COMBINATION OF STRATEGIES TO SUPPLY CALCIUM AND REDUCE BITTER PIT IN 'GOLDEN DELICIOUS' APPLES

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ABSTRACT

Calcium (Ca) sprays and Ca applications to soil throughout the growing season or Ca solution dips at post-harvest are widespread practices to supply Ca and decrease bitter pit in apples. However, published results conflict, and there is no information about the effectiveness of combining all these treatments. In the present study, the following treatments were assessed during four growing seasons: early-season (April) Ca soil applications applied 4 times, mid-season (May) $CaCl_2$ sprays applied 7 or 13 times, late-season (June) $CaCl_2$ sprays applied 7 times, and the combination of late-season sprays and soil applications. In addition, post-harvest dips were evaluated in the latter two growing seasons. Notably high bitter pit incidences were monitored for the first and fourth year of study (>20%), while the second and third year were almost without incidence. Post-harvest dips mitigated bitter pit incidence to a greater extent than pre-harvest treatments, and the sprays mitigated bitter pit to a greater extent than Ca soil applications. The combination of sprays and soil applications did not improve the results relative to Ca sprays alone. No detectable advantage for starting spray programmes earlier than June was observed. Our results showed a trend towards reduced bitter pit with an increasing number of $CaCl_2$ sprays, but this was not clearly an effect of maximizing fruit Ca. Finally, applying 13 $CaCl_2$ sprays in combination with a Ca solution dip at post-harvest appeared to be the most effective practice for minimizing the risk of bitter pit development.

Keywords: apple, calcium disorders, calcium supply, sprays, fertigation, post-harvest dips.

1. INTRODUCTION

Calcium (Ca) deficiency has been implicated in several disorders fruits, such as bitter pit in apples (*Malus* × *domestica* Borkh) (Casero et al., 2010; Ferguson et al., 1999; Lotze and Theron, 2007; Peryea et al., 2007). Although bitter pit develops during post-harvest storage, the underlying process stems from the period of fruit growth and development (Val et al., 2010). Ca supplied during the growing season through spray applications is currently the most common method of reducing bitter pit and/or increasing the Ca content of fruit. However, its effectiveness is irregular and can depend on the number of applications, salt type (nitrate, chloride or others) and period in which it is supplied (Val et al., 2008; Wojcik and Borowik, 2013; Wooldridge et al., 1998). The general consensus is that no Ca-based product has consistently shown greater efficacy in reducing bitter pit than $CaCl_2$ (Schönherr, 2001). Nevertheless, there is no general agreement on when is the best time to apply Ca sprays or on the efficacy of Ca supplied to the roots. Some studies have found that the best time for Ca sprays is immediately after full bloom, when fruits are small. Early applications may be advantageous because a less developed cuticle favours the penetration of Ca into the fruit (Neilsen et al., 2005; Wilsdorf et al., 2012). However, in a great number of studies, Ca sprays have been most effective in the second half of the fruit growth period (Benavides et al., 2001; Casero et al., 2010; Lotze et al., 2008; Peryea et al., 2007; Wilsdorf et al., 2012). According to Casero et al. (2002), starting Ca sprays at 10 days after full bloom (dafb) did not increase Ca accumulation in apples, while starting at 70 dafb increased the Ca absorption rate and accumulation in fruit. This effect is thought to occur because Ca is provided mainly by root absorption during the first period of fruit growth, whereas during the second period of growth, when fruit Ca absorption is reduced, Ca sprays may be more effective (Casero et al., 2002).

In this context, Ca root applications during the first period of fruit growth and Ca sprays during the second half of fruit growth appear to be the most effective management strategy for maximizing fruit Ca and minimizing the risk of bitter pit development. Nevertheless, there are no published reports about the effectiveness of Ca root applications in controlling bitter pit and very few reports about the effectiveness of combining Ca root applications with Ca sprays to increase the Ca concentration in fruit. Wilsdorf et al. (2012) evaluated the contributions of Ca root applications and sprays to increases in the Ca content of fruit. Ca sprays were more effective than soil Ca applications in increasing Ca in fruit, but the treatments could not be evaluated with respect to bitter pit because there was no incidence of the disorder. Although soil Ca application is a common practice and many studies have evaluated its effects on Ca content in fruits, there are no studies that specifically relate this practice to bitter pit reduction.

Another technique to control bitter pit in apples is to dip or drench fruit in Ca solutions post-harvest. This technique can also increase the Ca content of fruit and has shown effectiveness both in decreasing bitter pit and improving fruit quality (Manganaris et al., 2007). Guerra and Casquero (2010) observed a decrease in bitter pit of 16.7% with a post-harvest Ca treatment in the 'Reinette de Canada' cultivar.

Most of the published works about Ca fertilization have separately evaluated these approaches (soil application, sprays and dips) and have obtained insufficient results. The combination of these three practices might improve the efficacy of bitter pit control, but, as far as we know, there are no up-to-date published studies in which pre-harvest Ca application to soil, sprays and post-harvest dips have been combined, a strategy that may be the most effective practice for controlling bitter pit. Taking all these techniques into account, we investigated the effects of combining Ca applications pre-harvest (using soil and/or sprays) and Ca dips post-harvest on 'Golden Delicious', a cultivar that is susceptible to bitter pit (Moscetti et al., 2013). This is the first study combining these approaches to supply Ca to apples during several consecutive growing seasons.

2. MATERIALS AND METHODS

2.1. Plant material and crop technology

The trial was carried out in an apple tree (*Malus domestica* Borkh.) orchard of the IRTA Experimental Station of Lleida (Mollerussa, NE Spain) over four consecutive growing seasons (from 2007 to 2010). Mature, uniform, 'Golden Delicious' trees grafted onto 'M9' rootstock and planted in 1994 at 4 × 1.4 m spacing (1786 trees/ha) were selected from a bitter pit-prone orchard. Fertigation was applied through drip irrigation. The interrows were grassed down, but herbicide strips were kept along the rows. The soil was characterized as a calcareous loam with excellent drainage characteristics. During the four-year study, trees were managed according to the guidelines for apple integrated production, including the application of mineral fertilizers that were estimated to cover the nutrient requirements.

