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Calcium Sprays and Crop Load Reduction Increase Fruit Quality and Postharvest Storage in Sweet Cherry (*Prunus avium* L.)

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Abstract: In many fruit trees, the thinning of buds, flowers, or fruits is used to increase the leaf area-to-fruit ratio (LA:F) and reduce competition for carbohydrates. Meanwhile, calcium (Ca) sprays during fruit development are also used to increase fruit quality and postharvest storage. Such practices have been recommended to increase fruit firmness and reduce fruit cracking in sweet cherries. To understand the effects of foliar Ca sprays and crop load reductions in the combination 'Lapins'/'Colt', trained as the Kym Green Bush, a factorial experiment to determine the interactions between both managements was established in the Central Valley of Chile during the 2018/2019 growing season. Two levels of crop load (CL) were established—thinned (50% crop load) and unthinned (100% crop load) during Stage I of fruit development (31 days after full bloom, DAFB). Three timings of foliar applications of $CaCl_2$ (TFA; 0.8%) were evaluated: early 26 DAFB, later 39 DAFB, or late 62 DAFB. Natural fruit contents and concentrations of Ca were determined on unsprayed control trees. Fruit from the thinned trees were significantly larger and heavier and had a higher titratable acidity than unthinned trees did. Significant interactions between TFA and CL were observed for SSC, without a clear trend. Thinned trees were less affected by pedicel detachment, browning, and fruit decay after 45 d of storage (0 °C). In unthinned trees, a foliar $CaCl_2$ spray at Stage I allowed a higher fruit firmness than $CaCl_2$ sprays at Stage II and III of fruit development. The $CaCl_2$ applications at 39 or 62 DAFB reduced the incidence of cracking in thinned trees. Natural Ca concentrations decreased during fruit development, indicating a cessation of Ca import and a dilution by subsequent growth. Our results suggest that the early reduction of crop load has positive effects on fruit quality and condition during storage, and early Ca sprays (Stage I) improve fruit textural properties, even under high crop loads.

Keywords: cracking; fruit firmness; fruit quality; fruit thinning; growth; KGB; leaf area; photoassimilates; rheological properties

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1. Introduction

An optimal balance between fruits and leaves and a precise mineral nutrition strategy are essential for the production of high-quality sweet cherry fruit [1–3]. Compared with many tree crop species, the ontogeny of a sweet cherry fruit is quite short—just 60 to 80 days from bloom to harvest [4,5]. Over this period, fruits compete strongly with vegetative sinks for both carbohydrates (CHO) and macronutrients such as nitrogen (N) and Ca [6–9]. Fruit yield and fruit quality are positively related to the ratio of leaf area to fruit number (LA:F) [10–12]. The area of the fruiting spur leaves is too low to supply sufficient CHO to support fruit growth in the central axis, so additional CHO is required from the current season shoot (CSS) and from nonfruiting spurs (NFS) [7].

New 'pedestrian' training systems such as the Kym Green Bush (KGB) are sometimes used by sweet cherry growers to facilitate management (e.g., pruning, thinning, and harvest) and to reduce establishment and production costs. The KGB system comprises

multiple, temporary, vertical fruiting branches with spurs only, as lateral CSSs are removed during the growing season [13]. The protocol to produce high-quality fruit under the KGB requires periodic branch removal [14]. However, there is little information on the best strategies for crop load regulation, mineral nutrition, and irrigation supply for the KGB. It has been suggested that sweet cherry trees trained as a KGB may require fruit thinning to achieve high-quality fruit [15].

In most sweet cherry training systems, the main strategies for regulating crop load, and to optimize LA:F, include pruning and/or thinning of buds, flowers, and/or fruits [16]. Pruning is the most effective and less expensive strategy to control crop load in sweet cherries [17]. So far, chemical [18–20] and mechanical thinning [3] have been promising to reduce fruit number, but more studies are required. In this species, an optimal LA:F maximizes fruit quality parameters such as fruit mass, diameter, color, and soluble solids content [10,17,21] and minimizes mechanical damage (e.g., pitting) in some cultivars [22].

Other important fruit quality attributes include fruit color, size, firmness, and pedicel greenness [23,24]. Fruit texture is also an essential factor determining the maintenance of sweet cherry quality during handling shipping and storage. A firm texture is associated with reduced susceptibility to postharvest rots [23], higher resistance to mechanical damage, and greater consumer acceptance [25,26]. As well as firmness, instruments can also be used to analyze texture, which characterizes the rheological properties of the fresh fruit, based on detection of the mechanical parameters via a force–displacement curve [27]. This reports the elastic modulus and identifies the bio-yield point where high deformations will occur with quite small additional forces, with the strain and stress properties of the tissue being determined. Sweet cherries with high deformation and resistance values are more resistant to mechanical damage [28].

Different postharvest managements such as modified atmosphere packaging (MAP) [26], Ca-based products (e.g., Ca-acetate, Ca-chloride, Ca-formate, Ca-heptagluconate, Ca-lactate, Ca-nitrate. or Ca-propionate) [29], and edible coatings (e.g., alginate, gelatine, carboxy-methylcellulose, chitosan, whey protein isolate, shellac, calcium chloride, almond gum, gum arabic, Aloe vera gel, and β-aminobutyric acid) [24,30] have been used to improve storage life in sweet cherry and postharvest managements. In addition, other studies using foliar Ca-based products [31–33] and salts [34] during fruit development have shown positive effects on fruit quality. However, additional information about the relationships between preharvest foliar $CaCl_2$ application and crop load regulation (i.e., pruning and fruit thinning) is required to better understand their impact on the fruit quality, textural properties, and storage life of sweet cherries.

Calcium (Ca) is considered a critical nutrient in determining fruit quality in sweet cherries [9]. The physical and structural properties of fruit texture are influenced by Ca–pectin cross-links [35]. Although physiological disorders are not always associated with low fruit Ca concentration, some studies have shown that immersing fruit in $CaCl_2$ solutions of increasing concentration can reduce the proportion of cracked fruit [29,36]. Similarly, the effects of applying foliar Ca sprays have also reduced the incidence of preharvest, rain-induced cracking in fruit [37–39]. They have also increased fruit firmness and reduced pitting [31,37,39]. However, other authors have reported different results [32,40–42]. Measham [32] did not find differences in the cracking index, SSC, or TA in 'Sweet Georgia'/'F12/1', but fruit firmness increased after using Ca-based products. Similarly, Correia [40] did not find differences in the fruit cracking index in the combination 'Skeena'/'Gisela 6' after $CaCl_2$ sprays. In addition, Vangdal [42] did not find significant differences in color, firmness, or TA in cv. 'Merton Glory', 'Vega', or 'Sue' sweet cherries on 'F12/1' with foliar $CaCl_2$ sprays.

