In-season calcium-spray formulations improve calcium balance and fruit quality traits of peach

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Abstract

Experiments to evaluate the effect of in-season calcium (Ca) sprays on late-season peach (*Prunus persica* L. Batsch cv. Calrico) were carried out for a 2-year period. Calcium formulations (0.5% and 1.0% in 2008 and only 0.5% tested in 2009) supplied either as $CaCl_2$ or Ca propionate in combination with two or three adjuvants (0.05% of the nonionic surfactants Tween 20 and Break Thru, and 0.5% carboxymethylcellulose, CMC) were sprayed four to five times over the growing season. Peach mesocarp and endocarp Ca concentrations were determined on a 15-day basis from the beginning of May until the end of June. Further tissue analyses were performed at harvest. A decreasing trend in fruit Ca concentrations over the growing season was always observed regardless of the Ca treatments. Both in 2008 and 2009, significant tissue Ca increments associated with the application of Ca-containing sprays in combination with adjuvants were only observed in June, which may be coincident with the period of pit hardening. In 2008, both at harvest and after cold storage, the total soluble-solids concentration (° Brix) of fruits supplied with Ca propionate (0.5% and 1.0% Ca) was always lower as compared to the rest of treatments. The application of multiple Ca-containing sprays increased firmness at harvest and after cold storage, especially when $CaCl_2$ was the active ingredient used. Supplying the adjuvants Tween 20 and CMC increased fruit acidity both at harvest and after cold storage. Evaluation of the development of physiological disorders after cold storage (2 weeks at 0°C) indicated a lower susceptibility of Ca-treated fruits to internal browning. Fruits treated with multiple $CaCl_2$-, CMC-, and Break Thru®-containing sprays during the growing season were significantly less prone to the development of chilling injuries as compared to untreated peaches.

Key words: *Prunus persica* (L.) Batsch / fruit storage / physiological disorders / physiological calcium deficiency / foliar sprays

1 Introduction

The fresh-peach market in Spain and other countries in Europe, North Africa, and South America mostly relies on cling peach cultivars. The marketing season for fresh peaches in Spain is currently being extended by the production of very late maturing peaches, which, must be cold-stored for prolonged times. In parallel, new physiological disorders are being reported by growers, such as the so-called vitrescent dark spot of peach. Affected fruits exhibit darkened areas in the mesocarp, which are, in many cases, not visible through the exocarp (*Val*, 2007). Consequently, at harvest it is not always possible to discern affected from sound peaches as observed for bitter pit in apple (*Malus* × *domestica* Borkh.; *Saure,* 2005). Vitrescent dark spot has only been reported in late-ripening peach cultivars, and seems to be related to calcium (Ca)-nutrition imbalances (*Fernández* et al., 2009) like bitter pit in apple or blossom end rot in tomato (*Lycopersicum esculentum* Mill.; *Saure,* 2005; *Liebisch* et al., 2009).

The control of Ca-related disorders is often approached *via* applying Ca sprays to horticultural crops (*Ferguson* and *Watkins,* 1989; *Lurie* and *Crisosto,* 2005; *Kraemer* et al., 2009a). However, inconsistent results regarding the rate of penetration and distribution of surface-applied Ca and the subsequent storability of fruits have been often reported (*Lurie* and *Crisosto,* 2005; *Val* et al., 2008; *Bonomelli* and *Ruiz*, 2010; *Sotiropoulos* et al., 2010).

The uptake of nutrient solutions by plant surfaces is ruled by many processes which are currently not fully understood (*Fernández* and *Eichert*, 2009), but the key role of environmental factors such as relative humidity is crucial under the prevailing conditions in many semiarid and arid fruit-growing areas of the world. Regarding the effect of relative humidity on solution properties, *Schönherr* (2001) pointed out the relevance of the point of deliquescence (POD) of the Ca compounds. Working with isolated, adaxial, pear leaf cuticles, he observed that Ca nitrate and chloride (55% and 32% POD, respectively), penetrated at a higher rate than Ca acetate, Ca lactate, or Ca propionate (with PODs between 95% and 100%; *Schönherr*, 2001). However, the antifungal properties of Ca propionate may prove beneficial to preserve post-harvest fruit quality (*Blanco* et al., 2010). *Kraemer* et al (2009b) reported higher Ca penetration rates trough isolated tomato cuticles in association with the application of $CaCl_2$ *versus*

Ca acetate. Thereby, improving the performance of Ca sprays should be targeted via optimizing the rates of e.g. wetting, spreading, retention or rainfastness of the spray treatments (*Schmitz-Eiberger* et al., 2002a; *Fernández* and *Eichert*, 2009; *Kraemer* et al., 2009a).

