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Calcium applications on 'Fuji Suprema' and 'Maxi Gala' apple trees: fruit quality at harvest and after cold storage

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ABSTRACT: The aim of this study was to evaluate the effect of new Ca sources sprayed on 'Fuji Suprema' and 'Maxi Gala' apple trees on nutrient levels in leaves and fruit, as well as on fruit ripening features and quality at harvest time and after cold storage. Experiments were carried out in a commercial orchard planted with 'Fuji Suprema' and 'Maxi Gala' apple trees in Caçador, Santa Catarina state, Brazil. Application of different Ca sources and untreated trees were evaluated in each experiment. Fruit were harvested at two different ripening stages and analyzed based on the following variables: fruit ripening at harvest time, as well as fruit quality and incidence of physiological disorders after 210-day storage at 1 °C. Calcium applications did not change Ca levels in the leaves and of the whole fruit. Calcium levels in fruit peel increased in cultivars Maxi Gala and Fuji Suprema due to leaf Ca applications. 'Maxi Gala' apples recorded higher postharvest incidence of physiological disorders, such as greater loss of fruit firmness than 'Fuji Suprema' apples (Due to 1-methylcyclopropene [1-MCP] application on 'Fuji Suprema' apples). Calcium applications did not change fruit maturation (starch index and pulp firmness) or apple pulp firmness preservation in both cultivars, although they reduced the incidence of bitter pit disorder in 'Maxi Gala' apples. The new sources of Ca tested did not increase Ca contents, nor did they reduced the risk for physiological disorder compared to the standard $CaCl_2$ treatment that has been commercially used for decades as the main Ca fertilizer.

Key words: *Malus domestica* Borkh, mineral content, foliar spray, physiological disorder, postharvest.

INTRODUCTION

Apple (*Malus domestica* Borkh.) crops cover more than 30,000 hectares in Brazil and produce approximately 1.2 million tons of fruit per year (IBGE 2018). Rio Grande do Sul (RS) and Santa Catarina (SC) states are the largest apple producers in the country. Apple harvest period in orchards is relatively short: January and February for 'Maxi Gala' apples and its clones, and March and April for 'Fuji Suprema' apples and its clones (Gonçalves et al. 2017). However, apple fruit is available to consumers throughout the year, mainly due to postharvest cold storage technologies. However, high fruit loss rate can be observed during cold storage due to incidence of diseases (rot) and physiological disorders such as bitter pit, cork spot and lenticellar depression, among others (Watkins and Mattheis 2019). Crop losses due to Ca deficiency-associated disorders such as bitter pit can be higher than 60% (Mattheis et al. 2017).

Bitter pit is a physiological disorder caused by pulp cell collapse below the fruit peel, which creates small depressions that can be seen through dark color or small spots on fruit surface, often on the fruit calyx region (Amarante et al. 2018; 2020;

Ferguson et al. 1989; Freitas et al. 2010). This physiological disorder is associated with Ca deficiency or imbalance in K/Ca, Mg/Ca, N/Ca and K + Mg/Ca ratios (Amarante et al. 2006; Ferguson et al. 1989; Freitas et al. 2010; 2012; Miqueloto et al. 2018). In addition to bitter pit, other physiological disorders, such as pulp degeneration, have their incidence increased under conditions such as high Mg, K and N levels in fruit (Corrêa et al. 2017; Freitas et al. 2012; Neuwald et al. 2014). It happens because Ca^{2+} can produce two electrovalent bonds, whereas Mg, K and N can only produce one bond. Thus, membranes' sedimentation/stabilization power through Ca^{2+} is much greater than the one recorded for other nutrients; thus, Ca^{2+} enables greater membrane stability (Freitas et al. 2012). Accordingly, high K/Ca, Mg/Ca, N/Ca and K + Mg/Ca ratios make fruit more susceptible to present physiological disorders such as bitter pit and internal darkening. In addition, there are nutritional differences in fruit peel and pulp among different apple cultivars (Amarante et al. 2006).

Physiological disorders in apple fruit can be mitigated by strategies such as the application of fertilizer added with Ca on leaves and fruit, mainly on soils presenting low Ca content or on orchards planted with rootstocks and cultivars presenting low soil-nutrient absorption efficiency or low Ca transport capacity and redistribution within plants (Amarante et al. 2020; Blanco et al. 2010; Schlegel and Schönherr 2002). Fertilizers can be applied at different concentrations throughout plants' vegetative and fruit development periods (Amarante et al. 2020), since nutrients are preferentially absorbed by fruit. Therefore, Ca falling straight on fruit can reduce physiological disorders, since it is absorbed at higher rates, which increases its concentrations in the apoplast (Amarante et al. 2013).

Thus, application of $CaCl_2$ every 15 days, starting 30 days after full bloom, has been recommended as practice to reduce Ca deficiency risks and to prevent the emergence of physiological disorders associated with Ca deficiency (Sezerino 2018). However, new commercial products have been developed to meet the demand of Ca to apple plants. Currently, a large number of foliar fertilizers are available in the market, with formulations containing Ca and biostimulants, such as amino acids, sugar-alcohol, seaweed extracts, and humic acids. Although local and regional tests to prove the efficiency of these products have not been conducted by research. Despite this, apple growers have been using these products. The aim of the current study was to evaluate the effect of new Ca sources sprayed on 'Fuji Suprema' and 'Maxi Gala' apple trees on nutrient levels in leaves and fruit, as well as on fruit ripening features and quality at harvest time and after cold storage.

MATERIAL AND METHODS

Experiment description

Two experiments were carried out during the 2018/2019 crop season, in Caçador, Midwestern Santa Catarina state (Latitude: 26°42'36"S; Longitude: 51°03'28"W; Altitude: 1,025 m), Southern Brazil. Experiment 1 was carried out in a 'Maxi Gala' apple orchard planted in 2015 at 5 × 2.5 m spacing, under central leader system. 'Maxi Gala' trees were grafted on 'Marubakaido' rootstock with M9 as filter. Experiment 2 was carried out in an 'Fuji Suprema' apple orchard planted in 2011 at 5 × 2.5 m spacing, under central-leader system. 'Fuji Suprema' trees were grafted onto 'Marubakaido' rootstock. The physical-chemical featuring of the experimental soils is shown in Table 1. Levels of Ca and Mg in both experimental soils were classified as "high" (SBCS 2016).

In each cultivar (experiment) six treatments were applied, namely:

i) Control: water application, only;

ii) Treatment 1 (T1): standard application of $CaCl_2$ (p.c. with 18% of Ca), at dose of 0.5% Ca, 1st application at 30 days after full bloom (DAFB) and reapplication every 15 days—it totaled 10 applications;

iii) Treatment 2 (T2): product A, at dose of 0.25% Ca, 1st application at 30 DAFB and reapplication every 15 days—it totaled 10 applications;

iv) Treatment 3 (T3): Product A, at dose of 0.25% Ca, 1st application at 15 DAFB and reapplication every 15 days—it totaled 10 applications;

v) Treatment 4 (T4): Product A, at dose of 0.5% Ca, 1st application at 30 DAFB and reapplication every 15 days—it totaled 10 applications;

vi) Treatment 5 (T5): Product B, at dose of 0.5% Ca, 1st application at 30 DAFB and reapplication every 15 days—it totaled 10 applications.

Table 1. Physical and chemical attributes of soil cultivated with 'Maxi Gala' and 'Fuji Suprema' apple trees in the implementation of the experiments

Attributes of soil	Maxi Gala	Fuji Suprema
Clay ($g \cdot kg^{-1}$)	29	35
Organic matter (%)	1.8	3.0
pH in water (ratio 1:1. v/v)	6.0	5.6
K available ($mg \cdot dm^{-3}$)*	296	292
P available ($mg \cdot dm^{-3}$)*	47.5	8.0
Ca exchangeable ($cmolc \cdot dm^{-3}$)**	15.3	9.0
Mg exchangeable ($cmolc \cdot dm^{-3}$)**	5.0	3.0
CECpH7.0 ($cmolc \cdot dm^{-3}$)***	25.0	18.9
Cu ($mg \cdot dm^{-3}$)****	17.3	4.0
Zn ($mg \cdot dm^{-3}$)***	12.5	10.5
B ($mg.dm^{-3}$)****	> 5.0	> 5.0
Mn ($mg.dm^{-3}$)****	45.6	11.8

* Extracted by Mehlich-1 solution; ** Extracted by KCl 1.0 $mol \cdot L^{-1}$; *** cation exchange capacity; **** Extracted by HCl 1 $mol^{\cdot L-1}$.

Product A is registered as leaf fertilizer and presents the following composition: 5.3% of Ca complexed with sugar-alcohol, 7.0% of N and 2.6% of Mg. Product B is also registered as leaf fertilizer and presents the following composition: 18% of Ca (in CaO form) and 2% of B complexed with 10% of seaweed.

The two experiments have followed a complete randomized block design with four replications per treatment. Plots comprised 9 plants, where the 5 central plants were used for evaluations. Treatments were sprayed with an electric backpack sprayer on the entire canopy of the trees (new shoots and fruit), at spray volume equal to 1,000 $L \cdot ha^{-1}$.

