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Influence of Different Calcium Compounds and Fruit Development Stages on Yield, Fruit Quality and Shelf Life of 'Granny Smith' Apples

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Abstract

Calcium (Ca) plays major roles on the storage life and quality apples. In this study, the influence of calcium chloride ($CaCl_2$) and two organic calcium compounds including Calcicat and Folical at four different times of application on the quality and shelf life of 'Granny Smith' apples during two growing seasons. Quantitative and qualitative attributes were evaluated at harvest and after three- and six-months cold storage. In the first year, Folical all-season and mid-season sprays resulted in a significant increase in Ca concentration. In the second year, early-season spray resulted in significant Ca increase compared to control. In the first year, the early-season spray program of Folical had the highest yield and fruit weight. The use of calcium compounds in all timing programs reduced fruits soluble solids concentration (SSC) at harvest compared to control treatments. In both years, at harvest, no significant difference was observed among treatments regarding the 'Granny smith' apple firmness. In the first year, the chlorophyll content of 'Granny Smith' apple peels were affected by spray timing and the type of calcium compound. In both years, Fruits treated with Ca had lower ethylene concentration and brightness index (L*) than controls. In the second year, Folical spray decreased the percentage of superficial scald incidence for three months storage. During the first year, all-season Calcicat spray and in the second year early-season Folical spray showed the lowest percentage of scald disorder after six months storage. In the first year, the all-season spray program and in the second year Folical spray decreased the severity of scald after three months of storage.

Keywords: Fruit firmness; Internal ethylene concentration; Shelf life; Spray programs; Superficial scald

Introduction

Fruit quality attributes, including fruit finish are extremely important in any apple (*Malus domestica* Borkh.) industry, particularly when fruits are shipped for domestic and export markets. Apple fruit quality is a result of many management practices including pollination, pruning, thinning, plant nutrition and crop load, in addition to soil type and weather conditions [1,2]. A well-balanced nutrition program is crucial for high yield and fruit quality [3]. Many fruit quality attributes can be affected by application of calcium (Ca) fertilizers. Calcium (Ca) plays an extremely important role in plant growth and development and in maintaining and modulating various cell functions. It is necessary to maintain membrane stability and is an integral part of the cell wall where it provides rigidity [4]. Calcium also has direct influence on ripening attributes such as respiration, ethylene production, and flesh firmness [5-9]. Enhancing the calcium content of apple fruits can be very beneficial in maintaining fruit quality during storage [10]. Apple tree roots are poor at absorbing Ca from the soil [3]. Because of this, foliar sprays and apple dips are commonly used to increase the Ca content of the fruits. The effect of foliar spraying treatments depends on several factors including foliar spraying techniques, salt concentrations, time and number of treatments, type of fertilizer used and growing season [11].

Different calcium fertilizers are suggested to fruit growers. Experimental results showed different effects of applying calcium fertilizers. In addition, different results of foliar application of calcium compounds at different times have been reported. Some studies have suggested that early and late season spraying is effective in increasing fruit quality [12] and others have recommended late-season spraying [13]. On the other hand, Peryea et al. [14] reported that mid-season $CaCl_2$ spraying appears to be the most economical and effective spray management practice for increasing fruit Ca and reducing risk of bitter pit development. Lötze et al. [15] with the study of three Ca compounds including calcium nitrate, calcium acetate and Calcimax and three spraying programs (start of spraying at various developmental stages, early, mid and late of season) to increase Ca content and to decrease bitter pit stated that in addition to time, compound type is also effective in Ca absorption efficiency by fruits and bitter pit disorder decreasing. Given the above, the objective of this research was to study the pre-harvest foliar-applied effects of calcium chloride and two Ca-containing organic compounds (Calcicat® and Folical®) on fruit quality of 'Granny Smith' apples during two growing seasons.

Materials and Methods

This study was carried out during two growing seasons. In the first year, this study was conducted at the Haydari's Orcahrd located in Abhar, Zanjan-Iran and in the second year was conducted at the Taheri's orchard located in Takestan, Ghazvin-Iran. In both years, the experiment was set up in a commercial orchard of 'Granny Smith' apples, grafted on 'MM106' rootstock, planted at 2.5 x 4 m spacing in 2006. In this study, two organic Ca compounds namely Calcicat® including 20.5% of calcium as CaO, other elements such as nitrogen, magnesium, iron, manganese and zinc, and trace amounts of organic acids and Folical® including 17% calcium, organic acids and plant system amplifiers and calcium chloride as mineral compound with the control (water) in four different spray programs were compared. 5 g L-1 $CaCl_2$ solution was used [14] and the organic compounds were equal with $CaCl_2$ regarding of Ca content. The four different spray programs were: a) Early season (10-15 days after full bloom (DAFB)) involving 6 biweekly spray starting late May and Late-July, b) Midseason (35 DAFB) involving 6 biweekly spray starting early June and ending Mid-August, c) Late season (60 DAFB) involving 6 biweekly spray starting late June and ending late September and d) All-season (10-15 DAFB) late May and ending late September. In the second year, only Folical was examined during two spray programs including early-season and late-season. In the first year, the $CaCl_2$ solution was prepared in a trailer-mounted 100-L sprayer towed behind a tractor and in the second year, a ten-liter sprayer was used for this purpose. The experimental trees were sprayed to drip. Each experimental unit consisted of six trees distributed along the row, but only the central four plants were used for determinations. The three replicates were arranged in a randomized block design. The experiment area received all management practices used in the commercial orchard including winter and summer pruning, application of insecticides.

In both years, 80 fruits of uniform size and maturity level were randomly collected per replication at the harvest time on late September. The sample fruits were randomly collected from the outside of the middle third of the canopy from all treatment trees. The fruit remaining on the tree after the harvest samples had been removed were counted to determine total crop load. Trunk diameters were measured at same height above the soil line to allow calculation of No. of fruits per cm^2 trunk cross-sectional area (TCSA). A randomly sample of 30 fruits was used for measurements of mineral nutrient concentration and quality attributes at harvest. The remaining 50 fruits were stored in a commercial cold storage at 0°C and 95% of relative humidity (RH) located in Karaj region, Alborz province, for three and six months prior to determination of fruit quality attributes and storage disorders.

Samples were analyzed for flesh mineral content at harvest. A random sample of 10 fruit was also selected at harvest from each replication. Fruits were cut longitudinally and small wedges from 1cm below the skin were sampled. Mineral analyses were performed at the nutrient analysis laboratory. Ca and Mg content were determined by atomic absorption spectroscopy and K by flame emission [16]. All nutrient content values were expressed as gram per 100-gram dry mass basis. At harvest and after seven days ripening at room temperature following three- and six-months storage at 0°C, a randomly selected 10 apple subsamples from all plots was evaluated for flesh firmness, titratable acidity (TA), and soluble solids concentration (SSC). Flesh firmness was measured at two opposite points on the fruit equator (sunny and shady sides) using a penetrometer with an 11.1 mm diameter head and averaged. SSC was determined with a refractometer and TA was measured by titration of juice with 0.1 N NaOH to an 8.1 pH end point.

