

Texture and Other Quality Attributes in Olives and Leaf Characteristics after Preharvest Calcium Chloride Sprays

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Abstract. The effect of three preharvest sprays with water or 58.5 mM calcium chloride (CaCl_2) on texture and other quality attributes was investigated in ‘Konservolia’ olives harvested at the black-ripe stage and measured after 1 and 15 days of storage in air at 10 °C with 85% relative humidity. Effects on fruit calcium (Ca) and magnesium (Mg) concentrations during the period of spray applications were also studied. Concentrations of Ca, Mg, and chlorophyll and photosynthesis rate in leaves were measured on the day of final fruit harvest. No foliar or fruit toxicity was observed. In leaves, Ca sprays increased Mg but did not affect Ca and chlorophyll concentration and photosynthesis rate. In fruits, Ca sprays prevented the gradual decrease in Ca concentration that occurred in untreated fruits and maintained Mg at constant levels during ripening on the tree but did not affect fruit size and oil content as measured 1 day after storage. Ca-treated fruits were firmer with lower soluble pectin (SP) concentration and higher calcium pectate (CaP) than controls in both storage days, whereas the protopectin levels were similar in all fruits. Results showed positive linear correlation between Ca concentration and either firmness or CaP, whereas that between Ca and SP was negative as found in fruits stored for 1 day. Fruit production rates of carbon dioxide and ethylene, L* and chroma (C*) of peel, and hue angle (h°) of flesh were unaffected either by Ca treatment or storage time. Ca treatment did not affect fruit weight loss, the decreases in peel h° and flesh L*, but increased flesh C* during storage. The results indicate positive effects of preharvest calcium sprays on olive firmness without any negative effect on fruits or leaves.

Texture and color are the main quality attributes of black table olives (De Castro et al., 2007). Mafra et al. (2007) reported that the stage of ripening of olive fruit is determinant for a final product with adequate firm texture because decreases in firmness could be magnified after processing. Among the methods developed to maintain firmness during ripening and storage of fresh fruits were those that aim to increase fruit calcium (Ca) concentration by preharvest foliar and fruit sprays with Ca solutions (Ferguson and Watkins, 1989). Preharvest calcium chloride

(CaCl_2) treatments resulted in increased firmness in some fruit crops, like in apples (Recasens et al., 2004), cherries (Facteau et al., 1987), and peaches (Manganaris et al., 2005), although their effects on other fruit characteristics varied. However, to the best of our knowledge, there is no information regarding the impact of preharvest Ca applications on fresh olives.

The objective of this work was to investigate whether preharvest tree CaCl_2 sprays have any positive effect on olive firmness and also whether they affect other fruit or tree characteristics. The studied cultivar, Konservolia, is one of the most widespread table cultivars of olive trees in Greece and the fruits are used for the production of excellent-quality green table olives. However, black table ‘Konservolia’ olives are sold at relatively low prices or used for oil production, because they show low values of firmness in comparison with other black table olives of

the same size. Therefore, a better firmness maintenance of these olives would be significant.

Materials and Methods

Plant material. Self-rooted, 15-year-old olive trees (*Olea europaea* L. cv. Konservolia, Conservolia, or Amphissis) grown in Lamia (lat. 38°44′7″ N, long. 22°55′4″ W, altitude 7 m) were sprayed three times with 58.5 mM CaCl_2 (BDH Chemicals, Poole, UK) or with water (controls) up to the runoff point. The concentration of 58.5 mM (0.65% w/v) CaCl_2 was selected as equal or close to the highest used in other species with no toxic effects (Recasens et al., 2004; Tsantili et al., 2007) and after preliminary testing on ‘Konservolia’. The pH of the solutions was adjusted to 7.0 with 1 M NaOH. Calcium penetration depends mainly on natural peel openings and could vary according to the concentration and type of the surfactant used (Harker and Ferguson, 1991). Because there are no data concerning Ca sprays on olive trees, no surfactant was added in this work. Four trees were selected randomly for each treatment. Sprays were started 30 d before the final harvest and repeated at ≈10-d intervals. At the time of the first spray, nearly 55% of the fruits were at the black-ripe stage, whereas at the second and third sprays, this percentage increased to ≈70% and 80%, respectively. For each treatment concentration (0 or 58.5 mM CaCl_2), before each spray and at the final harvest on 24 Nov. 2004 (8 d after the third spray), 30 fruits per tree were collected to determine Ca and magnesium (Mg) concentrations. A few hours before the final fruit harvest, photosynthesis rate was measured on four single attached, mature leaves per tree. On the same day, ≈100 leaves per tree were selected for chlorophyll determination, Ca and Mg concentrations, and ≈350 olives per tree were collected for the rest of the fruit measurements. At this stage, all fruits were at the black-ripe climacteric stage. All fruits and leaves collected were macroscopically free of disorders and diseases. Sampling of fruits and leaves per tree, mixing samples from all four trees per Ca concentration on each sampling day, and sorting out samples in replicates were all carried out randomly. Fruits for storage were sorted out in groups of 10, placed in plastic pots, and stored in air at 10 °C with 85% relative humidity according to a completely randomized design. All measurements were carried out on four replicates. Fruit size and oil content were measured after 1-d storage. The rest of the measurements on stored fruits were carried out after 1- and 15-d storage and after temperature equilibration at 20 °C. Carbon dioxide (CO_2), ethylene (C_2H_4) production rates, and color were measured on the same replicates, whereas the rest of the measurements were each made on different replicates.

