Scientia Horticulturae 217 (2017) 179–188

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Combination of strategies to supply calcium and reduce bitter pit in 'Golden Delicious' apples

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form 11 January 2017
Accepted 13 January 2017
Available online 5 February 2017

Keywords:
Apple
Calcium disorders
Calcium supply
Sprays
Fertigation
Post-harvest dips

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.

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 effective-

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http://dx.doi.org/10.1016/j.scienta.2017.01.028
0304-4238/

ness 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 researches 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 °C, 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 °C 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 s 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 °C. After 90 days in cold storage, all samples were transferred to room temperature (20–23 °C) 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