These inconsistencies may be explained by some interactions between a range of horticultural practices whose effects on fruit quality were not distinguished from a foliar nutrition effect. Currently, there is a dearth of information on the influences of foliar $CaCl_2$ applications on fruit textural properties for sweet cherries. Furthermore, we are unaware of detailed studies that consider the combination of early crop-load reduction and foliar

$CaCl_2$ applications aimed at improving fruit quality and storage capacity for sweet cherry trees trained as a KGB. In this study, we hypothesize that fruit thinning and foliar Ca sprays during early fruit development in sweet cherries may interact positively to increase fruit quality and storage capacity in the combination 'Lapins'/'Colt', trained as a KGB.

2. Materials and Methods

2.1. Plant Material and Environmental Conditions

The experiment was carried out in an established commercial orchard using the sweet cherry combination 'Lapins'/'Colt' rootstock during the 2018/2019 growing season. The orchard was located at San Francisco de Mostazal, O'Higgins Region, Chile (34°0′ S, 71°41′ W). Trees were established in 2014 at a 3.5 × 2.0 m spacing and were trained as a KGB. The orchard had two dripper irrigation lines per row with four pressure-compensated emitters per tree (each emitter rated at 4 $L{\cdot}h^{-1}$). Trees were irrigated daily based on an estimate of crop evapotranspiration (100% replacement) to maintain nonlimiting soil water conditions. Irrigation requirements were determined according to daily crop reference evapotranspiration calculated using the Penman–Monteith equation [43]. According to Uribe et al. [44], the climate is warm-temperate with a prolonged dry season (200 to 250 days). The rainfall is concentrated between May and August with a mean annual precipitation in the range from 300 to 400 mm. The thermal regime is characterized by temperatures ranging between maximum values of 30 °C during the summer (January) and a minimum during the winter of 2.6 °C (July) [44]. For additional climatic data, see Supplementary Materials.

The soil has a sandy loam texture, flat topography, moderate depth (75–100 cm), and good drainage [45]. The main physical and chemical soil properties (0–30 cm) at the beginning of the season include an organic matter content of 1.93%, pH 6.5, and an electrical conductivity ($EC_{25\,°C}$) of 1 dS m^{-1}. The macronutrient availability was nitrogen (N) 16.5 $mg{\cdot}kg^{-1}$, phosphorous (P) (P-Olsen) 73 $mg{\cdot}kg^{-1}$, and potassium (K) 215 $mg{\cdot}kg^{-1}$. The exchangeable cations Ca and magnesium (Mg) were 13.9 and 3.2 meq 100 g^{-1}, respectively. Mineral nutrition consisted of 40 $kg{\cdot}ha^{-1}$ of N as $CO(NH_2)_2$ (35 $kg{\cdot}ha^{-1}$) and $NH_4NO_3{}^-$ (4 $kg{\cdot}ha^{-1}$), 20 $kg{\cdot}ha^{-1}$ of P as $H_3PO_4{}^-$, and 54 $kg{\cdot}ha^{-1}$ of K as K_2O (Aquamix full K®, Quimetal, Santiago, Chile). Macronutrients were provided through the irrigation system during the growing season. Foliar applications included 8 $kg{\cdot}ha^{-1}$ of boron (B) as $Na_2B_8O_{13}{\cdot}4H_2O$ (Solubor ®, Compo Expert, Santiago, Chile) at 100% of full bloom. The pest management program included monitoring and control: *Diaspidiotus perniciosus*, *Frankliniella occidentalis*, *Caliroa cerasi*, *Proeulia* spp., *Brevipalpus chilensis*, *Panonychus ulmi*, and *Drosophila suzuki*.

A 4 × 2 factorial design was used with eight treatments distributed in a completely randomized block design with five blocks (replications). Each block consisted of 16 trees, and each treatment was imposed on two trees as the experimental unit. The selected trees (16 × 5 blocks = 80 trees in total) were of similar size, vigor, and health.

The first factor 'A' was the timing of foliar $CaCl_2$ application and considered 4 levels (3 phenological stages + 1 control) and three phenological stages: 26 days after full bloom (DAFB) (Stage I of fruit development), 39 DAFB (Stage II), or 62 DAFB (Stage III). A single foliar application of $CaCl_2$ 0.8% (10.59 $g{\cdot}L^{-1}$ $CaCl_2{\cdot}2H_2O$; ACS grade, catalog no. 223506, Sigma-Aldrich Co., St. Louis, MI, USA) until runoff was carried out for each phenological stage (Stage I = 2.5 L, Stage II = 3.5 L, or Stage III = 4.5 L per tree) using a backpack sprayer (Nitro Tools, 14 L, 42 cc, Santiago, Chile). The control was sprayed with water only. The second factor 'B' was crop reduction and was of two levels: 100% crop load (i.e., natural crop load without fruit thinning) and 50% natural crop load (i.e., 50% of fruit were removed by hand) (Table 1). Fruit thinning left two fruits per spur and was carried out at 31 DAFB (Stage I). Full bloom was on September 7 (0 DAFB), fruit set was on September 27 (20 DAFB), and commercial harvest was on December 6 (90 DAFB).

Table 1. Sweet cherry ('Lapins'/'Colt') trained as Kym Green Bush (n = 80). Factorial design: Factor A: Timing of $CaCl_2$ foliar application. Factor B: Crop load.

Factor	Levels
Timing of $CaCl_2$ foliar application (A)	Days after full bloom
	Control (water)
	26
	39
	62
Crop load (B)	Unthinned trees (natural crop load)
	Thinned trees (50% fruit removed at Stage I, 31 DAFB)

2.2. Morphological and Leaf-to-Fruit Ratio Characterization

Before imposing treatments, a population of 80 uniform trees were characterized and one vertical branch was selected per tree with similar vigor and crop load. Trunk cross-sectional area was measured 20 cm above ground level (Table 2).

Table 2. Morphological characterization of individual vertical branches in the sweet cherry combination 'Lapins'/'Colt' trained as KGB during the 2018/19 growing season (n = 80).

