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ORIGINAL PAPER

EFFECT OF CALCIUM ON MINERAL NUTRIENT CONCENTRATIONS AND FRUIT QUALITY IN DIFFERENT APPLE TREE VARIETIES

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Abstract

Calcium is very important for the quality of fruit, especially apples. The goal of this study has been to determine the effect of calcium on the quality of apples and mineral nutrient concentration in different apple tree varieties. The determination of quality parameters can help to postpone fruit ripening. Golden Delicious, Pink Lady, Jonabres and Red Delicious apple varieties were used in this study. $CaCl_2$ was applied in a 0.5% solution sprayed twice, 15 days apart, after full bloom. Plant ion concentrations (Ca, K, Mg, Fe, Cu, Zn, Mn of the leaf tissue) were determined as well as the following quality parameters of apples: fruit weight, diameter, height, soluble solid content, pH, titratable acidity, fruit skin colour, firmness measured. Calcium application increased the Ca concentration in leaves and improved the quality parameters compared to the control conditions. Two applications of 0.5% Ca Cl_2 spray at a fifteen-day interval after full bloom were sufficient, and the foliar application of calcium was needed even when no deficiency symptoms were observed. While fruit weight, width and height increased, the respiration rate and ethylene production decreased. Variety differences were significant in this study. Jonabres and Golden Delicious apple varieties were affected more by the Ca treatments than the other varieties.

Keywords: calcium, apple cultivars, quality of fruit parameters, nutrients in leaves.

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INTRODUCTION

Calcium is one of the most important nutrients that affects fruit quality of such fruits as apple, pear and peach, where it reduces the incidence fruit diseases and improves fruit quality. Bitter pit and cork diseases are well-known calcium deficiency disorders. Calcium plays a key role in plant growth and development. Calcium is essential for the integrity of the plasma membrane of plant cells, specifically the selectivity of the transport of ions that they perform (Epstein, Bloom 2005). Calcium has a function in the regulation of the stomatal aperture by mechanisms only now being elucidated (NG et al. 2001). Calcium affects fruit quality and aging by altering cellular processes; calcium also affects the softness of fruit (Fallahi et al. 1997).

Apple is the most important fruit in horticulture and in human nutrition, as it is readily consumed by large numbers of people. According to data on apple yields, 3625960 tonnes of apples were produced in 2008 (FAO 2020). Fertilization is one of the most important measures to increase apple production and yield. Apple spray methods are important to consider when attempting to increase the fruit calcium content in order to control fruit diseases and improve fruit quality, and spraying the apples several times leads to an increase in the firmness of the fruit and delayed fruit ripening (Ferguson 1984, Oms-Oliu et al. 2010). The most effective application to increase the calcium concentration is foliar and fruit application. Plant tissues are mainly supplied with Ca^{+2} but when the rate of transpiration to fruits is poor, as may happen under humid conditions, inadequate levels of Ca^{+2} may be supplied to fruits and deficiency symptoms may result. Calcium application to fruit trees means preventing diseases of apples caused by Ca deficiency, but because of the limited Ca mobility, Ca containing sprays should be used (Raese, Drake 2000). During the storage of apples, pre-harvest Ca applications helps to maintain fruit firmness, prevent disorders like bitter pit, inhibit production of ethylene and reduce decay (Conway et al. 2002).

pH and Ca concentrations in soils, particularly uncultivated ones, can vary widely. During evolution, plant species have adapted to these changing pH and Ca conditions. For this reason, marked differences in tolerance occur between plant species and even varieties of a single species (Mengel, Kirkby 2001).

There are distinct differences between plant species in their ion uptake characteristics (Aznarte-Mellado et al. 2016). The Ca concentration of apple leaves changes within 1.20-1.60% (Jones et al. 1991) depending on the growing conditions, plant varieties, etc. Differences in Ca requirements between genotypes are related to Ca^{+2} binding sites in the cell walls (White, Broadley 2003). Not only species but also varieties can differ in plant nutrient uptake due to genotypic differences, such as the root structure, cation exchange capacity, leaf structure, etc. (Marschner 2012). Different varieties need to be known regarding their specific optimum nutrient levels and every variety needs a nutrition plan.

