Research Article

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Calcium applications throughout fruit development enhance olive quality, oil yield, and antioxidant compounds' content

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Abstract

BACKGROUND: Calcium is a preservative and firming agent largely used in the table olive industry. Foliar applications of calcium (as calcium chloride, $CaCl_2$) before harvest have been proposed in other fruits to increase firmness and reduce physiological disorders or internal damage. However, there is still a shortage of information regarding the source, the concentration, the number, and the period of calcium application onto the canopy to get an effective response of olive quality. In this study, we aimed to investigate the effect of two concentrations of $CaCl_2$ foliar treatments (0.5% and 1.0%), applied at different stages of fruit development (at the end of fruit set, end of pit hardening, and prior to harvesting), on olive quality for two varieties ('Manzanilla de Sevilla' and 'Ascolanta tenera'), cultivated in two different geographical areas (Spain and Italy respectively).

RESULTS: The calcium concentrations applied enhanced the fruit calcium content and decreased sodium and potassium. They also improved the mechanical properties without modifying fruit morphology or cuticle thickness; nor did they cause phytotoxicity. Foliar treatments increased the oil content in the pulp (dry weight basis) and the amount of hydroxytyrosol, tyrosol, and oleuropein, among other phenols.

CONCLUSION: Calcium foliar applications during fruit development effectively increase olive quality.

Keywords: 'Ascolana tenera'; 'Manzanilla de Sevilla'; firmness; fruit damage; phenolic compounds

INTRODUCTION

Calcium is a key plant nutrient that may significantly impact fruit development. It preserves the integrity and stability of the fruit cytoplasmic membranes, increasing the resistance of the cell wall by creating bonds with the pectin of the median lamella.[1] Physiological processes associated with ripening-related changes, fruit softening, browning reactions, and pathogenic disorders have been related to calcium concentrations.[2,3]

Postharvest treatments, such as dipping and impregnation in calcium salts, have been demonstrated to enhance the content of this nutrient and the fruit firmness, as well as extend the shelf life of many fruits.[4] Preharvest calcium sprays may also provide an extra supply of the mineral to prevent deficiencies,[3] improving fruit quality and its resistance to fungal disorders, posing an interesting alternative for integrated production systems.[5] Foliar applications are usually recommended to overcome temporary calcium deficiencies, mainly in the fruits. Calcium is a phloem immobile nutrient and its transport is via the xylem, so that the concentration in fruits depends on the transpiration rate and the vascular efficiency of the xylem. As the transpiration rate usually gets the highest values at fruit set and decreases significantly after that, the fruit calcium concentration also decreases by the mid-growth stage, in many cases to deficiency values.[6] Calcium absorption, transport, and storage in the fruit, as well as the impact on fruit quality at harvest and postharvest, are still poorly understood.[2]

The calcium sources, concentration, and number of applications in orchards are pivotal factors to increase its presence in the fruit or to avoid phytotoxicity, and they must be optimized for each species and cultivar.[3] Calcium chloride ($CaCl_2$) is currently used as a preservative and firming agent in many fruit industries.[4] $CaCl_2$ spraying during fruit development has also been demonstrated to

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be effective to enhance fruit quality by increasing firmness and decreasing postharvest decay in peach,[5] reducing fruit cracking in cherry,[7] and lowering the incidence of bitter pit and internal breakdown in apple[8] or the internal browning in peach.[9] Increases in antioxidant compounds, such as phenols, have also been reported.[10]

Calcium has been related to salinity tolerance,[11] cold acclimation,[12] and pathogen susceptibility[13] in olive. Different studies emphasized the potential of using $CaCl_2$ in the table olive industry to preserve black ripe olives in salt-free solution, delaying the softening during all the processing stages,[14] or for the production of 'Spanish-style' green table olives, promoting higher firmness and lower sodium content.[15] Nevertheless, the impact of $CaCl_2$ preharvest applications on olive quality has been scarcely studied. To our knowledge, only one paper[16] is available about this topic, reporting that fruits of the 'Konservolia' variety harvested at the black-ripe stage showed an increase of pulp firmness after three applications of $CaCl_2$ at 0.65% (w/v) over 30 days before harvest. This lack of literature may be partly due to the fact that calcium deficiency is not very common in olive,[17] but also to the difficulty in establishing the appropriate dates for the application onto the canopy to get an effective response of the fruit quality.

Our hypothesis is that foliar applications of calcium during fruit development can enhance the content of this macronutrient in olive fruit pulp, improving its quality by increasing the firmness. In this sense, the objective of this study was to analyse the impact of two $CaCl_2$ concentrations on the olive quality when applied onto the canopy at three important stages of fruit development, such as the end of fruit set, the end of pit hardening, and prior to harvesting. Leaf and fruit calcium levels, the interactions with sodium, potassium, and magnesium, and the possible changes in fruit quality, including firmness, cuticle and epidermal anatomy, mechanical damage, oil content, and phenol composition were investigated.

MATERIAL AND METHODS

Plant material

The study was carried out in 2017 at two locations, Morón de la Frontera (Spain) and Montalto delle Marche (Italy), with two different olive cultivars, 'Manzanilla de Sevilla' and 'Ascolana tenera' respectively. Both cultivars are currently destined for table olive or oil extraction industry.