Leaf sample and nutrient analysis

Leaves were sampled in the last week of January, as suggested by CQFS-RS/SC (SBCS 2016), in order to determine mineral nutrient concentrations in them. Twenty-five full ripe leaves, free from damage caused by insects and diseases, were sampled in the median part of shoots sprouted during the year, which were located in the median height of the crown and in the external part of each plant. Leaves were dried in oven equipped with forced air circulation at 65 ± 5 °C until they reached constant mass. Next, they were ground in Wiley mill and stored in paper bags. Macronutrient (N, P, K, Ca and Mg) levels in leaves were determined after sulfuric digestion, whereas micronutrient (Zn, Fe, Cu, Mn and B) levels in them were determined after nitroperchloric digestion (Silva 2009). Boron (B) level was determined through colorimetric method by using the azomethine H reagent, after incineration in muffle. Readings were performed in UV-visible spectrophotometer (Bell Photonics, 1105, Brazil), at 420 nm (Bataglia et al. 1983). Total N was determined in micro-Kjeldahl distiller (Tecnal, TE-0363, Brazil), based on Tedesco et al. (1995). Phosphorus (P) content was determined in UV-visible spectrophotometer (Bell Photonics, 1105, Brazil), at 882 nm (Murphy and Riley 1962). K, Ca, Mg, Zn, Fe, Cu and Mn readings were performed in atomic absorption spectrophotometer (Analyst 2000, PerkinElmer).

Fruit harvesting and mineral nutrient analysis

Apples of both cultivars were harvested at two different ripening stages that had been previously estimated based on starch index analysis. Fruit were harvested early (1st harvest) in order to induce disorders caused by Ca deficiency (Freitas et al. 2012), as well as harvested late (2nd harvest) to induce disorders associated with senescence and rot (Watkins and Mattheis 2019) during storage. Twenty mid-sized fruit (130 to 150 g) were sampled at both harvest times.

Mineral contents in fruit were determined in peel strips, as well as in pulp wedges with and without peel. Peel strips (approximately 1 cm wide) were taken in the longitudinal direction of each fruit, with the aid of fruit peeler, for mineral analysis. The longitudinal wedge of each fruit (approximately 1 cm thick in the peel) was taken for mineral analysis in the pulp and peel. Carpel tissues were removed from the wedge in the central region of the pulp. A wedge per fruit was taken, as described above, and the peel was removed beyond the carpel tissues to mineral content analysis in the pulp.

Fruit were divided for nutrient concentration analysis, as follows: i) peel: manually removed with the aid of peeler; ii) whole fruit (pulp + peel): wedge-shaped longitudinal slice (1 cm thick), without the central part of the carpel, extracted from each fruit; and iii) pulp: the same procedure adopted to analyze whole fruit, although without the epidermis. Tissue samples were dried in oven equipped with forced air circulation at 65 ± 5 °C until they reached constant mass, before they were ground. Subsequently, N, P, Ca and Mg concentrations were determined based on the same methodology used to determine leaf contents. N/Ca, K/Ca and K + Mg/Ca ratios were calculated, after nutrient content determination.

Fruit ripeness and quality analysis

Fruit ripeness and quality were analyzed at the day after harvest (at harvest), as well as after cold storage and 7 days on the shelf at 22 °C. Starch index (iodine-starch test, at 1–9 scale, wherein 1 indicates minimal starch degradation), pulp firmness, physiological disorder and rot assessed to each fruit, whereas total soluble solids (TSS) content and titratable acidity analyzed to 4 samples comprising 7 fruits per repetition as described by Argenta et al. (2020).

Physiological disorders, as well as external and internal rot, were visually analyzed based on severity scales ranging from 1 to 2, 1 to 3 or 1 to 4, wherein 1 indicates no damage, and 2, 3 or 4 indicate maximum severity, depending on the disorder (Table 2). Internal disorders were analyzed in four cross-sections of fruit (in the equatorial region, above the equatorial region and two cuts below the equatorial region). Disorder severity was determined based on fruit surface area, affected cross-section area or on the number of observed lesions, as described by Argenta et al. (2020).

Table 2. Severity scale of the physiological disorders described in Argenta et al. (2020).

Disorders	Measures	Severity score			
		1	2	3	4
Bitter pit e lenticellar depression	Number of spots on fruit epidermis and/or cortex	absent	1–4	5–9	> 9
Blotch pit	Area of the affected epidermis (cm2)	absent	< 1	1–3	> 3
Diffuse flesh browning*	Relative area (%) of cortex affected	absent	1–30	30–60	> 60
Browning epidermis and pulp**	Relative area (%) of cortex affected	absent	1–10	11–40	> 40
External rot	Area of the affected epidermis (cm2)	absent	≤ 1	> 1	-
Carpel rot	Area of the affected epidermis (cm2)	<50	50–100	-	-
Pulp cracking and shrivel	Present or absent	absent	present	-	-

*Assessed in the cross section of the equatorial region. **Evaluated in the cross section of the pistillary region (distal to the peduncle).

Treatment with 1-MCP, packaging and fruit storage

'Fuji Suprema' apples were treated with 1-MCP, as described by Amarante et al. (2010a), in order to avoid superficial scald, which leads to rot and makes it hard to identify bitter pit symptoms.

Apples were placed on cardboard trays and in cardboard boxes internally coated with low-density polyethylene plastic bag (20 µm thick) commercially used for apples to avoid dehydration. Packaged apples were transferred to cold storage chamber (1 ± 0.5 °C) one day after harvest. Apples were kept at cold atmosphere for 210 days; subsequently, they were kept at 22 ± 1 °C, for 7 days. Plastic bags were removed at the day fruit were removed from the cold storage chamber.

After 7 months of cold storage, apples were removed from the chamber and stored at 22 °C, for 7 days, in order to simulate transport and shelf time. 'Maxi Gala' fruit of 1st harvest were analyzed after 4- and 7-day shelf life while 'Maxi Gala' fruit of 2nd harvest and 'Fuji Suprema' of both harvests were assessed only after 7-day ripening.

Fruit firmness and TSS were evaluated after the storage period. Apples were sliced crosswise in order to visually identify and assess the incidence and severity of rot and physiological damage, based on severity degree. Rot assessment was based on the following scores: 1 for absence of rot; 2 and 3 for 1 or 2 lesions with total diameter smaller than 1 cm and larger than 1 cm, respectively. Lenticel breakdown and bitter pit disorders were analyzed based on the severity of symptoms such as dark brown cork-like spots on fruit epidermis and/or cortex, as follows: 1) lack of symptoms; 2) mild: 1 to 3 spots; 3) moderate: 4 to 9 spots; and 4) severe: more than 9 spots. Blotch pit was analyzed based on the severity of symptoms such as well-defined and depressed black spots on the epidermis, as follows: 1) lack of symptoms; 2) mild: 1 spot with diameter smaller than 1 cm; 3) moderate: 1 to 3 spots with total diameter ranging from 1 to 2 cm; and 4) severe: 1, or more, spots with total diameter larger than 2 cm. Carpel rot was evaluated based on the size of the injury in the internal cortex and pith tissues of apples subjected to cross section in the equatorial region, as follows: 1) lack of pith injury, 2) mild: injury smaller than 1 cm (in diameter); 3) moderate: injury with diameter ranging from 1 to 3 cm; 4) severe: injury with diameter larger than 3 cm. Superficial scald was analyzed based on the severity of symptoms such as well-defined dark brown spots on fruit surface (damage restricted to the epidermal tissue), as follows: 1) lack of symptoms; 2) 1 to 15% of total fruit surface affected by dark brown spots; 3) 15 to 40% of total fruit surface affected by dark brown spots; and 4) more than 40% of total fruit surface affected by dark brown spots. The senescent degeneration disorder is featured by browning pulp regions; it was evaluated based on scores ranging from 1 to 4 (1 = absent, 2 = mild, 3 = moderate, and 4 = severe).

Statistical analysis

Data were analyzed to check statistical assumptions. They were subjected to analysis of variance (ANOVA). Means were compared to each other through Tukey's test at 5% significance level ($p < 0.05$). All analyses were performed in R language (R Core Team 2020).

RESULTS AND DISCUSSION

Nutrients in leaves

Leaf N, P, K, Mg, Fe, Mn, Zn, B and Ca contents in the two cultivars (experiments 1 and 2) did not change due to Ca sources and doses applied via leaf fertilizer (Table 3). Results have shown that Ca applied via leaf was not absorbed by leaves. It may have happened due to unidirectionality of applications, according to which a small part of the applied Ca may have touched the outer surface of leaves (Kannan 2010; Schlegel and Schönherr 2002). In addition, part of Ca deposited on leaves after the application procedure may have been carried by the rain and ended up being deposited in the soil (Blanco et al. 2010; Kraemer et al. 2009) or in other plant organs.