Morphological Measurement	Season 2018–2019
	Mean [1] SEM
Number of nonfruiting spurs per branch	19.6 ± 0.9
Number of fruiting spurs per branch	25.7 ± 0.8
Number of fruit per fruiting spur at fruit set	9.6 ± 0.5
Number of branches per tree	26.2 ± 0.5
Length of nonfruiting spur section per branch (cm)	45.4 ± 0.4
Length of fruiting spur section per branch (cm)	104.5 ± 0.9
Length of terminal current season shoot (cm)	49.6 ± 0.7
Trunk cross-sectional area (cm)	93.0 ± 2.3

[1] Standard error of the mean (SEM) of 80 trees.

Five fruiting spurs (FS), NFS, and CSS were randomly sampled from four replicates per treatment to estimate the whole canopy leaf area (LA). Leaf measurements were made at 25, 50, and 88 DAFB using a leaf area meter (LI-COR, LI-3100, Lincoln, NE, USA). The LA:F was calculated according to Whiting and Lang [46]. Fruit diameter (mm) was measured weekly from 23 DAFB to harvest (90 DAFB) in 20 representative fruits per replicate using a digital caliper (model 500-196, Mitutoyo Corp., Kawasaki, Japan).

As expected, compared with the unthinned trees, the thinned trees had a higher LA:F at 50 DAFB (+33.5%) and 88 DAFB (+33.8%) (Table 3).

Table 3. Leaf area-to-fruit ratio (LA:F) in the sweet cherry combination 'Lapins'/'Colt' for two levels of crop load: (1) unthinned (natural crop load) and (2) thinned (50% of fruit removed, 31 DAFB).

		[1] LA:F (cm^2 $Fruit^{-1}$)				
		Days after Full Bloom				
		25	50		88	
Crop Load	Unthinned	27.8	45.8	b	83.4	b
	Thinned [2]	28.3	68.9	a	126.1	a
p-value		0.522	<0.0001		<0.0001	

[1] Different letters in the same column indicate significant differences between crop loads based on ANOVA ($p \leq 0.05$). [2] Fruit thinning was performed 31 DAFB.

The presence of leaves damaged by the $CaCl_2$ sprays was evaluated 15 d after each application on 10 shoots per replicate in each of the FS, NFS, and CSS. Leaves were placed

in plastic bags and transported to the laboratory in a portable cooler (Wenco S.A, Santiago, Chile) at 5 °C. Images were captured using a digital color camera (Canon PowerShot G10; Canon, Japan, Tokyo), to estimate the percentage of leaf area damage. Images were edited using Adobe Photoshop® 5.0.2. Software and IMAGE J (version 1.24t, Wayne Rasband, National Institute of Health MD). The percentage damage was expressed as the percent of brown area vs. the total area.

2.3. Fruit Quality at Harvest: Rheological Properties, Impact Damage Index, Cracking

The fruit was harvested at 90 DAFB between 6.00 and 10.00 h at air temperatures of 10 to 15 °C. Skin color was determined by a skin color chart (3–3.5 cherry color chart scale, Pontificia Universidad Católica de Chile).

Twenty fruits per replicate were evaluated for soluble solids concentration (SSC; %), using a digital thermos-compensated refractometer (PAL-1, Atago Co., Ltd., Tokyo, Japan), diameter (mm) was evaluated using a digital caliper (model 500-196, Mitutoyo Corp., Kawasaki, Japan), skin color (Chroma; C) was evaluated according to CIE (Commission International de l'Eclairage, Vienna, Austria) the system was evaluated using a Minolta colorimeter (Minolta, C 400, Japan), firmness (0–100, Shore units) was evaluated using a durometer (type A, Durofel, Agro-technologie, Tarascon, France), mass (g) was evaluated with a balance (UWE, ABM-60, Taipei Hsien, Taiwan), and titratable acidity (TA; % malic acid) was evaluated by titrating 10 mL of juice with 0.1 N NaOH to pH 8.1.

The rheological properties were measured in twenty fruits per replicate using a Texturometer TA.XT plus analyzer (Stable Micro Systems Ltd., Godalming, England). The test was visualized as a force/distance curve. The value of force (N) represents the force applied to the tissue by a cylindrical probe of 5 mm diameter, with a contact surface of 19.6 mm^2, where the distance refers to the distance traveled by the probe through the fruit surface. The force–distance curve is transformed into a stress/strain curve. The parameters: modulus of elasticity, stress, strain, and energy were obtained according to the protocol used by Param and Zoffoli [28] but considering a penetration depth of 10 mm.

Impact damage was induced one day after harvest on twenty fruits per replicate using a force of 0.10 N with a 5.41 mm diameter tip on one cheek at 8 cm from the fruit surface. To calculate the fruit impact damage index (FIDI), each fruit was graded visually according to the protocol used by Param and Zoffoli [28].

The cracking index (CI) was determined 24 h after harvest as described by Christensen [47] by immersion in tap water (unadjusted for pH; pH 7). Twenty fruits per replicate, each with an attached pedicel and no visible damage, were immersed in the water. The fruit was examined for cracking after 2, 4, and 6 h. At each time, the number of cracked fruits was recorded, and any cracked fruits were removed and discarded. The CI was calculated according to Equation (1), where N2h represents the number of cracked fruit after 2 h, N4h the number of cracked fruit after 4 h, N_{6h} the number of cracked fruits after 6 h of immersion, and NT the total number of fruits evaluated [38].

$$CI = \frac{(5N2h + 3N4h + N6h) * 100}{(5NT)} \quad (1)$$

2.4. Postharvest Storage

Within one hour of harvest at a pulp temperature of 16–24 °C, fruits were exposed to a shower-type hydrocooler using water at 0 °C, for 5 min, until fruits reached 4 to 6.5 °C pulp temperature.

Fruit samples (1.5 kg) were packed in modified atmosphere bags (5–8% CO_2, 10–15% O_2, San Jorge packaging, Chile) and held for 45 days at 0 °C (stored) and then for an additional 3 days at 20 °C (shelf life). The 1.5 kg samples were of selected fruit, distributed homogeneously from trees in each block (i.e., 750 g per tree per block) with five replicates. Half of each 1.5 kg of fruit was evaluated immediately after the 45 days storage period at 0 °C, and the other half immediately after the 3 days of shelf life at 20 °C. Individual fruits were ranked according to: the presence/absence of pedicel, cracking, and decay. Brown

pedicel, pebbling, and mechanical damage (pitting, bruising, etc.) followed the description of Zoffoli et al. [22]. The percentage of affected fruit was calculated within each ranking criterion. Fruit firmness was evaluated in 20 fruits per replicate after storage and again after shelf life.