2.2. Treatments

2.2.1. Pre-harvest treatments

Ca root applications were applied during the whole vegetative stage, whereas Ca sprays were applied during the mid- and/or late fruit growing stages according to each treatment. A total of 6 pre-harvest treatments were assessed: i) control with no calcium application; ii) early-season root applications (R_{Early}) consisted of four calcium applications to the soil through drip irrigation and were performed at full bloom, fruit set, the cell multiplication phase and the beginning of maturation (CaO total: 6.0 kg ha^{-1} per season); iii) Ca sprays from mid-season ($F7_{Mid}$) consisted of 7 Ca sprays every 12-14 days starting 30 days after full bloom (dafb) (CaO total: 5.9 kg ha^{-1} per season); iv) Ca sprays during late season ($F7_{Late}$) consisted of 7 Ca sprays every 5-10 days starting 60 dafb (CaO total: 5.9 kg ha^{-1} per season); v) Ca root applications and sprays ($R_{Early}F7_{Late}$) consisted of a combination of both R_{Early} and $F7_{Late}$ treatments (CaO total: 11.9 kg ha^{-1} per season); vi) additional mid-stage Ca sprays ($F13_{Mid}$) consisted of 13 Ca sprays every 5-10 days starting 30 dafb (CaO total: 11.0 kg ha^{-1} per season).

For the Ca root applications, a commercial $CaCl_2$ solution of 15% water-soluble CaO (Timac Agro, Spain) was used at a rate of 10 L ha^{-1} per application. For the Ca sprays, a commercial $CaCl_2$ solution of 16.9% water-soluble CaO (Yara Iberian SA, Spain) was used at 5 L ha^{-1} per application. Sprays were applied very early in the morning, when air temperatures were below 25 ºC, to minimize phytotoxicity, which could cause visible necrosis or marks on fruits or leaves. A high-pressure handgun sprayer (25 atm) was used at a rate of 1000 L ha^{-1}.

2.2.2. Post-harvest treatment

In each of the four growing seasons, in each elemental plot, 100 fruits of uniform size (70-75 mm in diameter) and without any disorder were picked at commercial harvest and placed in cold storage at 0 ºC for 90 days. In addition, within 24 h of harvest in 2009 and 2010, another 100 fruits per elemental plot were dipped into a solution with $CaCl_2$ (15%) for 30 seconds and received a dose of 3.5 L of $CaCl_2$ per 100 L of water. Immediately after, the dipped fruits were placed in cold storage at 0 ºC. After 90 days in cold storage, all samples were transferred to room temperature (20-23 ºC) for 7 days, during which time the samples were evaluated.

2.3. Bitter pit assessment

Bitter pit was evaluated using a category scale with 4 classes of bitter pit depending on the amount of pitted area (Torres et al., 2015): class 0 demonstrated no bitter pit symptoms; class

1 demonstrated slight symptoms, with fruit having 1-6 pits on the surface; class 2 demonstrated moderate symptoms, with less than one-third of the surface of the fruit affected (approximately 7-15 pits per fruit); and class 3 demonstrated severe symptoms, with more than one-third of the surface of the fruit affected (> 15 pits per fruit). Bitter pit incidence was calculated as the percentage of apples with at least one pit. In addition, a relative index of severity (S) was calculated for each sample using the formula $S = \sum_{n=1}^{N} \frac{I_n}{3 \times N}$, where S is the relative index of bitter pit severity (from 0 to 1), I_n is the severity class of each apple, N is the total number of apples assessed, and 3 is the maximum level of severity.

2.4. Fruit calcium content

In 2008, 2009 and 2010, 20 apples per elemental plot were collected at harvest for mineral analyses. The samples were taken from spurs on 2-year-old shoots. The apples were then carefully washed, and two longitudinal slices were cut from opposite sides of each fruit, excluding the cores and seeds. The complete sample from each elemental plot was weighed, dried, and then re-weighed to determine the percentage of dry mass. The dried tissue of each sample was wet digested with concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) in a microwave oven (Millestone MCR). The Ca content was then determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

2.5. Fruit yield parameters

Every season, all apples from each tree were separately harvested and weighed at commercial harvest by automatic fruit sorting equipment. The harvest and sampling were carried out when the starch index of the fruit was 6-7 (starch chart EC-Eurofru 1-10). Finally, for each treatment and season, fruit yield (kg tree^{-1}), fruit load (number of fruits tree^{-1}) and fruit weight (g fruit^{-1}) were calculated.

2.6. Fruit quality parameters

In 2008, 2009 and 2010, 20 apples from each elemental plot, of uniform size and without bitter pit symptoms, were taken for fruit quality assessments at harvest. The parameters measured were fruit firmness, starch index, soluble solid content and titratable acidity. Firmness was measured at two opposite sides on the fruit equator using a digital firmness tester (Penefel®; Ctifl, France). The starch index was visually measured using the EC-Eurofru scale (1-10). Soluble solid content (º brix) and titratable acidity (malic acid g L^{-1}) were determined using the freshly prepared juice of the whole subsample. Soluble solid content was measured using a digital temperature compensated refractometer (model PR-101, Atago Co. Tokyo Japan), and titratable acidity (expressed as malic acid) was determined by titrating 10 mL of juice with 1.0 M NaOH to pH 8.2. In the 2009 and 2010 seasons, fruit firmness at post-harvest was also measured.

2.7. Experimental design and statistical analysis

The experimental design of the trial was a randomized complete block with 4 blocks and 6 elemental plots per block. Each elemental plot consisted of 3 rows of 6 trees, and the measurements were carried out on the two central trees in the central row of each elemental plot.

Analyses were performed in SAS 9.2 (SAS Institute Inc., 2009). A two-way ANOVA was performed with the GLM procedure to test main effects of season and pre-harvest treatments, and their interaction, on bitter pit and the parameters analysed at harvest. Duncan's multiple range tests were used for the mean separation of significant effects and, if pre-harvest treatment effect from ANOVA models were significant ($P < 0.1$), single degree of freedom and polynomial contrast were performed to compare specifics groups of pre-harvest treatments. Besides, a three-way ANOVA was performed with the GLM procedure to test main effects of season, pre-harvest and post-harvest treatments, and their interaction, on bitter pit and the fruit firmness analysed at post-harvest.