The 'Manzanilla de Sevilla' drip-irrigated orchard (latitude 37° 09′ 39″ N; 5° 27′ 29″ W; altitude 163 m asl) was planted in 2011 with a tree density of 408 ha^{-1} (7.0 m × 3.5 m). The soil had a clay-loamy texture and a basic pH. Trees were trained to a vase system, and fertilizers were applied mainly by fertigation under non-limiting conditions. Full bloom and harvest occurred on 2 May and 15 September respectively. The 'Ascolana tenera' drip-irrigated orchard (latitude 42° 58′ 57″ N; longitude 13° 38′ 21″ E; altitude 310 m asl) was planted in 2009 with a tree density of 200 ha^{-1} (7.0 m × 7.0 m). The soil had a clay texture and a basic pH. Trees were trained to a vase system, and organic fertilizers were distributed on the soil at the end of winter according to the estimation of nutrients removal. Periodic shredding was applied to maintain a permanent and natural green cover. Full bloom and harvest occurred on 30 May and 14 September respectively. Both cultivars were harvested at the green-ripe stage. The climate is Mediterranean in both orchards. Yearly mean temperature and total rainfall were 19.1 °C and 460 mm respectively in the 'Manzanilla de Sevilla' orchard (CAP, Junta de Andalucía) and 13.4 °C and 820 mm respectively in the 'Ascolana tenera' orchard (Servizio Agrometeo ASSAM, Regione Marche). Total applied water was 1800 m^3 ha^{-1} from April to September and just 55 m^3 ha^{-1} from June to August.

Experimental design and treatments

The experimental design consisted of a randomized block with three replicates of five trees per each cultivar (total of 45 trees with similar volume and fruit load) surrounded by guard trees. Two calcium concentrations ($CaCl_2$ $2H_2O$, 99.0%; Labkem, Barcelona, Spain) were administered by foliar applications: 0.5% and 1.0% calcium. These were repeated three times during the fruit development. More precisely, 42 (end of fruit set) and 70 (end of pit hardening) days after full bloom (DAFB), and 2 weeks before harvesting (125 DAFB in 'Manzanilla de Sevilla' and 92 DAFB in 'Ascolana tenera'). Water was used as control (0%) and sprayed the same days calcium was applied. A commercial adjuvant (0.5% of Tween-20), at a concentration of 100 mL per 100 L was added to both calcium treatments and control on each application date. Foliar applications were carried out early in the morning to the point of runoff.

Two weeks after every calcium application, we determined the macronutrients content in both the leaves and fruits, as well as the oil content, phenol composition, mechanical properties of fruits, and the mean weight. At harvest, performed at the green-ripe stage, different fruit traits related to morphology, cuticle and epidermal cell anatomy, susceptibility to bruising, and respiration rate, were also measured. Leaves and fruits were randomly sampled around the canopy of the trees at 1.5 m height.

Leaf macronutrients analysis

The analysis were done in the laboratories of Scientific and Technological Research Center of Extremadura (CICYTEX). Fifty one-year-old leaves per block (10 per tree) were washed by immersing three times in distilled water, dried with filter paper, and crushed with a thermobeater. A 2 g aliquot was dried at 100 °C for 24 h and ashed in a muffle furnace at 550 °C (1° C min^{-1}). The ashes were dissolved in 20 mL of 4% nitric acid. The calcium, sodium, potassium, and magnesium contents were determined using a Perkin-Elmer 5300 DV inductively coupled plasma optical emission spectrometer at 317.933 nm, 766.490 nm, 285.213 nm, and 589.592 nm respectively in radial mode, with a concentric nebulizer and a cyclonic nebulization chamber. Results are expressed as milligrams per kilogram on a dry weight (DW) basis.

Pulp macronutrients analysis

Fifty healthy fruits per block (ten per tree) were carefully washed, by immersing three times in distilled water, and dried with filter paper. The olive pulp was removed from the pit with a knife and crushed with a thermobeater. A 2 g aliquot of the paste was taken for mineral analyses following the method described in the 'Leaf macronutrients analysis' section. Results are again expressed as milligrams per kilogram DW.

Moisture and oil content

The fruit moisture content was determined by crushing and weighing 10 g of olives per block after drying in an oven at 100 °C for 24 h until reaching a constant weight. For the oil content determination, the dry olives were extracted over 3 h in a Soxhlet apparatus using hexane as solvent. Results are expressed as grams per kilogram DW.

Determination of phenolic compounds

The phenolic profile determination was carried out in samples of 40 fruits per block (eight fruits per tree) with an Agilent 1100 (Hewlett-Packard, Waldbronn, Germany) high-performance liquid chromatography (HPLC) system following the method published by Cabrera-Bañegil *et al.*[18] '$\sum$ total phenols' was calculated as the sum of the individual phenols quantified by HPLC.

Mechanical properties of the fruits

Firmness was determined in samples of 40 fruits per block (eight fruits per tree) using a TA-TX2 texture analyser (Stable Micro Systems, Godalming, UK). Olive firmness was measured in two ways: (i) by a compression test, applying a force (newtons) through a 20 mm diameter compression probe to achieve a 6% deformation of the fruit diameter with a 100 mm aluminium plate; (ii) by a puncture test, measuring the maximum force needed to puncture the olive skin with a penetration of 2 mm in the centre of the fruit (expressed as breaking force in newtons).

