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Cooperative effects of pre-harvest calcium and gibberellic acid on tissue calcium content, quality attributes, and in relation to postharvest disorders of late-maturing sweet cherry

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ABSTRACT

Six $Ca(NO_3)_2$ sprays at 0.3% and 0.6% from pit hardening (PH) to 1 week before harvest (1WBH) most effectively increased Ca uptake in sweet cherry (*Prunus avium*). Low concentration (< 0.3%) did not affect the absorption of Ca; high concentration (> 1.6%) caused burning of leaf margins. Fruit treated with Ca had greater fruit firmness (FF), soluble solids content (SSC) and titratable acidity (TA) and fewer disorders than untreated fruit while retaining marketable color and size. A single, low concentration of GA_3 combined with Ca sprays enhanced Ca uptake, cracking resistance, and FF without delaying maturation. When Ca plus GA_3 sprays were reduced to four times, Ca uptake was retarded, but had an equal or greater benefit than Ca sprays alone. The 5-d harvest delay in combination-treated fruit did not affect skin color, but these fruit had reduced Ca levels at harvest and displayed stem browning after storage.

1. Introduction

Sweet cherries (*Prunus avium*) are increasingly popular with consumers worldwide for their attractive red/mahogany color, pleasing flavor, desirable taste, and high nutrient contents. In the United States (U.S.) Pacific Northwest (PNW) region, the 2016 sweet cheery harvest was 21.0 million of 20-pound equivalent boxes of fresh cherries. The volume of the 2017 harvest increased 25% over the 2016 harvest. Despite the increase in cherry production due in part to high consumers demand, significant postharvest issues such as dull skin color, softening, losses in acid and flavor, decay, splitting, surface pitting, and stem browning result in a rapid quality deterioration during storage and shipping (Wani et al., 2014; Chockchaisawasdee et al., 2016; Correia et al., 2017). It is clear that flesh firmness (FF) is an important fruit quality attribute impacted by handling and postharvest storage (Einhorn et al., 2013). Therefore, improved strategies to increase FF at harvest are essential for delivery of quality fruit to high-value export markets.

Calcium (Ca) is an essential plant nutrient that plays a fundamental role in cell wall binding, cell membrane systems integrity, and cellular signalling responses (White and Broadley, 2003; Hocking et al., 2016). Fruit with lower Ca levels are more susceptible to softening, pitting, and the decay processes that may follow damage to skin integrity (Thompson, 2003; de Freitas and Mitcham, 2012). Moreover, the low mobility of Ca in plants poses serious problems when Ca is applied to the soil with the intent of enhancing fruit Ca uptake (Bangerth, 1979; Conway et al., 2002). Pre-harvest application of Ca has been shown to directly improve fruit quality of sweet cherries. The most pronounced and consistent effect of Ca on fruit is higher FF and tissue Ca content, which may improve resistance to postharvest disorders without having a detrimental effect on skin color development (Martín-Diana et al., 2007; Tsantili et al., 2007; Wójcik et al., 2013; Wójcik and Wawrzyńczak, 2014; Michailidis et al., 2017). However, the response of fruit to Ca treatment depends on cultivar, source of Ca, and application frequency, timing and concentration (Tsantili et al., 2007; Wójcik et al., 2013; Wójcik and Wawrzyńczak, 2014; Michailidis et al., 2017).

Pre-harvest sprays of plant growth regulators such as gibberellic acid (GA_3) show a similar effect of improved quality of sweet cherries (Basak et al., 1998; Clayton et al., 2003; Lenahan et al., 2008; Zhang and Whiting, 2011a; Einhorn et al., 2013; Canli et al., 2015). In the PNW region of U.S. growers applied a single, a low concentration (10–25 mg L^{-1}) of GA_3 at the end of pit hardening to increase FF, and

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reduce stem browning and surface pitting of 'Skeena' and 'Sweetheart' (Kappel and Macdonald, 2002; Einhorn et al., 2013). However, skin color development and sugar accumulation are retarded by GA_3 (Choi et al., 2002; Kappel and Macdonald, 2002; Serrano et al., 2005; Webster et al., 2005; Ozkan et al., 2016). Indeed, these GA_3-treated fruit required additional maturation time. Ozkan et al. (2016) documented that GA_3 reduced the sugar level of cherries because of these ripening retardation effects. In addition to postponement of the harvest date, GA_3 significantly reduced phenolic compounds, anthocyanin accumulation and antioxidant capacity relative to untreated fruit. Therefore, a new strategy for the use of GA_3 in sweet cherry fruit must be investigated.