Determination of photosynthesis rate and chlorophyll concentration in leaves. Photosynthesis rate was measured by CO_2

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assimilation using a closed portable infrared gas (IRGA) analyzer (LI-6200; LI-COR, Lincoln, NE). The measurement was conducted between 9:00 AM and 11:00 AM when the air temperature was at 18 to 20 °C, the air CO₂ concentration at 370 to 380 µmol·L⁻¹ and the photosynthetically active radiation at 1800 to 2000 µmol·m⁻²·s⁻¹. The flow rate was 500 µmol·s⁻¹ and the rate of net photosynthesis was expressed in CO₂ as µmol·m⁻²·s⁻¹. Circular leaf disks of 5 mm diameter, one per leaf from a total of 14 leaves, were cut and used for chlorophyll extraction with 80% acetone in water (v/v). Chlorophyll determination was conducted according to Arnon (1949) using an ultraviolet-visible spectrophotometer (HeLios Gamma & Delta; Spectronic Unicam, Cambridge, UK) at 645 and 663 nm. Total chlorophyll (a + b) was expressed in g·m⁻² of leaf area.

Determination of calcium and magnesium in leaves and fruits. Ca and Mg determinations were made on leaves and fruits previously rinsed with 0.1 M HCl and then twice with distilled water. Leaves were dried at 70 °C for 3 d and fruits at 60 °C for 7 d. Powdered, dried samples of 0.5 g each were ashed at 500 °C for 4 h, digested with 5 mL of 65% HNO₃, filtered, and finally diluted in double-distilled water (v/v). Ca and Mg concentrations were determined by atomic absorption spectroscopy (SpectrAA 300; Varian Tecktron, Mulgrone, Victoria, Australia). Results were expressed in mg·g⁻¹ of dry weight.

Fruit dimensions, weight loss, firmness, and peel and flesh color. Fruit dimensions were measured using a digital caliper with accuracy of 0.03 mm. Fruit weight and weight loss (WL) during storage were determined using a balance with an accuracy of 0.01 g. Firmness values were recorded on two sides on the equatorial zone at 90° for each unpeeled fruit using a penetrometer (Chantillon DPP; J. Chatillon and Sons, New York, NY) with a scale of 0 to 1 kg (± 0.01) equipped with a conical probe (5 mm diameter × 5 mm height) at a descending speed of ≈50 mm·min⁻¹. Data are presented in Newtons (N). Color was measured by a chromatometer (CR-300; Minolta, Ahrensburg, Germany) and was expressed in L*, hue angle (h°), and chroma (C*). Three color measurements were made on each unpeeled fruit, two on opposite sides and one on the fruit top (Tsantili and Pontikis, 2004). Flesh color values were recorded on the other two opposite sides of fruits after peeling.

Fruit respiration and ethylene production rates. Both responses were determined on the same fruits at 20 °C according to Tsantili and Pontikis (2004). Respiration, as CO₂ production, was measured before C₂H₄ determination using a closed portable IRGA analyzer (LI-6200; LI-COR) connected to a 750-mL airtight jar. Each sample included 10 fruits and the flow rate was adjusted to 900 µmol·s⁻¹. The CO₂ production rates were expressed in µmol·kg⁻¹·h⁻¹. Ethylene production was measured after 2 h enclosure in 320-mL sealed jars. Ethylene was analyzed

by injection into a 120-cm × 0.2-cm i.d. column of 80 to 100 mesh activated alumina (Restek, Bellefonte, PE) in a gas chromatograph (Sigma 300; Perkin-Elmer, Norwalk, CT) equipped with a flame ionization detector. The detection limit was ≈10 nL·L⁻¹ and C₂H₄ production rates were expressed in nmol·kg⁻¹·h⁻¹.