2.5. Fruit Mineral Analyses

Fruit mineral analyses were performed at harvest according to the protocols of Sadzawkam et al. [48] and Pavicic et al. [49]. Four replicates from unthinned trees without foliar $CaCl_2$ sprays were used. Fresh fruit (500 g/replicate) without the pit were subjected to dry combustion to convert components to ash. The ash samples were dissolved in HCl (2 M), and mineral concentrations were determined by Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) (Agilent 720 ES axial, Varian, Mulgrave, Victoria, Australia). For water-soluble Ca, an additional 20 g of fresh flesh was homogenized with 40 mL of deionized water. From these measurements, Ca concentration (expressed as mg/100 g flesh) and total Ca content (expressed as mg of Ca in average individual fruit's flesh, without considering the pit) were calculated (the flesh:pit ratio changed with development: 6.7 at 26 DAFB; 3.0 at 33 DAFB; 5.7 at 46 DAFB; 16.7 at 70 DAFB; 18.8 at 84 DAFB; 18.6 at 90 DAFB).

2.6. Statistical Analyses

Data were first tested using a two-way ANOVA (two factors) to analyze the effect of the timing of foliar $CaCl_2$ application (first factor) and crop load (second factor) on fruit quality parameters. The timing of foliar $CaCl_2$ application (A) (4 levels = 3 timings + 1 control) considered three phenological stages or dates: 26 DAFB (Stage I), 39 DAFB (Stage II), and 62 DAFB (Stage III). The crop load (B) consisted of two levels: 100% crop load (i.e., natural crop load without fruit thinning) and 50% crop load (i.e., 50% of natural crop load removed, 31 DAFB). Significant differences among groups were tested with Tukey's honestly significant difference. Results were considered significant at $p \leq 0.05$. Data were presented as means ± standard error of the mean (SEM). All statistical analyses employed IBM SPSS Statistics v24 (New York, NY, USA).

3. Results

3.1. Morphological and Leaf:Fruit Ratio Characterization

The LA:F ratio was adjusted before pit hardening and when the natural fruit drop had ceased (31 DAFB). Differences observed for the timing of foliar $CaCl_2$ application and crop load reduction at 50 ($p < 0.0001$) and 90 DAFB ($p < 0.0001$) were due to the crop load reduction imposed at 31 DAFB. These results show that the crop load before pit hardening allowed a second treatment to be imposed (e.g., a Ca spray), which is useful for analyzing source–sink relationships in fruit trees.

No significant differences for the percentage of leaf damage in the NFS and CSS were observed at 26, 39, or 62 DAFB. Nevertheless, 3% leaf damage in FS due to the foliar $CaCl_2$ spray at 26 DAFB was detected ($p = 0.020$, data not shown, see Supplementary Material Table S1) with significant differences compared to the water control.

3.2. Fruit Quality at Harvest, Rheological Properties, Fruit Impact Damage Index, and Cracking

Fruit from thinned trees were of greater mass, larger diameter, and higher TA than fruit from unthinned trees. In addition, the $CaCl_2$ spray at Stage I of fruit development (26 DAFB) had a highly significant ($p < 0.0004$) effect on fruit firmness, with a 7% increase compared to the controls (Table 4). At harvest, unthinned trees had 15% more yield (9.0 kg·tree^{-1}) than thinned trees (for maturity index, see Supplementary Material Table S2).

Table 4. Fruit quality in the sweet cherry combination 'Lapins'/'Colt' at harvest, 90 days after full bloom (DAFB), was significantly affected by the timing of foliar $CaCl_2$ applications (a water control or a $CaCl_2$ spray at 26, 39, or 62 DAFB) and by crop load (natural unthinned vs. 50% thinned, 31 DAFB). There were no significant interactions between the timing of foliar $CaCl_2$ application and fruit thinning for: fruit fresh mass, diameter, TA, or firmness.

		[1] Fresh Mass (g)		[1] Diameter (mm)		[1] Titratable Acidity (%, Malic Acid)		[1] Firmness (0–100 Shore)	
[2] Timing of $CaCl_2$ foliar application (A)	Control	9.5	ab	27.0	ab	0.94		80.0	b
	26	9.3	b	26.8	ab	0.90		85.2	a
	39	9.2	b	26.5	b	0.88		81.1	b
	62	10.5	a	27.9	a	0.91		80.3	b
[2] Crop Load (B)	Unthinned	9.0	b	26.3	b	0.73	b	81.2	
	Thinned	10.3	a	27.8	a	1.08	a	82.1	
	p-value								
A	[2] TFA	0.002		0.005		0.068		0.0004	
B	[2] CL	<0.0001		<0.0001		<0.0001		0.347	
A × B	Interaction	0.817		0.728		0.099		0.104	

[1] Means of 20 fruits/replicate. Different letters in the same column indicate significant differences between the timing of $CaCl_2$ foliar applications (A) based on Tukey's test ($p \leq 0.05$) or between crop load levels (B) based on ANOVA ($p \leq 0.05$). Control with water only. [2] Timing of $CaCl_2$ foliar application (TFA) and Crop Load (CL).

Highly significant interactions between the timing of foliar $CaCl_2$ application and crop load were observed for SSC ($p = 0.0021$) and Chroma color ($p < 0.0001$) but with no clear trend or visual difference (Table 5). The lowest SSC and the highest chroma values were observed in fruit from unthinned trees after a foliar $CaCl_2$ spray during Stage I of fruit development.

Table 5. Fruit quality in the sweet cherry combination 'Lapins'/'Colt' at harvest, 90 days after full bloom (DAFB), was significantly affected by the timing of foliar $CaCl_2$ applications (a water control or a $CaCl_2$ spray at 26, 39, or 62 DAFB) and by crop load (natural unthinned vs. 50% thinned 31 DAFB). There were significant effects on SSC and Chroma at harvest and significant interactions between the timing of the foliar $CaCl_2$ applications and crop load.