After spray application, droplets of a certain spread area are deposited onto the plant surface and the active ingredient is distributed within the drops in relation to the physical-chemical properties of the formulation. Recent findings relating to the application of Ca-containing solutions ($CaCl_2$ and Ca acetate) to isolated cuticles of tomato fruit and apple leaves suggest that the rate of penetration is not related to the actual droplet spread area but rather to the area covered by Ca (*Kraemer* et al., 2009a, b). In conclusion, the authors suggest that the area ultimately covered by the active ingredient within the drop footprint should be considered in developing models to estimate the rate of penetration of surface-applied agrochemicals (*Kraemer* et al., 2009a, b, c).

Trials developed on apple trees showed that treatment with late Ca sprays was not more effective in preventing the occurrence of bitter pit as compared to early applications (*Lötze* and *Theron*, 2007). After applying multiple Ca sprays up to 1 month before harvest, *Val* et al. (2008) observed tissue Ca increments only on the apple skin. Peach treatment with multiple $CaCl_2$ and Ca-EDTA sprays led to increased peal and pulp Ca concentrations and lower rates of brown rot development over the cold-storage period, best results being obtained with $CaCl_2$ as active ingredient (*Manganaris* et al., 2005a). *Elmer* et al. (2007) also recorded lower rates of development of brown and post-harvest rots in peaches after the application of in-season Ca sprays.

One distinct feature of growing late-season peaches in the Bajo Aragón Area, Spain, is that fruits are bagged individually to avoid damage caused by the Mediterranean fly (*Ceratitis capitata* Wied.). This practice is generally implemented shortly after fruit thinning, and peaches grown in bags are marketed as top quality and agrochemical-free. However, the presence of the bag may limit the absorption of Ca sprays. Therefore, it is necessary to implement Ca-fertilization strategies that may maintain an adequate Ca status in the fruit until the time of harvest, such as the application of multiple sprays prior to bagging.

The aim of this study was to improve the performance of in-season Ca sprays as a means to prevent the development of post-harvest physiological disorders in late-season peaches. The exocarp of peaches is densely covered by trichomes, which pose a significant barrier for the absorption of surface-applied solutions (*Fernández* et al., 2009). Consequently, the effect of applying Ca treatments ($CaCl_2$ and Ca propionate) at different concentrations and in combination with different adjuvants was monitored for a 2-year period by assessing peach Ca budgets, quality traits and storability.

2 Material and methods

Two experiments were carried out in a commercial orchard located in the Mid-Ebro Valley (Caspe, Zaragoza, Spain) during the growing seasons of 2008 and 2009. Trials were performed on mature trees of cling late-maturing peach (*Prunus persica* L. Batsch cv. Calrico). Trees were selected to be as uniform as possible in terms of size and cropping, and had on average a trunk girth of $(25.3 \pm 2.3)\,cm^2$. In both trials, treatments were applied in randomized blocks with four replications, considering the single tree as experimental unit and comparing the results with those obtained for unsprayed, control trees.

2.1 Experiment 1

In this trial, the effect of applying multiple $CaCl_2$ (0.5% and 1% Ca; Panreac, Spain) or Ca propionate (0.5% and 1% Ca; Perstorp Ltd., Perstorp, Sweden) sprays containing 0.5% CMC (sodium carboxymethyl cellulose, ChemWorld S.A., Barcelona, Spain; an adjuvant with humectant and adherent properties) and 0.05% Tween 20 (polyoxyethylene sorbitane monolaurate, Panreac, Barcelona, Spain) was evaluated under field conditions. Ten treatments (four repetitions per treatment) were applied in the early morning (8 to 9 a.m.), using between 2 and 3 L of spray solution per tree. The experiment was designed as a 2×5 factorial (10 different treatments and a total of 40 trees), considering the adjuvant (*i.e.,* Tween 20 or CMC) and the Ca formulation used (0.5% and 1.0% Ca supplied as $CaCl_2$ or Ca propionate) as two factors.

The first sprays were applied on April 21, 2008, *i.e.,* 72 d before the date of bagging (July 2) supplying a total of five sprays at 2-week intervals until June 17. The 2nd, 3rd, 4th, and 5th treatments were sprayed to the trees 58, 43, 28, and 15 d before bagging, respectively. Leaf burn was severe in treatments containing 1% Ca^{+2} as $CaCl_2$, while those based on either 0.5% Ca supplied as $CaCl_2$ or 1.0% Ca in the form of Ca propionate were significantly less phytotoxic. No leaf-damage symptoms were observed in treatments containing 0.5% Ca as Ca propionate and also after the application of pure water solutions of 0.5% CMC and 0.05% Tween 20. When observed, leaf injuries were visible 3–4 d after the application of treatments and climatic conditions did not appear to influence the intensity of damage. Samples were collected approximately 2 weeks after each foliar treatment (*i.e.,* 58, 43, 28, 15, and 9 d after the date of bagging) and at the time of harvest (September 30).