Due to a very low mobility of Ca in the phloem, there is a risk of Ca falling below the critical level and deficiency-related disorders, e.g. bitter pit, can occur. A relatively small decrease in the Ca concentration of apples can cause economic losses due to storage disorders.

The aim of the study was to evaluate the effect of calcium on apple quality and mineral nutrient concentration in different apple varieties.

MATERIAL AND METHODS

Experimental area

This study was carried out at the Isparta University of Applied Sciences experimental station in 2019. Fifteen-year-old trees of four different apple varieties Golden Delicious, Jonabres, Pink Lady, Red Delicious grafted on M9 and planted at 1.5x4.5 m spacing were used in this study. The soil properties were as follows: pH 7.8 (1:2.5 soil: water, Jackson 1967), 1.2% organic matter (Black 1965), 17% $CaCO_3$ (Caglar 1949), 0.5 M $NaHCO_3$ 24 kg ha^{-1} P (Olsen 1982), NH_4OAc-exchangeable K 420 kg ha^{-1} (Thomas 1982). $CaCl_2$ (Merck) was used as a source of Ca applied in a 0.5% concentration solution, sprayed twice with a 15-day interval after full bloom. The 0.5% concentration was determined by taking into account the previous studies on apple growing practice. The fertilization regime was established according to the soil analysis results.

Horticultural process

Apple trees were fertilized by drip irrigation according to the soil analysis results. As basal fertilization composed of 25 kg ha^{-1} N, 25 kg ha^{-1} P, 20 kg ha^{-1} K, 10 kg ha^{-1} Mg ammonium sulphate, triple super phosphate, potassium nitrate, magnesium sulphate were used. The irrigation, pesticide application and pruning were carried out when needed.

Chemical analysis

Leaf samples were collected from middle shoots in July, 12 weeks after full bloom, by picking leaves around the tree. The sampled leaves were put in paper bags and brought to the laboratory, where they were washed with tap and distilled water and dried at 65 ±□ until stable weight. Afterwards, the dry leaves were ground. The ground leaves were wet digested with nitric acid (HNO_3) in a microwave-digestion system (CEMMars 5, manufactured by CEM Corp., USA). Plant ion concentrations (Ca, K, Mg, Fe, Cu, Zn, Mn of the leaf tissue) were determined by Atomic Absorbtion Spectrophotometry (AAS), while phosphorus concentrations were determined with the vanadate-molybdate colorimetric method (Kacar, İnal 2008).

Quality parameters

The quality parameters determined in this study were: fruit weight, diameter, height, soluble solid contents, pH, titratable acidity, fruit skin colour, and fruit firmness. Fruit flesh firmness was measured in each replicate on 15 fruits using a digital texture machine (Lloyd Instruments LF Plus), and by compression at harvest using a 1 kN load cell and a stainless steel, 11.1 mm diameter cylindrical probe, with a constant speed of 100 mm min^{-1}. The results were indicated in Newton units (N). A digital refractometer (Atago Pocket PAL^{-1}) was used for determination of the soluble solids content (SSC), expressed as percentage (%). Titratable acidity (TA) was determined by a digital pH meter (Hanna Instruments) and titrimeter (Digitrat, Isolab), and sexpressed as g of malic acid equivalent 100 g^{-1} fresh weight (g 100 g^{-1}). 15 apples were used for determinations of the soluble solids content (SSC) and titratable acidity (TA). In turn, 6 fruits for each replicate served to determine the respiration rate and ethylene production. Fruits were weighed and placed in 4 L airtight jars at 20°C. Then, a gas sample was taken from the jars and injected into gas chromatographs (Agilent GC-6890N U.S.A and Canada). Results were stated as the respiration rate mL CO_2 kg^{-1} h^{-1} and ethylene production μL C_2H_4 kg^{-1} h^{-1}. To make a pomological analysis, fruit weight was determined on a digital scale and the length and width of each fruit were measured with a digital calliper (Unior, No 270).