		Soluble Solids (%)				Chroma (C)			
		[1] Crop Load (B)				[1] Crop Load (B)			
		Unthinned		Thinned		Unthinned		Thinned	
Timing of $CaCl_2$ foliar application (A)	Control	19.5	a	17.8	abc	22.1	de	24.7	abc
	26	16.6	c	17.4	bc	26.9	a	25.7	ab
	39	16.8	c	19.1	ab	22.5	cde	22.6	cde
	62	18.2	abc	18.0	abc	20.9	e	23.8	bcd
A × B	*p*-value	0.002				<0.0001			

[1] Means of 20 fruits/replicate. Different letters indicate significant differences between A × B interaction based on Tukey's test ($p \leq 0.05$). Control with water only.

Of the rheological properties, the timing of the foliar $CaCl_2$ applications had significant effects on the modulus of elasticity ($p = 0.0001$) and energy at the maximum point ($p = 0.006$) (Table 6), while the crop load had significant effects on the modulus of elasticity ($p = 0.015$), energy at the maximum point ($p < 0.0001$), and FIDI ($p = 0.014$) (Table 6). Foliar Ca applied on 26 DAFB had a significant positive effect on the modulus of elasticity (2.36 MPa; $p < 0.0001$), while thinned trees showed a reduction in the modulus of elasticity ($p = 0.015$) of fruits (Table 6).

Table 6. Effect of the timing of foliar $CaCl_2$ applications (i.e., Control, and 26, 39, and 62 days after full bloom, DAFB) and crop load (i.e., natural crop load without fruit thinning; and 50% of fruit removal by hand, 31 DAFB) on the modulus of elasticity and fruit impact damage index in the sweet cherry combination 'Lapins'/'Colt' during the 2018–2019 growing season.

		[1] Reological Properties					
		Modulus of Elasticity (Mpa)		Maximun Energy (mJ)		[1,2] FIDI (0–4)	
[2] Timing of $CaCl_2$ foliar application (A)	Control	2.09	b	33.4	a	1.0	
	26	2.36	a	31.1	ab	0.9	
	39	2.15	b	29.5	b	1.0	
	62	2.10	b	31.1	ab	0.9	
[2] Crop Load (B)	Unthinned	2.20	a	29.3	b	0.8	b
	Thinned	2.12	b	33.2	a	1.1	a
	p-value						
A	[2] TFA	0.0001		0.006		0.847	
B	[2] CL	0.015		<0.0001		0.014	
A × B	Interaction	0.267		0.131		0.348	

[1] Means of 20 fruits/replicate. Different letters in the same column indicate significant differences between the timing of $CaCl_2$ foliar applications (A) based on Tukey's test ($p \leq 0.05$) or between crop load levels (B) based on ANOVA ($p \leq 0.05$). Control with water only. [2] Timing of $CaCl_2$ foliar application (TFA), Crop Load (CL), and fruit impact damage index (FIDI).

On the other hand, significant interactions between the timing of the foliar $CaCl_2$ application and the crop load level were observed at the bioyield point (stress $p = 0.021$; strain $p = 0.001$) (Table 7). Regardless of the crop load, the highest stress values were observed for the foliar $CaCl_2$ application at 26 DAFB (Stage I). In addition, strain values were similar among treatments, but the lowest value was registered in unthinned trees after a foliar $CaCl_2$ application during Stage II (39 DAFB).

Table 7. Interaction between the timing of foliar $CaCl_2$ applications (i.e., Control, and 26, 39, or 62 days after full bloom, DAFB) and crop load (i.e., natural crop load without fruit thinning vs. 50% of fruit removal 31 DAFB) on the rheological properties in the sweet cherry combination 'Lapins'/'Colt'.

		[1] Rheological Properties							
		Bioyield							
		Stress (kPa)				Strain (%)			
		[1] Crop Load (B)				[1] Crop Load (B)			
		Unthinned		Thinned		Unthinned		Thinned	
Timing of $CaCl_2$ foliar application (A)	Control	162.0	bcd	186.5	abc	8.1	ab	9.5	a
	26	214.7	a	198.4	ab	9.3	a	9.1	a
	39	141.4	d	179.0	abcd	6.8	b	9.3	a
	62	156.4	cd	168.1	bcd	8.2	ab	8.5	a
A × B	*p*-value	0.021				0.001			

[1] Means of 20 fruits/replicate. Different letters indicate significant differences between A × B interaction based on Tukey's test ($p < 0.05$). Control with water only.

Rain did not occur during the 2018/19 growing season, so rain-induced fruit cracking was not observed for any treatment. However, cracking induced (CI) in the lab showed significant interactions ($p = 0.044$) between the timing of the foliar $CaCl_2$ application and the crop load (Table 8).

Table 8. Interaction between the timing of foliar $CaCl_2$ application (i.e., Control, and 26, 39, or 62 days after full bloom, DAFB) and crop load (i.e., natural crop load without fruit thinning vs. 50% of fruit removed) on the Cracking Index (CI) in the sweet cherry combination 'Lapins'/'Colt'.

		[1] Cracking Index H_2O			
		Crop Load (B)			
		Unthinned		Thinned	
Timing of $CaCl_2$ foliar application (A)	Control	10.8	b	25.0	a
	26	13.8	ab	16.6	ab
	39	6.4	b	5.7	b
	62	4.8	b	4.4	b
A × B	*p*-value	0.044			

[1] Means of 20 fruits/replicate. Different letters indicate significant differences between A × B interaction based on Tukey's test ($p \leq 0.05$). Control with water only.

The highest CI (25%) was observed for thinned trees without $CaCl_2$ spray, while $CaCl_2$ applications during Stage II and III of fruit development induced a reduction in the CI values (4.4 to 6.4%) in thinned trees (Table 8).

3.3. Postharvest Storage

No interactions between the timing of $CaCl_2$ application and the crop load were observed after 45 days at 0 °C ($p > 0.05$). The timing of $CaCl_2$ application did not have a significant effect on any of the storage quality parameters (Table 9). However, crop load reduction positively influenced most of the quality parameters evaluated. Fruit from the thinned trees showed the lowest percentage for pedicel detachment ($p = 0.0001$), brown stem ($p < 0.0001$), and decay ($p = 0.03$).

Table 9. Effect of the timing of foliar $CaCl_2$ applications (i.e., Control, and 26, 39, or 62 days after full bloom, DAFB) and crop load (natural crop load without fruit thinning vs. 50% of fruit removed 31 DAFB) after cold storage (45 d at 0 °C) in the sweet cherry combination 'Lapins'/'Colt'.