2.2 Experiment 2

As leaf-burn problems were observed in association with the application of 1.0% Ca, a trial to evaluate the effect of supplying 0.5% Ca as $CaCl_2$ or Ca propionate in combination with three different adjuvants was carried out during the growing season of 2009. The adjuvants CMC (0.5%), Tween 20 (0.05%), and BreakThru® (0.05%, polyether-modified polysiloxane, Evonik Goldschmidt GmbH, Essen, Germany) were added to the Ca formulations. The experiment was designed as a 2×3 factorial, in which the Ca formulations ($CaCl_2$ or Ca propionate) and adjuvants (Tween 20, CMC, and BreakThru®) were the two factors analyzed. Four in-season sprays were applied at 2-week intervals, starting from April 24 and ending by June 9. Samples were collected approximately 2 weeks after each treatment, *i.e.,* 52, 35, 20, and 3 d prior to the date of bagging (June 29) and also at the time of harvest (September 10).

2.3 Mineral-element analysis

For mineral-element analysis, randomly selected fruits were collected around the tree canopy at the dates specified above. A total of 20 fruits was collected for each treatment, taking five fruits per tree (four trees per treatment). Peaches were thoroughly washed first in a 0.1% detergent solution (Mistol, Henkel, Germany), then rinsed in tap water, followed by a bath in 0.1N HCl and a final rinse in deionized water. A slice from the equator of the fruit was cut, and the exocarp was excised from the mesocarp using a sharp knife and removing the pulp from the exocarp (collecting skin samples of less than 1 mm in thickness) with a razor. Peach mesocarp and exocarp samples were analyzed separately on a fresh-weight (FW) basis. Mesocarp (1.0 g) and exocarp (0.5 g) tissues were wet digested (10 mL HNO_3 and 2 mL H_2O_2) until dryness in a hot plate. The dry deposit was redissolved in 10 mL of HCl and filled up with distilled water to reach a final volume of 25 mL. Calcium concentrations were determined using atomic-absorption spectroscopy (Unicam 929, Cambridge, UK).

2.4 Fruit quality traits

At harvest, fruits were collected and a random sample of ten fruits per tree was used to assess peach quality traits. Another randomly selected sample per experimental unit (50 fruits per tree) was stored under cold conditions (0.0°C–0.5°C), and fruit-quality parameters were again evaluated at different dates during the cold-storage period (after 4 and 2 weeks storage for 2008 and 2009, respectively). The fruit quality traits measured were: flesh firmness (Effegi penetrometer fitted with a 9 mm tip, and in 2009 also by an Aweta acoustic firmness sensor [AWETA G&P, Nootdorp, The Netherlands]), total soluble solids (ATAGO PR-101 digital refractometer), titratable acidity (TA; g malic acid [L juice]$^{-1}$ juice), and color (CR-200 colorimeter, Minolta Co., Osaka, Japan). After 2 weeks of storage at 0°C, the percentage of mesocarp browning and vitrescence in relation to the total area was visually estimated (*Brummell* et al., 2004; *Duan* et al., 2007). The browning and vitrescence indices of fruits were calculated as follows: 40 peaches were peeled to estimate the extent of development of vitrescent dark spot on the exocarp. Then, peeled fruits were sectioned following the longitudinal axis and the rate of internal browning was estimated on the mesocarp by assessing the total brown area per fruit using the following scale: 0 = no browning; 1 = slight browning (less than 1/4); 2 = 1/4–1/2; 3 = 1/2–3/4; and 4 = 100% of the area showing browning symptoms. The internal browning or external vitrescent indexes were calculated as (browning or vitrescent scale × percentage of corresponding fruit within each class) divided by the number of classes (*i.e.,* 5).

2.5 Statistical analysis

Data were statistically analyzed with ANOVA, and significant treatment effects were separated by Duncan's multiple range test.