Leaf Ca concentrations in both cultivars were higher than 8.0 $g \cdot kg^{-1}$; this content is considered sufficient for apple culture (SBCS 2016). The highest leaf Ca concentrations were observed in cultivar Fuji Suprema (12.5 $g \cdot kg^{-1}$) in comparison to 'Maxi Gala' (8.9 $g \cdot kg^{-1}$). This outcome can be explained by the fact that, overall, Ca translocation to apples tends to be higher in 'Maxi Gala' than in 'Fuji Suprema' (Nachtigall and Dechen 2006). Lower leaf contents were also verified in 'Maxi Gala' for N, Mg, Fe, Zn, Cu and, B (Table 3). Differences in plant architecture, orchard management, root system growth, with great influence of climatic variables, may explain lower leaf nutrient contents in orchards (Kalcsits et al. 2020). In any case, nutrient contents in leaves considered insufficient by CQFS-RS/SC (SBCS 2016) were not verified in this study.

Table 3. Nutrient concentrations in 'Maxi Gala' and 'Fuji Suprema' apple leaves with foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treatments	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	---------------- $g \cdot kg^{-1}$ ----------------					---------------- $mg \cdot kg^{-1}$ ----------------				
	'Maxi Gala'									
Control	12.2	2.5	22.6	8.4	2.2	28.6	504.9	121.3	5.6	38.1
T1	12.6	2.1	23.3	8.0	1.9	35.4	396.4	96.5	7.8	36.4
T2	13.3	3.0	26.3	9.1	2.4	27.5	511.4	94.7	7.8	37.9
T3	12.9	2.9	24.8	8.9	2.3	33.2	522.4	109.2	7.4	39.3
T4	12.3	2.7	23.8	8.8	2.2	27.7	479.0	109.7	9.1	38.4
T5	12.0	2.8	25.2	10.0	2.4	54.9	494.2	111.1	6.6	37.7
Mean	12.5	2.7	24.3	8.9	2.2	34.5	484.7	107.1	7.4	38
F-test	0.57	0.35	0.42	0.31	0.24	0.05	0.48	0.28	0.16	0.86
CV (%)	8.7	22.3	11.0	13.9	14.5	35.2	19.3	15.8	23.8	8.5
Treatments	'Fuji Suprema'									
Control	24.9	2.7	24.2	11.2	2.6	243.5	255.2	251.3	15.4ab	52.1
T1	24.7	3.0	23.0	13.8	2.7	67.2	242.5	215	12.8b	45.9
T2	24.2	2.5	22.8	12.9	2.5	62.8	255.8	246.6	16.3ab	51.9
T3	24.7	2.7	23.2	13.6	2.9	73.8	249.6	280.7	17.1a	49.2
T4	25.4	2.7	24.3	11.9	2.5	66.4	253.3	255	16.9a	52.6
T5	24.6	2.6	23.0	11.5	2.5	61.0	237.4	220.2	12.5b	48.3
Mean	24.7	2.7	23.4	12.5	2.6	95.8	249	244.8	15.2	50.0
F-test	0.99	0.17	0.91	0.18	0.49	0.44	0.96	0.36	0.00	0.52
CV (%)	9.7	8.8	10.5	13.2	12.2	150.4	13.7	18.2	11.7	11.4
Recommended contents	22.5	1.5	12.0	14.0	3.5	150.0	90.0	60.0	17.5	40.0

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Nutrients in fruit

Nitrogen, P, K and Mg concentrations in the peel, pulp and whole fruit of both cultivars were not affected by the application of doses and sources of leaf fertilizer added with Ca (Supplementary Material, Tables S1–S4). On the other hand, the lowest Ca concentrations in the peel of 'Maxi Gala' apples were observed for the control and T1, both in the 1st and 2nd harvest dates (Fig. 1a and b, respectively). The highest Ca concentrations in fruit peel were observed for T2, T3 and T5 in the 1st harvest, as well as for T5 in the 2nd harvest. On the other hand, K/Ca and K + Mg/Ca ratios did not change by treatments (Fig. 1c–f, respectively).

The Ca concentration, K/Ca and K + Mg/Ca ratios in the peel of 'Fuji Suprema' apple did not change due to treatment application in the 1st harvest (Fig. 2a, c and e). However, Ca contents in the peel of apples subjected to the control treatment (147.1 $mg \cdot kg^{-1}$) were significantly lower than those of T1 ($CaCl_2$) (176.1 $mg \cdot kg^{-1}$) and T4 (product A, 0.5% of Ca, at 15-day interval) (177.7 $mg \cdot kg^{-1}$) in the 2nd harvest (Fig. 2b, d and f). This outcome has evidenced Ca content increase by 16.5 and 17.2% in fruit subjected to treatments T1 and T4, respectively, in comparison to control. These higher Ca contents have also led to changes in K/Ca and K + Mg/Ca ratios in the peel of fruit subjected to these treatments; the highest ratios were observed in the control treatment, whereas the lowest ratios were observed in T1.

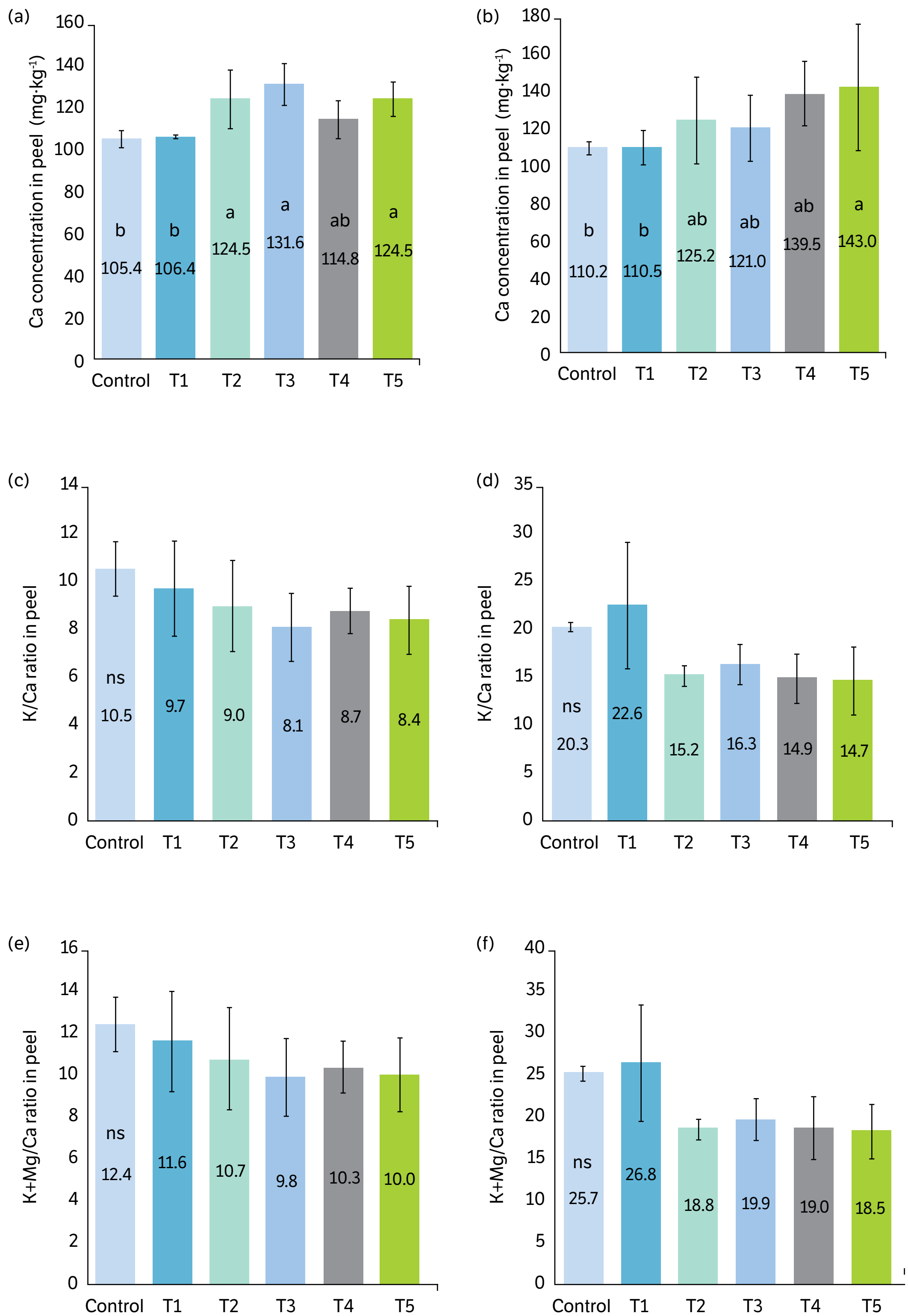

Figure 1. Calcium concentration in the 1st (a) and 2nd (b) harvest; K/Ca ratio in the 1st (c) and 2nd (d) harvest; and K + Mg/Ca ratio in the 1st (e) and 2nd (f) harvest in the fruit peel of 'Maxi Gala' apples submitted to foliar applications of different Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