Total chlorophyll content of apples peel was measured by spectrophotometer (T80+UV/VIS PG Instrument Ltd). Accurately weighted 0.5 g of apple peel was taken, homogenized in a mortar with the addition of 2 mL of 100% acetone as a solvent for extraction. The peel homogenate was centrifuged at 10000g for 10 min. The supernatant was removed and re-extracted. The absorption of the samples was read at two wavelengths of 645 and 670 nm [17]. Total chlorophyll content was calculated using the formula of Lichtenthaler and Buschmann [18] and expressed in milligrams per gram of fresh weight. The internal ethylene concentration (IEC) of Granny Smith apples was measured after three months storage. Five fruits from each replication were used to determine the IEC. For IEC measurement, 5 mL gas sample were withdrawn using a syringe. the samples were analyzed on a GC- HP 6890 GC with FID (2010 Shimadzu) detector and glass column. The temperature for the oven, injection port and detector were 100, 120 and 250°C, respectively and the column pressure was one atmosphere. Helium was used as the carrier gas at a flow rate of 30 mL/min. Ethylene gas concentrations were expressed in µL.L-1.

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At harvest, the color of the fruit peels was described using the CIE (L*, C*, and h°) color indicators defined by the International Commission of illumination, in which L* represents the brightness (between zero and one hundred), C* (chroma) expressing light and dark color and h° (color angle) are calculated using these three indicators. For this purpose, five fruits were selected from each replication and after cleaning the surface of the fruits, the skin color of the fruits was measured using a colorimeter (CR400-Minolta). After seven days ripening at room temperature following three- and six-months storage at 0°C, the samples were examined for the incidence of superficial scald incidence percentage and severity. For this purpose, 20 fruits from each replicate were examined. The incidence of disorder was expressed as percentage [19] and the index [20] was used to determine the severity of superficial scald. In the first year, Analysis of variance was performed on all fruit data as a split plot design and in the second year, a factorial design was used to analyze the data. All statistical analyses were undertaken using the general linear model (GLM) procedure of the SAS version 9.0. The Duncan's multiple range test ($P \leq 0.01$) was used to evaluate differences between treatments.

Results and Discussion

Mineral concentration of fruits, at harvest

In the first year, fruit Calcium (Ca), potassium (K) and magnesium (Mg) concentrations were significantly affected by the preharvest treatments. The all-season spray program in 'Granny Smith' was more effective in increasing the fruit Ca. Folical mid-season treatment significantly increased fruit Ca compared to control mid-season (Table 1). In the second year, early-season spray resulted in significant Ca increase compared to control (Figure 1). In the first year, all treated fruits received all- and early-season Folical and all-season $CaCl_2$ had higher content of K and fruits treated with Folical late-season and control late-season had higher concentration of Mg (Table 1). In the second year K and Mg concentration was not affected by the treatments.

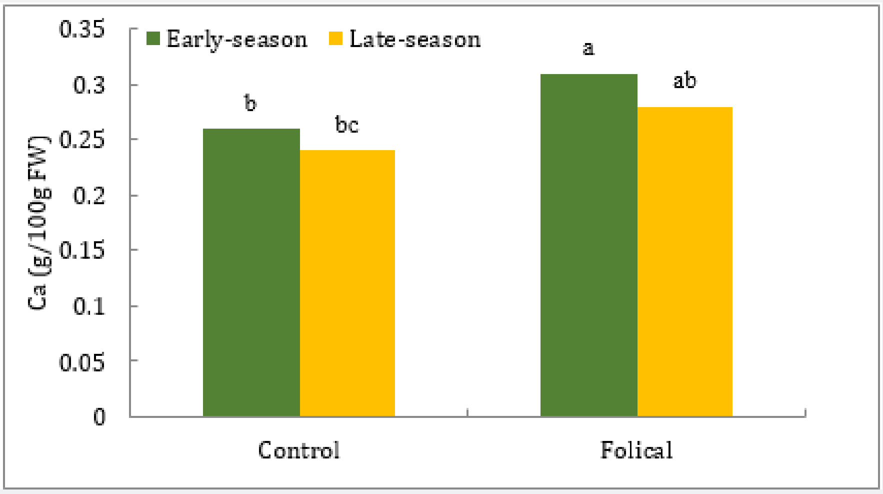

Figure 1: Ca Concentration (g/100g DW) in 'Granny Smith' Apple Fruits at Harvest, in the second year.

Table 1: Mineral concentration, yield and fruits weight of 'Granny Smith' apple fruits at harvest, in the first year.

Ca Solution	Spray program	Ca (g/100 g DW)	K (g/100g DW)	Mg (g/100g DW)	Yield (Kg/cm² TSCA)	Fruits Weight (g)
Control	All-Season	0.11de	0.65bc	0.092a	0.79ab	133.4de
	Early-Season	0.23a	0.91a	0.04c	0.49edf	141.4bcd
	Mid-Season	0.06g	0.67bc	0.092a	0.46f	140.5bcd
	Late-season	0.15bc	0.6c	0.1a	0.54b-f	141.7bcd
$CaCl_2$	All-Season	0.1def	0.9a	0.02de	0.73a-e	145.3b
	Early-Season	0.13cd	0.85a	0.04c	0.8ab	114.5h
	Mid-Season	0.08efg	0.93a	0.01e	0.75a-d	158.1a
	Late-season	0.07fg	0.72b	0.01e	0.52c-f	135.4cde

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Calcicat	All-Season	0.15bc	0.67bc	0.072b	0.76abc	118.7gh
	Early-Season	0.01h	0.65bc	0.076b	0.67a-f	127.5efg
	Mid-Season	0.05g	0.65bc	0.098a	0.72a-f	143.7bc
	Late-season	0.08eg	0.85a	0.031cd	0.65b-f	130.9ef
Folical	All-Season	0.21a	0.95a	0.04c	0.74a-d	145.1b
	Early-Season	0.18b	0.93a	0.02de	0.93a	153.7a
	Mid-Season	0.12cd	0.89a	0.03cd	0.47ef	124.1fg
	Late-season	0.17b	0.59c	0.106a	0.71a-f	127.5efg

Values of similar letters, within the same column, were not significantly different according to the Duncan's multiple range test (P≤0.01).

According to the findings of other studies [21-23], different calcium compounds had different effects on increasing of fruits Ca concentration. Application time of calcium compounds also affected the spraying results. The application of $CaCl_2$ and Calcicat had no effect on Ca enhancement, but Folical all-season and mid-season spray resulted in a significant increase in Ca concentration compared to the controls (Table 1). Peryea et al. [14] with the study of three calcium chloride foliar application programs (early, mid, and late season) for two years reported that calcium concentration in fruits after the mid- and late-season programs were higher than those treated earlier in the season. They found the mid-season foliar application was more economical and effective. In general, these results confirm the effect of type of calcium compounds and foliar application time on the rate of calcium uptake by fruits [11]. On the other hand, in the second year, foliar application of Folical during the early-season increased concentration of calcium in the "Granny Smith" fruits compared to the control and late-season program (Figure 1). These results are inconsistent with the study of Schlegel and Schönherr [24] results which indicated that the rate of calcium penetration into the fruits decreases with increasing fruit age during the growing season. These results may indicate the importance of fruit size in the amount of calcium absorption by the fruits [25].