3 Results

3.1 Experiment 1

Table 1 shows data corresponding to the Ca concentrations measured in the exocarp and mesocarp of Ca-sprayed peaches at key dates during the growing season. The initial (May 5, before the onset of treatments) and final (September 30, at harvest) Ca concentrations present in peach tissues provide evidence for the decrease of this element during the growing season. Regardless of the Ca formulations applied to the trees, exocarp Ca concentrations at the time of harvest were in general between 50% and 60% below the values determined in May, while mesocarp Ca concentrations decreased by 70% to 80% during the growing season (Tab. 1). Taking into account all treatments, the absolute Ca concentration per fruit increased linearly from the beginning of the experiment until the time of bagging (*i.e.,* approximately 7.12, 9.65, 12.66, and 14.75 g fruit^{-1} corresponding to May 5, June 4, June17, and June23, respectively). At harvest, the absolute Ca concentration per fruit (approximately 5.81 g fruit^{-1}) decreased below the levels recorded at the beginning of the trial.

The effect of the various Ca sprays was only noticeable during the 3rd week of June (*i.e.,* around the date of pit hardening and prior to fruit bagging), when increased Ca mesocarp and exocarp concentrations were determined in association with the application of all Ca-containing treatments. At this date, the highest Ca concentrations in peach tissues (mesocarp and exocarp) were recorded in fruits treated with 0.5% and 1.0% Ca supplied as $CaCl_2$, both in combination with 0.05% Tween 20 and 0.5% CMC. Calcium propionate sprays also promoted an increase in tissue Ca concentrations, leading to significant differences with the values determined in control fruits. Data regarding control 0.05% Tween 20 and 0.5% CMC sprays are not presented since they were similar to the ones obtained for untreated peaches.

Fruit quality traits at harvest were not affected by the adjuvant included in the spray solution (Tab. 2). However, the type of Ca compound applied to the trees had an effect on the total soluble solids (TSS) of fruits both at harvest and after cold storage (Tab. 2): peaches treated with $CaCl_2$ had in general higher TSS concentrations as compared to those sprayed with Ca propionate–containing formulations. The rest of the quality traits evaluated were not significantly affected by the Ca compounds applied to the fruits.

3.2 Experiment 2

In agreement with the results obtained in 2008, the application of Ca sprays prior to fruit bagging did not result in significant Ca increases at the time of harvest (Tab. 3). The initial

Table 1: Calcium concentrations (mg [100 g FW]$^{-1}$) at the beginning of the experiment (May 5, 14 d after the first treatment), prior to bagging (June 23, 9 d after the fifth treatment), and at time of harvest (Sep 30) in the mesocarp and exocarp of cv. Calrico peaches treated with multiple Ca sprays ($CaCl_2$ or Ca propionate) in combination with 0.05% Tween 20 or 0.5% CMC (Experiment 1; 2008). For each factor, means followed by different letters are significantly different (Duncan's multiple range test, $p \leq 5\%$, $n = 4$).

	Exocarp			**Mesocarp**		
Treatment	May 5	June 23	Sep 30	May 5	June 23	Sep 30
Untreated	28.46 a	22.90 a	13.26 a	13.26 b	7.68 a	2.64 a
Ca-treated	31.01 a	27.84 b	14.19 a	11.79 a	9.63 b	2.93 a
Ca forms						
$CaCl_2$	30.81 a	31.43 b	14.16 a	12.13 b	10.51 b	2.82 a
Ca propionate	31.20 a	24.26 a	14.22 a	11.45 a	8.75 a	3.04 a
Tween 20						
0.5% Ca ($CaCl_2$)	31.15 a	29.78 b	15.2 a	10.73 ab	10.14 bc	2.66 a
1.0% Ca ($CaCl_2$)	34.03 a	30.57 b	13.93 a	11.79 ab	11.16 c	2.20 a
0.5% Ca (Ca propionate)	31.68 a	23.49 a	14.94 a	9.91 a	8.30 ab	2.52 a
1.0% Ca (Ca propionate)	34.78 a	23.69 a	14.71 a	10.32 ab	8.43 ab	3.35 a
CMC						
0.5% Ca ($CaCl_2$)	29.5 a	28.80 bc	13.44 a	12.66 ab	9.47 bc	3.36 a
1.0% Ca ($CaCl_2$)	28.58 a	36.57 c	14.06 a	13.32 b	11.27 c	3.12 a
0.5% Ca (Ca propionate)	27.6 a	27.08 ab	14.54 a	12.24 a	8.70 ab	2.88 a
1.0% Ca (Ca propionate)	30.72 a	22.76 ab	12.69 a	13.34 b	9.59 bc	3.32 a

Table 2: Quality traits of cv. Calrico peaches at the time of harvest and after 4 weeks of cold storage (2008). Trees were treated with multiple sprays of $CaCl_2$ and Ca propionate (Ca-prop) (0.5% or 1.0% Ca) plus 0.05% Tween 20 or 0.5% CMC during the growing season. Values were averaged over adjuvant levels. Means followed by different letters are significantly different (Duncan's multiple range test, $p \leq 5\%$, $n = 10$).