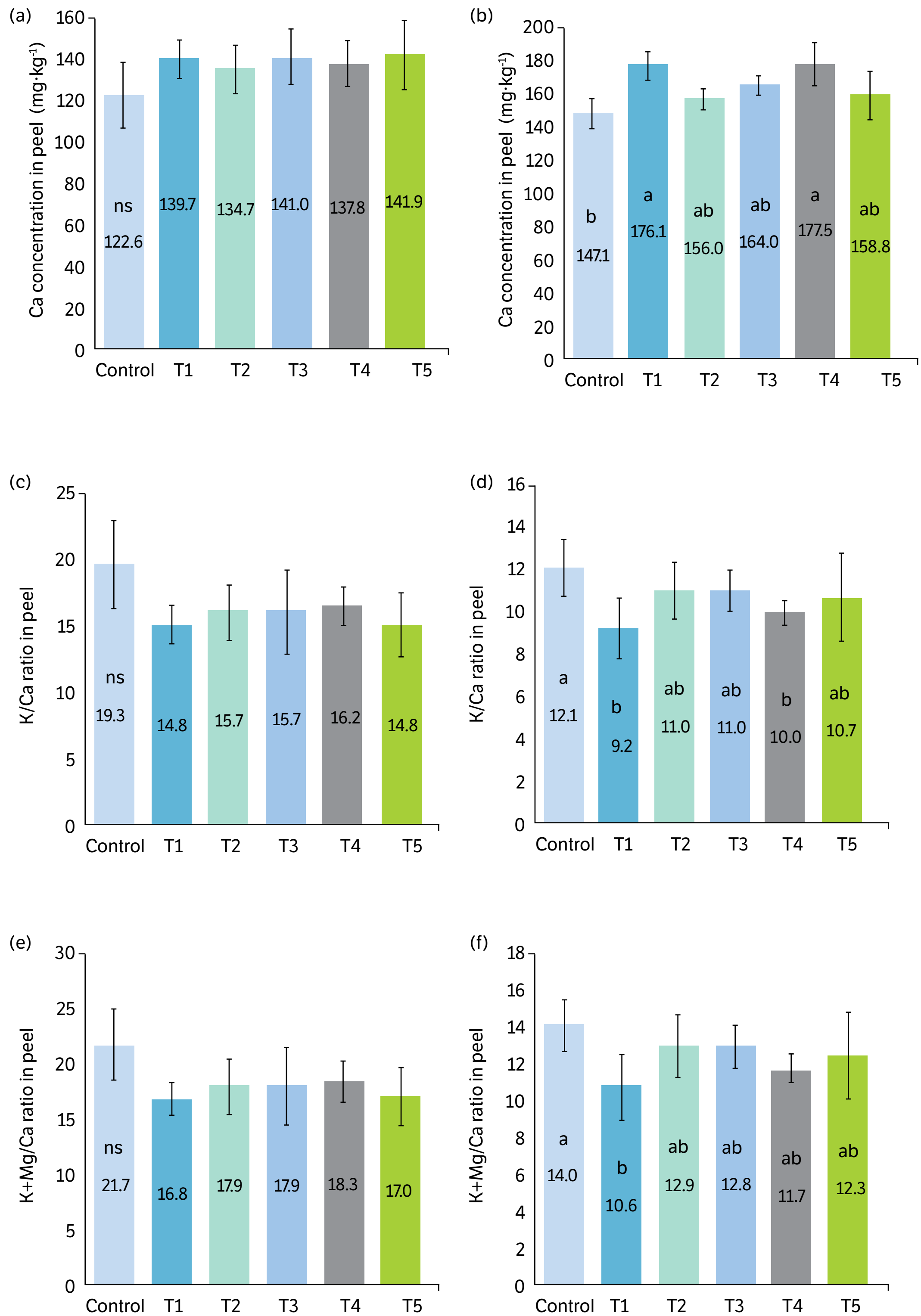

Figure 2. Calcium concentration in the 1st harvest (a) and 2nd harvest (b); K/Ca ratio in the 1st harvest (c) and 2nd harvest (d); and K + Mg/Ca ratio in the 1st harvest (e) and 2nd harvest (f) in the fruit peel of 'Fuji Suprema' submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

The 'Maxi Gala' apples have shown the lowest Ca concentrations in the pulp at T1 (32.3 mg·kg^{-1}) at 2nd harvest date (Fig. 3b). On the other hand, T4 fruit have shown the highest Ca concentrations in the pulp (45.0 mg·kg^{-1}), which led to decreased K/Ca and K + Mg/Ca ratios (Fig. 3d and f, respectively). According to Neuwald et al. (2008) and Freitas et al. (2010), K content in the pulp higher than 950 mg·kg^{-1}—such as the one herein observed in apples of all treatments in the present study including untreated control (Supplementary Material, Tables S3 and S4)— affects K/Ca ratio increase and can be harmful to stored fruit. It happens because K excess reduces Ca absorption and transport to fruit (Freitas et al. 2012) and competes with Ca for the binding sites in the plasma membrane (Freitas et al. 2010). Increased Ca concentration in the pulp of T4 fruit (product A at 0.5% Ca applied every 15 days) has indicated that part of the Ca falling on fruit may have been redistributed to the pulp (Saure 2005). This phenomenon mainly happens at early fruit development stage—between 4 and 6 weeks after flowering—when Ca absorption is fast and linear, although it presents notable decline until harvest (Cline et al. 1991; Saure 2005).

There was no difference between Ca concentrations in the whole fruit, if one takes into consideration treatment application in the two harvest times and both evaluated cultivars (Supplementary Material, Tables S1 and S2). According to Suzuki and Basso (2006), adequate nutrient concentrations used to feature apple fruit with nutritional quality comprise N < 500, P > 100, K = 800–1,200, Ca > 40, Mg > 40 mg·kg^{-1}, as well as nutrient ratios such as N/Ca < 14, K/Ca < 20 and K+ Mg/Ca < 30. Therefore, there were adequate Ca contents in fruit of all treatments, including control, to both cultivars and both harvest times. This outcome may indicate that Ca contents in the soil, or even in plants, were enough to supply Ca demanded by fruit. However, K concentrations higher than the recommended one (800–1200 g·kg^{-1}) were observed at both 'Fuji Suprema' harvest times, mainly at the 2nd harvest (Supplementary Material, Tables S1 and S2). The aforementioned high K values have increased the K/Ca and K + Mg/Ca ratios to levels above the recommended (< 20 and < 30, respectively) in all evaluations conducted at the 2nd 'Maxi Gala' harvest. Unbalanced mineral ratios increase the likelihood of developing physiological disorders during cold storage and fruit commercialization, which would lead to product depreciation. According to Argenta and Suzuki (1994) and Amarante et al. (2010b), K + Mg/Ca ratio higher than 27 and 32, respectively, lead to greater risks of bitter pit incidence in 'Gala' apples.

Fruit ripening stage at harvest time

'Maxi Gala' apples have shown pulp firmness higher than 20 lb, TSS content higher than 12 °Bx at both harvest times, as well as starch index 2 at the first harvest and 3.4 at the second one (Supplementary Material, Table S6). 'Fuji Suprema' apples have shown pulp firmness equal to 19.9 and 16.1 lb, as well as starch index 3.4 and 6.2 at the 1st and 2nd harvests, respectively. Apples of both harvest date presented ripening indices corresponding to that of commercial harvest time (Gonçalves et al. 2017), although apples of both cultivars were at more advanced ripening stage at the 2nd harvest date than at the 1st one. 'Maxi Gala' apples of both harvest dates were at the proper ripening stage recommended for long-term storage; 'Fuji Suprema' apples collected at the 1st and 2nd harvests were at suitable maturation stage recommended for long- and mid-term storages, respectively.

'Fuji Suprema' fruit have shown higher pulp firmness in T1 than in the control at the 2nd harvest (Fig. 4b). Increased pulp firmness observed in T1 fruit was associated with better nutritional balance (higher Ca content; lower K/Ca and K + Mg/Ca ratios – Fig. 2), which may have increased fruit cell membrane and wall stabilization and enabled greater pulp firmness (Amarante et al. 2012; Fallahi et al. 2010). Brackmann et al. (2010) have also observed increased Ca contents in the peel of 'Fuji Suprema' apple fruit, which led to fruit ripening delay due to low ethylene production, as well as enabled pulp firmness maintenance and higher TSS levels, after 9 $CaCl_2$ (0.6%) applications. The incidence of rot in the present study was remarkably low (Fig. 5a and b) and presented minimal differences between treatments.