Fruit morphology and cuticle characteristics

Maturity index and fruit morphology

The maturity index, based on the skin/pulp colouring level, was determined according to Ferreira[19] in a subsample of 100 fruits per block (20 fruits per tree). Later, the mean values of fresh weight (grams), volume by water displacement in a graduated cylinder (millilitres), and the pulp-to-pit ratio were measured (the mean pit weight was estimated after manual removal of the pulp) for the same sample. Equatorial and polar diameters (millimetres) were determined in 30 fruit samples, as well as the shape index as the polar/equatorial diameter ratio.

Mechanical damage

Mechanical damage was induced in samples of 50 uninjured fruits per block (ten per tree). They were dropped one by one from a height of 1 m, without applying energy, on a plastic box with chipboard inside, surrounded by cotton, according to the methods described by Jiménez *et al.*[20] The percentage of non-bruised fruits was determined 2 h later.

Cuticle and epidermis anatomy

The study of cuticle and epidermis anatomy was made as described by Jiménez *et al.*[20] in five uninjured fruits per block (one per tree) that were sampled only from the south-exposed portion of the canopy, and fixed in formalin:acetic acid:95% ethanol:distilled water (10:5:50:35 v/v/v/v) 2 h after harvest. The cuticle thickness (micrometres), cuticle area/cell (micrometres squared), and epidermal cell area (micrometres squared) were determined following the methodology described by Hammami and Rapoport[21] in three tissue portions per fruit of successive ten-cell groups, with a Nikon (Eclipse 80i DSRi1) binocular microscope and a Leica Qwin (Leica, Cambridge, UK) camera connected for image capture, then processed using Nis-Elements AR 3.2 image analysis software.

Fruit respiration intensity

The respiration rate (mL CO_2 kg^{-1} h^{-1}) was determined only for 'Manzanilla de Sevilla' induced-damage fruits. Measurements were made with a G100 portable gas analyser (Geotechnical Instruments Ltd, Leamington Spa, UK) at 23 °C in samples of 500 g per replicate that had been previously placed into 3.7 L glass jar hermetically sealed for 2 h.

Statistical analysis

Significant differences were established among treatments and homogeneous groups of means using a variance analysis. When the difference among the mean values was significant, a test comparison of means using the Tukey test (univariate analysis, $P < 0.05$) was performed. Data were processed using IBM SPSS software version 19 for Windows.

RESULTS

Leaf and fruit macronutrients

The average leaf calcium content showed an increase during fruit development (Fig. 1). Higher calcium contents were recorded in the leaf of calcium-treated trees for both varieties and on each sampling date, with the only exception being 'Ascolana tenera' on 56 DAFB. Leaf calcium content increased when applied Ca concentrations of 0.5% and 1.0% were compared 84 DAFB and at harvest: 2.5% and 9.9% respectively for 'Manzanilla de Sevilla' and 9.2% and 5.9% respectively for 'Ascolana tenera'.

A slight decrease of the average leaf sodium content was observed during fruit growth. Moreover, the nutrient content for both varieties was higher in the control between 84 DAFB and harvest. In contrast, when considering the average potassium content, a strong decreasing trend was registered in the leaves from 84 DAFB to harvest time, and no significant differences were recorded for the potassium content between the applied calcium concentrations and the control, except for 'Manzanilla de Sevilla' at 56 DAFB, where a significantly higher potassium content in the leaves was registered for 1.0% calcium foliar application. On the other hand, a trend was observed of an increasing average leaf magnesium content during fruit growth, whereas no differences were recorded between the applied treatments and the control for both varieties on each sampling date for potassium.

The average pulp calcium content showed an increase between 56 and 84 DAFB and a decrease between 84 DAFB and harvest time (Fig. 2). Moreover, the pulp showed a higher amount of calcium for calcium-treated trees on the three sampling dates, and significant differences between the applied calcium concentrations were observed for 'Manzanilla de Sevilla' at 56 DAFB and for both varieties at 84 DAFB and harvest.

The average pulp sodium content of both varieties decreased during fruit development. Conversely, the potassium content increased steeply between 56 and 84 DFAB for 'Manzanilla de Sevilla' and between 84 DAFB and harvest for 'Ascolana tenera' (Fig. 2). For both varieties, potassium was the most cumulative nutrient in the pulp at harvest, followed by sodium, calcium, and magnesium. Sodium and potassium contents in the pulp were significantly lower in calcium-treated trees than in control on 84 DAFB and at harvest. Significant differences were recorded for the different calcium concentrations applied only in the pulp calcium and potassium contents at harvest. Concerning magnesium, a slightly decreasing trend was observed for the content in the pulp during fruit growth for both varieties, whereas significant differences for the different calcium concentrations applied and the control were recorded only at harvest for both varieties.