	CMC						**Tween 20**					
	firmness N	L	a	b	TSS / °Brix	acidity / g L^{-1} MA	firmness N	L	a	b	TSS / °Brix	acidity / g L^{-1} MA
At harvest												
Control	35.54 a	72.00 a	6.80 a	51.66 a	14.11 b	3.82 a	32.18 a	72.15 a	7.65 a	52.23 a	14.27 b	3.52 a
0.5% Ca ($CaCl_2$)	35.38 a	71.63 a	7.97 a	51.56 a	14.72 b	3.67 a	36.38 a	71.54 a	8.19 a	51.92 a	15.08 c	3.59 a
1.0% Ca ($CaCl_2$)	38.07 a	72.08 a	6.06 a	52.03 a	14.11 b	3.87 a	37.29 a	72.59 a	6.77 a	51.29 a	14.43 b	3.86 a
0.5% Ca (Ca-prop)	31.40 a	71.37 a	7.87 a	52.19 a	13.96 ab	3.46 a	34.66 a	72.06 a	6.47 a	52.00 a	14.16 b	3.73 a
1.0% Ca (Ca-prop)	40.02 a	71.63 a	6.68 a	51.71 a	13.72 a	3.97 a	31.93 a	70.77 a	7.26 a	51.32 a	13.69 a	3.62 a
Four weeks after harvest												
Control	31.02 a	70.93 a	7.76 a	50.89 a	14.74 b	3.36 a	29.99 a	70.73 a	8.04 a	51.00 a	15.15 b	3.20 a
0.5% Ca ($CaCl_2$)	29.12 a	70.55 a	9.09 a	52.15 a	15.24 b	3.28 a	30.23 a	70.85 a	9.19 a	51.60 a	14.90 b	3.12 a
1.0% Ca ($CaCl_2$)	30.07 a	70.55 a	9.12 a	52.01 a	14.81 b	3.30 a	29.82 a	70.65 a	8.41 a	50.80 a	15.11 b	3.38 a
0.5% Ca (Ca-prop)	25.87 a	70.37 a	8.29 a	51.30 a	13.98 a	3.23 a	27.88 a	70.96 a	8.02 a	50.53 a	14.21 a	3.04 a
1.0% Ca (Ca-prop)	32.94 a	70.54 a	6.87 a	50.10 a	14.10 a	3.50 a	30.68 a	70.11 a	7.50 a	50.02 a	14.31 a	3.14 a

(May 8, before the onset of treatments) and final (at harvest) Ca concentrations present in peach tissues again followed a decreasing trend throughout the growing season. At the time of harvest, exocarp Ca concentrations of nontreated fruits decreased by 40% *versus* 14% determined for Ca-treated peaches. By contrast, similar mesocarp Ca-concentration decreases ranging from 55% to 65% were recorded at the time of harvest regardless of the Ca compound or the adjuvant type included in the spray formulation.

The effect of the different Ca sprays concerning the Ca compound and adjuvant type used was only noticeable in June. Significantly higher mesocarp Ca concentrations were measured after spraying Ca formulations containing the surfactant Break Thru® (both for data corresponding to fruits collected on June 9 and 26). By the end of June, significantly higher mesocarp Ca concentrations were determined in peaches treated with $CaCl_2$ formulations in comparison with the values measured for Ca propionate–sprayed fruits, which

Table 3: Calcium concentrations (mg [100 g FW]$^{-1}$) of cv. Calrico peach tissues after treatment with multiple in-season 0.5% Ca sprays ($CaCl_2$ or Ca propionate; Ca-prop.) in combination with 0.05% Tween20, 0.05% Break Thru® or 0.5% CMC (Experiment 2, 2009). Values were averaged over adjuvant and Ca-compound levels. Means followed by different letters are significantly different (Duncan's multiple range test, $p \leq 5\%$, $n = 4$).