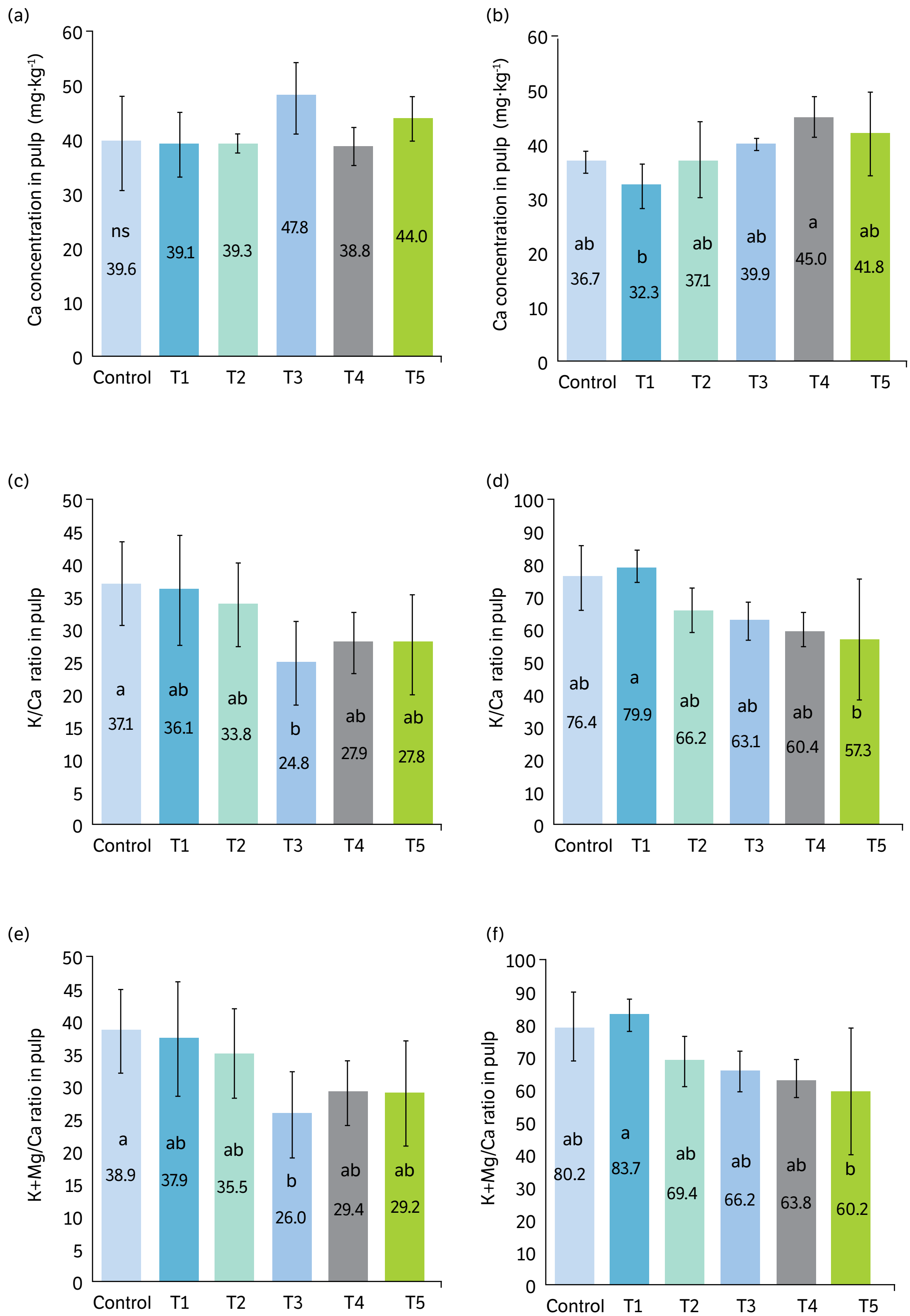

Figure 3. Calcium concentration in the 1st harvest (a) and 2nd harvest (b); K/Ca ratio in the 1st harvest (c) and 2nd harvest (d); and K + Mg/Ca ratio in the 1st harvest (e) and 2nd harvest (f) in the fruit pulp of 'Maxi Gala' submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

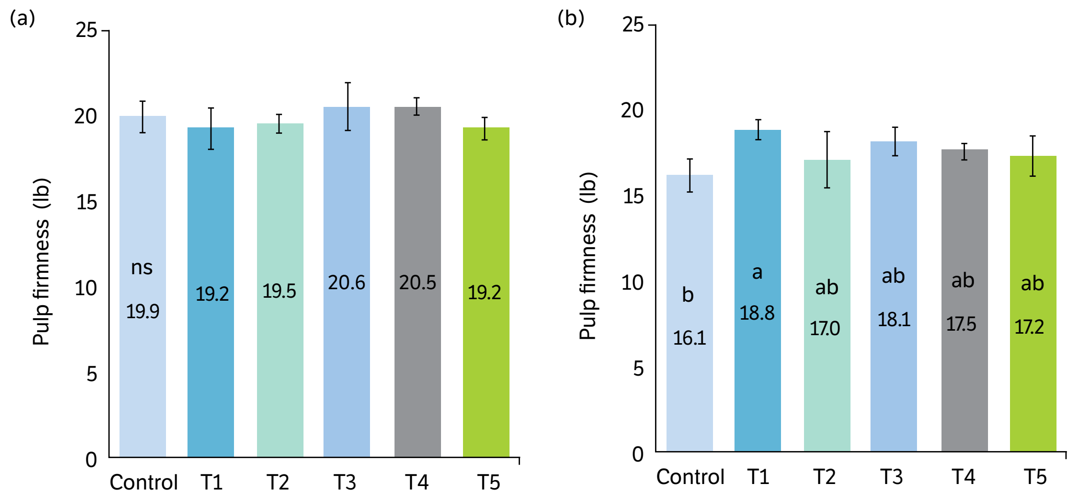

Figure 4. Pulp firmness in the 1st harvest (a) and in the 2nd harvest (b) in 'Fuji Suprema' apples submitted to foliar applications of different Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

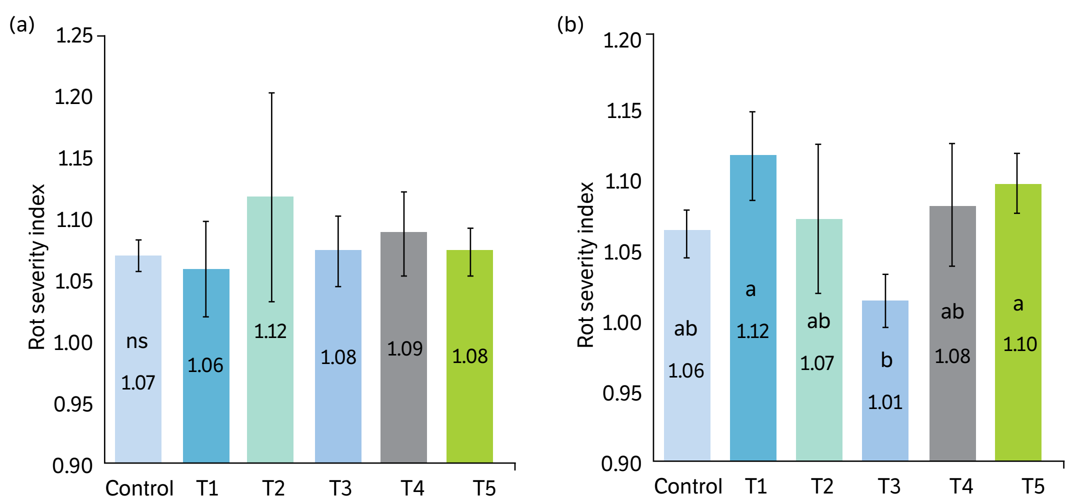

Figure 5. Rot severity index in the fruit 7 months after the 1st harvest (a) and 2nd harvest (b) in 'Fuji Suprema' apples submitted to foliar applications of different Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

Fruit quality and physiological disorders after storage

Calcium treatments did not affect ripening and physiological disorders in 'Fuji Suprema' apples after cold storage (Table 4). Pulp firmness values after 7-month cold storage (19.0 lb, on average, in all treatments) were virtually the same as those observed at the beginning of storage (19.8 lb, on average, in all treatments) (Table 5). In addition, there was low incidence of physiological disorders in 'Fuji Suprema' apples, which indicated the good nutritional status of this cultivar, for storage purposes (Amarante et al. 2012; Fallahi et al. 2010). Overall rot and core rot were the most observed postharvest disorders, although at low levels (Tables 4 and 5).