Oil content and phenolic composition

Oil content in the pulp increased during fruit development, particularly 84 DAFB (Fig. 3). Both varieties showed that the oil content was always lower in the control, and no significant differences were found for the different calcium concentrations applied at harvest. The increase in the calcium-treated trees compared with

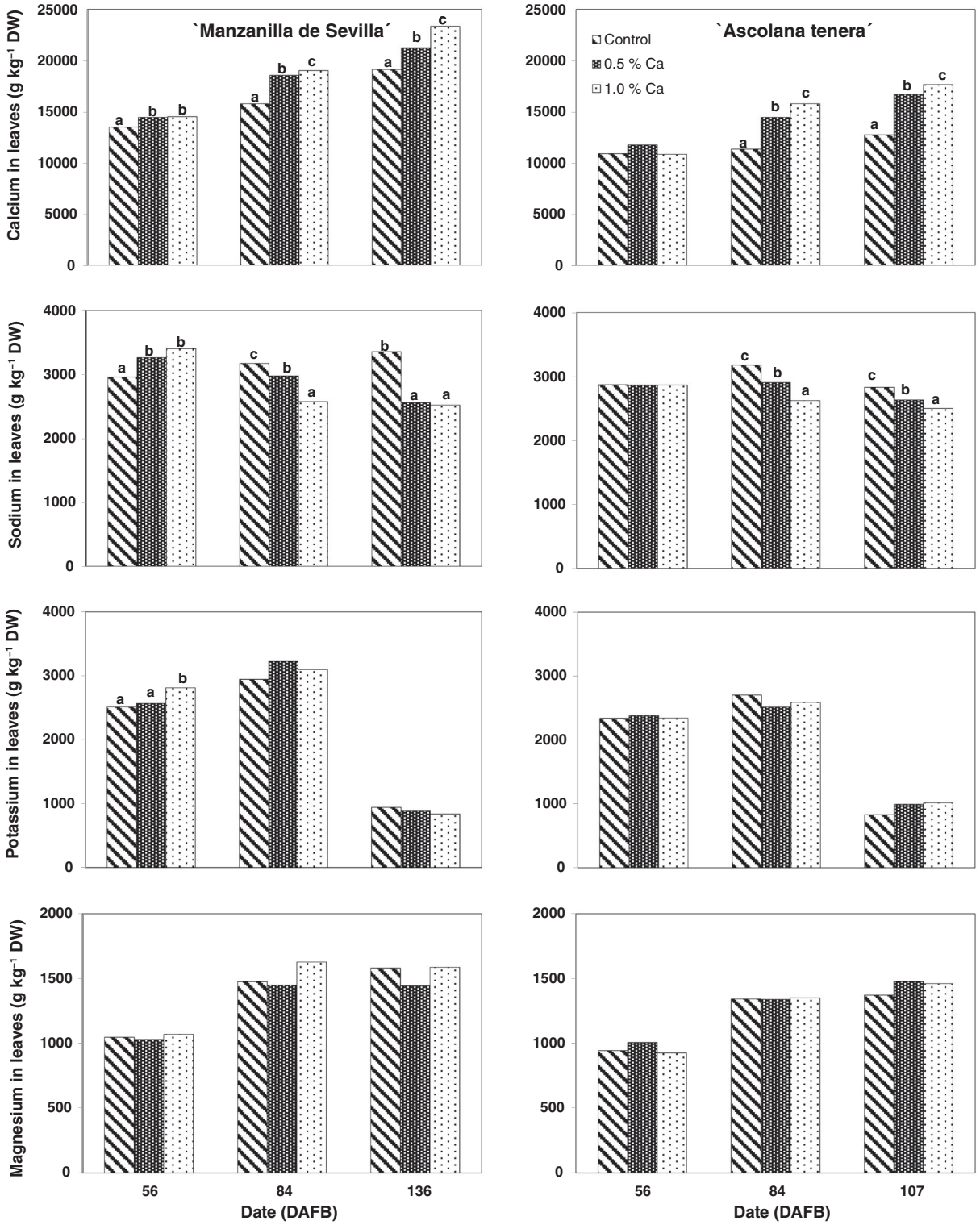

Figure 1. Leaf macronutrients after applying two calcium treatments during fruit development. Different lower-case letters indicate significant differences between treatments (Tukey's test, $P \leq 0.05$). DAFB, days after full bloom.

control was 2.8% for 'Manzanilla de Sevilla' and 1.2% for 'Ascolana tenera' on this late date.

The concentration of phenolic compounds decreased from 84 DAFB to harvest (Table 1). The most abundant phenols were hydroxytyrosol, oleuropein, verbascoside, and tyrosol. On each sampling date, the application of calcium increased the amount of the main phenols, compared with the control. At harvest, the amount of $\sum$ total phenols was 10% higher in the 1.0% calcium concentration than in the 0.5%; this result was due to the increase in content of some of the phenols analysed, such as hydroxytyrosol, oleuropein, and verbascoside.