Fruit oil content. The oil content, as percent of fresh weight, was determined by the Soxhlet method after 1-d storage. Fruits were dried at 60 °C for 7 d and then at 105 °C for 3 h. Three grams of dried sample were extracted with 50 mL petroleum ether for 6 h.

Fruit pectic fractionation and analysis. Pectic fractionation was carried out according to Gallardo-Guerrero et al. (2002) after some modifications. In particular, the alcohol-insoluble solids (AIS) were extracted by homogenizing 50 g of destoned olives in an Ultra-Turrax homogenizer (T 25; Kika Labortechnik, Saufen, Germany) with 60 mL of 70% ethanol in water (v/v) and the homogenate was centrifuged at 3500 g_n for 5 min. The extraction procedure was repeated three more times in succession followed by two extractions with 60 mL of acetone. The final residue (AIS) was dried at room temperature under N₂. The different pectic fractions were obtained from 200 mg dried AIS. In particular, soluble pectins (SP) were extracted with 20 mL of distilled water by stirring vigorously in a supersonic bath for 20 min and centrifuging at 27,000 g_n (Sorval Ultra Pro 80, New Town, CT) for 15 min. The SP extraction procedure was repeated four times totally. All four supernatants combined were used for SP determination, whereas the final pellet was used for the extraction of EDTA-soluble pectin fraction or calcium pectate (CaP). The CaP was extracted following the SP procedure, but using 0.1 M buffer Tris-HCl and 0.2% EDTA at pH 6.2. The protopectin (PP) was extracted once from the final pellet after CaP extraction with 20 mL of 0.05 N NaOH. The amount of each pectic fraction was assessed colorimetrically for its galacturonic acid (GA) concentration according to Blumenkrantz and Asboe-Hansen (1973).

Data analysis. The significance of treatment effects was assessed by applying one-way analysis of variance for all leaf characteristics, fruit dimensions, and fruit weight loss. Split plot analysis was used for the other fruit parameters with Ca treatment selected as the main plot and the storage days as the subplot

apart from fruit Ca and Mg concentrations in which the time of sprays was selected as the subplot. Correlation analyses were applied between parameters considered to be linked with fruit texture. To assess the significance of the correlations, the correlation coefficient *r* was determined from the best fit.

Results and Discussion

Leaf characteristics. No foliar toxicity was observed during the whole experiment. Calcium treatment increased only Mg concentrations (Table 1). However, all values found in this work were close to those measured in untreated 'Konsevolia' leaves by Loupassaki et al. (2002). Alcaraz-Lopez et al. (2004) found increases in leaf Ca but small and nonsignificant decreases in Mg in plum leaves after foliar sprays containing only Ca.

Calcium treatment had no effect on either chlorophyll concentration or on photosynthesis rate (Table 1). Similar values for both attributes have been found in untreated 1-year-old leaves of the same cultivar measured in November by Hagidimitriou and Pontikis (2005).

Fruit dimensions, oil content, weight loss, and peel and flesh color. There was no fruit toxicity observed during the whole experiment. Preharvest Ca sprays did not affect fruit weight (9.81 g on average), length and width (29.92 mm and 23.76 mm on average, respectively; *P* > 0.05 for the three attributes; data not shown) measured after 1-d storage. On cherries, preharvest Ca sprays at an early stage during fruit development may result in decreased fruit size that was probably the result of cell wall strengthening by Ca (Facteul et al., 1987).

Oil is a main constituent of olives and its content increases during ripening (Fernandez Diez, 1971). In this work, oil content did not change after Ca sprays (Table 2) and was close to the highest value for ripe 'Konsevolia' (Garrido Fernandez et al., 1997).

During storage, WL was not influenced by Ca treatment and averaged 6.55% (Table 2). Nanos et al. (2002) reported that WL was 3% to 5% for the same cultivar stored at 10 °C for 25 d, but storage was in humidified air flow.