The objectives of this study were (1) to determine the response of tissue Ca content, quality attributes and postharvest disorders of 'Lapins' to pre-harvest application of $Ca(NO_3)_2$ by concentration (0.3–1.6%) and frequency of application (2 or 6 times) at harvest and after 4 weeks of cold storage; (2) to evaluate the effects of $Ca(NO_3)_2$ and GA_3, separately and in combination, on Ca content, quality attributes, and postharvest disorders of 'Lapins' and 'Regina' cherries; and (3) to evaluated the effects of reduced Ca-spraying frequency or delayed harvested date in a combination treatment.

2. Materials and methods

2.1. Plant material

Experiments were performed in three consecutive years on 'Lapins' and 'Regina' trees from the orchard of the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, OR, USA. 'Lapins' trees were 19-years old and on Mazzard rootstock; 'Regina' trees were 17-years old and on Gisela 6 rootstock. All trees were maintained with standard cultural, fertilizer, herbicide and pesticide practices.

Calcium nitrate ($Ca(NO_3)_2$, Sigma-Aldrich Inc., MO, USA) or gibberellic acid (GA_3, ProGibb, Valent USA Corp., Walnut Creek, CA) was supplemented with 0.1% (v/v) nonionic surfactant (Silwet L-77, Helena Chemical Co., Collierville, TN, USA) and applied to achieve complete coverage. Whole canopies were sprayed with a CO_2 pressurized hand sprayer (Model D Less Boom; Bellspray Inc., Opelousas, LA, USA). Spraying was conducted when outdoor temperature were below 27 °C and application was avoided before rainfall. Fruit were harvested 1 d before the commercial harvest commenced, and packed in commercial zip-lock polyethylene bags (1-kg) with a 2% perforation ration, then stored at 0 °C (Wang and Long, 2014) for up to 4 weeks.

2.2. Experimental designs

Experiment 1: In 2015, a total of sixty-three 'Lapins' trees were randomly selected and divided into seven treatments with three replicates of 3 each, then treated as follows: (1) Control, untreated; (2) Water, H_2O plus 0.1% (v/v) nonionic surfactant; (3) 0.3% $Ca(NO_3)_2$ 2 times, 0.3% (w/v) $Ca(NO_3)_2$ applied twice at pit hardening (PH, 33 days after full bloom (DAFB)) and 1 week before harvest (1WBH, 83 DAFB); (4) 0.3% $Ca(NO_3)_2$ 6 times, 0.3% (w/v) $Ca(NO_3)_2$ applied six times at 10-day intervals from PH to 1WBH; (5) 0.6% $Ca(NO_3)_2$ 2 times, 0.6% (w/v) $Ca(NO_3)_2$ applied twice at PH and 1WBH; (6) 0.6% $Ca(NO_3)_2$ 6 times, 0.6% (w/v) $Ca(NO_3)_2$ applied six times at 10-day intervals from PH to 1WBH; (7) 1.6% $Ca(NO_3)_2$ 2 times, 1.6% (w/v) $Ca(NO_3)_2$ applied twice at PH and 1WBH. After 40, 50, 60, 70, 80, or 90 DAFB, sixty fruit from each replicate were sampled and measured for tissue Ca content. Fruit quality was evaluated at harvest and after 4 weeks of storage at 0 °C.

Experiment 2: In 2016, thirty-six trees of 'Lapins' or 'Regina' were selected and divided into four treatments with three replicates of 3 each, then treated as follows: (1) Control, untreated; (2) $Ca(NO_3)_2$, 0.6% (w/v) $Ca(NO_3)_2$ applied six times at 10-day intervals from PH to 1WBH; (3) GA_3, a single application of 25 mg L^{-1} GA_3 applied at PH; (4) $Ca(NO_3)_2$ + GA_3, 0.6% (w/v) $Ca(NO_3)_2$ applied six times at 10-day intervals from PH to 1WBH + a single application of 25 mg L^{-1} GA_3 at PH. Tissue Ca content and fruit cracking were measured at harvest. Fruit quality was evaluated at harvest and after 4 weeks of storage at 0 °C.

Experiment 3: In 2017, thirty-six trees of 'Lapins' or 'Regina' were selected and divided into four treatments with three replicates of 3 each, then treated as follows: (1) Control, untreated; (2) $Ca(NO_3)_2$ 6 times, 0.6% (w/v) $Ca(NO_3)_2$ applied six times at 10-day intervals from PH to 1WBH; (3) GA_3 + $Ca(NO_3)_2$ 4 times, a single application of 25 mg L^{-1} GA_3 applied at PH + 0.6% (w/v) $Ca(NO_3)_2$ applied four times at 15-day intervals from PH to 1WBH; (4) GA_3 + $Ca(NO_3)_2$ 6 times, a single application of 25 mg L^{-1} GA_3 applied at PH + 0.6% (w/v) $Ca(NO_3)_2$ applied in six times at 10-day intervals from PH to 1WBH. After 5 days, the trees of treatment 4 were harvested as treatment 5 (GA_3 + $Ca(NO_3)_2$ 6 times (5 d delayed harvest)). Tissue Ca contents were sampled and measured at harvest. Fruit quality was evaluated at harvest and after 4 weeks of storage at 0 °C.