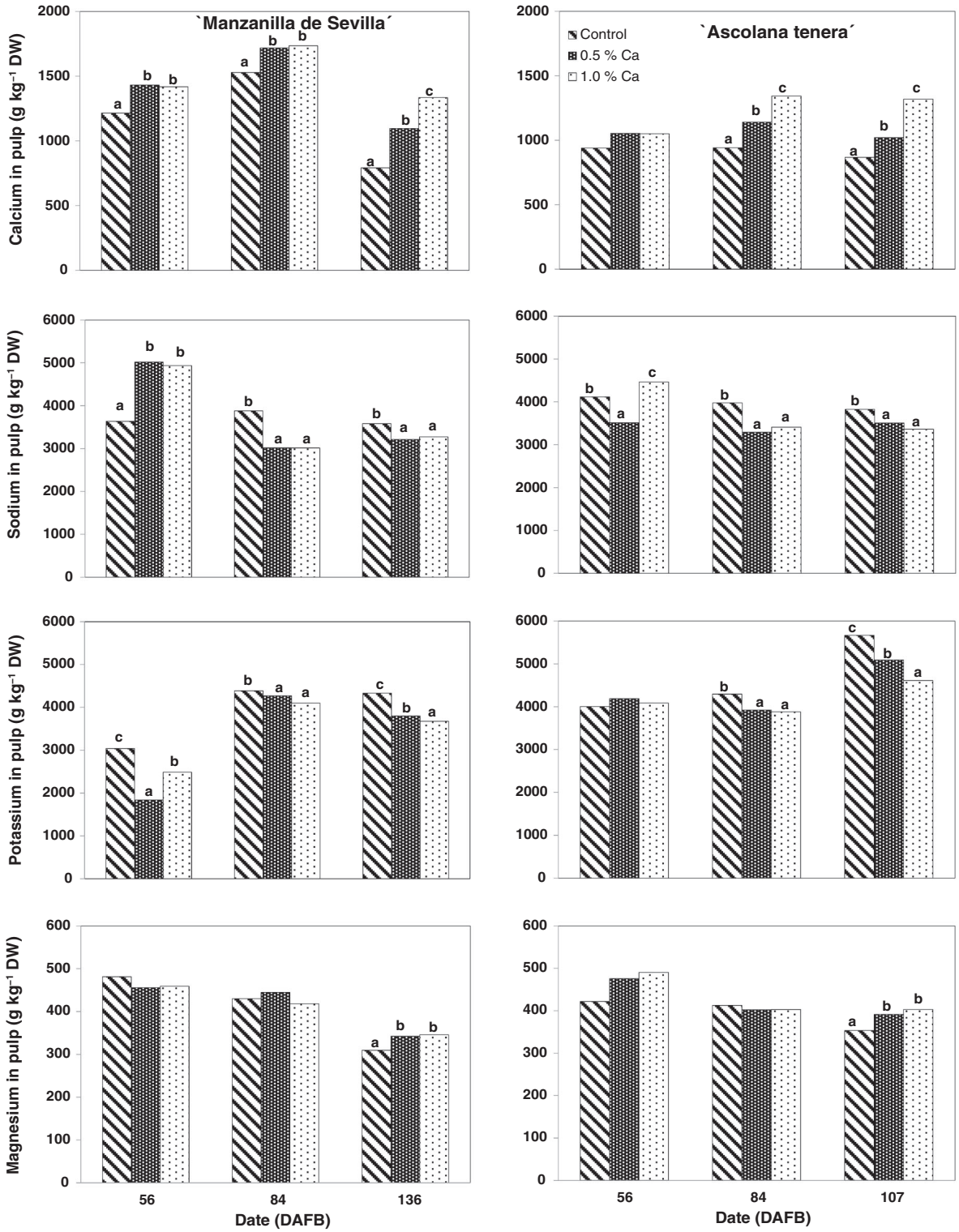

Figure 2. Pulp macronutrients after applying two calcium treatments during fruit development. Different lower-case letters indicate significant differences between treatments (Tukey's test, $P \leq 0.05$). DAFB, days after full bloom

Fruit firmness

The compression force decreased during fruit development for both varieties, in particular from 84 DAFB (Fig. 3). Calcium treatments increased the compression force compared with the control, with the highest values being found for the 1.0% calcium treatment. The skin breaking force decreased during the fruit development, although to a lesser extent in 'Ascolana tenera', and the highest values were also showed by the 'Manzanilla de Sevilla' fruits (Fig. 3). Starting from the first sampling date, the breaking force increased with the calcium concentration, except for the fruits of 'Manzanilla de Sevilla' at harvest, where no significant differences between calcium concentrations were found.