3. RESULTS AND DISCUSSION

3.1. Effect of pre-harvest treatments

3.1. Effect on bitter pit

The two-way ANOVA indicated that bitter pit was strongly related to the season of growth (Table 1). The incidence of bitter pit was higher than 20% in two seasons (2007 and 2010) and almost inappreciable in other two seasons (2008 and 2009) (Figure 1). The pre-harvest treatments also showed a significant effect on bitter pit incidence and severity. The interaction season × pre-harvest treatment was non-significant.

A contrast confirmed a significant difference between the control and the pre-harvest treatments. In general, the sprays decreased bitter pit to a greater extent than root applications (Figure 1). The root applications alone (R_{Early}) did not show a significant decrease of bitter pit with respect to the control treatment. A contrast confirmed significant differences between the R_{Early} treatment vs. the sprays treatments ($F7_{Mid}$, $F7_{Late}$ and $F13_{Mid}$). The combination of root applications and sprays ($R_{Early}F7_{Late}$) did not bring any significant improvement with respect to the rest of treatments (Table 2). Considering all these findings, it appears not to be detectable advantage on bitter pit control as a result of applying Ca through fertigation.

No significant differences were observed between applying 7 and 13 sprays, although the $F13_{Mid}$ treatment showed a trend towards a lower incidence of bitter pit in comparison to the other treatments (Figure 1). These results are in agreement with those obtained by Casero et al. (2010), who did not observed a significant decrease in bitter pit by increasing the number of applications of $CaCl_2$ from 6 to 12 sprays using similar doses to those used in the present trial. Lotze et al. (2008) did not obtained either a significant improvement by increasing from

6 to 8 the number of sprays. Significant differences were neither observed between starting the sprays at mid-season and at late-season (Table 2). Casero et al. (2002), Peryea et al. (2007), Lotze et al. (2008), and Lotze and Theron (2007) did not observed either any improvement by beginning sprays before June. Instead, we observed a trend towards a lower incidence of bitter pit when the applications were made later in the season. In line with this, only the $F7_{Late}$, $R_{Early}F7_{Late}$ and $F13_{Mid}$ treatments significantly decreased bitter pit severity in comparison to the control treatment (Figure 1). Thus, our results suggest that increasing the number of sprays from June until harvest could more effectively minimize the risk of bitter pit than starting spray programmes earlier. However, not only the number or moment of applications can be important but also the concentration of $CaCl_2$ in solution. Yuri et al. (2002) achieved significant efficacy in controlling bitter pit by carrying out only 6 $CaCl_2$ sprays (an 81% reduction with respect to the control treatment) but with a higher total amount of CaO per hectare than the amount used in the present study (18 vs. 11 kg CaO ha^{-1}).

3.1.2. Fruit calcium concentration

The Ca content of fruit was significantly different among growing seasons (Table 1). The highest Ca content was recorded in 2008 (5.1 mg 100 g^{-1} fw) when it was not observed any incidence of bitter pit (Figure 2). On the other hand, no differences in the Ca content were observed between 2009 and 2010 (3.9 mg 100 g^{-1} fw) although the bitter pit incidence was significantly different between these seasons (1% vs. 19%).

There was no clear relationship between Ca content and bitter pit incidence. Generally, a threshold of 5-6 mg Ca 100 g^{-1} fw in apples at harvest is required to minimize bitter pit, but there are many papers reporting the failure of predictions based on this level of Ca content (Lotze et al., 2008) which is in line with our results. To explain this lack of relationship, some researchers have suggested that bitter pit is dependent on abnormal Ca partitioning and distribution in the cell (Pesis et al., 2010). According to Pavicic et al. (2004), the water-soluble Ca fraction is a more significant factor than the water-insoluble Ca fraction or the sum of these fractions. De Freitas et al. (2010) reported that the development of bitter pit is a complex process that involves not only the total input of Ca into the fruit but also Ca homeostasis at the cellular level, Ca accumulation into storage organelles and Ca binding to the cell wall. Some researchers have achieved better results when analysing skin instead of tissue from fleshy fruit (Amarante et al., 2013; Peryea et al., 2007; Val et al., 2008). Saure (2014) reported that stress was the main cause of 'Ca disorders' in different fruits and vegetables. Stress increases the production of reactive oxygen species (ROS), which causes lipid peroxidation and an increase in the leakiness of membranes, leading to the rapid vacuolation of parenchyma cells and the loss of ions, such as apoplastic Ca. Therefore, in the context of bitter pit development, the final deficiency of Ca might be considered a result rather than a cause. Ca applications could increase antioxidant capacity, including the total content of phenols and ascorbic acid. This effect would explain the efficacy of treatments in the reduction of bitter pit symptoms. Based on our results, a low Ca concentration in fruit (<4 mg 100 g^{-1} fw) is not enough to develop bitter pit. Considering the differences observed between seasons in the present study, it is important for future research to expand the knowledge on the relationship between weather conditions and bitter pit to gain a better understanding of bitter pit triggers.

All Ca treatments led to a significant average increase of 14% in the Ca content of fruit compared to the control treatment. No interaction effect was found between season and pre-harvest treatments (Table 1). Increasing fruit Ca content was more successfully achieved with Ca sprays than with root applications. Only the increase in the $F7_{Late}$ and $F7_{Early}$ treatments was significant in comparison to the control treatment (Figure 2). Advancing the start date of sprays, increasing the number of sprays or combining root applications and sprays did not improve the fruit Ca content (Table 2). Wilsdorf et al. (2012) also observed a greater effect of sprays in increasing Ca in comparison with root applications. Some research has found that the Ca reserves from the permanent structure of the trees make an important contribution to new growth during the period of rapid shoot extension (Himelrick and McDuffie, 1983; Wilsdorf et al., 2012). Along these lines, Wilsdorf et al. (2012) observed that root applications in the previous season, after harvest, were more efficient than root applications during the growing season. We should note that our study was carried out on calcareous soil, and thus, there were high levels of Ca in the soil. However, in most of the growing season, the Ca concentration in fruit is lower than the minimum Ca requirement to avoid the risk of bitter pit (5-6 mg 100 g^{-1} fw) (Johnson et al., 1987; von Bennewitz et al., 2015). Leaves do not experience such a low Ca concentration, possibly because water and mineral moves from roots to leaves via xylem through transpiration and the leaves have a higher transpiration rate than the fruits. Therefore, under the conditions of our study (calcareous soil and/or high temperatures), fruit sprays appeared to be the most effective practice for maximizing fruit Ca.