2.3. Determination of tissue Ca content

After harvest, thirty fruit per replicate per treatment were washed, oven-dried at 65 °C, then ground to pass through a 1-mm sieve. Samples were digested in a MARS Express CEM microwave using nitric acid and hydrogen peroxide. Prepared samples were analyzed for Ca content by a Thermo 6500 duo ICP (Thermo and Fisher Scientific, Waltham, MA, USA). Tissue Ca content is expressed on a dry weight basis as mg kg^{-1}.

2.4. Evaluations of FF, fruit size, SSC, TA, and skin color

After harvest or after 4 weeks of storage at 0 °C, sixty fruit per replicate per treatment were placed in the laboratory at 20 °C for 4 h. Fruit size expressed as fruit diameter (FD) at the widest point of the fruit opposite the suture. Flesh firmness (FF) was determined non-destructively using a FirmTech-2 fruit firmness instrument (BioWorks Inc., Stillwater, OK, USA). After determination, one hundred grams of cherries were juiced for 3 min using a juicer (6001, Acme Juicer Manufacturing Co., Sierra Madre, CA, USA) equipped with a uniform milk filter strip (Schwartz Manufacturing Co., Two Rivers, WI, USA). Soluble solids content (SSC) was determined using a refractometer (PAL-1, Atago, Tokyo, Japan). Titratable acidity (TA) was determined by titrating 10 mL juice plus 40 mL distilled water to pH 8.1 with 0.1 N NaOH using a titrator (DL-15, Mettler-Toledo, Zurich, Switzerland) and expressed as the equivalent percentage of malic acid. Skin color was rated on a scale of 1–7, where 1 is equivalent to light pink and 7 is dark mahogany, using a CTIFL (Centre technique interprofessionel de fruits et légumes, Paris, France) color chart (Einhorn et al., 2013).

2.5. Evaluations of fruit cracking, decay, stem browning, and surface pitting

Prior to fruit harvest in 2016, two hundred fruit per replicate per treatment were assessed for fruit cracking. Cracked fruit were expressed as a percentage of two hundred fruit showing any apical, stem, or side cracks. Decay was expressed as a percentage of fruit from one hundred fruit samples showing any type of decay after 4 weeks of storage; however, the decay organisms were not identified. Stem browning was evaluated after 4 weeks of storage and recorded as a percentage of one hundred fruit samples pedicels showing > 30% of the entire surface browned (Clayton et al., 2003). Surface pitting was evaluated after stem browning evaluation. Grading was standardized using a 4-point scale (Toivonen et al., 2004): 1, superficial pitting, pit diameter 1 mm or less, very shallow depression of skin with edges being diffuse; 2, minimal pitting, pit diameter 1 to 2 mm; 3, moderate pitting, pit diameter 2 to 3 mm, deeper and wider with clearly distinct edges; 4, severe pitting, pit diameter 3 mm or greater, very deep, edges of pits sunken into pulp

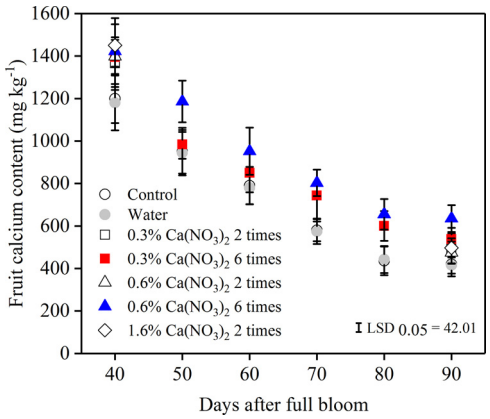

Fig. 1. Effect of application rate and frequency of $Ca(NO_3)_2$ on fruit calcium content in 'Lapins' cherries during fruit development in 2015. Values are presented as the means ± standard deviation (SD), $n = 3$.

tissue. Surface pitting was calculated as the sum of the number of fruit in each of the four categories multiplied by the four factors 1, 2, 3, and 4, and the whole divided by the one hundred fruit.

2.6. Statistical analysis

Experiments were performed using a completely randomized design. One-way analysis of variance (ANOVA) was carried out to determine the significance of differences between means using Fisher's protected least significant difference (LSD) test ($P < 0.05$). The data were subjected to analysis using IBM SPSS Statistics (IBM Co., Armonk, NY, USA).