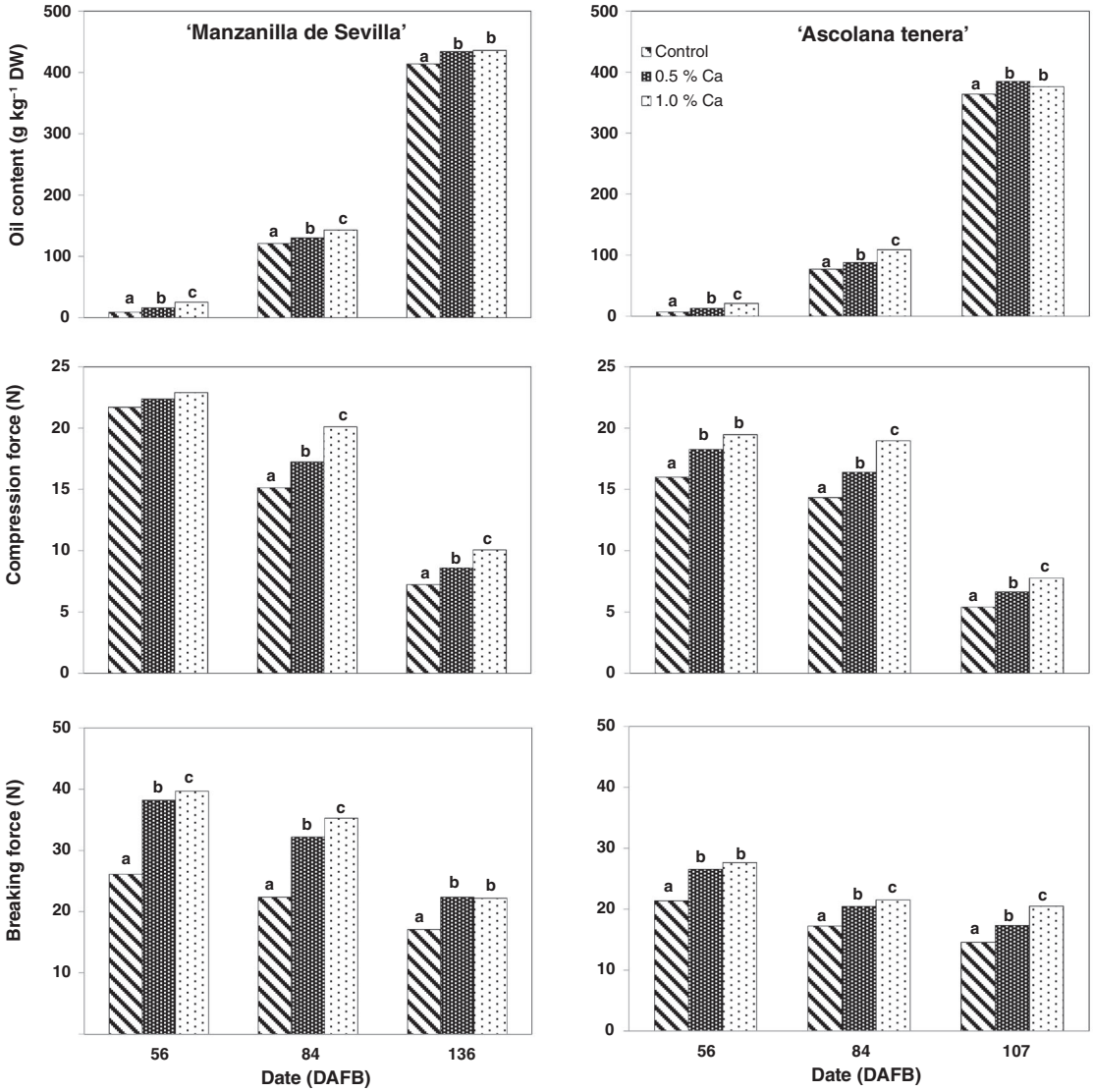

Figure 3. Physico-chemical parameters of the fruits after applying two calcium treatments during fruit development. Different lower-case letters indicate significant differences between treatments (Tukey's test, $P \leq 0.05$). DAFB, days after full bloom

Fruit morphology and cuticle characteristics

Fruit weight increased from 1 g to 3 g between 56 DAFB and harvest in 'Manzanilla de Sevilla' and from 3 g to 5 g in 'Ascolana tenera' (Table 2), with no differences between treatments on any date. At harvest, the calcium treatments did not affect the maturity index, the volume, or the fruit pulp-to-pit ratio; the mean values were rather similar for both varieties. Fruit shape was also similar, as well as the polar diameter, and a small but significant increase of the equatorial diameter was found with the application of 0.5 and 1.0% calcium concentrations.

The average values of all the traits studied regarding the cuticle and epidermal cells were higher in 'Ascolana tenera' (Table 2). In both varieties, the area of the cuticle per epidermal cell was lower in the control, but no significant differences between calcium treatments were found.

The respiration rate measured in 'Manzanilla de Sevilla' fruits (Table 2) decreased in calcium-treated trees. It was significantly lower for the 1.0% calcium concentration than for the control. On the contrary, the percentage of non-bruised fruits after the induced damage increased significantly in calcium-treated trees, from 1.0% to 4.5%. An increase of non-damaged fruits in the calcium treatments was also found in 'Ascolana tenera', but differences between treatments were not significant.

The water content in the fruits of 'Manzanilla de Sevilla' (Table 2) increased significantly with the calcium treatments, by 1%, compared with the control. In contrast, in 'Ascolana tenera' it decreased by 2.0% and 5.0% in the 0.5% and 1.0% calcium concentrations respectively.

DISCUSSION

Effect of calcium application on leaf and fruit macronutrients

Leaf calcium content increased during the experimental period, remaining above 1%, the threshold for sufficiency.[22] The leaf calcium accumulation (Fig. 1) registered in our study matches the notion that this nutrient is not re-translocated to other organs through the phloem transport. Calcium supplied via foliar

Table 1. Polyphenol content (mg kg^{-1}) in olive fruits. Different lower-case letters indicate significant differences between treatments (Tukey's test, $P \leq 0.05$)