A colour chromometer was used (Minolta Cr 300, Ramsey, NJ, USA) to determine fruit skin colour on 15 apples in each replicate. The device was first calibrated according to the standard white calibration plate (Y=92.3, x=0.3136 and y=0.3194). The chromaticity of colour was calculated from the formula:

square root of ($a^{*2}+b^{*2}$) and the hue = tan inverse (b^*/a^*)

Statistical analysis

The results on the plants' nutritional status were compared with the results of Jones et al. (1991). This study was set up according to a randomized design, with four varieties and three replicates. The statistical analysis of data was supported by the SAS package program and the differences were grouped in the LSD test at 5% and 1% significance levels.

RESULTS AND DISCUSSION

Plant nutrient concentrations

As seen in Table 1, Ca concentrations increased with Ca applications compared to control conditions. Ca concentrations in leaves of all the varieties increased owing to Ca applications, and the differences among varieties

Table 1

The effect of Ca application on Ca (g kg^{-1}), K (g kg^{-1}) and Mg (g kg^{-1}) concentrations of leaves of different apple tree varieties

Varieties	Control	Ca	Mean	Control	K	Mean	Control	Mg	Mean
Golden D.	19.6	21.5	20.5A*	20.3*ab******	20*ab*	20.2	3.2	3.0	3.1AB
Jonabres	20.4	23.6	22.0A	18.5*abc*	17.1*bc*	17.8	3.2	3.2	3.2AB
Pink Lady	14.4	16.2	15.3B	19.2*ab*	21.6*a*	20.4	2.6	3.1	2.8B
Red D.	15.7	16.9	16.3B	18.5*abc*	15.5*c*	17.0	3.9	3.0	3.5A
Mean	17.5b**	19.6a		19.1	18.6		3.2	3.1	

* Capital letters shows the differences among varieties;
** lowercase letters shows the application differences;
*** italic letters shows the difference between the application and variety interaction.

were found to be significant. An increase in the concentration of Ca in the solution often leads to an increase in the Ca concentration in the leaves (Marshner 2012). Studies on Ca results are consistent with our results (Moor et al. 2006). While Jonabres apple variety had the highest Ca concentration (23.6 g kg^{-1}), Pink Lady had the lowest Ca concentration (16.2 g kg^{-1}). When the potassium concentrations were evaluated among varieties, contrary results were found. Pink Lady had the lowest Ca concentration while having the highest K concentration. Jonabres had the lowest K concentration (17.8 g kg^{-1}) among all the varieties. The variety and application interaction was important, as Pink Lady, for example, achieved 21.6 g kg^{-1} Ca concentration after the Ca application. Although K^+ channels are similar to Ca^{+2} channels, their functions are different because potassium ions act directly as solutes, changing the osmotic potential in the compartments and thereby the cell's turgor (Sanders et al. 2002). According to Jones et al. (1991), calcium concentrations (16 g kg^{-1}) were higher than determined in our study, and there were no cases of no calcium deficiency; however, we studied four different varieties. Even when there was no calcium deficiency, we could see more increase in the calcium content in apples. Calcium was supplied by foliar application and there were no toxicity symptoms. The results of this study indicate that two applications of 0.5% calcium spray with a 15-day interval should be recommended. Magnesium concentrations did not change in apple trees with Ca application compared to the control conditions. Among the tested varieties, Pink Lady (2.8 g kg^{-1}) had the lowest Mg concentrations.