Table 4. Maturation attributes and physiological disorders in 'Fuji Suprema' fruit from the 1st harvest after seven months of storage, submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treatments	Pulp firmness (lb)	TSS (°Bx)[1]	ER[2]	Cracking	LD[3]	BP[4]	CR[5]
Control	19.16	13.1	1.07	1.00	1.00	1.06	1.08
T1	19.04	13.1	1.06	1.00	1.00	1.12	1.01
T2	19.40	12.9	1.12	1.00	1.01	1.02	1.09
T3	18.85	12.9	1.08	1.00	1.00	1.01	1.11
T4	18.64	13.0	1.09	1.01	1.00	1.04	1.06
T5	18.91	13.3	1.08	1.00	1.00	1.06	1.08
Mean	19.00	13.05	1.08	1.00	1.00	1.05	1.07
F-teste	0.68	0.83	0.55	0.45	0.45	0.11	0.08
CV (%)	3.5	3.69	4.29	0.82	0.82	5.25	4.21

[1]TSS = total soluble solids; [2]ER = external rot; [3]LD = lenticellar depression; [4]BP = bitter pit; [5]CR = carpel rot; CV = coefficient of variation. Means were analyzed by Tukey's test at $p < 0.05$. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table 5. Maturation attributes and physiological disorders in 'Fuji Suprema' fruit from the 2nd harvest after seven months of storage, submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treatments	Pulp firmness (lb)	TSS (°Bx)[1]	ER[2]	LD[3]	BP[4]	HDS[5]	CR[6]	SS[7]	SD[8]
Control	17.49	13.7	1.06ab	1.00	1.00	1.00	1.09	1.01	1.00
T1	17.40	13.4	1.12a	1.03	1.04	1.01	1.16	1.01	1.00
T2	17.61	13.6	1.07ab	1.02	1.01	1.00	1.14	1.00	1.00
T3	17.25	13.4	1.01b	1.01	1.04	1.00	1.10	1.00	1.01
T4	17.17	13.6	1.08ab	1.01	1.02	1.00	1.10	1.00	1.00
T5	17.40	13.8	1.10a	1.03	1.04	1.00	1.09	1.00	1.01
Mean	17.39	13.58	1.07	1.02	1.03	1.00	1.11	1.00	1.00
F-teste	0.96	0.87	0.02	0.27	0.26	0.45	0.62	0.45	0.60
CV (%)	4.16	3.72	3.56	2.28	2.86	0.41	6.61	0.52	0.60

[1]TSS = total soluble solids; [2]ER = external rot (cm^2); [3]LD = lenticellar depression; [4]BP = bitter pit. [5]HDS = hard deep scald (blotch pit or deep scald) without corky below the epidermis; [6]CR = carpel rot (cm^2); [7]SS = superficial scald. [8]SD = senescent degeneration; CV = coefficient of variation. Means were analyzed by Tukey's test at $p < 0.05$. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

'Maxi Gala' apples did not show any effect of Ca applications on fruit ripening after 7-month cold storage, in fruit of both harvest times (Tables 6 and 7). 'Maxi Gala' apples recorded a greater reduction in pulp firmness than 'Fuji Suprema'; the average firmness of the 'Maxi Gala' pulp recorded for early-harvested fruit reached 22.7 lb, whereas that of fruit of 2nd harvest reached 20.5 lb. Pulp firmness has decreased to 11.02 and 8.85 lb, after 7-month cold storage, respectively. The lower firmness loss rate recorded for 'Fuji Suprema' apples during storage, in comparison to that of 'Maxi Gala' apples, was closely associated with the treatment based on ethylene inhibitor 1-MCP application to 'Fuji Suprema' apples and with genetic differences between cultivars. The lowest fruit firmness value recorded for 'Maxi Gala' apples may be associated with fruit ripeness, since this cultivar is more sensitive to ethylene than 'Fuji Suprema' (Brackmann et al. 2000).

Although there was no difference between Ca sources and doses in the applied treatments, pulp browning disorder recorded the most significant incidence, both at the 1st and 2nd harvests (Tables 6 and 7). Rot and bitter pit have also recorded considerable incidence at both harvests. However, it is worth mentioning that the disorder incidence observed in the current study was overall low in comparison to that observed in similar studies (Freitas et al. 2012; Watkins and Mattheis 2019).

After 7-month cold storage of 'Maxi Gala' fruit from 1st harvest, it was possible seeing that fruit subjected to the Ca applications decreased the bitter pit index compared to the control, which had the highest index (1.14) (Fig. 6b). Low Ca concentrations in fruit peel and pulp, as well as high Mg/Ca, K + Mg/Ca and K + Mg + N/Ca ratios, have led to increased bitter pit severity (Amarante et al. 2006). In addition, fruit size has influenced its susceptibility to bitter pit, since exponential fruit size increase also increased nutrient content dilution—such as Ca—in fruit and enabled the emergence of this disorder (Brackmann and Ribeiro 1992). In the present study, the relationship of nutrient dilution in larger fruit was not verified. The fruit had an average weight of 108.4 and 115.6 g for 'Maxi Gala' and 'Fuji Suprema', respectively, without treatment effect, which was also verified for yield (24.7 and 29.1 $t \cdot ha^{-1}$, respectively) (data not shown). Apple fruit presenting Ca deficiency have shown higher bitter pit incidence in the postharvest period (Corrêa et al. 2017; Miqueloto et al. 2018), and it can get worse when fruit present high Mg, N and K levels and its relationship with Ca (Amarante et al. 2020), as the ones observed in the present study. On the other hand, fruit presenting high Ca concentrations often show greater pulp firmness, both at harvest and during storage (Fallahi et al. 2010). Thus, leaf applications of fertilizers added with Ca, regardless of its source, have shown positive effect on decreasing the incidence of bitter pit disorder. This outcome corroborates the study by Brackmann et al. (2010), who observed that 9 and 10 $CaCl_2$ (0.6%) applications helped avoiding physiological disorders in fruit belonging to 'Fuji Suprema'.

Table 6. Maturation attributes and physiological disorders in 'Maxi Gala' fruit from the 1st harvest after seven months of storage, submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treat	Fir.[1]	TSS[2]	ER[3]	SSD[4]	ISD[5]	Crack	LD[6]	BP[7]	Shrivel	CR[8]	SDB[9]	DBSC[10]	HDS[11]
Control	11.46	13.6	1.09	1.67	1.02	1.07	1.09	1.14	1.01	1.00	1.01	1.15	1.04
T1	11.09	13.9	1.07	1.68	1.08	1.09	1.12	1.26	1.05	1.01	1.01	1.09	1.00
T2	9.08	13.8	1.16	2.00	1.06	1.27	1.14	1.13	1.02	1.02	1.00	1.19	1.01
T3	11.95	13.5	1.09	1.49	1.00	1.07	1.12	1.07	1.08	1.02	1.04	1.06	1.00
T4	11.32	13.8	1.09	1.52	1.04	1.07	1.12	1.12	1.03	1.00	1.03	1.12	1.02
T5	11.20	13.7	1.12	1.53	1.06	1.04	1.20	1.18	1.01	1.00	1.03	1.15	1.03
Mean	11.02	13.7	1.10	1.65	1.04	1.10	1.13	1.15	1.03	1.00	1.02	1.13	1.02
F-teste	0.20	0.87	0.62	0.10	0.21	0.19	0.43	0.79	0.19	0.52	0.21	0.33	0.53
CV (%)	14.0	3.1	7.01	15.4	4.4	11.5	6.7	15.9	3.9	1.8	2.3	7.3	3.2

[1]Fir = PULP firmness (lb); [2]TSS = total soluble solids (°Bx); [3]ER = external rot; [4]SSD = superficial senescent degeneration; [5]ISD = internal senescent degeneration; [6]LD = lenticellar depression; [7]BP = biter pit; [8]CR = carpel rot; [9]SDB = superficial diffuse browning; [10]DBSC = dark brown spots in the carpel chalice. [11]HDS = hard deep scald (blotch pit or deep scald) without corky below the epidermis; CV = coefficient of variation. Means were analyzed by Tukey's test at $p < 0.05$. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table 7. Maturation attributes and physiological disorders in 'Maxi Gala' fruit from the 2nd harvest after seven months of storage, submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treat	Fir[1]	TSS[2]	ER[3]	Crack	SSD[4]	SDB[5]	LD[6]	BP[7]	Shrivel	ISD[9]	CR[10]
Control	9.54	15.1	1.07	1.21	1.82	1.20	1.07	1.14a	1.00	1.03	1.00
T1	8.29	14.8	1.27	1.40	2.26	1.06	1.10	1.02b	1.00	1.13	1.00
T2	8.74	14.6	1.07	1.23	2.15	1.06	1.10	1.02b	1.01	1.07	1.00
T3	8.06	14.7	1.27	1.47	2.37	1.12	1.19	1.01b	1.00	1.05	1.00
T4	8.77	14.5	1.14	1.32	1.96	1.12	1.15	1.01b	1.00	1.03	1.01
T5	9.68	15.0	1.04	1.15	1.78	1.08	1.19	1.00b	1.00	1.06	1.01
Mean	8.85	14.78	1.14	1.30	2.06	1.11	1.13	1.02	1.00	1.06	1.00
F-teste	0.09	0.32	0.46	0.46	0.10	0.46	0.94	0.01	0.45	0.15	0.45
CV (%)	10.67	3.11	11.74	17.63	17.24	7.22	9.91	4.66	0.41	6.79	0.41

[1]1Fir = Pulp firmness (lb); [2]TSS = total soluble solids (°Bx); [3]ER = external rot; [4]SSD = superficial senescent degeneration; [5]SDB = superficial diffuse browning; [6]LD = lenticellar depression; [7]BP = bitter pit; [8]ISD = internal senescent degeneration; [10]CR = carpel rot; CV = coefficient of variation. Means were analyzed by Tukey's test at $p < 0.05$. Control = water application, only; T1 = CaCl2 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Calcium translocation to fruit only takes place through the xylem, which functionality in apple fruit is relatively short (Dražeta et al. 2004; Miqueloto et al. 2014). Therefore, it is important emphasizing that supplementary Ca sources must be applied in order to guarantee fruit quality, whenever there is competition for this nutrient between organs such as leaves and fruit, with emphasis on 'Fuji Suprema', whose fruit present high nutritional imbalance (Amarante et al. 2012).