3. Results

3.1. Optimal Ca application in 'Lapins'

Pre-harvest $Ca(NO_3)_2$ sprays twice at PH and 1WBH in 'Lapins', at 0.3%, 0.6%, and 1.6% concentrations tissue increased Ca content from 420.6 (control) or 415.9 mg kg^{-1} (water) to 493.5, 475.0, and 497.1 mg kg^{-1} at the time of harvest, respectively (Fig. 1). Application $Ca(NO_3)_2$ concentrations below 0.3% did not affect Ca uptake. When Ca $(NO_3)_2$ concentration was as higher as 1.6%, no additional benefit was observed in increasing tissue Ca content, but this higher concentration caused burning of leaf margins (data not shown). Compared to spraying Ca twice, both 0.3% and 0.6% $Ca(NO_3)_2$ spays applied 6 times significantly increased tissue Ca content. At harvest, 0.3% or 0.6% Ca $(NO_3)_2$ sprays for 2 or 6 times did not affect fruit size or skin color development (Table 1). However, Ca significantly increased FF, SSC, and TA values in cherries. After 4 weeks of storage at 0 °C, both 0.3% and 0.6% $Ca(NO_3)_2$ sprays for 6 times reduced decay, stem browning, and surface pitting disorders compared to 2 spraying Ca.

3.2. Effect of Ca combined with GA3 on 'Lapins' and 'Regina'

Significant increase of tissue Ca levels were observed in 'Lapins' or 'Regina' when Ca was applied alone or in combination with GA_3 (Fig. 2A). In 2016, a rainfall event totaling 8.6 mm occurred 9 d before the commercial harvest. Percent fruit cracking were 20% and 18% for 'Lapins' and 9% and 12% for 'Regina' in the control and GA_3 treatments, respectively (Fig. 2B). The percent of cracked fruit in Ca or combination treatments was lower than untreated fruit, and no significant differences were found between these two treatments in either cultivar. At harvest, fruit size was not affected by Ca and GA_3 treatment, separately or in combination. Fruit color (Fig. 3) and SSC development were delayed by GA_3 in 'Lapins', but not in 'Regina' (Table 2). Although GA_3 increased FF in both cultivars, TA were lower than other treatments. A combination treatment in either cultivar did not affect fruit color development or SSC and TA accumulation, and maintained higher FF at harvest than untreated fruit. After 4 weeks of cold storage, Ca and GA_3 treatments, separately or in combination, impacted higher FF and less decay, stem browning, and surface pitting.

3.3. Effects of reducing Ca sprays frequency or delaying harvested date by using a combination treatment

In this study, combination sprays provided the highest tissue Ca content (Fig. 4). When reducing Ca sprays to 4 times, the combination treatment had an equal tissue Ca content to Ca sprays alone in 'Lapins', but a higher tissue Ca content in 'Regina'. Ca alone or 4–6 times combination applications to either cultivar did not affect color development, fruit size, or SSC, but did significantly increased FF and TA while reducing postharvest disorders compared to the control (Table 3). At harvest or 4 weeks of storage, there were no differences in tissue Ca content, FF, SSC, TA, skin color, or decay fruit treated with GA_3 and Ca-combination treatment when fruit were harvested at a commercial date or when harvest date was delayed. However, late harvest resulted in the increase of stem browning.

4. Discussion

Ca sprays as a way to reduce fruit cracking in sweet cherry fruit has been previously demonstrated (Lidster et al., 1979; Facteau et al., 1987; Tsantili et al., 2007; Wójcik et al., 2013; Erogul, 2014; Michailidis et al., 2017). However, there are inconsistent interpretations of the effectiveness of Ca spays to increase tissue Ca content and to improve fruit quality (Val et al., 2008; Sotiropoulos et al., 2010). To increase Ca uptake and obtain consistent efficacy, factors such as Ca source, application concentration, timing, and frequency must be considered to develop a commercial protocol for sweet cherry cultivars. Two-year evaluation of 'Lapins' treated with different Ca sources ($CaCl_2$, Ca $(NO_3)_2$, calcium citrate, $Ca(OH)_2$ + organic acid, amino acid chelated calcium, calcium carbonate, or calcium carbonate silicon) indicated that $Ca(NO_3)_2$ treatment is strongly preferred to increase tissue Ca content and improve fruit quality without affecting return bloom or fruit set (Wang, Einhorn and Dong, unpublished data). Therefore, Ca $(NO_3)_2$ was selected for use in this study. Our study further indicated that the optimum of application $Ca(NO_3)_2$ was determined to be 0.3–0.6% applied 6 times from PH to 1WBH. At this recommended application, cherries directly absorbed more Ca into fruit that reinforced tissue rigidity and contributed to resistance to postharvest disorders.