3.1.3. Fruit yield parameters

The pre-harvest Ca treatment did not affect fruit yield parameters. Contrariwise, there was a significant effect of growing season (Table 1). The 2007 and 2010 seasons, which had the lowest fruit yield, also had the highest incidences of bitter pit. Therefore, the fruit yield may affect bitter pit incidence, but we did not observe a statistically significant relationship between fruit yield and bitter pit incidence (data not shown). In general, heavy crop load relates toes a lower incidence of bitter pit (Ferguson and Triggs, 1990). A possible explanation for the higher bitter pit incidence observed in trees with light crops is that such trees have a higher proportion of large fruits (Schumacher et al., 1980). Large fruits generally have lower Ca concentrations and a higher risk of bitter pit than small fruits (Peryea et al., 2007). Nevertheless, our results showed higher Ca concentration in the season with the largest fruits and a higher incidence of bitter pit in the seasons when fruit were smaller. Generally, trees with lower crop load provide larger fruits (Corollaro et al., 2015; Delong et al., 2006), but this did not occur in 2007 and 2010 (Figure 3). In both growing seasons, there were more cloudy days and days with cooler temperatures during the bloom period. These conditions may have resulted in poor pollination and, consequently, a decrease in crop load and poor quality of set fruit, as poor pollination leads to the development of fewer seeds (De Freitas et al., 2015) . Bramlage et al. (1990) and Buccheri and Di Vaio (Buccheri and Di Vaio, 2004) showed a negative relationship between the number of seeds and the incidence of bitter pit; unfortunately, we did not count the number of seeds for this purpose.

3.1.4. Fruit quality parameters

All tested fruit quality parameters were significantly different among growing seasons. The starch index was significantly higher in 2008 and 2010 than in 2009 (Figure 4). Most reports have shown that, in general, bitter pit is more severe in early-picked than in later-picked apple fruit (Delong et al., 2006; Ferguson et al., 1999). However, we found no consistent effect of fruit maturity on the incidence of bitter pit. The sugar content was significantly lower in 2009 than in 2008 or 2010 as expected, according to the results of starch index. Generally, sugar content in fruits tends to increase with maturation due to the hydrolysis of starch. The highest content of malic acid content was observed in 2008, whereas the lowest content was observed in 2010. In 2010, fruit firmness was significantly higher than in 2008 and 2009 and no consistent relation could be established between fruit firmness and starch index (Figure 5).

The pre-harvest Ca treatment did not affect any fruit quality parameter. According to other authors, the application of Ca might delay the changes associated with ripening and senescence in fruits (Beavers et al., 1994; Fallahi et al., 1997; Mason and Drought, 1975; Vicente et al., 2007). Therefore, applications of Ca can decrease the sugar content and increase starch index in apples by slowing senescence. In the same way, Ca treatments can increase acidity in fruit and/or affect perception of fruit flavour compounds (El Hadi et al., 2013; Recasens et al., 2004) and, consequently, affect in sensorial acceptability.

The relationship between fruit Ca concentration and fruit firmness is well documented (Glenn and Poovaiah, 1990; Vicente et al., 2007). However, in 2008, fruit firmness was significantly lower than in 2010 and 2009, and fruit had a significantly higher Ca content. There are many papers reporting the failure of Ca applications to increase or keep fruit firmness (Dris and Niskanen, 1999; Glenn and Poovaiah, 1990; Recasens et al., 2004). Casero et al. (2010), when applying similar amounts of Ca to the amounts applied in the present study, achieved an increase in fruit firmness at harvest only in one out of three growing seasons. The Ca application rate may play an important role. In accordance with this hypothesis, Weis et al. (1980) and Wojcik et al. (2001) obtained significant effects on fruit firmness by applying 68% and 50% more Ca, respectively, than in the present study, but damage to apple skin was observed.

Based on our results, there are likely factors other than minerals that affect the physiological and biochemical changes that take place during fruit development. However, little is known about the contributions of pre-harvest factors to fruit quality.

3.2. Effect of post-harvest treatment

3.2.1. Effect on bitter pit

Dipping in $CaCl_2$ solution significantly decreased the incidence and severity of bitter pit (Table 3). Globally, dips at post-harvest showed a higher efficacy in decreasing bitter pit incidence and severity relative than the pre-harvest treatments. No interaction between Ca applications at pre-harvest × dips at post-harvest was observed. Therefore, the pre-harvest treatments $F7_{Late}$ and $F13_{Mid}$ might contribute to improve the effectiveness of the dips like when there was no treatment at post-harvest.

There was a significant interaction between season × dips at post-harvest since the effect of dips was higher in the season with higher incidence of bitter pit (Figure 6). In 2010, the growing season with higher bitter pit incidence, the dips significantly decreased the incidence and severity of bitter pit whereas no significant effect was observed in 2009, the growing season with low bitter pit. An effective prediction even close to harvest time could help to packing houses to decide whether to use $CaCl_2$ dips, which would mean an important saving (Torres et al., 2015).

Other researchers have also reported significant effects of Ca dips in decreasing bitter pit. Mignani et al. (1983), and more recently Guerra and Casquero (2010) and Guerra et al. (2011), obtained a reduction of 17%, but they did not compare their results with Ca applied in pre-harvest treatments. As the first example of a combination of pre-harvest treatments and post-harvest dips, our results showed that spraying later in the season, especially with a high number of sprays, in combination with dips at post-harvest, was the most effective approach to mitigate bitter pit, with a reduction of 30% with respect to the untreated control.