		'Manzanilla de Sevilla'			'Ascolana tenera'		
Phenol	DAFB	Control	0.5% Ca	1.0% Ca	Control	0.5% Ca	1.0% Ca
Hydroxytyrosol	56	7142.6	7005.6	7255.4	9755.8	9871.2	9902.4
	84	3419.0 a	3960.3 b	3941.3 b	4055.1 a	4541.3 b	4964.2 c
	136/107	2017.5 a	2311.7 b	2904.0 c	2123.2 a	2229.4 b	2541.0 c
Tyrosol	56	643.5	754.2	764.2	821.6	864.5	827.9
	84	227.3 a	277.7 b	262.6 b	314.6 a	387.9 b	425.6 c
	136/107	151.1 a	183.1 b	196,6 b	175.7 a	194.5 b	258.0 c
Oleuropein	56	6755.1 a	7234.1 b	7864.0 c	7864.2 a	7900.6 a	8024.6 b
	84	2252.1 a	2674.3 b	3416.4 c	2655.9 a	3021.6 b	3297.4 c
	136/107	1514.0 a	1810.8 b	2655.8 c	1352.4 a	1505.0 b	1726.1 c
Verbascoside	56	1755.2	1800.9	1855.7	2514.3 a	2655.4 b	2814.7 c
	84	865.2 a	921.6 b	963.7 b	1262.3 a	1595.4 b	1864.7 c
	136/107	553.9 a	627.4 b	828.6 c	440.0 a	641.2 b	655.9 b
$\sum$ total phenols	56	20 428.2 a	20 877.8 a	22 242.5 b	27 322.0	28 102.6	28 424.4
	84	8440.1 a	9497.4 b	10 585.7 c	10 513.3 a	12 505.8 b	13 918.4 c
	136/107	5366.6 a	6132.9 b	7814.2 c	5133.2 a	6023.1 b	6814.2 c

DAFB, days after full bloom.

Table 2. Fruit morphology, cuticle and epidermis anatomy, bruising after induced damage, and respiration rate in olive fruits at harvest. Different lower-case letters indicate significant differences between treatments (Tukey's test, $P \leq 0.05$)

	'Manzanilla de Sevilla'			'Ascolana tenera'		
	Control	0.5% Ca	1.0% Ca	Control	0.5% Ca	1.0% Ca
Maturity index[a]	1.0	1.0	1.0	0.8	0.9	0.9
Fresh weight (g)	2.9	2.9	2.8	5.3	5.5	5.5
Volume (mL)	2.9	3.0	2.9	5.3	5.4	5.5
Pulp-to-pit ratio (fresh weight)	5.2	5.2	5.0	5.3	5.3	5.5
Polar diameter (mm)	18.6	18.9	19.1	23.4	23.4	23.6
Equatorial diameter (mm)	15.4 a	16.0 b	15.9 b	19.7 a	20.0 ab	20.3 b
Shape index	1.2	1.2	1.2	1.3	1.2	1.2
Cuticle						
Thickness (μm)	11.9	12.3	12.7	13.5	13.6	13.5
Area/cell (μm^2)	288.2	300.7	303.6	334.8	345.2	343.5
Epidermal cell area (μm^2)	320.7	327.5	319.8	386.3	392.2	383.9
Respiration intensity (mL CO_2 kg^{-1} h^{-1})	85.4 b	79.0 ab	69.5 a	—	—	—
Non-bruised fruits (%)	1.0 a	4.8 b	4.3 b	1.0	7.0	4.0
Water content (g/100 g)	60.8 a	62.0 b	62.0 b	62.8 c	61.1 b	57.6 a

[a] Yellowish-green colour of olive skin/pulp.

applications was absorbed by the leaves, and the accumulation at harvest was higher for the highest calcium concentration (1.0%) for both varieties. These results are in accordance with those obtained by Tsantili *et al.*[16] for 'Konservolia', even though no significant differences were reported in that study between the applied calcium concentration and the control. An opposite trend was observed for the sodium, which decreased with increasing applied calcium concentrations from 84 DAFB. Therefore, an antagonistic behaviour can be assumed for the leaf accumulation of this nutrient when compared with calcium. Potassium content in leaf strongly declined between 84 DAFB and harvest, confirming the results reported by Xiloyannis *et al.*[23] for kiwifruit, whereas the magnesium content of leaf increased during the experimental period, but its accumulation does not seem to be related to the applied calcium concentrations, in accordance with Alcaraz-López *et al.*[24] and in disagreement with Tsantili *et al.*[16]

The average calcium pulp concentration (Fig. 2) showed a decrease at harvest time for both varieties. The effect of calcium treatments was clear: it promoted a significant increase of this nutrient content starting from a concentration of 0.5%. In this sense, different studies found an accumulation of calcium in the pulp of grape during ripening and storage,[25] of peach,[5] and of olive[16] when pre-harvest calcium sprays were carried out. On the contrary, Xiloyannis *et al.*[23] observed that calcium content in

kiwifruit increased until 60 days after fruit set when fruit transpiration was high. Dichio *et al.*[26] proved that, 8–9 weeks after flowering, a sharp reduction in fruit transpiration due to xylem vessel dysfunction induced a decline of calcium accumulation in the kiwifruit. Our results showed that it is important to apply calcium throughout the entire fruit development, from fruit set to harvest, in order to allow accumulation in the pulp.

Regarding the other macronutrients, the sodium content of pulp decreased during fruit development for both varieties. Furthermore, the fruits treated with the highest calcium concentration showed less sodium content; thus, an antagonistic behaviour of these nutrients was observed. Nonetheless, these results are interesting for consumers with awareness about the benefits of a diet rich in calcium[4] and poor in sodium.[14] However, higher average amounts of potassium in the pulp were registered at harvest, confirming the fruit as a powerful sink for this element absorbed from the leaves during the final stage of fruit growth.[23] Nevertheless, potassium also appeared to have an antagonistic effect with calcium. The magnesium content did not appear to be influenced by calcium treatments. Tsantili *et al.*[16] found that calcium-treated fruits had similar magnesium levels during ripening, and Alcaraz-López *et al.*[24] observed that the magnesium content did not change in peach after sprays containing only calcium.