		[1] Fruit Quality after 45 d at 0 °C (%)											
		[3] Pedicel Detachmnet		[4] Brown Stem		[3] Cracking	[4] Pebbling	[4] Mechanical Damage		[3] Decay			
[2] Timing of $CaCl_2$ foliar application (A)	Control	6.4		32.1		0.0	61.5	22.3		6.0			
	26	3.6		21.4		0.5	64.0	18.0		5.5			
	39	6.8		21.5		0.8	63.8	16.5		4.5			
	62	8.0		23.0		0.8	63.0	16.0		4.0			
[2] Crop Load (B)	Unthinned	11.4	a	34.5	a	0.6	66.4	16.6		6.4	a		
	Thinned	2.3	b	14.5	b	0.4	59.8	19.8		3.6	b		
p-value													
A	[2] TFA	0.408		0.361		0.641	0.990	0.164		0.673			
B	[2] CL	0.0001		<0.0001		0.548	0.194	0.0996		0.031			
A × B	Interaction	0.823		0.441		0.942	0.324	0.304		0.191			

[1] Means of 100 fruits/replicate. Different letters in the same column indicate significant differences between the timing of $CaCl_2$ foliar application (A) based on Tukey's test ($p \leq 0.05$) or between crop load (B) based on ANOVA ($p \leq 0.05$). Control with water only. [2] Timing of $CaCl_2$ foliar application (TFA) and Crop Load (CL). [3] Individual fruits were ranked according to: the presence/absence of pedicel, cracking, and decay (i.e., rotten fruit). Rotten fruit: *Monilinia* spp., *Botrytis* spp., and *Alternaria* sp. [4] Brown stem, pebbling, and mechanical damage (pitting and bruising) follows the description of Zoffoli et al. [22].

No significant interaction between the timing of the foliar $CaCl_2$ application and crop load was observed for fruit firmness after 45 days at 0 °C ($p = 0.135$, data not shown). The highest fruit firmness after cold storage was obtained with foliar Ca applied during Stage I (26 DAFB; $p = 0.0031$). No significant differences were observed with respect to the

control. Fruit firmness was similar between thinned and unthinned trees ($p = 0.365$, data not shown).

Interactions between the timing of foliar $CaCl_2$ application and crop load ($p = 0.0043$) were observed after 45 days at 0 °C plus 3 days at 20 °C (Table 10). In general, fruit from thinned trees showed higher firmness than fruit from unthinned trees under the same conditions (i.e., water or $CaCl_2$), except for the early application of $CaCl_2$ (Stage I) in unthinned trees. Calcium chloride applications to unthinned trees at Stage I increased fruit firmness to similar values of fruit from thinned trees.

Table 10. Effect of the timing of foliar $CaCl_2$ application (i.e., Control, and 26, 39, or 62 days after full bloom, DAFB) and crop load (natural crop load without fruit thinning vs. 50% of fruit removal 31 DAFB) on fruit firmness under cold storage (45 d at 0 °C in modified atmosphere packaging) in the sweet cherry combination 'Lapins'/'Colt'.

		[1] Firmness (0–100 Shore) 45 Days AM 0 °C + 3 Days 20 °C			
		Crop Load (B)			
		Unthinned		Thinned	
Timing of $CaCl_2$ foliar application (A)	Control	80.2	bc	81.7	abc
	26	85.2	a	83.7	ab
	39	80.6	bc	84.1	ab
	62	77.8	c	82.8	ab
A × B	*p*-value	0.004			

[1] Means of 20 fruits/replicate. Different letters indicate significant differences between A × B interaction based on Tukey's test ($p \leq 0.05$). Control with water only.

3.4. Calcium Mineral Analysis from Unthinned Trees without Ca Sprays

The dynamics of Ca content and concentration in fruits of unthinned trees without foliar $CaCl_2$ application (i.e., natural evolution of Ca in fruits) is shown in Figure 1. The highest Ca concentration (31.8 mg/100 g flesh) in fruits was observed 26 DAFB. Later, Ca concentrations decreased as the fruit's volume increased, reaching the lowest average values (9.4 mg/100 g flesh) at Stage III of fruit development (76 DAFB) (Figure 1B). The total Ca content showed the lowest values at Stage I of fruit development (0.21 mg in flesh, 36 DAFB). The total Ca content increased until Stage III, reaching its highest values 70 DAFB (1.21 mg in flesh) (Figure 1B). In addition, the soluble Ca concentration (4.74 mg/100 g flesh) as well as the total soluble Ca content (0.04 mg in flesh) showed their lowest values at Stage I (26 DAFB). The total soluble Ca content increased constantly until 84 DAFB, reaching 0.55 mg in flesh, while the soluble Ca concentration reached a peak value of 8.65 mg/100 g of flesh at the end of Stage II of fruit development (54 DAFB), decreasing until 6 mg/100 g of flesh at harvest (Figure 1C).