3.2.1. Effect on fruit firmness

As expected, the firmness of apples decreased during storage. Significant differences were observed between seasons (Table 3). Fruit firmness at harvest and post-harvest was significantly lower in 2009 than in 2010 (Figure 7). There are likely pre-harvest factors other than fruit Ca content that affect the physiological and biochemical changes that take place during storage. Some research has found that the regulation of water transpiration from ripe fruits may represent an important strategy to prolong fruit firmness (Vicente et al., 2007). Neither pre-harvest nor post-harvest treatments had an effect on retention of fruit firmness during storage (Table 3). Casero et al. (2010) did not either retain the firmness of apples during storage when applying at pre-harvest similar amounts of Ca to the amounts applied in the present study. The $CaCl_2$ concentration application rate and dipping duration may play an important role in the post-harvest dips. Ibadullah et al. (2016) found that the higher these parameters, the firmer the apples were. Besides, they observed a significant effect of dips on other quality parameters, such as ascorbic acid or weight loss. According to the authors, the retention of firmness can be attributed to the formation of Ca pectate, which leads to increased rigidity of the cell wall and improved turgor pressure. In the present study, the Ca concentration and the dipping duration were equal and lower, respectively, than the lowest rates using by Ibadullah et al. (2016). Our results showed that dips into a 3.5% $CaCl_2$ solution for a period of 30 seconds is enough to significantly reduce the bitter pit incidence in seasons with high incidence, but with no effect on fruit firmness. It is therefore important for future research to investigate how dipping duration can affect fruit quality parameters during post-harvest storage of fruits.

4. CONCLUSIONS

Although the literature concerning calcium application is extensive, there are very few works dealing with the combination of different techniques for applying Ca to reduce bitter pit and increase fruit Ca content, and no previous studies have combined the three main techniques of root applications, sprays and dips at post-harvest. In this work, these three practices were tested during four consecutive years. Year had more influence on tested parameters than Ca application. This finding highlights the need to increase our knowledge of the effects of environmental factors on the triggering of bitter pit, the maturation and development of fruits. However, Ca applications had a significant effect on bitter pit and on Ca concentration in fruit. When the different techniques were compared globally, Ca dips at post-harvest decreased bitter pit to a greater extent than pre-harvest treatments (sprays or root applications). No interaction was observed between the pre- and post-harvest treatments. Regarding the pre-harvest treatments, sprays decreased bitter pit and increased Ca content in fruit to a greater extent than root applications. Combining root applications and sprays did not provide an improvement with respect to sprays alone. Increasing the number of sprays or applying closer to harvest resulted in an improvement in bitter pit control, but they did not lead to an increase in fruit Ca content in fruit with respect to earlier sprays. Neither pre-harvest treatments nor post-harvest treatments had an effect on fruit quality parameters. From our findings, we concluded that increasing the number of sprays that occurred closer to harvest can improve bitter pit control and that their combination with dips at post-harvest results in the most efficient way to reduce the bitter pit incidence.

ACKNOWLEDGEMENTS

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FIGURES AND TABLES

Table 1. *P*-values from two-way ANOVA of factors season and pre-harvest Ca applications, and its interaction, on bitter pit incidence and severity and on the parameters analysed at harvest (Ca concentration in fruit, number of fruit per tree, kg tree^{-1}, fruit weight, starch index, acidity sugar content and fruit firmness).

NS indicates *P*-value > 0.1.

Factor	Season	Pre-harvest treatment	Season × Pre-harvest treat.
Bitter pit incidence	<.0001	0.0651	NS
Bitter pit severity	<.0001	0.0156	NS
Ca concentration	<.0001	0.0624	NS
No. fruits per tree	<.0001	NS	NS
Fruit yield	<.0001	NS	NS
Fruit weight	<.0001	NS	NS
Starch index	<.0001	NS	NS
Acidity	<.0001	NS	NS
Sugar content	<.0001	NS	NS
Firmness harvest	<.0001	NS	NS
Firmness post-harvest	<.0001	NS	NS

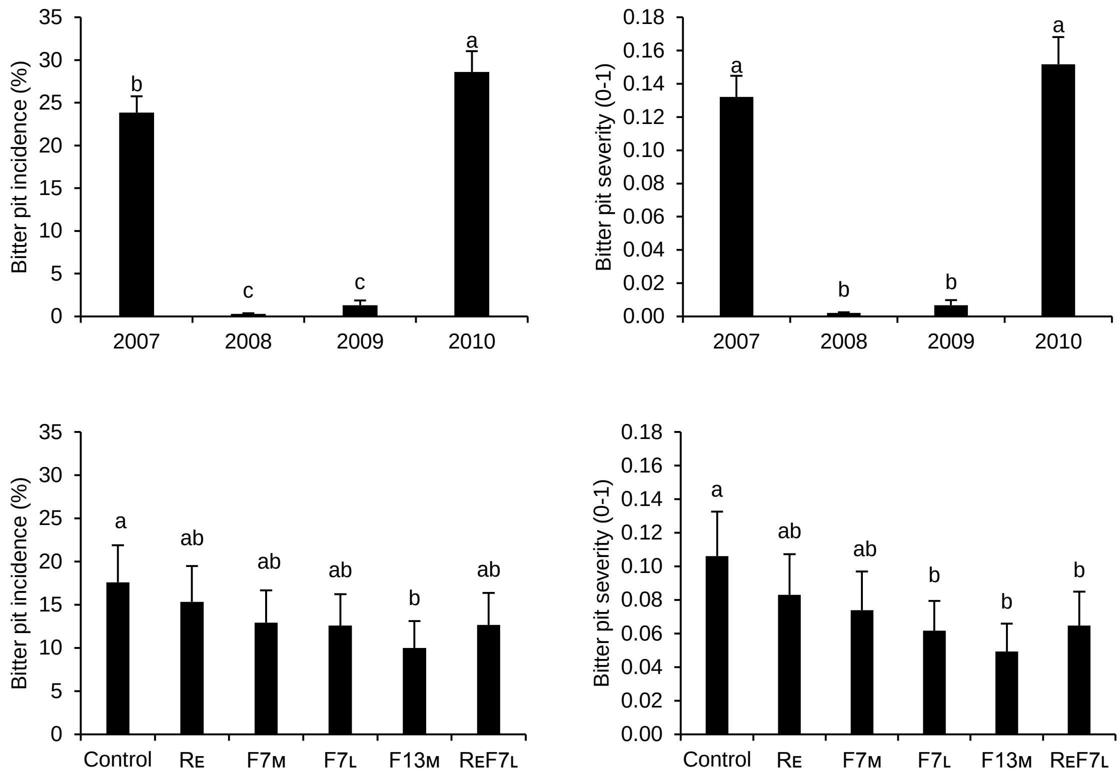

Figure 1. Mean value of bitter pit incidence (left) and relative index of severity (right) for each season (above) and pre-harvest treatment (below).