According to the results of the first year, foliar application during the all-season (which included a greater number of foliar applications) did not have an effect on increasing the concentration of calcium compared to other foliar application programs. As a result, it can be stated that the time of spraying is more important than the number of sprays.

Yield and fruits weight

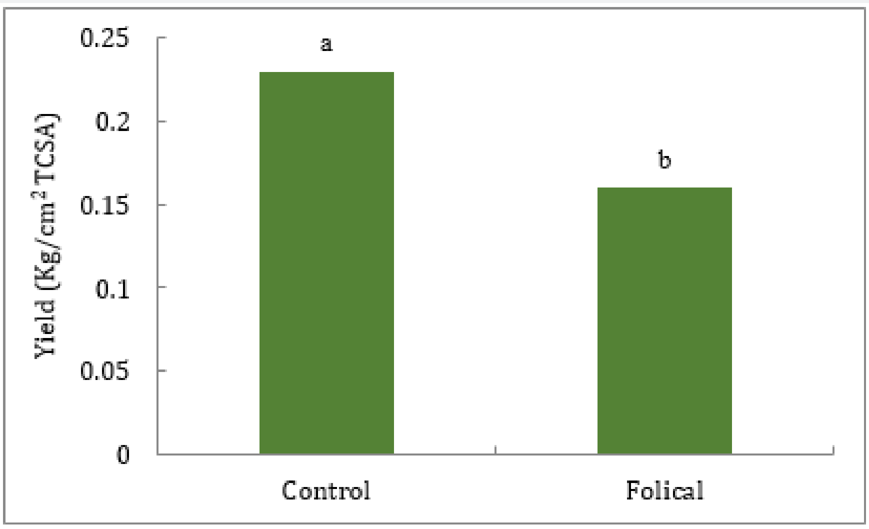

Figure 2: Effect of Ca compounds on yield of 'Granny Smith' apple trees, in the second year.

Yield efficiency (Kg/cm^2 TSCA) and fresh fruit weight at harvest affected by used calcium compounds and spray programs. According to table 1, Folical early-season treatment had highest yield and control mid-season had the lowest. In each compound, different spray programs had no significant difference regarding yield. Foliar application of three Ca compounds during all-season

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showed no significant variation compared to all-season control. While early-season spray programs increased tree yield rather than related control. Application of calcium chloride and Calcicat during the mid-season increased yield. Calcicat and Folical spray during late of season increased tree yield; however, this difference was not statistically significant (Table 1). In the second year, yield of the trees was affected by Ca compounds. As Figure 2 shows, Folical foliar application reduced the yield of trees compared to control, while in the first year the opposite was observed. In Rosenberger et al. [26] study, the yield of trees decreased under the influence of calcium foliar application, which confirms our findings during the second year. The early-season spray program of Folical and $CaCl_2$ had the highest and lowest fruit weight respectively (Table 1), whereas in the second year, late-season spray program had higher weight than early-season spray (Figure 3). Various results have been reported on the effect of calcium on fruit size [25-27].

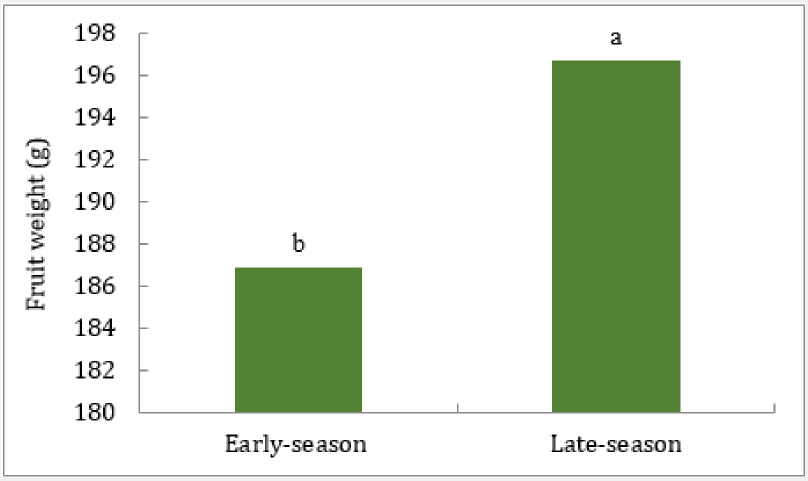

Figure 3: Effect of spray programs on the weight (g) of 'Granny Smith' apple fruits at harvest, in the second year.

Soluble solids concentration at harvest

In the first year, SSC was affected by Ca application and spray programs at harvest and during three- and six-months storage. According to the results, Folical mid-season, control early and late-season and $CaCl_2$ late-season treatments showed the highest SSC and $CaCl_2$ all-season showed the lowest concentration (Table 2). In the second year, fruits SSC was affected by spray programs and spraying during the late season increased the fruits SSC (Figure 4).

Table 2: Soluble solids concentration and titratable acidity of 'Granny Smith' apple at commercial harvest time and during three- and six-months storage were affected by Ca treatments and spray programs, in the first year.

Ca Solution	Spray program	SSC (%)			TA (g/L)		
		At harvest	After 3 months storage	After 6 months storage	At harvest	After 3 months storage	After 6 months storage
Control	All-Season	12.3be	12.9bc	12.6abc	11cd	6.3bcd	5.3de
	Early-Season	12.8ab	12.2de	11.9c-f	11.7abc	6.3bcd	5.9cd
	Mid-Season	12.3b-e	12.5cde	12.9ab	7.4f	4.7h	5.8cd
	Late-season	12.8ab	13.4a	11.8def	9.7e	5.3f	5.1de
$CaCl_2$	All-Season	10.9h	12.4d-e	12.2b-e	9.6e	5.8e	7ab
	Early-Season	12.2c-f	12.8bc	11.7ef	9.9de	4.8gh	5.3de
	Mid-Season	11.8fg	12.6cde	11.4fg	11.1bcd	5.1fg	4.8e
	Late-season	12.7abc	13.5a	12.4ad	12.1ab	5.2f	4.5e
Calcicat	All-Season	12.1d-g	12.1e	12.3b-e	10.3de	6.6ab	5.8cd
	Early-Season	12efg	13.1ab	12.6abc	10.7cde	6.2cd	4.9de
	Mid-Season	11.6g	12.1e	11.3fg	9.9de	5.3f	5.4de
	Late-season	11.7g	12.2e	12.7abc	12.4a	6.8a	7.6a

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Folical	All-Season	11.9efg	12.3de	10.7g	11.6abc	6.5abc	5.4de
	Early-Season	11.6g	12.7bcd	12.6abc	10.2de	5.4f	5.9cd
	Mid-Season	12.9a	12.6cde	13.1a	10.4de	4.7h	6.3bc
	Late-season	12.5acd	13.1ab	12.8ab	12.6a	6.1de	5.5de

Values of similar letters, within the same column, were not significantly different according to the Duncan's multiple range test (P≤0.01).