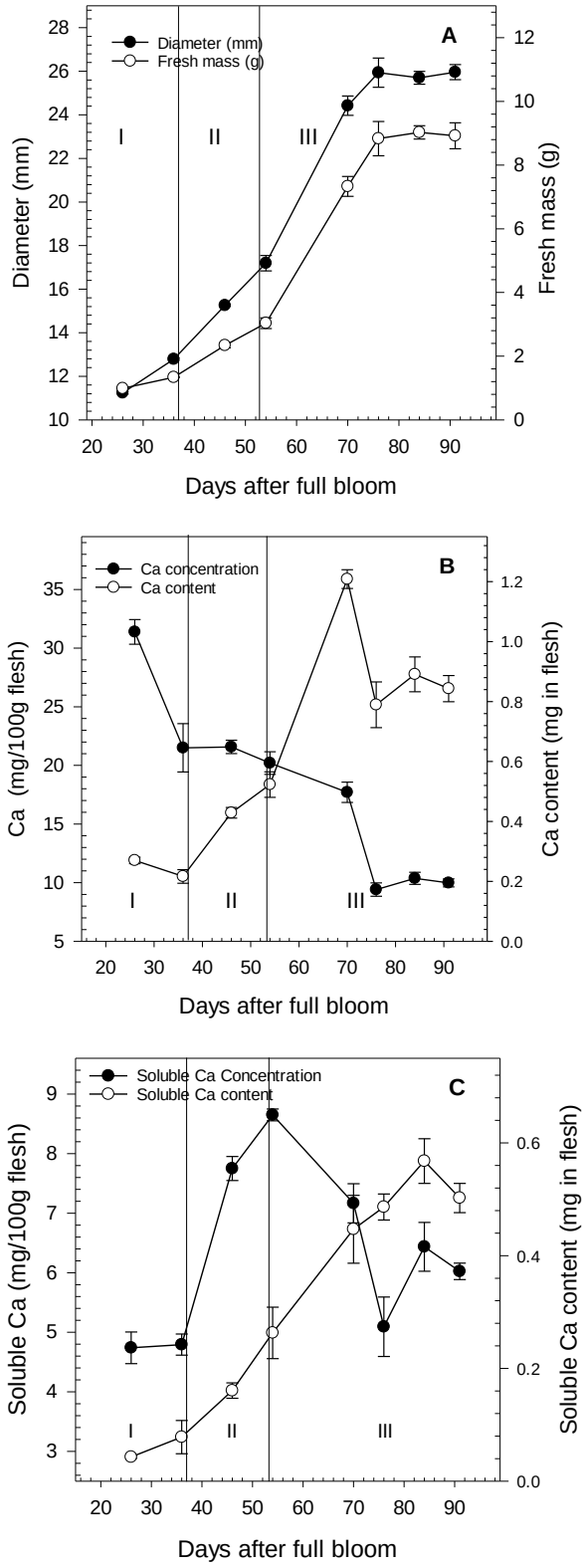

Figure 1. Calcium (Ca) accumulation patterns in fruits from trees with natural crop load and without foliar calcium chloride applications in the sweet cherry combination 'Lapins'/'Colt'. Each data point represents the mean ± SEM (n = 4). (**A**) Equatorial diameter and fresh mass; (**B**) calcium concentration and total Ca content, and (**C**) soluble Ca concentration and total soluble Ca content.

4. Discussion

The crop load reduction in sweet cherry trees trained as a KGB increased fruit quality at harvest and storage quality. The early $CaCl_2$ spray (Stage I) improved fruit firmness (i) at harvest, (ii) firmness in cold storage, and (iii) the modulus of elasticity. Later $CaCl_2$ foliar applications reduced induced fruit cracking in thinned trees.

Fruit thinning has induced a higher SSC in sweet cherries [10,17]; however, in this study, we did not observe an evident increment after crop load regulation (Table 5). Despite that, fruit thinning resulted in heavier and larger fruit with higher acid than unthinned trees (Table 4). Such results after crop load reductions have been reported previously for several cherry cultivars [12,18,34,41,50,51]. In cherries, an increased LA:F increases CHO availability, which, in turn, increases fruit mass, quality, and postharvest life. It is likely that the smaller fruit on the unthinned trees were the result of a lower LA:F (83.4 cm^2 $fruit^{-1}$), indicating source limitation during fruit development (Table 3). Usenik et al. [10] proposed an optimum LA:F of 98.9 cm^2 $fruit^{-1}$ for the highly productive dwarfing combination 'Lapins'/'Gisela 5' trained as a spindle axe, while Whiting and Lang [46] proposed a minimum of 244 cm^2 $fruit^{-1}$ for the combination 'Bing'/'Gisela 5' trained as a standard multiple-leader. In this study, trees from the combination 'Lapins'/'Colt' trained as a KGB, with a LA:F ratio of 126.1 cm^2 $fruit^{-1}$ after early fruit thinning, produced higher-quality fruit compared to unthinned trees.

The crop load reduction did not significantly affect firmness ($p > 0.05$). Inconsistent results for LA:F and firmness have been described previously [17,21,22,46]. Thinning of dormant reproductive buds (20 fruit·m^2 LA) on 'Bing'/'Gisela 5', trained as a multiple-leader, did not present significant differences in firmness [46]. Similarly, Whiting and Ophardt [17] reported no effects on fruit firmness of the removal of 50% of flowers or the removal of 50% of spurs in the combination 'Bing'/'Gisela 5′ and 'Bing'/'Gisela 6' trained as a multiple-leader. In contrast, Zoffoli et al. [22], using 'Van'/'Mericier', trained as a central leader, found a significant reduction in fruit firmness using spur thinning (LA:F = 2:1), while fruit thinning (LA:F = 3:1) increased it.

In addition to the effect of crop load on fruit quality, foliar $CaCl_2$ applied at Stage I increased fruit firmness and reduced fruit softening at harvest and after cold storage (Tables 4 and 10). Commercial Ca sprays are used to increase firmness and reduce cracking in sweet cherries [52,53]. However, multiple Ca sprays do not always improve fruit quality [32,40–42]. The effectiveness of foliar Ca-based products varies according to cultivar and season [41], soil characteristics [53], training system [38], stage of fruit development [32,40], formulation of the product [37], concentration [39], and number and type (dipping or spray) of application, among a range of factors [36,42,51,54]. Michailidis et al. [54] reported significant changes in primary metabolites (i.e., sugars, soluble alcohols, organic, and amino acids) following Ca sprays during fruit development and after harvest in sweet cherries.

Calcium sprays are commonly recommended from the beginning of Stage II (pit hardening) [37,38,53] as, at this time, susceptibility to cracking increases [47]. However, in this study, we found a beneficial effect on fruit firmness after a $CaCl_2$ application during Stage I (i.e., cell division). The uptake mechanisms of Ca by leaves and fruit and its subsequent distribution among fruit are not well understood. Ca uptake in sweet cherries may occur via two parallel pathways: (1) Ca is taken up by the roots from the soil solution and delivered to the shoot and fruits via the xylem, driven by evapotranspiration [55]; and/or (2) for foliar application, Ca follows polar pathways through the fruit exocarp via the cuticular membrane [56]. Stomatal and cuticular pathways are effective for nutrient uptake by leaves and fruits [57]. According to Saure [58], Ca uptake across the fruit skin is correlated with fruit transpiration, which declines as fruits mature. Furthermore, the type of Ca source affects its uptake and transport [39]. Calcium salts with low deliquescent relative humidity or deliquescence point (DP), such as $CaCl_2$ used in this study, tend to be more available for uptake after foliar applications. If the relative humidity is lower than

the DP, the salt-solution evaporates completely, leaving a dry residue on the leaf surface, from which uptake becomes impossible [59].