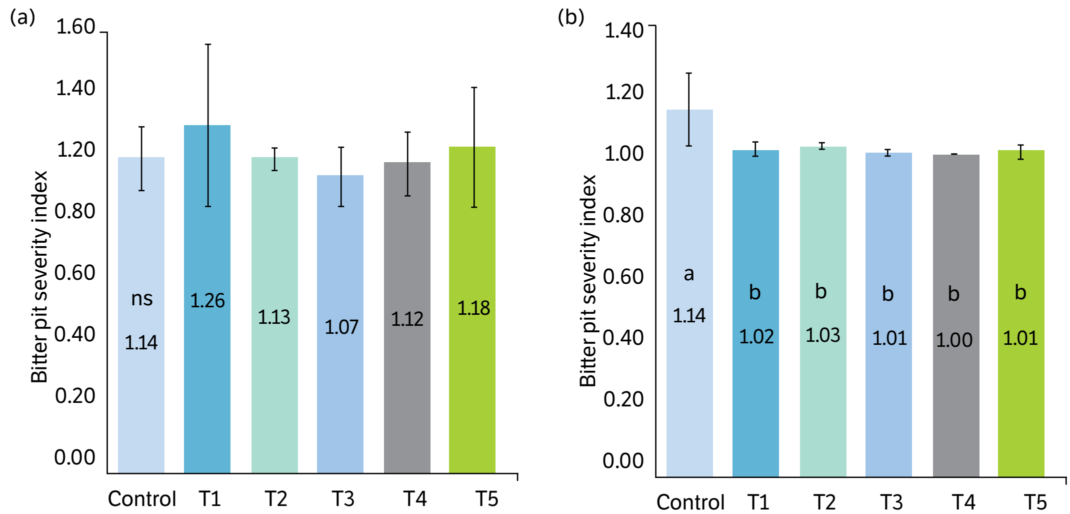

Figure 6. Bitter pit severity index (scale 1 to 4) on apples 7 months after the 1st (a) and 2nd (b) harvest in 'Maxi Gala' apple trees submitted to foliar applications of different Ca sources starting at 30 DAFB and reapplication every 15 days.

Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB. Means followed by the same letter do not differ by the Tukey's test at $p < 0.05$. ns = nonsignificant.

In general, the new sources of Ca tested did not increase Ca contents, nor did they improve N/Ca or K + Mg/Ca ratios, nor did they reduced the risk for physiological disorder compared to the standard $CaCl_2$ treatment that has been commercially used for decades as the main Ca fertilizer for apple trees. An exception occurred for the Ca concentration in the peel of the cultivar Maxi Gala in the 1st harvest, where T2, T3, and T5, as well as for T5 in the 2nd harvest, which showed a higher Ca concentration compared to the standard $CaCl_2$ treatment.

CONCLUSION

Calcium fertilizers $CaCl_2$, product A (Ca complexed with sugar-alcohol plus N and Mg) and product B (CaO plus B complexed with seaweed) sprayed on apples trees did not change Ca levels in leaves.

All three Ca-source fertilizers have increased Ca levels in the fruit peel of both 'Maxi Gala' and 'Fuji Suprema' apple trees. However, the pulp was influenced to a lesser extent, whereas whole fruit were not affected by these fertilizers.

Calcium sources have decreased the incidence of bitter pit in fruit of 'Maxi Gala', depending on harvest time.

The new sources of Ca tested did not increase Ca contents, N/Ca or K + Mg/Ca ratios, nor did they reduced the risk for physiological disorder compared to the standard $CaCl_2$ treatment that has been commercially used for decades as the main Ca fertilizer.

AUTHORS' CONTRIBUTION

Conceptualization: Hahn L. and Argenta L. C.; **Methodology:** Hahn L., Suzin, D. L. and Argenta L. C.; **Investigation:** Hahn L. and Suzin, D. L.; **Writing – Original Draft:** Hahn L., Tiecher T. L., Thewes F. R., Moura-Bueno, J. M. and Brunetto

G., **Writing – Review and Editing:** Hahn L., Argenta L. C., Tiecher T. L. and Moura-Bueno, J. M., Brunetto, G.; **Funding Acquisition:** Hahn L.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

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SUPPLEMENTARY MATERIAL

Table S1. Concentrations and relationships between nutrients in the peel, pulp and whole fruit of 'Fuji Suprema' apples in the 1st harvest with foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Parts	Treatments	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
		mg·kg^{-1}							
Peel	Control	1039.0	730.7	2338.0	122.6	291.5	8.6	19.3	21.7
	T1	989.9	734.5	2040.4	139.7	273.0	7.1	14.5	16.5
	T2	1058.6	755.1	2117.4	134.7	295.0	7.8	15.7	17.9
	T3	1075.4	690.3	2211.2	141.0	307.5	7.7	15.7	17.9
	T4	1046.8	737.3	2232.6	137.8	302.6	7.6	16.2	18.3
	T5	961.7	731.2	2095.0	141.9	307.1	6.8	14.8	17.0
	Mean	1028.6	729.9	2172.4	136.3	296.1	7.6	16.0	18.2
	F-test	0.74	0.84	0.87	0.41	0.97	0.19	0.10	0.10
	CV (%)	11.5	9.2	16.6	10.2	22.1	11.5	13.7	12.8
Pulp	Control	321.7	286.9	1689.1	41.2	54.1	8.1	41.4	42.7
	T1	361.5	308.4	1783.1	47.3	59.7	7.7	37.8	39.1
	T2	298.0	271.0	1590.7	42.7	52.7	7.0	37.7	39.0
	T3	386.2	286.6	1461.5	41.9	49.1	9.3	35.1	36.3
	T4	367.5	303.8	1547.9	43.7	52.1	8.4	35.6	36.8
	T5	352.7	281.4	1543.9	45.6	54.3	7.8	34.1	35.3
	Mean	347.9	289.7	1602.7	43.7	53.7	8.1	37.0	38.2
	F-test	0.07	0.73	0.28	0.38	0.63	0.19	0.25	0.26
	CV (%)	11.4	13.0	12.2	10.0	15.5	15.2	11.6	11.6
Whole fruit	Control	421.5	313.9	1525.8	44.7	76.1	9.6	34.5	36.2
	T1	382.9	319.3	1424.5	48.8	74.3	8.0	29.7	31.2
	T2	383.6	315.2	1326.5	46.3	70.8	8.3	28.7	30.3
	T3	444.3	305.8	1232.8	46.9	64.9	9.5	26.1	27.5
	T4	443.6	331.5	1413.3	50.6	73.1	8.9	28.0	29.4
	T5	371.2	317.7	1282.8	53.4	68.1	7.3	24.7	26.0
	Mean	407.9	317.2	1367.6	48.5	71.2	8.6	28.6	30.1
	F-test	0.13	0.96	0.32	0.62	0.52	0.44	0.11	0.11
	CV (%)	11.1	12.0	13.8	15.5	12.4	20.5	15.9	15.7

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table S2. Concentrations and relationships between nutrients in the peel, pulp and whole fruit of 'Fuji Suprema' apples in the 2nd harvest with foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Parts	Treatments	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
		mg·kg^{-1}							
Peel	Control	788.0	78.2	1771.3	147.1 b	273.1	5.4	12.1 a	14.0 a
	T1	889.3	68.8	1621.0	176.1 a	248.5	5.0	9.2 b	10.6 b
	T2	829.6	68.5	1715.2	156.0 ab	284.1	5.3	11.0 ab	12.9 ab
	T3	780.0	69.0	1800.4	164.0 ab	296.9	4.8	11.0 ab	12.8 ab
	T4	804.1	67.5	1773.9	177.7 a	294.3	4.6	10.0 b	11.7 b
	T5	785.1	68.0	1682.4	158.8 ab	257.7	5.0	10.7 ab	12.3 ab
	Mean	812.7	70.0	1727.4	163.2	275.8	5.0	10.7	12.4
	F-test	0.65	0.84	0.63	0.00	0.59	0.48	0.04	0.04
	CV (%)	12.5	18.3	9.4	5.2	16.3	12.8	11.0	11.1

Continue...

Table S2. Continuation...