	Exocarp					Mesocarp				
Ca treatments	May 8	May 25	June 9	June 26	Sep 10	May 8	May 25	June 9	June 26	Sep 10
Untreated	43.4 a	47.2 a	42.8 a	69.2 a	25.6 a	21.5 a	16.4 a	12.4 a	15.7 a	9.1 a
Ca-treated	41.1 a	56.0 a	44.4 a	67.4 a	35.4 a	20.7 a	17.0 a	13.8 a	18.4 a	7.9 a
Ca compound										
Ca propionate	42.2 a	53.1 a	46.7 b	61.1 a	38.0 a	21.2 a	15.7 a	14.2 a	16.3 a	8.6 a
$CaCl_2$	40.0 a	59.0 a	42.1 a	73.6 b	32.5 a	20.1 a	18.2 a	13.4 a	20.4 b	7.3 a
Adjuvant										
Tween20	41.4 a	54.6 a	47.1 a	61.5 a	33.2 a	20.6 a	16.3 a	13.4 a	15.6a	8.0 a
CMC	40.6 a	60.5 a	39.9 a	66.4 ab	36.3 a	20.5 a	18.8 a	13.2 a	18.1ab	7.2 a
Break Thru®	41.4 a	52.9 a	46.3 a	74.2 b	35.3 a	21.0 a	15.8 a	14.8 b	21.3b	8.6 a

Table 4: Quality traits of cv. Calrico peaches at the time of harvest and after 2 weeks of cold storage. Trees were treated with multiple sprays of $CaCl_2$ and Ca propionate (0.5% Ca) plus 0.05% Tween20, 0.05% Break Thru®, or 0.5% CMC during the growing season (2009). Values were averaged over Ca treatment, Ca compound, and adjuvant levels. Means followed by different letters are significantly different (Duncan's multiple range test, $p \leq 5\%$, $n = 10$).

Ca treatments	**Firmness** (N)	(Aweta)	**TSS** / ° Brix	**Acidity** / g L^{-1} MA	**Humidity** / %	**L**	**a***	**b***
				At harvest				
Untreated	29.24 a	10.95 a	17.0 a	4.11 a	82.1 a	70.5 a	13.7 a	54.94 a
Ca-treated	30.21 a	12.6 b	16.4 a	4.68 a	83.2 a	70.3 a	13.6 a	54.59 a
Ca compound								
Ca propionate	29.36 a	12.0 a	16.9 a	4.50 a	82.8 a	70.1 a	13.9 a	54.56 a
$CaCl_2$	31.06 a	13.2 b	15.9 a	4.85 a	83.7 a	70.4 a	13.3 a	54.6 a
Adjuvant								
Tween20	30.9 a	12.5 a	16.4 a	4.75 b	83.3 a	70.4 a	13.5 a	53.9 a
CMC	31.7 a	13.0 a	16.6 a	5.16 b	83.2 a	70.3 a	13.8 a	55.6 a
Break Thru®	28.0 a	12.3 a	16.1 a	4.12 a	83.1 a	70.0 a	13.5 a	54.2 a
After 2 weeks cold-storage								
Untreated	17.73 a	5.15 a	18.0 a	3.08 a	81.6 a	67.8 a	16.1 a	54.9 a
Ca-treated	23.79 b	8.13 b	17.2 a	3.17 a	82.4 a	68.1 a	15.1 a	54.6 a
Ca compound								
Ca propionate	21.7 a	6.44 a	17.7 b	3.02 a	82.3 a	67.3 a	15.3 a	49.3 a
$CaCl_2$	25.8 b	9.82 b	16.6 a	3.32 a	82.6 a	69.0 a	14.9 a	51.6 a
Adjuvant								
Tween20	21.8 a	6.7 a	17.3 a	3.51 b	83.4 a	67.6 a	15.2 a	49.9 a
CMC	26.4 b	9.0 b	17.1 a	3.40 b	82.1 a	68.9 a	15.2 a	51.5 a
Break Thru®	23.2 ab	8.7 b	17.1 a	2.60 a	81.2 a	68.0 a	14.9 a	49.8 a

Table 5: Evaluation of injuries (vitrescent dark spot or browing) developing in cv. Calrico peaches after 2 weeks of cold storage (0°C). Trees were treated with multiple sprays of $CaCl_2$ and Ca propionate (0.5% Ca) plus 0.05% Tween 20, 0.05% Break Thru®, or 0.5% CMC during the growing season (2009). Values were averaged over Ca treatment, Ca compound, and adjuvant levels. For each factor, means followed by different letters are significantly different (Duncan's multiple range test, $p \leq 5\%$, $n = 40$).

	Vitrescence		**Browning**	
Ca treatments	%	index	%	index
Untreated	74.38 a	41.10 a	43.75 b	15.0 b
Ca-treated	59.23 a	29.71 a	22.08 a	8.59 a
Ca compound				
Ca propionate	67.28 a	34.13 a	23.75 a	10.63 b
$CaCl_2$	51.17 a	25.29 a	20.42 a	6.56 a
Adjuvant				
Tween20	64.40 a	35.09 b	32.50 b	13.28 b
CMC	51.81 a	21.84 a	19.38 ab	7.34 a
Break Thru®	61.46 a	32.20 b	14.38 a	5.16 a

did not differ significantly from those obtained for untreated trees.

