treatment allowed a decrease (P ≤0.05) on day 9 of 6.7 % compared to control on day 7. The 1st application of gluconate (T5) reduced (P ≤0.05) dehydration by 3.2 %, 9.7 %, and 9.6 % on the 5th, 7th, and 9th storage days. Additionally, this treatment allowed a decrease (P ≤0.05) on day 9 of 9.1 % compared to the control evaluated on day 7. The other applications did not generate a significant effect. In Agua Bonita, there were no significant differences, registering an average between 7.4 % to 9.3 % for the 1st application, 3.7 % to 4.8 % for the 2nd, and 3.6 % to 7.2 % for the 3rd. In this regard, Ayala Sánchez *et al*. (2013) in the samples evaluated of andean blackberry at 2 °C registered a progressive increase in weight loss due to transpiration; with 2 %, 3 %, 6 %, 8 %, and 9.6 % on the 2nd, 3rd, 4th, 5th, and 6th storage days; respectively. According to the authors, this high perishability is due to its high-water content, making it very susceptible and fragile to handling and the time that elapses after harvest. In Laurel alto, there were differences in fruits that received the 3rd application of T1, T2, and T3: T1 reduced (P ≤0.05) dehydration by 4.0 % on day 7, T2 reduced it (P ≤0.05) by 4.8 % and 5.1 % and T3 reduced it (P ≤0.05) by 4.7 % and 4.5 % in the days 5 and 7, compared to the control. Romero-Gomezcaña *et al*. (2006) found that, after nine days, control fruits and those that received $Ca(NO_3)_2$ presented similar weight losses.

Fruit damage. The 1st application of nitrate (T2) in El Colegio reduced (P ≤0.05) the damage by 20 % on the 5th day concerning control fruits that presented 61 %. The 2nd application of chelate (T1), nitrate (T2), KCaB (T4), and gluconate (T5) allowed reductions (P ≤0.05) on day 2 between 25.6 % and 42.0 % in relation to control fruits with 72.7 %. In contrast, the 3rd application of chelate (T1), oxide (T3), and gluconate (T5) generated increases (P ≤0.05) in damage on the 5th day, with values between 27.4 % and 30.5 % for the control that registered 21.9 %. There were no significant differences for Agua Bonita; damage values were between 23 % to 47 % for the 2nd day, 26 % to 65 % for the 5th day and between 52 % to 88 % for the 7th day. Romero-Gomezcaña *et al*. (2006) found that, after nine days, control fruits and fruits that received $Ca(NO_3)_2$ presented weight losses between 4.8 % to 5.9 %. In Laurel alto, an increase (P ≤0.05) of 18.1 % of fruits damage that received T2 and evaluated on day 6 was presented after the 1st application, while the fruits that received T5 presented an increase (P ≤0.05) of 27.3 % with respect to control fruits that registered damage of 41.5 %. After the 2nd application there were increases (P ≤0.05) on the 2nd storage day in fruits that received T2, T3, and T5 with values between 47.1 % and 59.6 % to control fruits without damage. After the 3rd application, there were no significant differences. Di Miro *et al*. (2005) found that peach fruits from trees treated with calcium chelate had fewer fungal attacks after 11 storage days than control fruits.

Calcium is closely related to meristematic activity, influences the enzyme systems regulation, phytohormone activity, and increases tissue resistance to pathogens. It increases postharvest shelf life and nutritional quality (Paniagua *et al*. 2013; Aghdam *et al*. 2012).

Soil and plant tissue analysis. To identify the effect of calcium foliar fertilization in the nutrient contents of soil and plant, soil and plant tissue analyses were carried out in each locality. Table 3 shows variable fertility soils with low ECEC and contrasting aluminum and calcium contents. Regarding the concentration of macro and micronutrients in andean blackberry crops, there were no differences

Table 3. Analysis of soils cultivated with andean blackberry in the municipalities of Silvania, San Bernardo, and El Colegio.

Parameter	Unity	Silvania (Agua Bonita)	San Bernardo (Laurel alto)	El Colegio (El Carmelo)
pH	Dimensionless	5.03	5.43	5.04
Electric conductivity	$dS\ m^{-1}$	0.21	0.22	-
Organic matter	g/100g	13.68	8.1	3.82
P	$mg\ kg^{-1}$	6.98	32.21	41.32
S	$mg\ kg^{-1}$	6.62	7.28	13.82
ECEC	$cmol_{(+)}\ kg^{-1}$	2.22	9.16	4.21
B	$mg\ kg^{-1}$	0.21	0.24	0.62
AI	$cmol_{(+)}\ kg^{-1}$	1.03	1.03	-
Al	$cmol_{(+)}\ kg^{-1}$	0.83	0.82	0.56
Ca	$cmol_{(+)}\ kg^{-1}$	0.75	5.75	2.14
Mg	$cmol_{(+)}\ kg^{-1}$	0.22	1.73	0.44
K	$cmol_{(+)}\ kg^{-1}$	0.17	0.55	0.96
Na	$cmol_{(+)}\ kg^{-1}$	<0.05	<0.14	0.12
Fe	$mg\ kg^{-1}$	254.33	168.77	30.25
Cu	$mg\ kg^{-1}$	2.27	<1.00	0.51
Mn	$mg\ kg^{-1}$	1.43	11.69	6.48
Zn	$mg\ kg^{-1}$	1.67	4.34	3.14