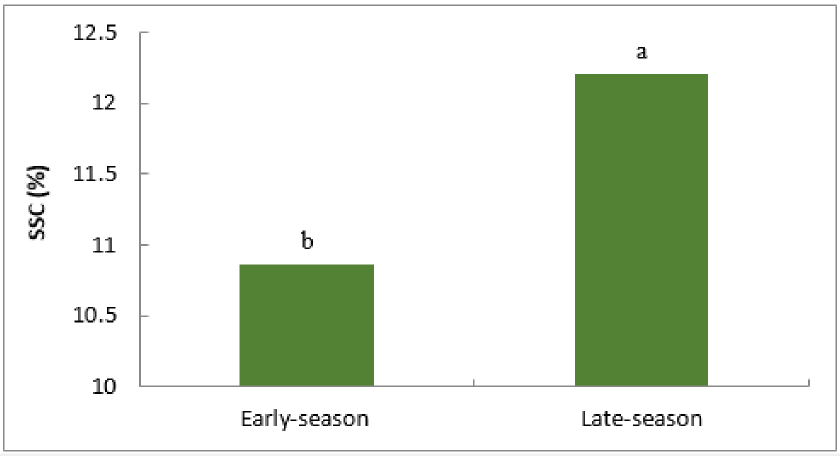

Figure 4: Effect of spray programs on soluble solids concentration (SSC) of 'Granny Smith' apple fruits at harvest, in the second year.

Table 2: Soluble solids concentration and titratable acidity of 'Granny Smith' apple at commercial harvest time and during three- and six-months storage were affected by Ca treatments and spray programs, in the first year. During the first year, no specific pattern was observed in terms of the effect of calcium compounds on reducing or increasing SSC at harvest. The use of calcium compounds in all timing programs reduced the amount of SSC at harvest compared to control treatments, but this reduction was not significant for all treatments (Table 2). Various results have been reported on the effect of calcium on SSC levels [28-31]. Our results agree with the findings of Dris and Niskanen [31] on reducing fruits SSC following the use of calcium compounds. Nielsen et al. [25] found that the SSC value of apple fruit was not affected by different spray programs (early, mid, and late season) of calcium chloride. Decreased SSC in treated fruits is probably due to the fact that calcium decreases respiration and metabolic activity, thus delay the process of fruits ripening [32,33].

Soluble solids concentration during storage

In the first year, significant variation observed among treatments regarding of SSC after three- and six-months storage. $CaCl_2$ late-season and Folical mid-season had the highest SSC after three- and six-months storage, respectively (Table 2). In the second year, fruits SSC was affected by Ca and spray programs after six months storage and Folical late season showed the highest content of it (Figure 5). For three months storage, the fruits SSC increased, but in some treatments, after six months storage, SSC decreased compared to fruits SSC at harvest and after three months storage (Table 2). This trend was also observed in the second year. Mahajan [34] and IhI et al. [35] reported similar results in the 'Red Delicious' and 'Granny Smith' apples. They stated that the initial increase in SSC values is due to the hydrolysis of starch into sugars, which cannot increase after the end of starch hydrolysis. Thus, it can be stated that in this storage conditions, six months storage is not recommended for 'Granny Smith' apples, and the maximum storage period in these conditions needs to be studied.

Titratable acidity at harvest

In the first year, Folical late-season and control mid-season showed the highest and the lowest TA concentration at harvest (Table 2). In the second year, late-season spray had the higher TA compared to the early-season spray (Figure 6). Various results have been reported on the effect of calcium on the amount of TA [25,36,37]. In our study (Table 2), no specific trend was observed and the effect of compounds on TA levels varied depending on the foliar application time.

Titratable acidity after storage

In the first year, fruits TA was affected by the studied treatments after three- and six-months storage (Table 2). In the second year, the effect of Ca solution on fruits TA was significant after three months storage and fruits received Folical had more TA

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than control (Figure 7). As previous findings have reported [35, 36] the amount of TA in the fruits reduced during storage. This reduction did not have a clear trend between control and treated fruits. However, as the data suggest, fruits TA decreased sharply during storage, which, according to the results of Jinhe et al. [38], fruits that have a high TA at harvest time show a further decrease during storage.

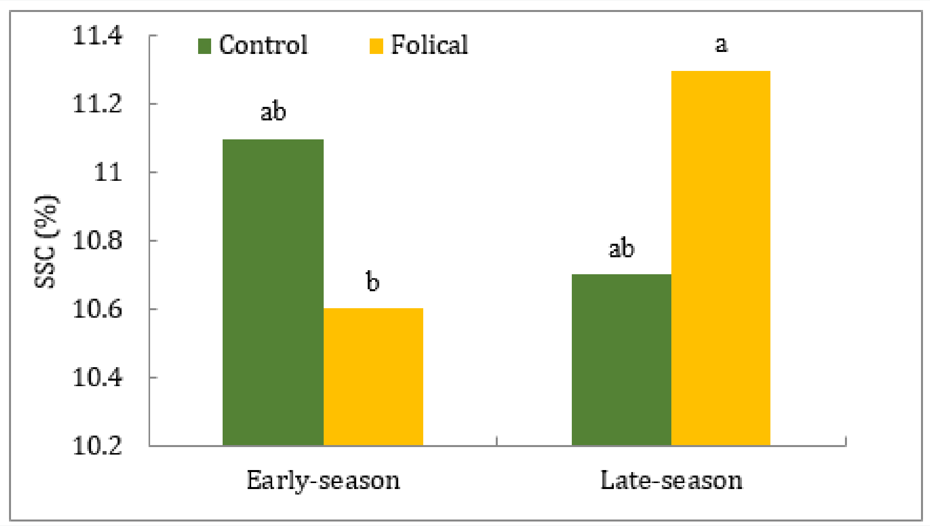

Figure 5: Effect of Ca Compounds and spray programs on SSC of Granny Smith apple fruits for six months storage, in the second year.

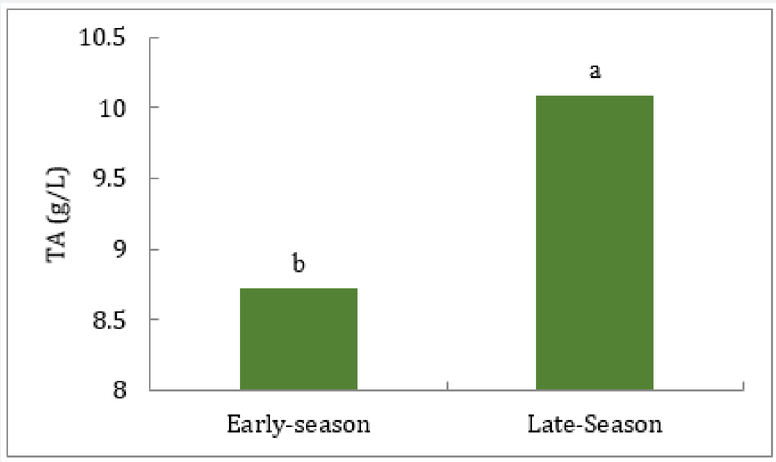

Figure 6: Effect of spray programs on titratable acidity (TA) of 'Granny Smith' apple fruits at harvest, in the second year.