Parts	Treatments	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
		------------------ mg·kg^{-1} ------------------							
Pulp	Control	287.1	34.7	1412.9	34.4	47.9	8.4	41.7	43.1
	T1	319.4	28.5	1543.3	38.4	53.9	8.4	40.5	41.9
	T2	277.9	35.1	1469.4	30.6	48.8	9.4	49.9	51.6
	T3	276.4	50.6	1528.2	36.3	53.9	7.7	43.1	44.6
	T4	266.8	34.0	1490.9	35.4	48.8	7.8	43.2	44.7
	T5	266.9	34.0	1592.9	34.4	56.4	8.0	47.2	48.9
	Mean	282.4	36.1	1506.3	34.9	51.6	8.3	44.3	45.8
	F-test	0.58	0.41	0.27	0.24	0.26	0.81	0.61	0.62
	CV (%)	15.6	40.0	7.0	12.1	11.4	22.2	18.9	19.1
Whole fruit	Control	341.4	51.1	1452.8	42.9	85.5	8.0	34.0	36.0
	T1	335.6	58.5	1376.9	51.7	78.4	6.5	26.9	28.4
	T2	307.5	54.0	1267.4	43.4	71.9	7.2	29.5	31.2
	T3	334.5	73.8	1309.5	49.2	85.4	7.0	27.5	29.3
	T4	308.3	47.2	1410.1	47.4	75.6	6.5	29.8	31.3
	T5	344.2	49.2	1435.1	47.8	79.3	7.3	30.4	32.1
	Mean	328.6	55.6	1375.3	47.1	79.4	7.1	29.7	31.4
	F-test	0.42	0.33	0.26	0.19	0.70	0.55	0.23	0.25
	CV (%)	9.7	31.2	8.8	11.0	17.5	17.7	13.7	13.9

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table S3. Concentrations and relationships between nutrients in the peel, pulp and whole fruit of 'Maxi Gala' apples in the 1st harvest with foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Parts	Treatments	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
		------------------ mg·kg^{-1} ------------------							
Peel	Control	696.5	577.1	1106.5	105.4 b	201.4	6.6	10.5	12.4
	T1	627.8	552.8	960.5	106.4 b	184.0	6.3	9.7	11.6
	T2	701.5	580.9	1115.8	124.5 a	225.3	5.6	9.0	10.7
	T3	641.0	561.8	1043.4	131.6 a	229.8	4.9	7.9	9.7
	T4	684.5	584.3	1004.3	114.8 ab	184.8	5.9	8.7	10.3
	T5	674.3	568.6	1039.2	124.5 a	198.3	5.4	8.4	10.0
	Mean	670.9	570.9	1044.9	116.7	203.9	5.8	9.0	10.8
	F-teste	0.91	0.96	0.59	0.00	0.65	0.18	0.12	0.19
	CV (%)	17.0	9.4	13.0	6.7	23.5	15.1	13.7	13.9
Pulp	Control	273.6	178.0	1457.2	39.6	73.0	7.2	37.1 a	38.9 a
	T1	289.4	160.4	1378.2	39.1	69.0	7.5	36.1 ab	37.9 ab
	T2	279.9	162.4	1324.9	39.3	66.7	7.1	33.8 ab	35.5 ab
	T3	281.5	162.2	1163.2	47.8	57.7	6.0	24.8 b	26.0 b
	T4	280.8	152.5	1073.5	38.8	57.6	7.3	27.9 ab	29.4 ab
	T5	287.8	161.2	1203.3	44.0	63.2	6.6	27.8 ab	29.2 ab
	Mean	282.2	162.8	1266.7	41.4	64.5	6.9	31.3	32.8
	F-teste	0.99	0.86	0.10	0.22	0.70	0.64	0.04	0.04
	CV (%)	12.3	16.7	15.2	14.0	24.9	19.4	18.5	18.5
Whole fruit	Control	327.7	385.4	1171.2	48.3	65.8	7.0	24.3	25.7
	T1	334.2	358.0	1186.2	39.7	66.3	8.4	30.7	32.4
	T2	354.2	384.7	1330.8	51.8	84.9	6.9	25.9	27.6
	T3	303.2	365.1	1102.0	52.8	68.2	5.9	21.6	22.9
	T4	290.2	378.3	1225.1	46.0	67.6	6.3	26.6	28.1
	T5	302.4	375.6	1378.2	47.3	90.2	6.6	28.7	30.6
	Mean	318.6	374.5	1232.3	47.6	73.8	6.9	26.3	27.9
	F-teste	0.10	0.83	0.31	0.24	0.30	0.13	0.22	0.23
	CV (%)	8.1	9.0	14.7	16.1	25.4	17.7	19.2	19.2

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table S4. Concentrations and relationships between nutrients in the peel, pulp and whole fruit of 'Maxi Gala' apples in the 2nd harvest with foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Parts	Treatments	N	P	K	Ca	Mg	N/Ca	K/Ca	K+Mg/Ca
		------------------		$mg \cdot kg^{-1}$		------------------			
Peel	Control	748.6	419.0	2239.0	110.2b	586.0	6.8	20.3	25.7
	T1	576.8	435.7	2074.7	110.5b	392.6	5.3	18.9	22.4
	T2	659.1	402.4	1889.2	125.2ab	447.5	5.4	15.2	18.8
	T3	614.6	394.7	1960.9	121.0ab	440.7	5.1	16.3	19.9
	T4	767.9	414.9	2046.6	139.5ab	510.1	5.7	14.9	19.0
	T5	703.9	393.2	2019.4	143.0a	547.0	5.0	14.7	18.5
	Mean	678.5	410.0	2038.3	124.9	487.3	5.5	16.7	20.7
	F-teste	0.25	0.49	0.26	0.03	0.13	0.39	0.22	0.45
	CV (%)	18.3	8.3	9.6	16.8	21.0	23.1	18.3	16.8
Pulp	Control	284.2	259.8	2551.6	36.7ab	129.0	7.5	76.4ab	80.2 ab
	T1	280.9	231.5	2429.0	32.3b	117.8	7.9	79.9a	83.7 a
	T2	256.8	258.5	2521.5	37.1ab	124.2	6.4	66.2ab	69.4 ab
	T3	301.0	266.8	2701.7	39.9ab	152.2	6.7	63.1ab	66.2 ab
	T4	269.4	266.8	2295.0	45.0a	117.0	6.8	60.4ab	63.8 ab
	T5	296.2	271.9	2788.2	41.7ab	138.3	8.1	57.3b	60.2 b
	Mean	280.9	231.5	2429.0	37.1	117.8	7.9	67.2	70.6
	F-teste	0.40	0.63	0.11	0.04	0.46	0.12	0.01	0.02
	CV (%)	12.3	13.3	9.6	12.7	21.4	20.5	13.5	13.4
Whole fruit	Control	339.9	257.6	2765.7	45.8	183.3	7.9	62.9	67.0
	T1	317.1	258.1	2333.9	47.4	152.3	6.7	49.3	52.5
	T2	327.5	226.8	2031.6	48.4	135.6	6.9	43.0	45.8
	T3	303.6	242.6	2407.2	49.1	143.1	6.2	49.2	52.1
	T4	331.2	237.7	2149.9	43.1	148.8	7.8	50.4	53.9
	T5	299.2	257.1	2288.7	48.7	140.7	6.3	47.5	50.5
	Mean	319.8	246.7	2329.5	47.1	150.7	7.0	50.4	53.6
	F-teste	0.48	0.85	0.22	0.87	0.59	0.60	0.25	0.24
	CV (%)	10.3	16.9	17.4	16.0	25.8	24.4	21.6	21.6

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table S5. Pulp firmness values, total soluble solids (TSS), and iodine-starch index in 'Fuji Suprema' in the 1st and 2nd harvest of fruit submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 day.

Treatments	Pulp firmness (lb)		TSS (°Bx)		Iodine-starch index	
	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest
Control	19.94	16.14b	12.1	13.9	3.4	6.2
T1	19.23	18.77a	12.0	13.3	3.9	6.6
T2	19.58	17.28ab	11.6	13.3	3.3	6.7
T3	20.50	18.09ab	12.0	13.8	3.6	6.8
T4	20.55	17.53ab	11.7	13.4	2.9	6.0
T5	19.26	17.23ab	12.3	13.4	3.3	7.1
Mean	19.84	17.50	11.95	13.5	3.4	6.6
F-teste	0.68	0.01	0.76	0.89	0.45	0.75
CV (%)	6.23	5.79	17.78	4.93	22.42	12.86

Means followed by the same letter, in the columns, do not differ by the Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.

Table S6. Pulp firmness values, total soluble solids (TSS), and iodine-starch index in 'Maxi Gala' in the 1st and 2nd harvest of fruit submitted to foliar applications of Ca sources starting at 30 DAFB and reapplication every 15 days.

Treatments	Pulp firmness (lb)		TSS (°Bx)		Iodine-starch index	
	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest	1st Harvest	2nd Harvest
Control	23.15	20.60	12.1	14.50	2.50	3.4
T1	21.43	18.93	12.4	13.90	2.10	3.9
T2	22.07	19.57	13.2	13.70	2.00	3.3
T3	23.19	21.55	13.6	13.60	2.20	3.6
T4	23.31	20.97	11.7	13.65	1.60	2.9
T5	23.40	21.41	11.8	14.65	1.98	3.3
Mean	22.76	20.50	12.5	14.0	2.06	3.4
F-teste	0.82	0.56	0.16	0.13	0.69	0.45
CV (%)	8.83	8.63	14.24	4.03	45.61	22.42

Means were analyzed by Tukey's test at $p < 0.05$. CV = coefficient of variation. Control = water application, only; T1 = $CaCl_2$ 0.5% Ca every 15 days after full bloom (DAFB); T2 = Product A 0.25% Ca every 15 DAFB; T3 = Product A 0.25% Ca product every 30 DAFB; T4 = Product A 0.5% Ca every 15 DAFB; T5 = Product B 0.5% Ca every 15 DAFB.