In our study, foliar $CaCl_2$ applications during the period of fruit development had a positive effect on fruit firmness and the rheological properties of fruit tissue, indicating some degree of uptake after fruit set. It is possible that during early spring, sweet cherry fruit and leaves both absorbed foliar Ca, and some competition for this nutrient occurred. Stomata are functional in young cherry fruit, losing functionality as the fruit matures [60]. Recently, Winkler and Knoche (29) found that Ca uptake by a cherry fruit during Stage II and III was through the epidermis and the junction between the fruit and the pedicel.

Texture is one of the main targets for genetic material selection in sweet cherries [61]. Firm fruit with good mechanical properties is required to cope with the rapid handling in most packing lines; otherwise, pitting appears later [62]. Fruit mechanical damage is the failure of the internal structure and is closely dependent on fruit mechanics [27]. Brüggenwirth and Knoche [63] believed that physical and chemical differences of the cell wall can explain differences in mechanical properties and cracking susceptibility in cherry cultivars such as 'Regina' (less susceptible) and 'Burlat' (more susceptible). Calcium has effectively maintained firmness in some crops by preventing morphological changes of pectin and/or reducing the activity of pectin degradation enzymes [64,65]. Previous studies indicated that pectin constitutes up to 60% of wall mass in fruits [66].

Considering the above, we believe that the improvement of the mechanical properties of sweet cherry fruit is important to reduce postharvest losses. The higher FIDI observed in fruit from thinned trees may be explained by a lower modulus of elasticity, indicating a lower value of tissue resistance. On the other hand, the foliar $CaCl_2$ application at Stage I (26 DAFB) induced a higher modulus of elasticity (i.e., higher tissue resistance) compared with later applications. Param and Zoffoli [28] reported that rheological properties and structural differences in mesocarp and epidermal cells in sweet cherries are related to the susceptibility to mechanical damage in cv. 'Bing', 'Regina', 'Lapins', and 'Sweetheart'. Hence, fruit with high values of modulus of elasticity together with high values of stress and strain at the bioyield point are likely to be more resistant to mechanical damage.

During this study, no cracking was observed, as no rain events occurred. Evaluation for cracking susceptibility using Christensen's protocol [47] shows that CI increased in control fruit from thinned trees. In addition, no differences in CI between sprayed and control trees were detected at Stage I, regardless of crop load. Measham et al. [32] reported no differences in CI with $Ca(NO_3)_2$ applied in the early stages of fruit development (4, 11, 20, 26, 29, and 32 DAFB). However, $CaCl_2$ sprayed in Stages II and III reduced CI significantly in thinned trees and showed lower levels than the control for thinned trees but with no differences. Similar results using foliar Ca sprays based in sweet cherries have been observed by several authors [37,38,51,53,54,67]. Recently, Breia et al. [68] in the sweet cherry combination 'Skeena'/'Gisela 6' reported that exogenous applications of $CaCl_2$ upregulated the expression of PaPIP1;4 two-fold (a water-permeable aquaporin in mature fruit skins). This allows water distribution between the symplast and the apoplast, which reduces stress and strain in the skin of a growing cherry. The expressions of the plasma membrane intrinsic proteins (PIPs), PaPIP1;2 and PaPIP1;4, increases during early Stage I and continues at relatively high levels throughout Stage III in the combination 'Regina'/'Gisela 5' [69]. The aquaporins PIPs are involved in transcellular water movement or apoplast to symplast water transport [70].

A lower percentage of pedicel detachment, brown stem, and fruit decay after cold storage was observed in fruit from thinned trees (Table 9), while no differences in the susceptibility to those defects were observed among the timings of foliar $CaCl_2$ applications. Similar results were reported by Zoffoli et al. [22], with fruit thinning (LA:F 3:1) using the sweet cherry combination 'Van'/'Mericier', with a reduction of ~23% for pitting and ~53% for decay with respect to the untreated controls.

The sweet cherry fruit growth followed a double sigmoid curve, as described by Tukey and Young [4] (Figure 1A). According to our results, the Ca content in the fruit in

the combination 'Lapins'/'Colt' increased to the end of stage I (36 DAFB) and rapidly reached a maximum value (70 DAFB), remaining stable for the last two weeks before harvest, similar to the trend described for cv. 'Regina' [71] (Figure 1B). The rate of xylem water flow (Ca^{2+} is phloem immobile) influences Ca delivery and distribution in sweet cherry fruit tissues [71]. In the combination 'Sam'/'Gisela 5', xylem flows exceeded phloem flows and remained relativity constant during Stage II and early Stage III. At mid Stage III, xylem flows decreased steadily to near zero by harvest [72], which, in turn, reduced the Ca concentration in the fruit. Our results agree with Winkler et al. [71], as the Ca concentration decreased during fruit growth. The highest Ca concentration in fruit was observed at Stage I (26 DAFB; 31.8 mg/100 g flesh), but Ca dilution by fruit growth was observed at Stage III, 76 DAFB with the lowest average values (9.4 mg/100 g flesh). Studies have shown that the incidence of bitter pit in apple [49] and blossom end root in tomatoes [73] may be a consequence of an abnormal cellular Ca partitioning and distribution. Both authors indicated that a low soluble Ca concentration in fruit might explain localized Ca deficiencies within the cell. In our study, the soluble Ca concentration in control fruit showed the lowest values at Stage I (4.74 mg/100 g flesh) with a slight negative effect on fruit quality.

5. Conclusions

In summary, fruit thinning in sweet cherry trees trained as a KGB increased fruit quality. In addition, a foliar spray of $CaCl_2$ at Stage I of fruit development improved firmness, the modulus of elasticity, and the tissue resistance at the bioyield point, indicating a beneficial effect of foliar $CaCl_2$ applications in KGB trees with heavier crop loads. Otherwise, $CaCl_2$ applications at Stage II or III at fruit development reduced the susceptibility to induced cracking in thinned trees. Additional studies are required to determine the optimal concentration of $CaCl_2$ for foliar applications during early fruit development and how best to manipulate the uptake of calcium by the fruit using preharvest practices.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12040829/s1, Figure S1. Climatic data during 2018–2019 growing season; Table S1: Percentage of leaf damage area in NFS and FS. Table S2: Maturity index (MI), was determined as the ratio of SSC (%) to TA (%; Malic acid) at harvest.

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