Firmness at harvest

In both years, at harvest, no significant difference was observed among treatments regarding firmness of 'Granny smith' apples.

Firmness during storage

In the first year, after three- and six-months storage, flesh firmness of apple fruits was influenced by preharvest treatments as recorded in Table 3. Control late-season showed the highest flesh firmness and $CaCl_2$ all-, early- and mid-season showed the lowest, after three months storage. For six months storage, Folical and control early-season had the highest firmness and Calcicat early-season had the lowest of it (Table 3). In the second year, apple tissue firmness was not affected by Ca treatments during three- and six-months storage. There are conflicting reports regarding the relationship between calcium levels and fruit firmness. According to some studies, firmness of fruit tissue increases with

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increasing calcium content of fruit [39,40], and according to some other studies, firmness of fruit tissue is not affected by pre-harvest foliar application of calcium chloride or other calcium solutions [41]. In this investigation, calcium solution did not increase the tissue firmness of the fruits compared to the control. During the second year, the firmness of the 'Granny Smith' apple fruits was not affected by the Folical foliar application and the spraying times. Ernani et al. [1] and Wojcik et al. [29] also reported that the use of calcium compounds had no effect on the firmness of 'Gala' and 'Granny Smith' fruits. As the data show, the firmness of the fruit tissue in the second year was less than in the first year, which can be attributed to the bigger size of apple fruits during the second year.

According to Table 3, The firmness of the fruits tissue reduced during storage. Mature apples undergo changes of ripening process during storage, which involve cell wall degradation by the enzymes polygalacturonase and pectin methyl esterase [42, 43]. The result of these changes is a reduction in the firmness of the fruit tissue and, as a result, an increase in susceptibility to fungal pathogens [44]. In fact, storage at low temperatures, due to the degradation of the middle membrane and cellular separation, leads to a reduction in the fruit tissue firmness [8,45]. As the data show (Table 3), calcium treatments reduced fruit tissue firmness less than control fruits. Calcium reduces the sensitivity of the cell wall to enzymatic hydrolysis by inhibiting the activity of the enzymes polygalactornase and pectin methyl esterase, as well as by binding to cellular polymers, and prevents the breakdown and disintegration of cells [46-49].

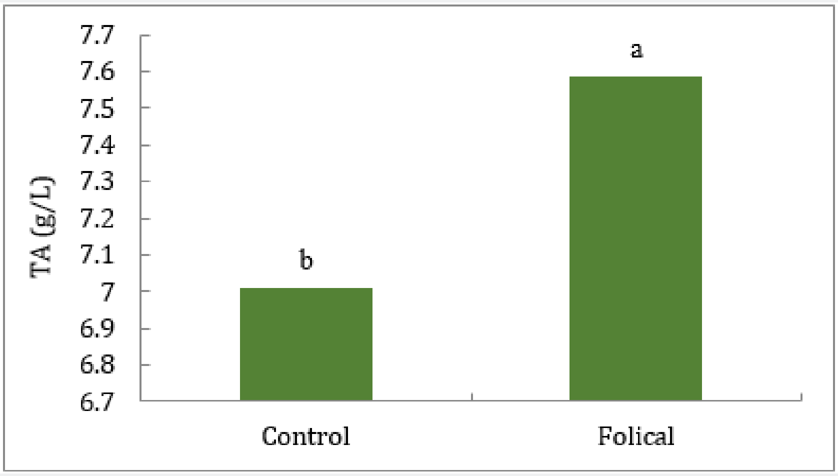

Figure 7: Effect of spray programs on TA of 'Granny Smith' apple fruits for three months storage, in the second year.

Table 3: Firmness and total chlorophyll concentration of 'Granny Smith' apple at commercial harvest time and after 3- and 6-months storage were affected by Ca treatments.

Ca Solution	Spray program	Firmness (N)			Chlorophyll (µg/g FW)		
		At harvest	After 3 months storage	After 6 months storage	At harvest	After 3 months storage	After 6 months storage
Control	All-Season	107.8a	72.1ab	69.6a-e	39.5d-g	52.03abc	50.82b-e
	Early-Season	116.4a	68.1b-e	74.6a	44.19b-e	53.37ab	43.09e
	Mid-Season	110a	66.3dce	72.6abc	45.65 a-e	55.2ab	50.46b-e
	Late-season	111.6a	73.4a	72.3a-d	47.47a-d	57.66a	59.08ab
CaCl2	All-Season	98.4a	65.4e	65.1e	33.8g	51.87abc	62.23a
	Early-Season	109.5a	65.4e	65.1e	43.46b-f	38.06d	49.66cde
	Mid-Season	98.9a	65e	70.8a-e	38.59efg	54.11ab	55.02ac
	Late-season	99.9a	71.1abc	74.4a	42.75b-f	39.54d	50.52b-e
Calcicat	All-Season	105.8a	68.1b-e	68a-e	39.94c-g	56.24a	58.87ab
	Early-Season	103.7a	68.4b-e	65e	42.97b-f	53.2ab	45.45de
	Mid-Season	101.4a	67c-e	66.8cde	49.97ab	58.29a	61.52a
	Late-season	102.3a	66e	70.1a-e	47.43a-d	42.84cd	48.37cde

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Folical	All-Season	102.2a	66.2de	73.8ab	35.94fg	55.77ab	54.17abc
	Early-Season	106.2a	72.2ab	75a	53.32a	52.82ab	44.53de
	Mid-Season	105a	67.2c-e	65.5de	48.01abc	54.9ab	52.36bcd
	Late-season	107.4a	70.7a-d	66.9b-e	39.74d-g	45.99bcd	56.08abc

Values of similar letters, within the same column, were not significantly different according to the Duncan's multiple range test (P≤0.01).

Total chlorophyll concentration at harvest

According to the results, significant difference was observed among treatments regarding total chlorophyll of apple peels, in the first year. In the second year, there was no significant difference among fruits. According to the data (Table 3), the effect of each calcium compound was different depending on the time of application. The highest concentration of chlorophyll found in fruits treated with Folical during early of fruits growth steps and the lowest found in $CaCl_2$ all-season treatment. The application of different calcium solutions during all, mid and late-season did not have a significant effect compared to the corresponding control fruits. Only the Folical early-season spray increased the concentration of chlorophyll relative to the control early-season, significantly.

Total chlorophyll concentration during storage

In the first year, chlorophyll concentration of apple peels was affected by preharvest application of Ca and spray programs after three- and six- months storage. After six months storage, the highest chlorophyll found in $CaCl_2$ all-season and Calcicat mid-season and the lowest chlorophyll concentration found in control early-season (Table 3).