Columns not sharing the same letter indicate significant differences according to the Duncan test ($P = 0.05$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

Table 2. *P*-values of the contrasts to compare specific groups from pre-harvest treatments on parameters bitter pit incidence, bitter pit severity and Ca concentration in fruit (parameters with significant effect according the two-away ANOVA).

NS indicates the *P*-value was greater than 0.1.

Contrasts	Bitter pit incidence	Bitter pit severity	Ca content in fruit
Control vs. Ca-treatments (R_E, $F7_M$, $F7_L$, $F13_M$ and R_EF7_L)	0.0138	0.0022	0.0097
Root (R_E) vs. sprays ($F7_M$, $F7_L$ and $F13_M$)	0.0909	0.1068	0.0977
Mid ($F7_M$) vs. late-season ($F7_L$)	NS	NS	NS
13 ($F13_M$) vs. 7 Ca sprays ($F7_M$ and $F7_L$)	NS	NS	NS
Combination (R_EF7_L) vs. separately (R_E and $F7_L$)	NS	NS	NS

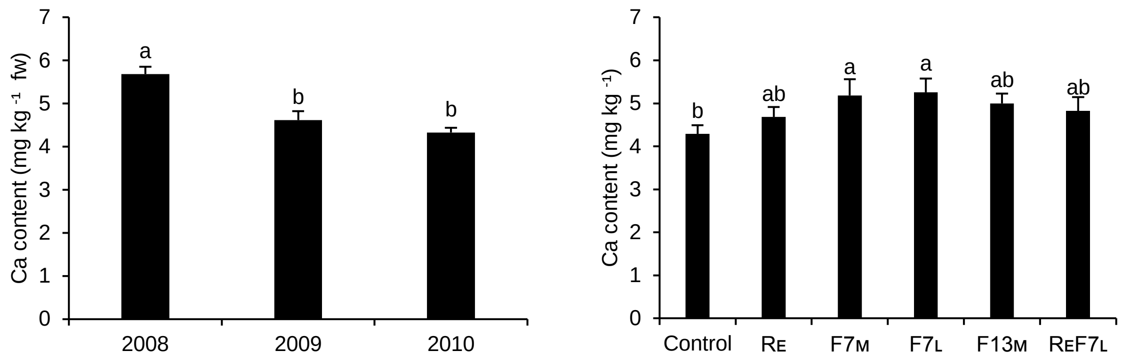

Figure 2. Mean value of calcium content in fruit for each season (left) and pre-harvest treatment (right).

Columns not sharing the same letter indicate significant differences according to the Duncan test ($P = 0.05$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

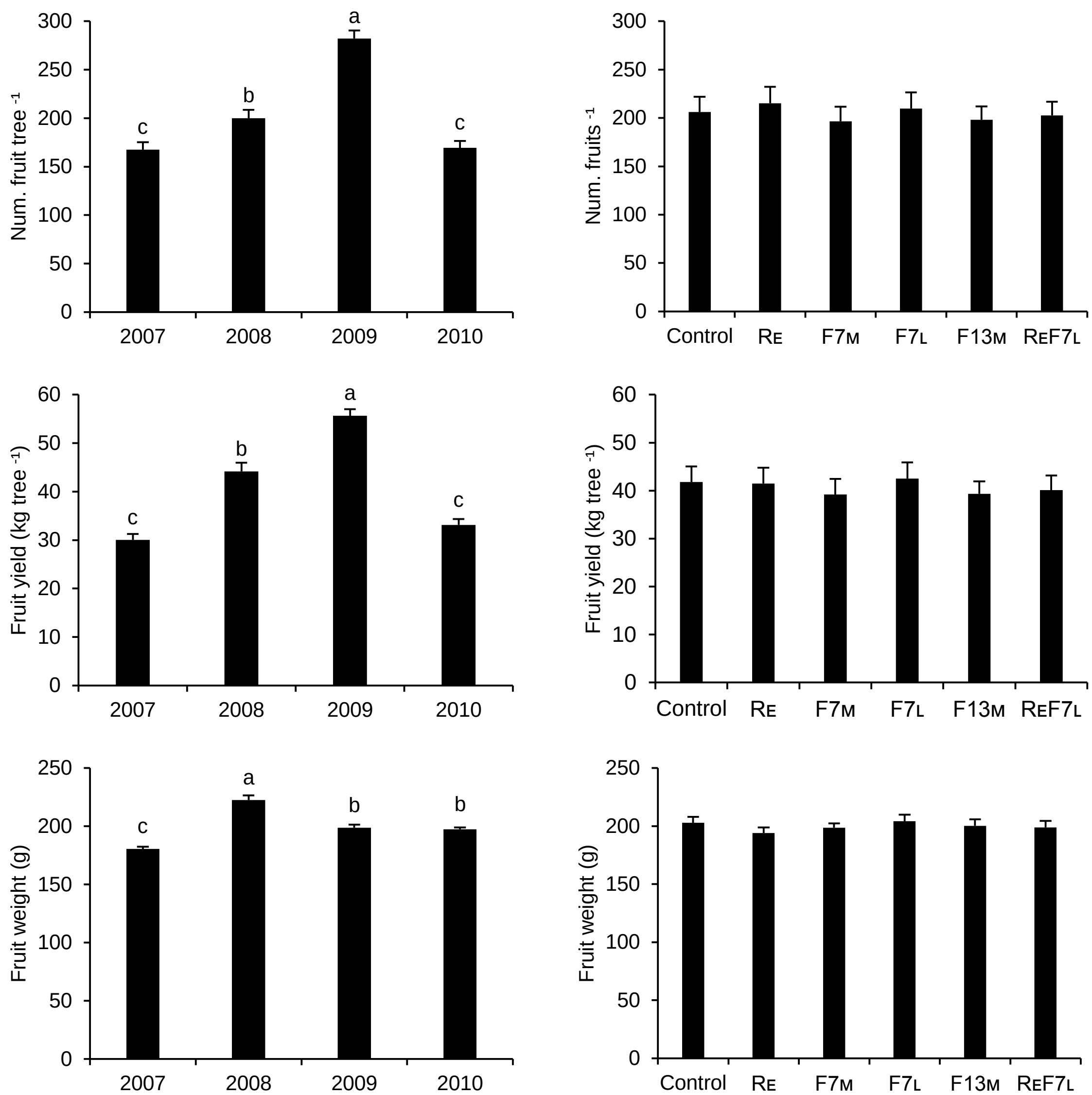

Figure 3. Mean value of number of fruit per tree (above), fruit yield (center) and fruit weight (below), for each season (left) and pre-harvest treatment (right).