Measurement of fruit quality traits at the time of harvest (Tab. 4) showed that flesh firmness (Aweta acoustic firmness sensor) increased significantly in Ca-treated fruits, especially when $CaCl_2$ was applied *versus* Ca propionate. Spray formulations containing the adjuvants CMC and Break Thru® led to increased fruit acidity at the time of harvest. Fruit-quality-traits evaluation after 2 weeks of cold storage indicated the occurrence of significant differences regarding fruit firmness, TSS, and acidity in relation to the different formulation components. Increased fruit firmness was determined in association with the application of Ca-containing sprays, chiefly when $CaCl_2$ was used as Ca source and when 0.5% CMC and Break Thru® were added to the formulations. Calcium propionate–treated peaches showed higher TSS levels as compared to those sprayed with $CaCl_2$-based formulations. As observed at harvest, the application of sprays containing CMC or Break Thru® led to increased acidity values as compared to untreated or Tween 20 sprayed peaches.

A beneficial effect of in-season Ca sprays was observed concerning the development of fruit physiological disorders after 2 weeks of cold storage (Tab. 5). However, none of the Ca formulations applied to the trees led to decreased vitrescence development rates in peaches. The lowest vitrescence index was recorded for fruits treated with CMC as compared to Tween 20 and Break Thru®. In relation to internal browning (Tab. 5), the proportion of fruits exhibiting damages significantly decreased in Ca-treated fruits as compared to untreated ones. Supplying Ca as $CaCl_2$ provided the lowest browning index, and the addition of adjuvants also had an effect on the storability of peaches. The lowest rates of browning were determined when Ca was applied in association with 0.05% Break Thru® followed by 0.5% CMC.

4 Discussion

In this investigation, the effect of applying in-season Ca sprays to peaches under field conditions was evaluated for two growing seasons, in terms of assessing fruit quality traits, storability, and Ca status. The application of multiple Ca sprays to fruit crops as a strategy to prevent the development of Ca-related disorders is largely recommended in many fruit-producing areas of the world. However, inconsistent results concerning the effectiveness of Ca sprays to increase the Ca budget of the fruit and limit the development of Ca-related disorders have been reported for various fruit crops (*Manganaris* et al., 2005a, b; *Val* et al., 2008; *Sotiropoulos* et al., 2010).

Due to the limited mobility of Ca within the plant, the efficiency of Ca sprays will be largely associated with the rate of Ca absorption by the fruit surface and its distribution within the organ (*Fernández* et al., 2009; *Manganaris* et al., 2005a). Thereby, the development of optimized Ca formulations is a key factor to improve the performance of Ca sprays, especially in arid and semiarid areas of the world where, in the summer, average temperatures are high and relative humidities are low. Avoiding the occurrence of Ca imbalances in fruits during the growing season is important to prevent the incidence of post-harvest physiological disorders, such as chilling injuries (*Manganaris* et al., 2005a, b; *Val*, 2007).

To increase the chance for penetration of the plant surface by a nutrient solution, factors such as wetting, spreading, retention, and the physical-chemical properties (*e.g.,* point of deliquescence, molecular weight, solubility) of the applied formulation should be considered as suggested by *Kraemer* et al. (2009a, b). Given the hydrophobic nature of the peach surface, trials to assess the effectiveness of multiple, in-season Ca sprays based on $CaCl_2$ or Ca propionate as active ingredients were carried out under field conditions. The study was chiefly aimed at assessing: (1) the effect of supplying Ca as two different Ca compounds (Ca propionate or $CaCl_2$), (2) the effect of supplying two different Ca concentrations (0.5% and 1.0% Ca), and (3) the influence of including in the spray formulation the adjuvants CMC and two nonionic surfactants (Tween 20 and Break Thru®). The Ca compounds used were selected on the basis of the low point of deliquescence of $CaCl_2$ (32% *versus* 95% for Ca propionate, *Schönherr*, 2001) and the antifungal properties of Ca propionate (*Biggs* et al., 1997; *Fernández* et al., 2009).

In general, the Ca concentration in peach tissues followed a decreasing trend from May until the time of harvest. However, in 2009 Ca-treated peaches experienced only a 14% decrease in exocarp Ca concentrations *versus* 40% determined in untreated fruits. Tissue Ca increases associated with the various treatments were recorded by the end of June, a date which is probably coincident with the period of pit hardening. It can be hypothesized that preserving an adequate Ca balance until the time of pit hardening may have an influence on the quality and storability of fruits. However, this suggestion should be evaluated in more detail.