Calcium treatment, as the main factor, did not affect any of the peel and flesh color parameters. During storage, peel color became darker resulting in a substantial decrease in h°, but L* and C* values were unaffected (Table

Table 1. Ca, Mg, chlorophyll concentrations, and CO₂ assimilation in leaves of olive trees untreated or treated with preharvest CaCl₂ sprays.

CaCl ₂ treatment (mM)	Leaf characteristics			
	Ca concn (mg·g ⁻¹ DW)	Mg concn (mg·g ⁻¹ DW)	Chlorophyll concn (g·m ⁻²)	CO ₂ assimilation (µmol·m ⁻² ·s ⁻¹)
0.0	12.17	1.75	1.21	7.61
58.5	14.31	2.10	1.21	7.85
SE _{Ca} (n = 4) ^z	1.22	0.06	0.007	0.52
<i>P</i> _{Ca} ^y	NS	≤0.01	NS	NS

^zSE_{Ca} (n=4) = SE of means.

^y*P*_{Ca} = Probability of the effect of CaCl₂ treatment.

Ca = calcium; Mg = magnesium; DW = dry weight; NS = nonsignificant.

Table 2. Weight loss, oil content, and peel and flesh color of olive fruit during storage from olive trees untreated or treated with preharvest CaCl₂ sprays.

CaCl ₂ treatment (mm)	Storage (d)	Attribute							
		Whole fruit		Peel			Flesh		
		Wt loss (%)	Oil content (%)	Color parameter			Color parameter		
				L*	h°	C*	L*	h°	C*
0.0	1	—	23.53	30.86	65.24	22.43	64.94	346.32	4.87
58.5	1	—	23.65	31.40	70.14	22.51	64.89	338.55	4.35
0.0	15	6.46	—	31.10	24.60	22.59	44.00	341.91	3.80
58.5	15	6.64	—	30.09	29.35	21.36	46.49	345.57	5.96
SE _{Ca} (n=4) ^z	—	0.22	0.40	0.30	3.73	0.81	2.06	5.62	0.38
SE _D (n=4) ^z	—	—	—	0.43	5.28	1.15	2.91	7.95	0.53
P _{Ca} ^y	—	NS	NS	NS	NS	NS	NS	NS	NS
P _D ^y	—	—	—	NS	≤0.001	NS	≤0.001	NS	NS
P _{Ca × D} ^y	—	—	—	NS	NS	NS	NS	NS	≤0.05

^zSE_{Ca} (n=4) = SE of means for CaCl₂ treatment; SE_D (n=4) = SE of means for days in storage.

^yP_{Ca} = probability of the effect of CaCl₂ treatment; P_D = probability of the effect of storage days; P_{Ca × D} = probability of interaction between CaCl₂ treatment and storage days.

h° = hue angle; C* = chroma; NS = nonsignificant.

2). Concerning the flesh color, L* value decreased, whereas h° and C* were unaffected by storage. However, after 15-d storage, controls had decreased and Ca-treated fruits had increased flesh C* as compared with 1-d stored fruits, but the effect was not considered negative. Most of the color changes during storage could be possibly ascribed to the ripening process and/or WL as observed in other cases (Tsantili et al., 2007).

Fruit respiration and ethylene production rates. The levels of CO₂ and ethylene production rates indicate that fruits were at the climacteric stage (Maxie et al., 1960; Tsantili and Pontikis, 2004) during storage (Table 3). Similar production rates of ethylene and relatively higher of CO₂ were found in untreated black-ripe 'Konservolia' olives stored at 20 °C for 1 d (Nanos et al., 2002). In this work, Ca sprays had no effect on either CO₂ or ethylene production rates during storage. These results disagree with many studies in which Ca decreased both responses (Recasens et al., 2004) because Ca delays ripening (Poovaiah et al., 1988). However, there were cases in which Ca did not affect respiration rates (Duque et al., 1999; Tsantili et al., 2007) or both responses (Manganaris et al., 2005). Moreover, the present data, although limited, indicated a Ca effect on increased firmness rather than a general delay of ripening.

Fruit calcium and magnesium concentrations. In fruit such as the apple, Ca concentrations usually decline during fruit development, whereas that of Mg may be constant or increase (Ferguson and Watkins, 1992). In this study, there was a gradual decrease in Ca concentration in untreated fruits during fruit development (Fig. 1A). The levels of Ca in Ca-treated olives showed a reduction after the first spray but then remained almost constant and higher than controls (Table 4). The difference in Ca concentration between controls and Ca-treated fruits increased gradually and started to be significant after the second spray. Increased Ca concentrations in peel and flesh have been observed in peaches (Manganaris et al., 2005) and plums (Alcaraz-Lopez et al., 2004) after preharvest Ca sprays.