Columns not sharing the same letter indicate significant differences according to the Duncan test ($P = 0.05$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

Table 3. *P*-values from three-way ANOVA of factors season, pre-harvest and post-harvest treatment, and its interaction, on bitter pit incidence and severity and fruit firmness at post-harvest.

NS indicates the *P*-value > 0.1.

Factor	Bitter pit incidence	Bitter pit severity	Firmness post-harvest
Season	<.0001	<.0001	<.0001
Pre-harvest	0.0737	0.0337	NS
Season × Pre-harvest	NS	NS	NS
Post-harvest	<.0001	<.0001	NS
Season × Post-harvest	<.0001	<.0001	NS
Pre- × Post-harvest	NS	NS	NS
Season × Pre- × Post-harvest	NS	NS	NS

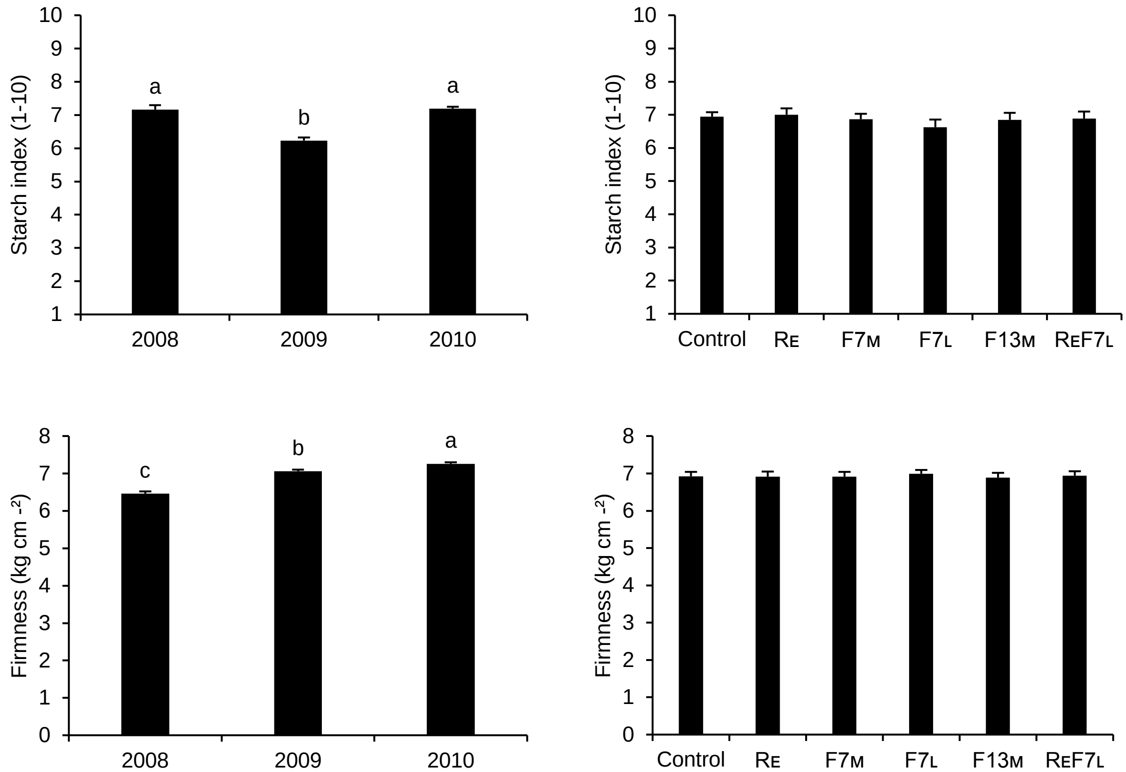

Figure 4. Mean value of number of starch index (above) and fruit firmness (below), for each season (left) and pre-harvest treatment (right).

Columns not sharing the same letter indicate significant differences according to the Duncan test ($P = 0.05$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