When compared to the control conditions, phosphorus concentrations in leaves increased with Ca applications, but the effects depended on the varieties, which fell into two groups: Golden Delicious with the highest phosphorus content (5.7 g kg^{-1}) in one, and the other one with the three remaining varieties, which were determined to have much lower concentrations of this element (Table 2). Apparently, there is a relationship between the effects of Ca and P on plant growth under different conditions, with species-specific responses. At high Ca supply, most P accumulated in the epidermal

Table 2

The effect of Ca application on P (g kg^{-1}), Cu (mg kg^{-1}) and Mn (mg kg^{-1}) concentrations of leaves of different apple tree varieties

Varieties	Control	P	Mean	Control	Cu	Mean	Control	Mn	Mean
Golden D.	5.7	5.7	5.7A*	10.5	6.0	8.3A	68	64	66A
Jonabres	5.4	5.4	5.4B	8.3	9.2	8.8A	62	68	65A
Pink Lady	5.2	5.4	5.3B	7.4	8.4	7.9A	42	50	46B
Red D.	5.1	5.3	5.2B	5.9	6.1	6.0B	50	47	49B
Mean	5.3b**	5.5a		8.0	7.4		55	57	

* Capital letters shows the differences among varieties;
** lowercase letters shows the application differences.

cells of L. Cosentinii. Researchers found that high Ca supply alleviated the P-deficiency symptoms of lupin species, and Ca also had a positive effect on shoot biomass (Ding et al. 2008). Calcium can interact with the uptake of other ions, for instance Ca increases the P-absorption rate of some legumes (Bell et al. 1989), and aggravates P-toxicity symptoms of Grevillea cv. 'Poorinda Firebird' (Proteaceae) (Nichols, Beardsell 1981). While calcium applications did not vary Cu and Mn concentrations, the varieties were found to differ statistically significantly. The concentrations of Cu decreased from 8 mg kg^{-1} to 7.4 mg kg^{-1} compared to the control, and all the other varieties except Red Delicious (6 mg kg^{-1}) were in the same group (Table 2). Manganese concentrations divided the tested varieties into two groups: Golden Delicious and Jonabres, which had higher manganese concentrations, and Pink Lady and Red Delicious, which had lower concentrations. When all the nutrients were evaluated, Jonabres and Golden Delicious apple varieties had higher mineral nutrient concentrations generally. Genotypic differences should be taken into consideration while preparing fertilization plans.

Similar results were obtained as regards the other micronutrients, Zn concentrations were different in the different varieties, whereas Ca applications did not affect them. The effects of Ca applications on Fe concentrations were found to be significant in terms of differences between both varieties and applications. Jonabres variety (90 kg mg^{-1}) had the highest Fe concentrations, Red Delicious variety (57 kg mg^{-1}) had the lowest Fe concentrations. With Ca application, Fe concentrations increased from 69 kg mg^{-1} (control) to 75 kg mg^{-1} (Table 3). The leaf zinc concentrations were lower while Fe concentrations were optimal according to Jones et al. (1991). Even when apple leaf zinc concentrations were under 20 mg kg^{-1}, zinc deficiency symptoms were not seen in any of the tested varieties. Malakouti et al. (1999) reported that micronutrients were not affected by $CaCl_2$ treatments, and our results are consistent with this report.

Table 3

The effect of Ca application on Fe (mg kg^{-1}) and Zn (mg kg^{-1}) concentrations of leaves of different apple tree varieties

Varieties	Control	Fe	Mean	Control	Zn	Mean
Golden D.	79	79	79B*	16	16	16AB
Jonabres	84	96	90A	17	18	18A
Pink Lady	57	66	62C	14	16	15B
Red D.	55	60	57C	11	12	12C
Mean	69b**	75a		15	16	

* Capital letters shows the differences among varieties;
** lowercase letters shows the application differences.

Table 4

The effect of Ca application on fruit weight (g), width (mm) and height (mm) of apples from different apple tree varieties

Varieties	Control	FW	Mean	Control	Width	Mean	Control	Height	Mean
Golden D.	192	195	194A*	69	70	69B	64	64	64AB
Jonabres	146	156	151C	77	78	78A	64	67	66A
Pink Lady	177	179	178B	69	71	70B	64	65	65A
Red D.	146	153	149C	66	69	67B	59	59	59B
Mean	165	171		70	72		63	64	

* Capital letters shows the differences among varieties.