In the present study, the initial Mg concentration in controls was higher than in Ca-treated (Fig. 1B). However, Mg concentration in controls decreased after the first spray and then remained almost stable (Table 4). Ca-treated olives had similar Mg levels during the whole experiment but consistently lower than controls. Correlation analysis between Ca and Mg in fruits during Ca treatment showed no relationship (Table 5). Alcaraz-Lopez et al. (2004) observed that Mg concentration did not change in plums after sprays containing only Ca.

Table 3. Ethylene and CO₂ production rates in olive fruit during storage from olive trees untreated or treated with preharvest CaCl₂ sprays.

CaCl ₂ treatment (mm)	Storage (d)	Attribute	
		Ethylene production (nmol·kg ⁻¹ ·h ⁻¹)	CO ₂ production (μmol·kg ⁻¹ ·h ⁻¹)
0.0	1	20.11	1.63
58.5	1	17.75	1.71
0.0	15	20.10	1.71
58.5	15	19.89	1.59
SE _{Ca} (n=4) ^z	—	2.48	0.21
SE _D (n=4) ^z	—	2.97	0.29
P _{Ca} ^y	—	NS	NS
P _D ^y	—	NS	NS
P _{Ca × D} ^y	—	NS	NS

^zSE_{Ca} (n=4) = SE of means for CaCl₂ treatment; SE_D (n=4) = SE of means for days in storage.

^yP_{Ca} = probability of the effect of CaCl₂ treatment; P_D = probability of the effect of storage days; P_{Ca × D} = probability of interaction between CaCl₂ treatment and storage days.

NS = nonsignificant.

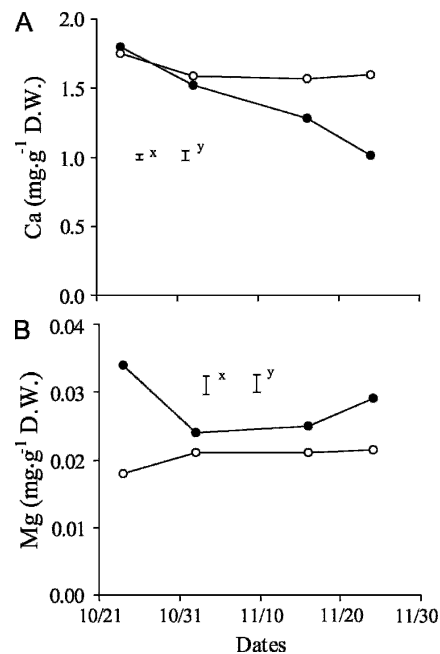


Fig. 1. Calcium (Ca) and magnesium (Mg) concentrations in olive fruits from olive trees untreated or treated with preharvest CaCl₂ sprays. Solid points, 0.0 mM CaCl₂; open points, 58.5 mM CaCl₂. (A) Ca concentration; (B) Mg concentration. Vertical bars are the averaged SE (n=4); x bar for concentration of CaCl₂ sprays and y bar for number of sprays.

Firmness and pectic fractions. Calcium treatment was effective on increased firmness in olives from the first measuring day (1-d storage) (Fig. 2A; Table 4). Positive linear correlation with high *r* (+0.979) was found between fruit Ca concentration and firmness (Table 5). The present results agree with studies on cherries in that the Ca effect was observed after 1-d storage (Tsantili et al., 2007), whereas on peaches it was after 15-d storage (Manganaris et al., 2005). In the present work, further changes in firmness during storage were not significant (Table 4) confirming that the positive Ca effect on increased firmness in olives remained after 15-d storage. The Ca-related firming effect is generally explained by the impact of Ca on complexation of cell wall and middle lamella polygalacturonate (Morris, 1980). Particularly in olive cell walls, Ferreira et al. (2006) suggested that oligogalacturonides are held by Ca and pectic polysaccharides occurred as Ca-bridged macromolecules.