according to Dunnett's test (P ≤0.05), where crops from Laurel alto (San Bernardo) and Agua Bonita (Silvania) localities presented N and P high contents, K, Ca, S, Mn, and Zn normal contents; and magnesium and copper low contents (Da Silva *et al.* 2004). Using PCA, three components explained 73.6 % of the variability. In component 1, P and Mg were grouped and negatively related to micronutrients. In component 2, Ca and S were grouped and negatively related to foliar K. However, considering the antagonistic relationship between Ca with Mg and P and the high correlation presented in PC 1, Ca was reassigned to this component. Variable fertility (Table 3) coincides with the differential response of the calcium foliar applications between localities, presenting a greater effect in crops without acidity problems and adequates calcium saturation, and where foliar calcium fertilization is complementary and not supplementary. Regarding the relationships between Ca and Mg with potassium (data not shown), a cationic imbalance was found that did not favor the latter's availability, results that contrast with the low and adequate cationic relationships presented in Cundinamarca soils (Cardona & Bolaños-Benavides, 2019). The above makes it necessary for the andean blackberry producer to reinforce the supply of K by edaphic route to optimize the plant response to foliar fertilization. The soil organic matter content low of El Carmelo with respect to Laurel alto and Agua Bonita contents (Table 3) could increase the plant response to foliar fertilization (Jantalia & Halvorson, 2011). CaBZn gluconate applications allowed increases of 11.6 % in plant tissue Ca concentration of andean blackberry crop by Agua Bonita (1.54 %) and did not affect the Laurel alto macronutrient concentration. Calcium chelate applications increased the macronutrient foliar concentration (except K), highlighting a 22.1 % calcium increase from the crop in El Carmelo (0.95 %). Ca chelate applications in El Carmelo made it possible to obtain sufficiency levels like those of the other localities. The macro and micronutrient concentrations in andean blackberry crops did not present significant differences, like those reported by Cardona & Bolaños-Benavides (2019). In contrast, calcium applications in pomegranate, kiwi, and apple trees increased the concentration of this nutrient in leaves, compared to control trees (Davarpanah *et al*. 2018; Koutinas *et al*. 2010), K decreased in apple trees (Danner *et al*. 2015) and did not generate significant effects on N, P, K, and Mg in grape and kiwi (Bonomelli & Ruiz, 2010; Koutinas *et al*. 2010).

Calcium foliar fertilization in the andean blackberry crops with thorns is a viable alternative to improve fruit firmness, weight, and diameters. In contrast, chemical and color parameters in andean blackberry fruits could depend on the edaphoclimatic conditions inherent in each zone, which weren't evaluated in the current research. This fertilization should be considered as a complement in soils without acidity problems and with balanced cationic saturations.

There are calcium sources with different levels of efficiency and effect at the local level and according to the nutritional status of the andean blackberry crop, and based on the results obtained in this research, the application in commercial doses of chelate, nitrate, and calcium oxide is recommended in crops in production with pruning management and in the phenological stages of flower bud, fruit set, and red fruit.

During storage, El Colegio and Laurel alto fruits which received calcium during flower bud and fruit set, showed less weight loss due to transpiration. Regarding the effect of calcium on the appearance of damage, there was a decrease in El Colegio, a null effect in Agua Bonita, and an increase in Laurel alto.

Conflicts of interest: The manuscript was prepared and reviewed with the participation of all the authors, who declare that no conflict of interest puts the validity of the results presented at risk. Authors contribution: William Andrés Cardona, Blanca Lucía Botina Azain y Clara Viviana Franco Flórez curated and analyzed the data for writing the draft of the manuscript. Research and resource management in the field by William Andrés Cardona, María Cristina García Muñoz, Blanca Lucía Botina Azain y Pablo Edgar Jiménez Ortega. María Cristina García Muñoz led the contextualization and methodology of the work. All authors participated in writing, reviewing, and editing the manuscript. Funding: This study was funded by the Universidad de los Andes, the Corporación Colombiana de Investigación Agropecuaria – AGROSAVIA and the Ministerio de Agricultura y Desarrollo Rural de Colombia.

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