Raising Ca content of fruit to a level sufficient to create the desired results is difficult. It has been postulated that an ideal Ca tissue content exceeds 250 mg kg^{-1} dry weight (DW), which bestows the capacity to control Ca-related physiological disorders (Meheriuk and Moyls, 1989). In order to affect FF significantly, it is necessary to raise tissue Ca levels to 800–1000 mg kg^{-1} DW, however, tissue Ca levels higher than 1000 mg kg^{-1} DW may cause fruit surface injury (Conway and Sams, 1985). In this study, tissue Ca content increased to 650 and 860 mg kg^{-1} for 'Lapins' and 922 and 972 mg kg^{-1} for 'Regina' in Ca alone and combination treatments, respectively, indicating that GA_3 had an additional benefit of boosting Ca uptake while protecting fruit from surface injury. Additionally, spraying Ca or Ca plus GA_3 improved cracking resistance, perhaps due to more Ca^{2+} bonded to pectins and forming bridges between pectic acid molecules, stabilizing wall structures, and resulting in reduced cracking (Madani et al., 2016).

There are three distinct periods during sweet cherry fruit development: stage I, rapid enlargement beginning at full bloom; stage II, retarded pericarp development; and stage III, rapid pericarp development prior to fruit ripening (Tukey, 1936). At the end of stage II, named pit hardening (PH), a high rate of cell division that will determined the

Table 1
Effect of application concentration and frequency of $Ca(NO_3)_2$ on fruit quality attributes in 'Lapins' at harvest and after 4 weeks of storage at 0 °C in 2015.

Treatments	Harvest					Postharvest						
	FF (N)	FD (mm)	SSC (%)	TA (%)	Skin color (CTIFL[b])	FF (N)	SSC (%)	TA (%)	Skin color (CTIFL)	Decay (%)	SB (%)	SP (1-4)
Control	2.66 b	31.3 a	16.4 c	0.80 b	5.5 a	3.61 c	20.3 bc	0.68 b	5.5 a	5.2 b	32.3 a	3.2 a
Water	2.63 b	31.1 a	16.5 bc	0.79 b	5.5 a	3.48 d	20.2 bc	0.66 b	5.5 a	6.0 a	31.9 a	3.1 a
0.3% $Ca(NO_3)_2$ 2 times[a]	2.61 b	31.1 a	16.6 bc	0.81 ab	5.4 a	3.63 bc	20.1 c	0.68 b	5.5 a	4.8 b	31.9 a	2.8 a
0.3% $Ca(NO_3)_2$ 6 times	2.73 a	30.7 a	17.3 a	0.83 a	5.3 a	3.97 a	20.9 a	0.73 a	5.3 a	2.5 c	22.5 b	2.3 b
0.6% $Ca(NO_3)_2$ 2 times	2.67 b	30.9 a	16.8 b	0.79 b	5.5 a	3.70 b	20.5 b	0.68 b	5.5 a	4.8 b	30.8 a	2.7 a
0.6% $Ca(NO_3)_2$ 6 times	2.81 a	31.1 a	17.4 a	0.84 a	5.3 a	4.03 a	21.3 a	0.73 a	5.3 a	2.1 c	19.8 b	2.0 b
1.6% $Ca(NO_3)_2$ 2 times	2.69 b	30.9 a	16.7 b	0.78 b	5.4 a	3.59 c	20.3 bc	0.68 b	5.5 a	5.0 b	31.3 a	3.0 a

FF, flesh firmness; FD, fruit diameter; SSC, soluble solids content; TA, titratable acidity; SB, stem browning; SP, surface pitting.
Means were separated within columns by Fisher's protected least significant difference test (LSD), whereby means associated with different letters are significantly different at $P < 0.05$.

[a] All $Ca(NO_3)_2$ treatments were initiated at 33 days after full bloom (DAFB, pit hardening, PH). When $Ca(NO_3)_2$ application were limited to 2 treatments, the second application was made at 83 DAFB (1 week before harvest, 1WBH). When $Ca(NO_3)_2$ was applied six times, application were made at 10-day intervals from PH to 1WBH.

[b] Skin color was evaluated using CTIFL color chat on a scale of 1–7 (light pink to dark mahogany).