Concerning the pectic fractions measured in this work, the concentrations of SP, CaP, and PP in controls after 1-d storage were 4.58 g·kg⁻¹ fresh weight, 1.18, and 1.16, respectively (Fig. 2B–D). In 'Gordal' olives, lower values of SP and CaP and higher of PP than in 'Konservolia' were found (Gallardo-Guerrero et al., 2002), but the cultivar was studied at the green stage. Decreases in firmness and losses in uronic acids in cell wall material were observed in 'Hojiblanca' olives during ripening (Jimenez et al., 2001).

All Ca-treated 'Konservolia' olives had reduced SP concentration and increased CaP

Table 4. Probabilities of the effects of number of sprays on olive Ca and Mg concentrations (Fig. 1 data) and of storage days on firmness and pectic fraction concentrations in olives from trees untreated or treated with preharvest CaCl_2 sprays (Fig. 2 data).

Probability (P)	Fruit Ca	Fruit Mg	Firmness (F)	Soluble pectin (SP)	Calcium pectate (CaP)	Protopectin (PP)
P_{Ca}^z	≤ 0.001	≤ 0.05	≤ 0.05	≤ 0.001	≤ 0.05	NS
P_S^z	≤ 0.001	≤ 0.001	—	—	—	—
$P_{Ca \times S}^z$	NS	≤ 0.001	—	—	—	—
P_D^z	—	—	NS	≤ 0.001	≤ 0.001	NS
$P_{Ca \times D}^z$	—	—	NS	≤ 0.05	NS	NS

$^zP_{Ca}$ = probability of the effect of CaCl_2 concentration; P_S = probability of the effect of number of sprays; $P_{Ca \times S}$ = probability of interaction between CaCl_2 concentration and number of sprays; P_D = probability of the effect of storage days; $P_{Ca \times D}$ = probability of interaction between CaCl_2 treatment and storage days. Ca = calcium; Mg = magnesium; NS = nonsignificant.

Table 5. Correlation analyses between attributes related to olive texture from olive trees untreated or treated with preharvest CaCl_2 sprays.

Correlated attributes	P^z	r^z
Fruit Ca^y	NS	—
Fruit Ca^x	≤ 0.001	+0.979
Fruit Ca^x	≤ 0.001	-0.958
Fruit Ca^x	≤ 0.05	+0.916
Fruit Ca^x	NS	—
Firmness (F) ^w	≤ 0.01	-0.629
Firmness (F) ^w	≤ 0.001	+0.749
Firmness (F) ^w	NS	—

zP = probability of correlation between the attributes; r = linear correlation coefficient.

y Data from all harvest days.

x Data after 1-d storage.

w Data after 1-d and 15-d storage.

Ca = calcium; Mg = magnesium; NS = nonsignificant.