Effect of calcium application on fruit quality

Calcium application increased the oil content in both varieties studied (Fig. 3). This is an interesting result if the fruits are destined for oil extraction, since a slight oil increase implies a higher yield, which, in turn, translates into higher company commercial benefits. Therefore, the cost of calcium foliar applications would be perfectly viable and justified by the higher oil yield obtained from treated trees. Tsantili *et al.*[16] did not find any effect when applying calcium in 'Konservolia' olives; however, as mentioned earlier, they sprayed during the 30 days before black-ripe stage of maturity, not during the entire fruit development.

On the other hand, the consumption of table olives with their healthy fat is beneficial for human health because of the oil and antioxidants compounds.[27] The increase of oil content does not negatively affect the table olives' elaboration process, as long as the ripening of the fruits was in the very early stage (maturation index 0–1). If the ripening stage had been more advanced, the olives would break during the elaboration, releasing oil into the tank and provoking the formation of a protective oil film around the skin that would make the elaboration more difficult, both in the 'Spanish' and 'Californian' styles. It is also reported that both 'Manzanilla de Sevilla' and 'Ascolana tenera' are classified as low oil content varieties.[28,29]

The phenolic compounds also increased with the application of calcium. The highest contents were found with the highest calcium concentration. These results agree with a previous study[10] that reported significant changes in the antioxidant capacity or in the content of antioxidant compounds, such as phenols and ascorbic acid, in calcium-treated fruits compared with untreated ones. The increase in phenolic compounds may be due to a greater synthesis caused by calcium treatments,[25] or to an improved integrity of the cell tissues and compartmentation.[30] As mentioned herein, the increase in fruit firmness may have caused the phenols to remain compartmentalized in the cells, preventing contact with the polyphenol oxidase enzyme, thus preventing their degradation.[31]

In any case, the greater increase in olive phenol content implies that the consumption of the fruit, after the elaboration process, or the extracted oil, will maintain higher amounts of these compounds and will provide health benefits, thanks to the antioxidant properties of these compounds, among others. In fact, the European Food Safety Authority has indicated that the consumption of 5 mg day^{-1} of hydroxytyrosol contributes to the protection of human beings from oxidative damage.

Despite the differences in fruit cumulative calcium between varieties, which may depend on genetic factors and environmental conditions, the increase of this nutrient led to a higher firmness (Fig. 3). This result is particularly interesting for table olive production, as fruit firmness is an important quality attribute. It may decrease significantly at the green- or black-ripe processing stage, during immersion in a sodium hydroxide solution, and during the fermentation process, as these processes can cause olive softening, which reduces the quality and limits table olive commercialization.[14] On the other hand, the highest fruit firmness probably favoured a decrease in the level of mechanical damage, as suggested by the percentage of non-bruised fruits after induced damage, in particular in 'Manzanilla de Sevilla'. In this sense, foliar $CaCl_2$ applications could decrease fruit damage after mechanical harvesting and therefore increase table olive and oil quality.[32] Moreover, the susceptibility to pathogens and diseases could also decrease with the highest fruit firmness. Different studies have proved that calcium reduces the incidence of fungal infection by strengthening the fruit cell walls and increasing the resistance to degradation by fungal enzymes.[33] The chemical structures of the phenolic compounds also play an important role in the olive trees overall, protecting the fruit against pests and diseases.[34]

Our results show that the cuticle firmness increased, registering the highest values of breaking force in calcium-treated trees. This higher cuticle firmness was probably caused by changes in its composition rather than in its thickness (Table 2). Tsantili *et al.*[16] applied three $CaCl_2$ pre-harvest sprays with positive effects on firmness in 'Konservolia' olives. It is known that the application of calcium maintains cell turgor and tissue firmness.[3,5] Also, these researchers indicated that the increase of calcium concentrations in both peaches and olives makes the cell walls more rigid, reduces their flexibility, and provokes a delay of pulp softening, because it slows down the degradation of cell-wall polymers. In fact, calcium ions act by modifying the pectin solubilization, forming bridges that reinforce the selective permeability and integrity of the cell wall structure.[35]

Concerning fruit damage, the addition of calcium led to a decrease of bruising incidence (Table 2). This was particularly true for 'Manzanilla de Sevilla' fruits, and it could be related to the increased firmness, but also to the lower respiration intensity of the fruits from calcium-treated trees, in agreement with Segovia-Bravo *et al.*,[36] who showed that this trait is significantly higher in bruised fruits. In any case, the calcium treatments applied are not expected to drastically reduce the level of this damage after mechanical harvesting.

CONCLUSION

Foliar application of calcium ($CaCl_2$ formulation) at concentrations of 0.5 or 1.0% during fruit development (at the end of fruit set, at the end of pit hardening, and 2 weeks before harvesting) is able to enrich the pulp's calcium content and to enhance olive quality by increasing fruit firmness, oil content, and antioxidant compounds. These interesting results provide helpful information to improve field nutrition management in order to increase fruit

quality at harvest. Further studies are required to understand which stage of fruit development is more active in adsorbing and accumulating calcium, how allocation of this nutrient occurs in pulp, pits, and seed, how less susceptible to damage calcium-enriched fruits can be when mechanical harvest is performed, and how fruit characteristics are affected during processing.

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