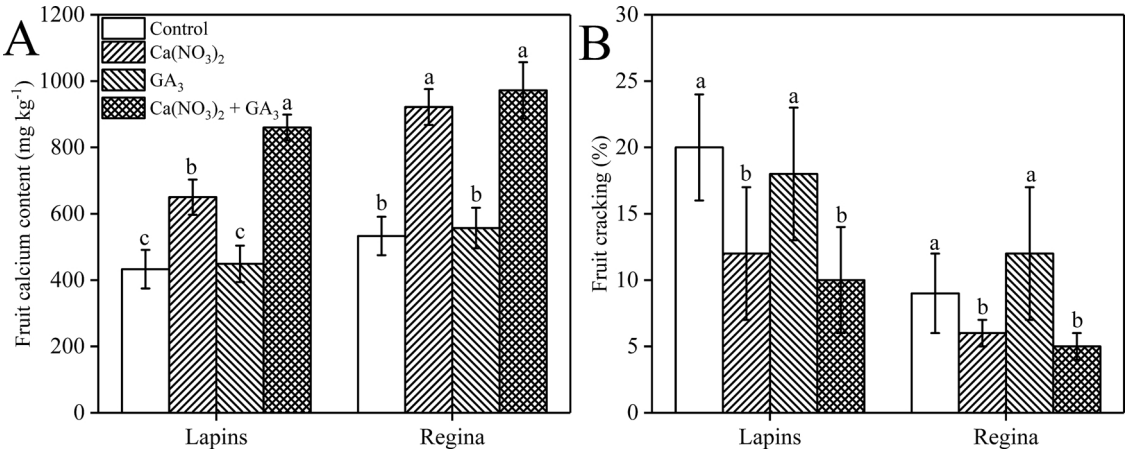

Fig. 2. Effects of pre-harvest $Ca(NO_3)_2$ and GA_3, applied separately or in combination, on fruit calcium content (A) and cracking (B) of 'Lapins' and 'Regina' cherries at harvest in 2016. Values are presented as the means ± SD, $n = 3$. Different letters indicate significant differences among treatments according to Fisher's protected LSD ($P < 0.05$), $n = 3$.

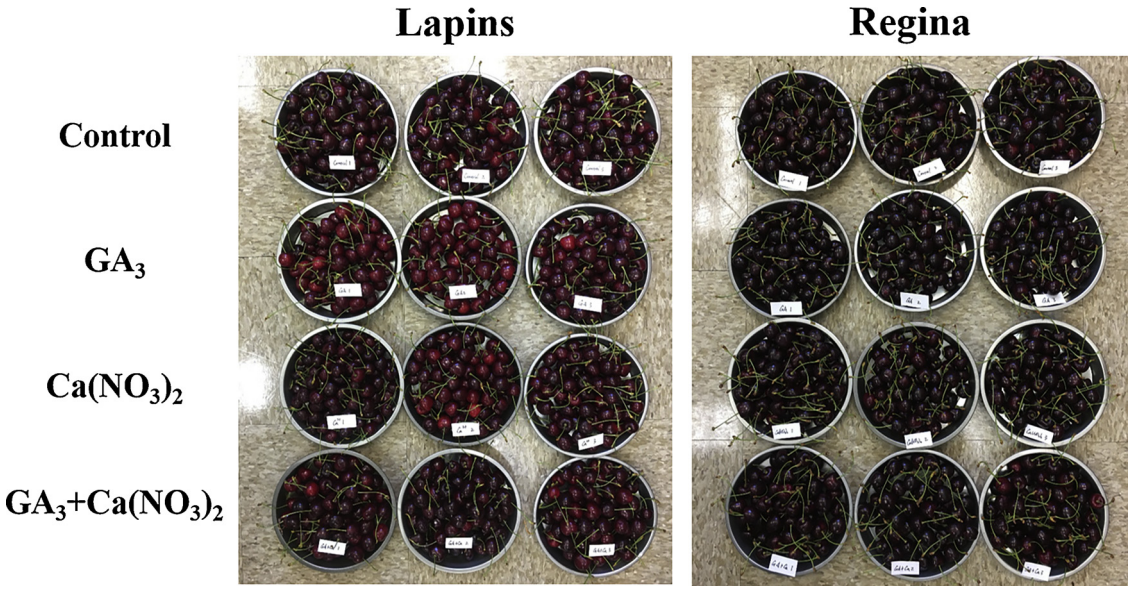

Fig. 3. Effects of pre-harvest $Ca(NO_3)_2$ and GA_3, applied separately or in combination, on 'Lapins' and 'Regina' cherries at harvest in 2016.

Table 2
Effects of pre-harvest $Ca(NO_3)_2$ and GA_3, applied separately or in combination, on fruit quality attributes of 'Lapins' and 'Regina' at harvest and after 4 weeks of storage at 0 °C in 2016.