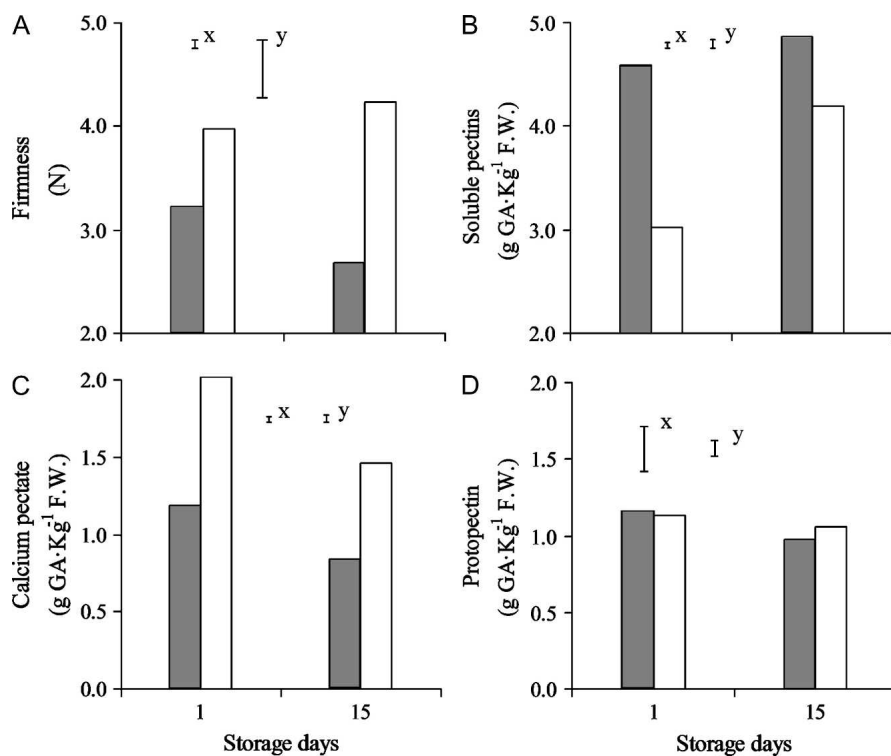


Fig. 2. Firmness and pectic fractions in olive fruits during storage from olive trees untreated or treated with preharvest CaCl_2 sprays. Checked columns, 0.0 mM CaCl_2 ; open columns, 58.5 mM CaCl_2 ; (A) fruit firmness; (B) soluble pectins; (C) calcium pectate; (D) protopectin. Vertical bars are the averaged SES ($n=4$), x bar for concentration of CaCl_2 treatment and y bar for storage days.

compared with controls, whereas Ca sprays did not affect PP either after 1-d or 15-d storage (Table 4). Storage time resulted in increases in SP in Ca-treated fruits and decreases in

CaP in all samples but did not affect the minor changes in PP. Prevention of increases in SP during storage after preharvest CaCl_2 sprays has also been found in peaches and

cherries (Manganaris et al., 2005; Tsantili et al., 2007).

Furthermore, as far as correlation between fruit Ca and firmness, correlation analyses between some fruit attributes that were likely to influence fruit texture directly or indirectly showed linear correlations (Table 5). The correlation coefficient (r) between fruit Ca and SP was -0.958 and that between Ca and CaP was $+0.916$, indicating that Ca prevented pectin solubilization. Firmness was positively correlated with CaP ($r = +0.749$) and negatively with SP ($r = -0.629$), indicating that at least part of the softening is related to pectin solubilization in 'Konservolia'. The increased cell wall-bound Ca with decreased cell wall degradation has been well demonstrated in Ca-treated apples, suggesting mobility of Ca to the cell wall (Chardonnet et al., 2003). A decrease in methyl esterification of olive pulp cell wall pectic polysaccharides during ripening (Mafra et al., 2001) could result in increased polymeric GA residues available for complexation with Ca (Ferreira et al., 2006). This is where Ca from Ca treatment could possibly bind to form Ca bridges and delay olive softening in the present work.

Conclusions

Three preharvest sprays with 58.5 mM CaCl_2 each seemed to be beneficial to fruit texture of 'Konservolia' olives harvested at the black-ripe stage without any harmful effect on fruits or leaves as assessed by some physiological parameters and under our particular experimental conditions. The main results were that Ca treatment prevented the decreases in Ca concentration and retained Mg levels in fruits during the spraying period, prevented softening and increases in SP concentration, and retained higher levels of CaP in fruits during storage. These results were obtained by a simple management tool (foliar and fruit application of Ca) that growers could readily adopt.

Literature Cited

- Alcaraz-Lopez, C., M. Botia, C.F. Alcaraz, and F. Riquelme. 2004. Effects of calcium-containing foliar sprays combined with titanium and algae extract on plum fruit quality. *J. Plant Nutr.* 27:713-729.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24:1-15.
- Blumenkrantz, N. and G. Asboe-Hansen. 1973. New method for quantitative determination of uronic acids. *Anal. Biochem.* 54:484-489.
- Chardonnet, C.O., C.S. Charron, C.E. Sams, and W.S. Conway. 2003. Chemical changes in the cortical tissue and cell walls of calcium-infiltrated 'Golden Delicious' apples during storage. *Postharvest Biol. Technol.* 28:97-111.
- De Castro, A., P. Garcia, C. Romero, M. Brenes, and A. Garrido. 2007. Industrial implementation of black-ripe olive storage under acid conditions. *J. Food Eng.* 80:1206-1212.
- Duque, P., M.G. Barreiro, and J.D. Arrabaca. 1999. Respiratory metabolism during cold storage of apple fruit. I. Sucrose metabolism and glycolysis. *Physiol. Plant.* 107:14-23.