With regard to the climatic variables during the growing seasons (April 1 to October 30) of 2008 and 2009, similar temperatures were recorded, but 2009 was more dry (139 mm) as compared to 2008 (209 mm). Nevertheless, water was supplied to the trees *via* fertigation based on the evapotranspiration values calculated for the particular location and the orchard and crop characteristics. Therefore, no apparent relationship between tissue Ca concentrations during the growing season could be ascribed to climatic differences between the two experimental seasons.

The highest June tissue Ca concentrations were recorded in association with the application of $CaCl_2$ *versus* Ca propionate. Application of foliar sprays containing 1.0% Ca during 2008, led to leaf burn especially when $CaCl_2$ was supplied to the trees. Subsequently and to avoid the risk of phytotoxicity, only 0.5% Ca–containing sprays were supplied to the trees in 2009. Regarding the effect of spraying $CaCl_2$ *versus* Ca propionate on fruit quality and storability, it was observed that peaches treated with the mineral Ca salt had increased firmness values and showed a lower degree of chilling injury as compared to those treated with Ca propionate. Similar were obtained by *Manganaris* et al. (2005a) after the application of $CaCl_2$ and Ca EDTA.

An important and complex cause of quality deterioration and customer dissatisfaction regarding the consumption of peaches and nectarines is the presence of flesh browning, flesh mealiness, darkened pit cavity, flesh translucence, red-pigment accumulation (bleeding), and loss of flavor (*Lurie* and *Crisosto,* 2005). Peaches exposed to conventional cold storage at 0°C–1°C for up to 2 weeks, normally suffer from chilling injuries, although the occurrence of the disorder strongly depends on the cultivar and maturity stage at harvest (*Fernández-Trujillo* and *Artés*, 1998). In this work, both Ca treatments and two of the adjuvants used (CMC and Break Thru®) were found to be effective in decreasing the incidence of chilling injuries in cv. Calrico peaches after 2 weeks of cold storage. Best results were recorded after the application of multiple Ca sprays containing 0.05% Break Thru® followed by 0.5% CMC. However, the incidence of vitrescent dark spot, which is actually the main peach disorder affecting late-season cultivars grown in Northeast Spain (*Val*, 2007; *Fernández* et al., 2009), remained unaffected by the Ca treatments (Tab. 5), which might be related to the random pattern of appearance of this disorder within and between orchards and different growing seasons. Currently, the causal factors leading to the occurrence of vitrescent dark spot remain unclear and should be investigated in the future.

To lower the surface tension of the Ca-containing solutions, two commercial surface-active agents having surface tensions of approximately 43 (Tween 20) and 25 mN m^{-1} (Break Thru®) were added to the formulations, in addition to an adjuvant with sticker and humectant properties (CMC). Application of Ca sprays containing 0.5% CMC in combination with 0.5% and 1.0% $CaCl_2$ led to increased mesocarp and exocarp Ca concentrations by the end of June 2008. Increased mesocarp and exocarp Ca concentrations were determined for a similar date when the organosilicon surfactant Break Thru® was added to the formulations. The lowest degree of chilling injury was actually recorded for fruits treated with 0.5% Break Thru® followed by 0.5% CMC as adjuvants. Results can be related to the improved penetration rate of surface-applied Ca due to changes in the area of distribution of Ca within the spray droplet as suggested by *Kraemer* et al. (2009a, b), to the increased penetration rates associated with the low surface tension of Ca solutions containing the organosilicon surfactant (*Fernández* et al., 2008), and to the properties of CMC in terms of increasing the retention and humectancy and maybe reducing the wash-off of foliar Ca sprays by rain as suggested by *Schmitz-Eiberger* et al. (2002a) or *Kraemer* et al. (2009a). The transient Ca increases detected in June prior to fruit bagging may indicate that up to this time, peaches are more permeable to Ca sprays as compared to more mature physiological stages. The lower surface tension of Break Thru®–containing solutions and the higher retention and humectancy provided by CMC may facilitate the absorption of Ca by the peach surface. Such transient Ca increases achieved after the application of four to five sprays up to June may play a major role for Ca homeostasis and the potential development of Ca-related disorders at fruit maturity. Thus, in agreement with several studies (*Haefs* et al., 2001; *Schmitz-Eiberger* et al., 2002b; *Manganaris* et al., 2005a) evidence for the beneficial effect of applying in-season Ca sprays to fruit crops was obtained in this investigation.

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