Cultivar	Treatments	Harvest					Postharvest						
		FF (N)	FD (mm)	SSC (%)	TA (%)	Skin color (CTIFL)	FF (N)	SSC (%)	TA (%)	Skin color (CTIFL)	Decay (%)	SB (%)	SP (1-4)
Lapins	Control	3.58 c	30.2 a	16.7 a	0.82 a	5.5 a	4.13 c	16.1 a	0.69 a	5.5 a	6.5 a	29.9 a	3.2 a
	$Ca(NO_3)_2$	3.67 b	30.0 a	16.2 b	0.80 a	5.2 a	4.62 a	15.8 a	0.67 a	5.3 a	2.8 c	21.1 c	2.5 c
	GA_3	3.63 bc	30.2 a	14.8 c	0.73 b	4.6 b	4.30 b	14.0 b	0.60 b	4.5 b	4.8 b	25.3 b	2.8 b
	$Ca(NO_3)_2$ + GA_3	3.87 a	30.3 a	16.1 b	0.79 a	5.2 a	4.66 a	15.7 a	0.68 a	5.3 a	2.1 d	17.6 d	2.2 d
Regina	Control	3.54 c	31.1 a	19.0 a	0.83 a	5.5 a	4.02 c	18.4 a	0.67 a	5.5 a	8.3 a	33.9 a	2.9 a
	$Ca(NO_3)_2$	4.19 a	31.4 a	19.3 a	0.81 a	5.4 a	4.48 a	18.0 a	0.66 a	5.3 a	5.2 c	25.6 c	2.4 b
	GA_3	3.81 b	31.0 a	18.9 a	0.76 b	5.3 a	4.17 b	18.2 a	0.56 b	5.3 a	6.7 b	28.4 b	3.0 a
	$Ca(NO_3)_2$ + GA_3	4.21 a	30.9 a	18.8 a	0.83 a	5.3 a	4.47 a	18.4 a	0.64 a	5.3 a	4.1 d	20.1 d	2.2 b

All treatments were initiated at PH. For GA_3 or $Ca(NO_3)_2$ + GA_3 applications, GA_3 was applied a single time at PH, separately or combined with $Ca(NO_3)_2$, then afterwards the combination treatment sprayed 0.6% $Ca(NO_3)_2$ at 10-days intervals ending 1WBH.
Means were separated within columns by Fisher's protected LSD, whereby means associated with different letters are significantly different in each cultivar at $P < 0.05$.

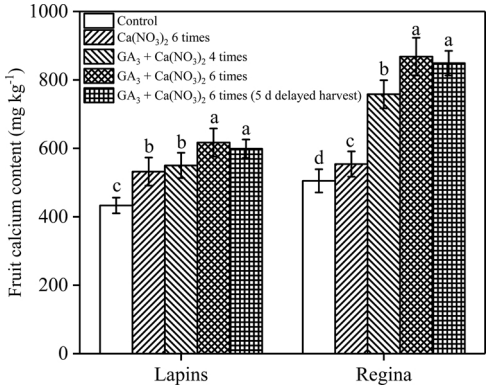

Fig. 4. Effects of application frequency of $Ca(NO_3)_2$ in combined with GA_3 on fruit calcium content of regular- or late-harvest 'Lapins' and 'Regina' cherries at harvest in 2017. Values are presented as the means ± SD, $n = 3$. Different letters indicate significant differences among treatments according to Fisher's protected LSD ($P < 0.05$), $n = 3$.

final cell number within fruit is near completion (Tukey and Young, 1939), and GA level decrease (McAtee et al., 2013). If the GA biosynthesis inhibitor, prohexadione-Ca (calcium 3-oxido-4-propionyl-5-oxo-cyclohexene), is applied once at that same time, smaller fruit size and delayed color development would be evident at harvest (Zhang and Whiting, 2011b). In this study, more Ca was absorbed into the fruit, perhaps in part due to the exogenous GA sprays, which caused cell wall elongation and increased Ca demand in order to synthesize new cell walls. Compared to 'Regina', the combination treatment in 'Lapins' likely promoted increased Ca uptake by the fruit, indicating that the cuticle or epidermis structure of the cultivars affected Ca absorption (Wang et al., 2014).

Fruit skin color is one of essential attributes affecting overall quality of sweet cherry after postharvest storage and marketing. Consumer purchase decisions for sweet cherry is highly related to the darkening of skin color. Application of GA_3 can increase fruit FF and size and reduce fruit cracking, but it delays fruit color change (Correia et al., 2017). Our results further verified that a low concentration and single application of GA_3 at PH improved FF and fruit quality, but delayed maturation (Einhorn et al., 2013). Thus, growers are reluctant to apply GA_3 on blush and mid- or late-maturing cultivars in the PNW region. Interestingly, Ca alone or in combination with GA_3 did not retard fruit ripening, but higher FF was obtained, indicating that pre-harvest spraying Ca or Ca + GA_3 to increase tissue Ca content would appear to be effective strategies to improve fruit quality, protect against postharvest disorders, and extend postharvest fruit quality.

Table 3
Effects of reducing $Ca(NO_3)_2$ sprays frequency or delaying harvested date with a combination treatment on fruit quality attributes of 'Lapins' and 'Regina' at harvest and after 4 weeks of storage at 0 °C in 2017.