- Facteau, T.J., K.E. Rowe, and N.E. Chestnut. 1987. Response of 'Bing' and 'Lambert' sweet cherry to preharvest calcium chloride applications. *HortScience* 22:271–273.
- Ferguson, I.B. and C.B. Watkins. 1989. Bitter pit in apple fruit. *Hort. Rev. (Amer. Soc. Hort. Sci.)* 11:289–355.
- Ferguson, I.B. and C.B. Watkins. 1992. Crop load affects mineral concentrations and incidence of bitter bit in 'Cox's Orange Pippin' apple fruit. *J. Amer. Soc. Hort. Sci.* 117:373–376.
- Fernandez Diez, M.J. 1971. The olive. p. 255–279. In: Hulme, A.C. (ed.). *The biochemistry of fruits and their products*. Academic Press Inc., London, UK.
- Ferreira, J.A., I. Mafrá, M.R. Soares, D.V. Evtuguin, and M.A. Coimbra. 2006. Dimeric calcium complexes of arabin-rich pectic polysaccharides from *Olea europaea* L. cell walls. *Carb. Polym.* 65:535–543.
- Gallardo-Guerrero, L., D. Hornero-Mendez, and M. Minguez-Mosquera. 2002. Pectins as possible source of the copper involved in the green staining alteration of cv. Gordal table olives. *J. Agr. Food Chem.* 50:6746–6751.
- Garrido Fernandez, A., M.J. Fernandez Diez, and M.R. Adams. 1997. *Table olives*. Chapman & Hall, London, UK.
- Hagidimitriou, M. and C. Pontikis. 2005. Seasonal changes in CO₂ assimilation in leaves of five major Greek olive cultivars. *Scientia Hort.* 104:11–24.
- Harker, F.R. and I.B. Ferguson. 1991. Effects of surfactants on calcium penetration of cuticles isolated from apple fruit. *Scientia Hort.* 46:225–233.
- Jimenez, A., R. Rodriguez, I. Fernandez-Caro, R. Guillen, J. Fernandez-Bolanos, and A. Heredia. 2001. Olive fruit cell wall: Degradation of pectic polysaccharides during ripening. *J. Agr. Food Chem.* 49:409–415.
- Loupassaki, M.H., K.S. Chartzoulakis, N.B. Digalaki, and I.I. Androulakis. 2002. Effects of salt stress on concentration of nitrogen, phosphorus, potassium, calcium, magnesium, and sodium in leaves, shoots, and roots of six olive cultivars. *J. Plant Nutr.* 25:2457–2482.
- Mafrá, I., A.S. Barros, and M.A. Coimbra. 2007. The combined effects of black oxidizing table olive process and ripening on the cell wall polysaccharides of olive pulp. *Carb. Polym.* 68:647–657.
- Mafrá, I., B. Lanza, A. Reis, V. Marsilio, C. Campestre, M. De Angelis, and M.A. Coimbra. 2001. Effect of ripening on texture, microstructure and cell wall polysaccharide composition of olive fruit (*Olea europaea*). *Physiol. Plant.* 111:439–447.
- Manganaris, G.A., M. Vasilakakis, I. Mignani, G. Diamantidis, and K. Tzavella-Klonari. 2005. The effect of preharvest calcium sprays on quality attributes, physicochemical aspects of cell wall components and susceptibility to brown rot of peach fruits. *Scientia Hort.* 107:43–50.
- Maxie, E.C., P.B. Catling, and H.T. Hartmann. 1960. Respiration and ripening of olive fruits. *Proc. Amer. Soc. Hort. Sci.* 75:275–291.
- Morris, E.R. 1980. Physical probes of polysaccharide conformations and interactions. *Food Chem.* 6:15–39.
- Nanos, G.D., E. Agtsidou, and E.M. Sfakiotakis. 2002. Temperature and propylene on ripening of green and black 'Conservolea' olives. *Hort-Science* 37:1079–1081.
- Poovaiah, B.W., G.M. Glenn, and A.S.N. Reddy. 1988. Calcium and fruit softening: Physiology and biochemistry. *Hort. Rev. (Amer. Soc. Hort. Sci.)* 10:107–152.
- Recasens, I., A. Benavides, J. Puy, and T. Casero. 2004. Pre-harvest calcium treatments in relation with respiration rate and ethylene production of 'Golden Smoothee' apples. *J. Sci. Food Agr.* 84:765–771.
- Tsantili, E. and C. Pontikis. 2004. Response to ethylene and its interactive effects with N⁶-benzyladenine (BA) in harvested green olives during ripening. *Postharvest Biol. Technol.* 33:153–162.
- Tsantili, E., D. Rouskas, M.V. Christopoulos, V. Stanidis, J. Akrivos, and D. Papanikolaou. 2007. Effects of two pre-harvest calcium treatments on physiological and quality parameters in 'Vogue' cherries during storage. *J. Hort. Sci. Biotechnol.* 82:657–663.