In the second year, chlorophyll concentration was affected by Ca solution for three months storage and fruits received Folical had higher chlorophyll compared to the control (Figure 8). After six months storage, it was affected by Ca and spray programs and Folical late-season had the highest concentration (Figure 9). Various results have been reported regarding the effect of calcium on the synthesis and accumulation of chlorophyll. Knypl and Rennert [50] stated that potassium and calcium play a prominent role in chlorophyll accumulation. Calcium spraying on wheat has increased chlorophyll levels [51], while Calcium spraying on apple fruits, has reduced chlorophyll [30]. As the data show, unlike Rutkowski et al. [52] report, the amount of chlorophyll in the peel of fruits fluctuates during three and six months of storage, but in general, the chlorophyll content of fruits peel increased with the exception of Folical early-season (Table 3). The results of the second year also confirmed the observed results during the first year.

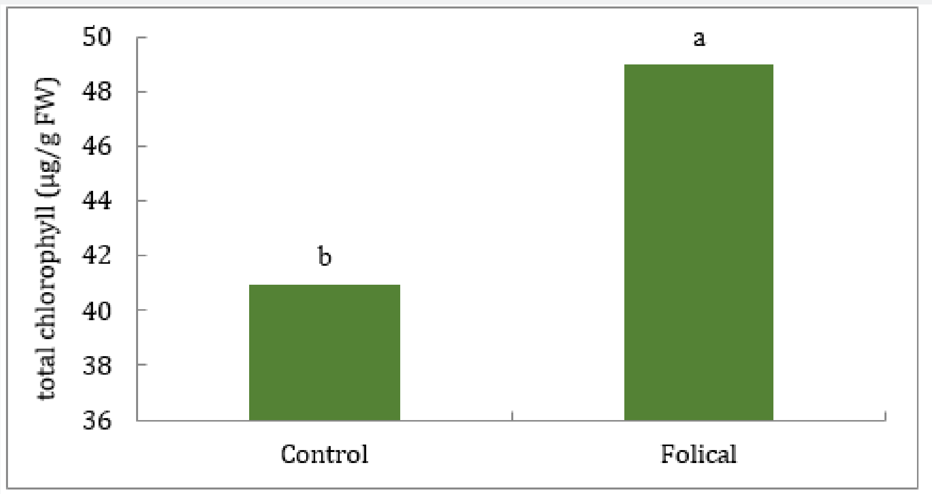

Figure 8: Effect of Ca compounds on total chlorophyll concentration of 'Granny Smith' apple peels for three months storage, in the second year.

Internal ethylene concentration (IEC) for three months storage

In both years, there was a significant variation among treatments regarding internal ethylene concentration, after three months storage. As the data show (Table 4), calcium compounds had different effects on fruit ethylene production depending on the time of application. In the first year (Table 4), foliar application of calcium compounds during all-season did not have a significant effect on ethylene concentration. Early-season $CaCl_2$ and Folical spray significantly reduced ethylene levels compared to control early-season treatment. The use of the studied calcium compounds during the mid-season reduced the concentration of internal

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ethylene in fruits compared to control, significantly. About late-season spray program, only Folical solution significantly affected the production of internal ethylene concentration and reduced the concentration of ethylene compared to control fruits. The use of Folical late-season in the second year had the same effect (Figure 10). The reduction in the ethylene concentration of the fruits was consistent with the findings of Fallahi et al. [53], Saftner et al. [54], Sams and Conway [55]. Calcium affects the structure and function of cell membranes, and by binding enzymatic and non-enzymatic proteins to cell membrane phospholipids, reduces the activity of ethylene-producing enzymes that have a protein structure and are attached to the cell membrane and thereby slightly retarding the climacteric rise and reducing the climacteric maximum and preventing softness [53,56].

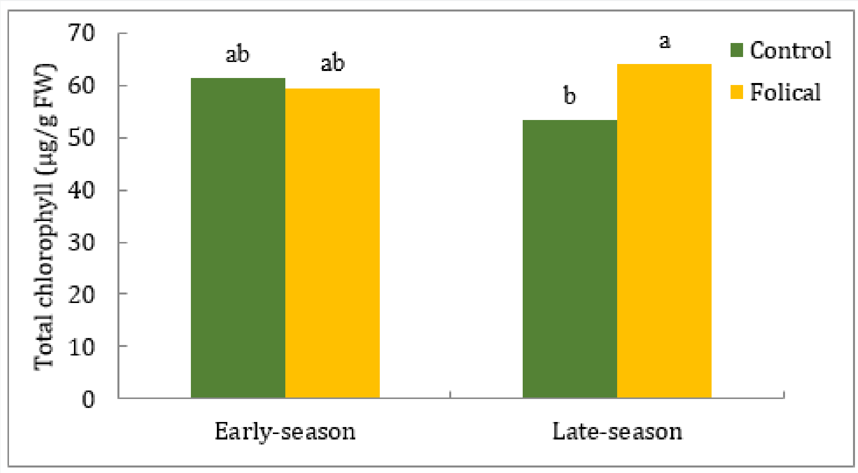

Figure 9: Effect of Ca compounds and spray programs on total chlorophyll concentration of 'Granny Smith' apple peels for six months storage, in the second year.

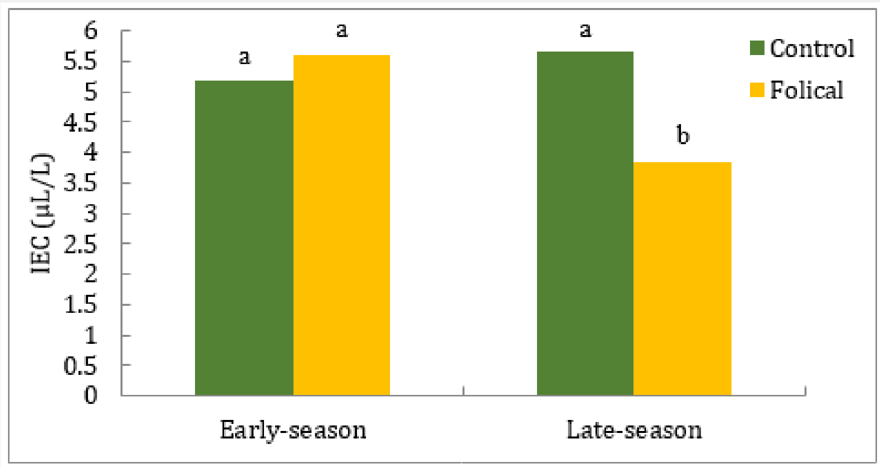

Figure 10: Effect of Ca compounds and spray programs on internal ethylene concentration of 'Granny Smith' apple fruits for three months storage, in the second year.