Cultivar	Treatments	Harvest					Postharvest						
		FF (N)	FD (mm)	SSC (%)	TA (%)	Skin color (CTIFL)	FF (N)	SSC (%)	TA (%)	Skin color (CTIFL)	Decay (%)	SB (%)	SP (1-4)
Lapins	Control	3.25 b	28.3 a	16.0 a	0.74 b	5.5 a	3.29 b	15.6 a	0.55 b	5.4 a	5.5 a	33.2 a	3.2 a
	$Ca(NO_3)_2$ 6 times	3.45 a	28.5 a	16.2 a	0.83 a	5.3 a	3.61 a	15.7 a	0.63 a	5.2 a	3.3 b	25.6 c	2.5 b
	GA_3 + $Ca(NO_3)_2$ 4 times	3.48 a	28.0 a	16.3 a	0.82 a	5.3 a	3.65 a	15.8 a	0.62 a	5.2 a	2.6 c	25.2 c	2.5 b
	GA_3 + $Ca(NO_3)_2$ 6 times	3.41 a	27.8 a	16.1 a	0.80 a	5.3 a	3.63 a	16.1 a	0.64 a	5.3 a	2.6 c	21.2 d	2.0 c
	GA_3 + $Ca(NO_3)_2$ 6 times (5 d delayed harvest)	3.38 a	28.1 a	16.2 a	0.80 a	5.3 a	3.61 a	16.0 a	0.62 a	5.3 a	2.8 c	29.9 b	2.4 b
Regina	Control	3.32 c	28.6 a	19.1 a	0.75 b	5.5 a	3.61 c	18.8 a	0.56 b	5.5 a	12.1 a	38.5 a	2.9 a
	$Ca(NO_3)_2$ 6 times	3.44 b	28.6 a	19.5 a	0.88 a	5.3 a	3.69 b	18.8 a	0.64 a	5.4 a	7.7 b	27.9 c	2.2 b
	GA_3 + $Ca(NO_3)_2$ 4 times	3.59 a	28.5 a	19.3 a	0.87 a	5.3 a	3.81 a	18.6 a	0.63 a	5.4 a	7.8 b	22.1 d	2.0 b
	GA_3 + $Ca(NO_3)_2$ 6 times	3.58 a	28.3 a	19.1 a	0.85 a	5.3 a	3.77 a	18.2 a	0.61 a	5.3 a	7.5 b	21.6 d	2.1 b
	GA_3 + $Ca(NO_3)_2$ 6 times (5 d delayed harvest)	3.58 a	28.5 a	19.3 a	0.84 a	5.4 a	3.78 a	18.6 a	0.60 a	5.3 a	7.6 b	32.6 b	2.2 b

All treatments were initiated at PH. When GA_3 + $Ca(NO_3)_2$ were applied four or six times, GA_3 was applied a single time combined with $Ca(NO_3)_2$ at PH, then afterwards $Ca(NO_3)_2$ was sprayed at 10-day or 15-day intervals ending 1WBH.
Means were separated within columns by Fisher's protected LSD, whereby means associated with different letters are significantly different in each cultivar at $P < 0.05$.

Due to limited mobility of Ca within plants, Ca sprays on plant aerial parts are recommended routinely to prevent the occurrence of Ca deficiency and improve fruit quality in many crops (Conway et al., 2002). However, the costs of labor and supplies for Ca sprays reduce farmers' income. In this study, it made sense to reduce Ca application frequency to 4 times in combination with GA_3 because the combination treatment had an effect on fruit Ca uptake. However, it is unclear whether less than four times of Ca application in combination sprays would impact a similar effect on improving fruit quality.

In this study, our results showed that GA_3 combined with six Ca applications could expand the harvest window and support storage quality without affecting fruit maturation. However, later harvest resulted in decreased stem browning resistance during cold storage, as previously shown in 'Lapins' (Drake and Elfving, 2002). Thus, a comparative genetics research approach to revealing the relationship between Ca and stem browning might provide insight into controlling postharvest disorders.

5. Conclusion

This research provided a commercial protocol for application of Ca $(NO_3)_2$ sprays at 0.3-0.6% for 6 times from pH to 1WBH or in combination with 25 mg L^{-1} GA_3 a single time at PH in late-maturing sweet cherry cultivars. The protocol was effective in enhancing fruit Ca uptake, improving quality attributes, and reducing postharvest disorders without delaying maturation. Data further revealed that increased tissue Ca content incrementally improved fruit cracking resistance and might strengthen cell wall structures. Therefore, pre-harvest spraying of $Ca(NO_3)_2$ or in combination with GA_3 may have high potential for improving storage or shipping quality of commercial sweet cherry.

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