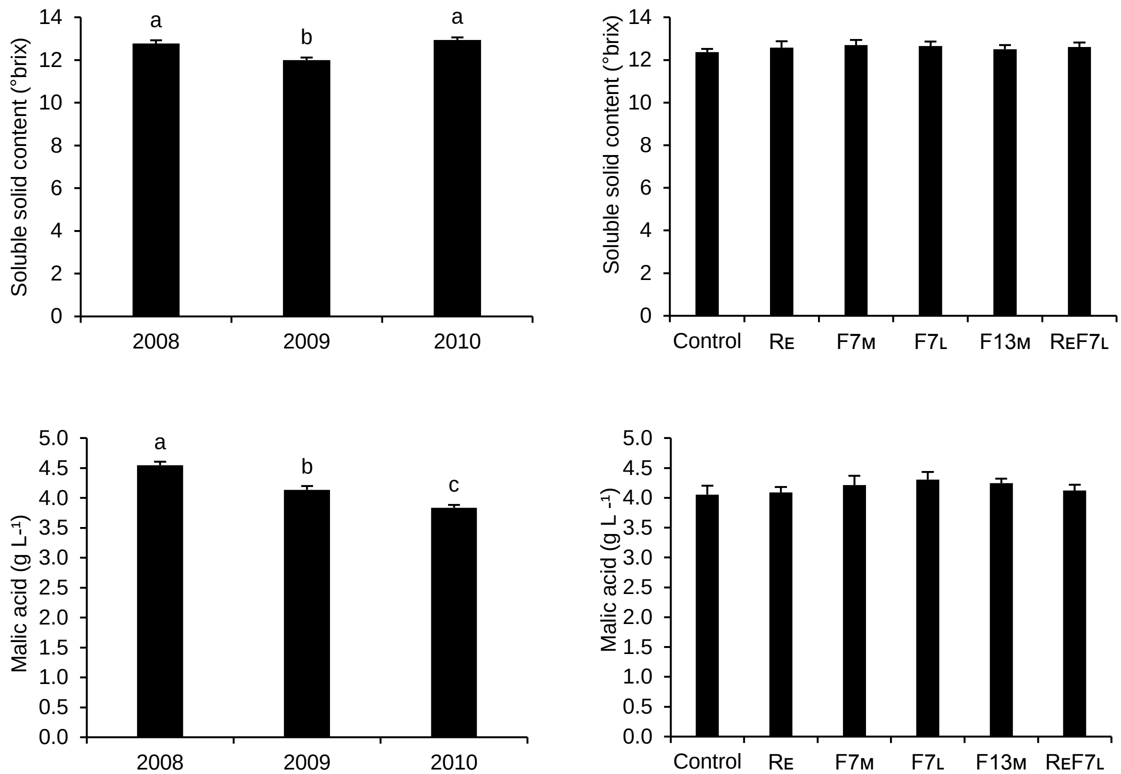

Figure 5. Mean value of number of soluble solid (above) and malic acid (below) content in fruit, for each season (left) and pre-harvest treatment (right).

Columns not sharing the same letter indicate significant differences according to the Duncan test ($P = 0.05$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

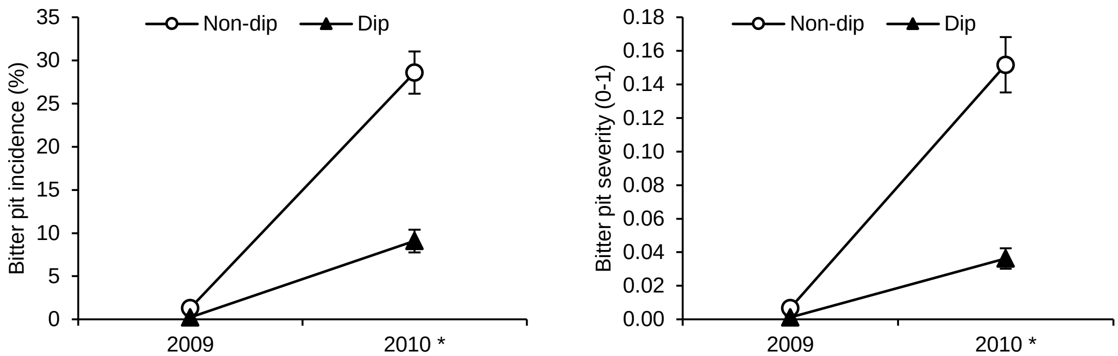

Figure 6. Interaction effect of season × post-harvest treatments on bitter pit incidence (left) and relative severity index (right).

The asterisk denotes a significant difference between the two treatments (ANOVA, $P < 0.001$). The error bars represent the standard error of the mean. Error bars indicate the standard error of the mean.

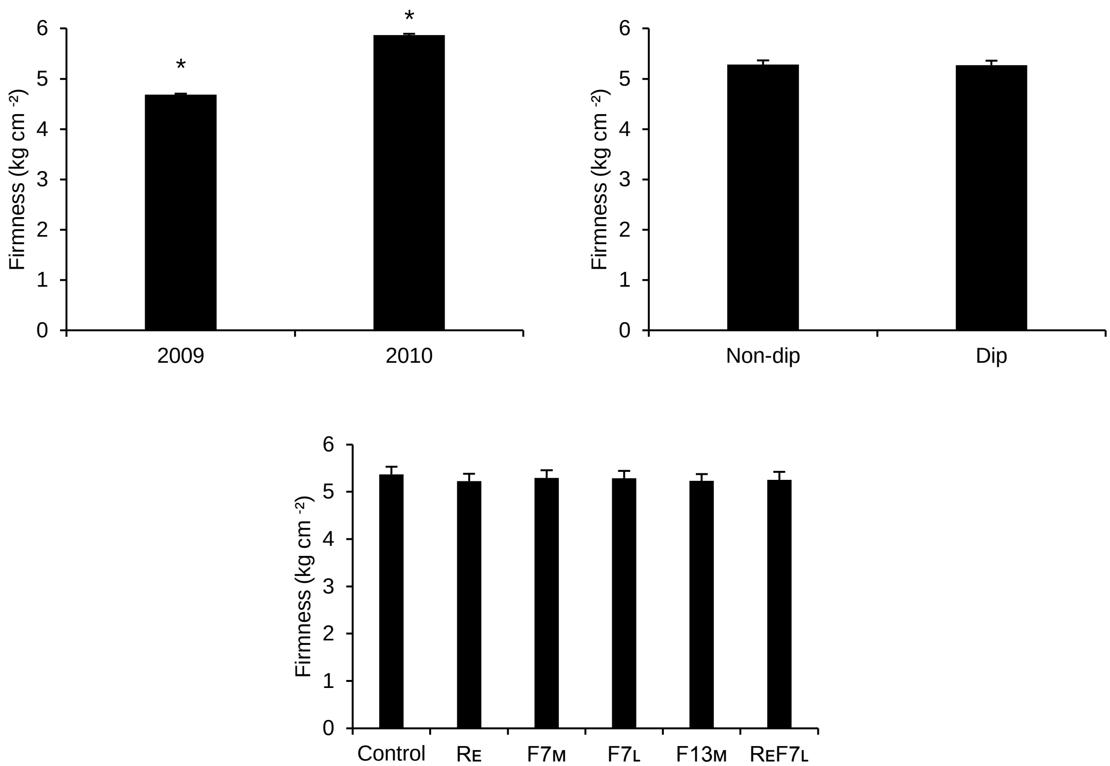

Figure 7. Mean value of fruit firmness at post-harvest for each season (above), pre-harvest treatments (centre) and pre-harvest treatments (below).

The asterisk denotes a significant difference between the two seasons (ANOVA, $P < 0.001$). Error bars indicate the standard error of the mean.

R_E: early-season root applications; $F7_M$: 7 Ca sprays from mid-season (every 12-14 days starting 30 dafb); $F7_L$: 7 Ca sprays during late season (every 5-10 days starting 60 dafb); $F13_M$: 13 Ca sprays from mid-season (every 5-10 days starting 30 dafb); R_EF7_L: combination of both R_E and $F7_L$ treatments.

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