Color Indices of fruits peel at harvest

In the first year, the L* index of fruits peel was affected by the simple effect of calcium solution and the interaction of calcium solution × spray programs. C* and h° indices were not affected by any of the treatments. As the data show (Table 4), fruits treated with calcium solutions had a lower brightness index (L*) than control fruits. In the second year, only the interaction of calcium solution and spray programs was significant regarding L* index (Figure 11). The two indices C* and h° were not affected by the studied treatments. The L* reduction as a result of the use of calcium compounds is consistent with the findings of Sharma et al. [57].

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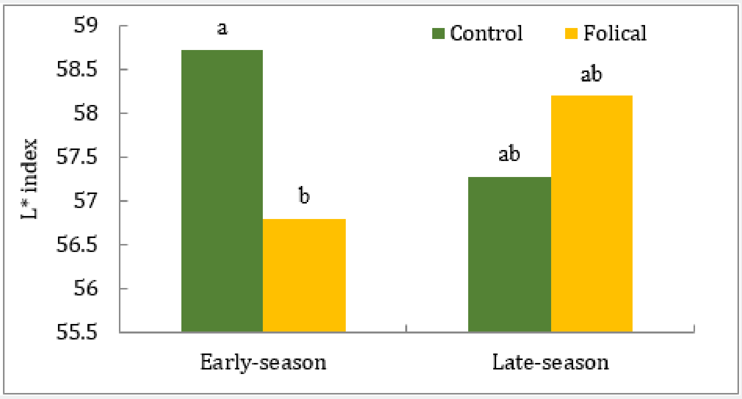

Figure 11: Effect of Ca compounds and spray programs on L* color index of 'Granny Smith' apple peels at harvest, in the second year.

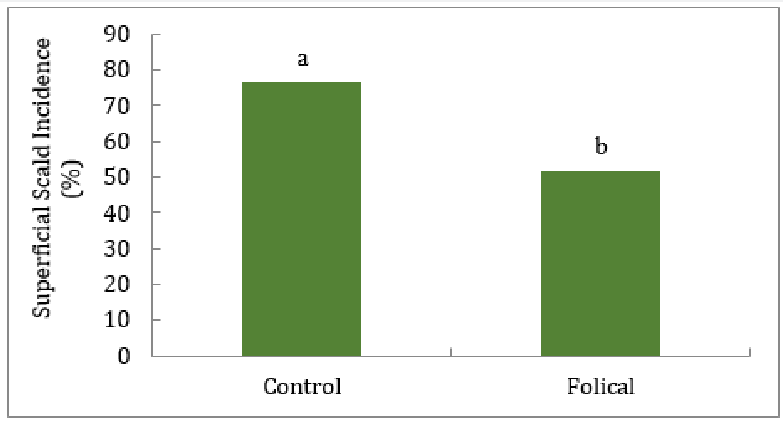

Figure 12: Effect of Ca compounds on superficial scald incidence (%) for three months storage, in the second year.

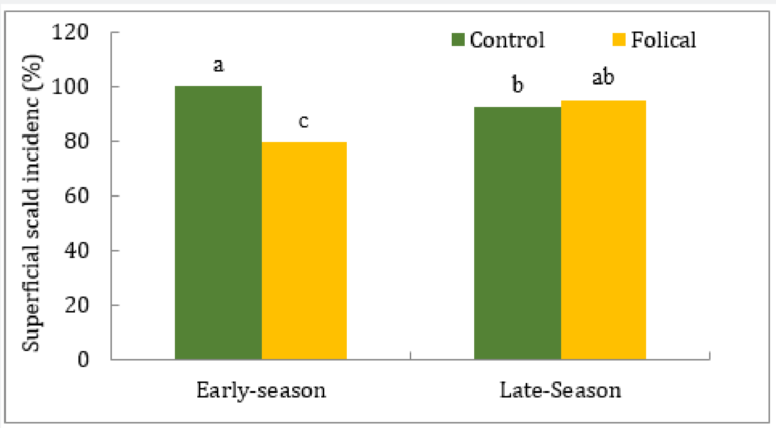

Figure 13: Effect of Ca compounds and spray programs on superficial scald incidence (%) for six months storage, in the second year.

Incidence of percentage of superficial scald for three- and six-months storage

In the first year, the percentage of superficial scald incidence was not affected by preharvest treatments for three months storage. But in the second year, the effect of Ca solution on incidence of superficial scald was significant. Folical application reduced the incidence of this storage disorder after three months storage (Figure 12). In both years, there was significant variation

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among treatments regarding scald incidence for six months storage. During the first year, the incidence percentage was 100 with the exception of Calcicat all-season (Table 4). In the second year, Folical early-season showed the lowest percentage of scald incidence compared to the control and Folical late-season and the highest percentage of disorder was observed in the control early-season fruits (Figure 13).

Scald severity for three- and six-months storage

In the first year, scald severity was affected by spray programs for three months storage and in the second year was affected by Ca solution. In the first year, the severity of the scald disorder was not affected by the different calcium solutions. But the severity of scald in the treated fruits during all-season was significantly lower than other three spraying programs (Figure 14). However, during the second year, the severity of this disorder was significantly lower in the treated fruits with Folical compared to control fruits (Figure 15). According to the results, after six months storage, there was significant difference among calcium compounds regarding scald severity. The severity of scald was significantly lower in the fruits treated with Calcicat solution compared to control and fruits treated with calcium chloride and Folical (Figure 16). In the second year, scald severity was affected by spray programs and Ca. the scald severity was significantly lower in the Folical early-season treatment than others (Figure 17). Previous studies have shown that scalds are more in products harvested after a hot-dry summer than fruits harvested during the humid summer. But in the present study, during the first year when the weather was cooler and the humidity was higher, the percentage and severity of the disorder was higher than in the second year (especially after three months storage).

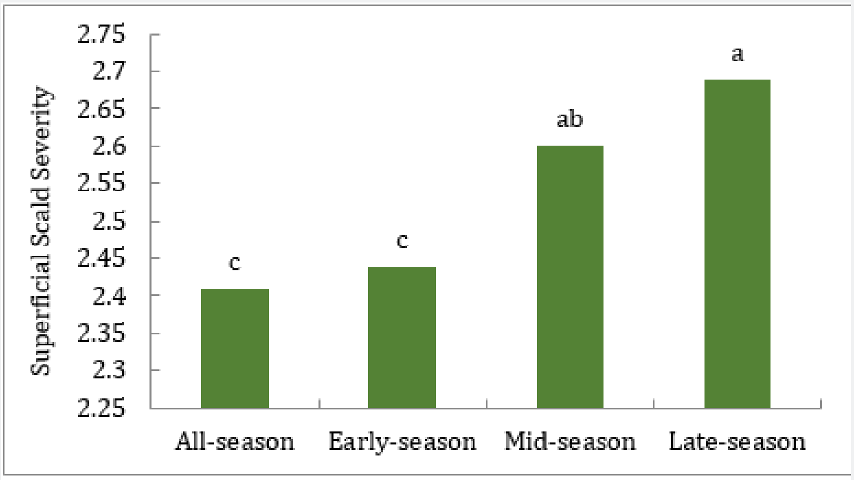

Figure 14: Effect of spray programs on superficial scald severity of 'Granny Smith' apple fruits for three months storage, in the first year.

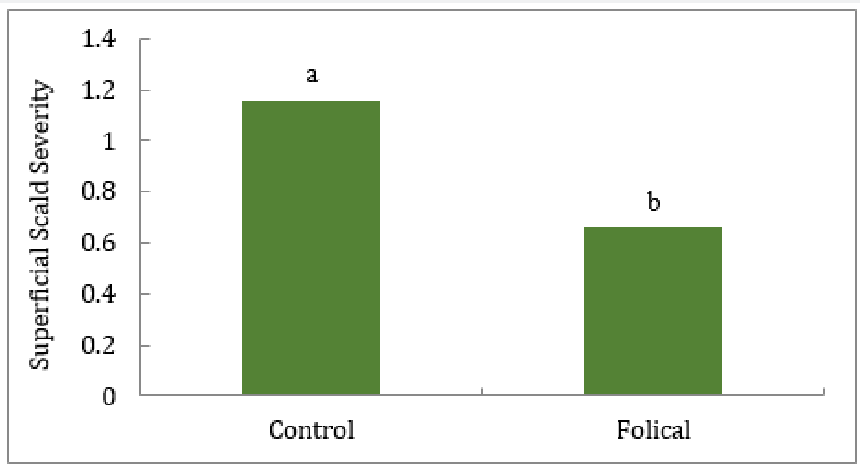

Figure 15: Effect of Ca compounds on superficial scald severity of 'Granny Smith' apple fruits for three months storage, in the second year.

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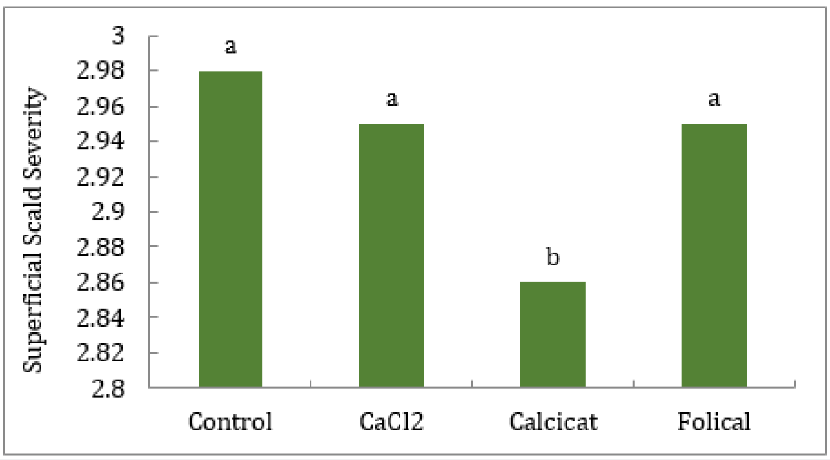

Figure 16: Effect of Ca compounds on superficial scald severity of 'Granny Smith' apple fruits for six months storage, in the first year.

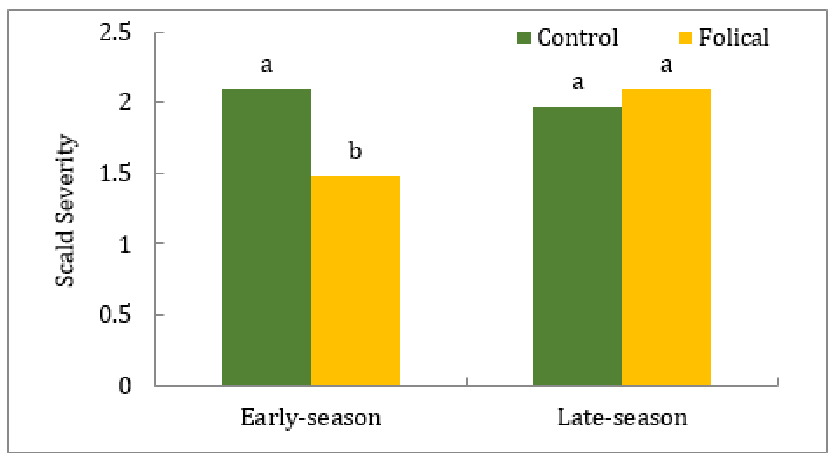

Figure 17: Effect of Ca compounds and spray programs on superficial scald severity of 'Granny Smith' apple fruits for six months storage, in the second year.

Table 4: Color indices of fruits peel, internal ethylene concentration and superficial scald incidences of 'Granny Smith' apple fruits were affected by Ca treatments.

Ca Solution	Spray program	Internal ethylene Concentration (μL/L)	Color Indices			Superficial Scald Incidence (%)
		After 3 months storage	At harvest			After 6 months storage
			L*	C*	h°	
Control	All-Season	43.49d-g	61.8a	46.39a	114.31a	100a
	Early-Season	50.43bcd	60.86ab	46.41a	114.23a	100a
	Mid-Season	64.08a	59.62b-e	46.75a	114.5a	100a
	Late-season	55.39abc	60.36abc	47.28a	114.67a	100a

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$CaCl_2$	All-Season	47.22b-e	57.97def	46.86a	115.02a	100a
	Early-Season	36.1efg	59.49b-e	46.88a	115.13a	100a
	Mid-Season	34.65fgh	59.003b-f	46.28a	115.08a	100a
	Late-season	45.12c-f	58.68c-f	45.84a	115.52a	100a
Calcicat	All-Season	43.69d-g	57.31f	46.91a	115.74a	90b
	Early-Season	57.86ab	57.35f	45.45a	115.94a	100a
	Mid-Season	47.18b-e	60.14abc	45.81a	114.92a	100a
	Late-season	51.77bcd	58.80b-f	46.13a	115.22a	100a
Folical	All-Season	36.35efg	58.02def	44.86a	115.37a	100a
	Early-Season	24.9h	58.73c-f	46.91a	115.21a	100a
	Mid-Season	32.54gh	59.97a-d	46.56a	114.94a	100a
	Late-season	43.31d-g	57.83ef	46.10a	115.23a	100a

Values of similar letters, within the same column, were not significantly different according to the Duncan's multiple range test (P≤0.01).

Conclusions

According to this study, various calcium compounds, stages of fruit development and time of application had different effects on fruit quality attributes of 'Granny Smith' apple. The impact of time of spray was more important than the number of sprays on fruit quality. Application of calcium chloride and Calcicat during the mid-season increased yield. In the second year, Folical foliar application reduced the yield of trees, while in the first year, different result was observed. According to yield data in both years (Table 1 & Figure 1), trees yield in the first year was higher than the second year. Different results in both years regarding SSC, TA and Firmness can be due to differences in trees yield during the studies years [39]. In both years, Fruits treated with Ca had lower ethylene concentration and brightness index (L*) than controls. According to the both years results, Folical and Calcicat compounds (Figure 15-17) and early-season spray program (Figure 14 & 17) were more effective in improving of superficial scald disorders, which needs further study.

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