Quality parameters

As seen in Table 4, fruit weight, width and height increased compared to the control. Mean values of fruit weight increased from 165 g to 171 g, width increased from 70 to 72 mm and height increased from 63 to 64 mm. Our results are similar to the ones obtained by Solhjoo et al. (2017), who reported that fruit weight, length and diameter increased with $CaCl_2$ spray in Red Delicious apple. Golden Delicious apple variety had the highest fruit weight of all the varieties. Jonabres apple variety had the biggest width and height increment of the varieties. The soluble solid content of apples decreased from 14.1% to 13.7% (Table 5). Our results showed that while the soluble solid content decreased, pH and titratable acidity increased following Ca applications compared to the control. Moor et al. (2008) reported that fruit the dry matter content at harvest reduced significantly after Ca applications. The soluble solid content reduction may be due to the inverse relationship between fruit yield and SSC (Scalisi et al. 2021). $CaCl_2$ enhanced fruit quality parameters. 1% $CaCl_2$ should be studied in next experiments. Application of 2% Ca can cause foliage burns in Red Delicious apple leaves (Malakouti et al. 1999).

When fruit skin colour evaluated, L, chrome and hue values were considered, in this study we had red coloured, and yellow coloured apples. Thus,

Table 5

The effect of Ca application on SSC (%), pH and T-acidity (%) of apples from different apple tree varieties

Varieties	Control	SSC	Mean	Control	pH	Mean	Control	T-acidity	Mean
Golden D.	13.9	13.6	13.7B*	3.5	3.6	3.6AB	0.60*c***	0.61*c*	0.60
Jonabres	16.5	15.8	16.1A	3.6	3.4	3.5B	0.65*c*	0.86*b*	0.75
Pink Lady	12.7	12.1	12.4C	3.3	3.4	3.4B	1.16*a*	1.18*a*	1.17
Red D.	13.3	13.4	13.3B	3.6	3.8	3.7A	0.41*d*	0.45*d*	0.43
Mean	14.1	13.7		3.5	3.6		0.70	0.77	

* Capital letters shows the differences among varieties;
** italic letters shows the difference between application and variety interaction.

Table 6

The effect of Ca application on L, chrome and hue of apples from different apple tree varieties

Varieties	Control	L	Mean	Control	chrome	Mean	Control	Hue	Mean
Golden D.	73.9	73.4	73.7A*	46	48	47A	113	110	111A
Jonabres	66.6	66.2	66.4B	40	36	38B	80	70	75B
Pink Lady	54.3	54.2	54.2C	28	24	26C	36	30	33C
Red D.	50.0	54.8	52.4C	30	28	29C	48	40	44C
Mean	61.2	62.4		36	34		69	62	

* Capital letters shows the differences among varieties.

Table 7

The effect of Ca application on respiration rate (mL CO_2 kg^{-1}), ethylene (μL C_2H_4 kg^{-1} h^{-1}) and firmness (N) of apples from different apple tree varieties

Varieties	Control	Respiration	Mean	Control	Ethylene	Mean	Control	Firmness	Mean
Golden D.	5.4	4.5	4.9A*	2.5	2.3	2.4B	51	56	54C
Jonabres	2.2	1.3	1.7B	2.7	2.9	2.8B	44	50	47C
Pink Lady	6.5	6.1	6.3A	3.6	3.6	3.61B	70	72	71A
Red D.	2.5	2.4	2.5B	6.0	5.0	5.5A	62	64	61B
Mean	4.1	3.5		3.7	3.4		57	61	

* Capital letters shows the differences among varieties.

all the varieties had different skin colour values, and variety-specific effect was found to be significant. Yellow coloured apple Golden Delicious had higher values compared to red coloured apples, as seen in Table 6. The skin colour values decreased compared to the control, but the red skin coloration increased with Ca application. Raese, Drake (2000) and Solhjoo et al. (2017) reported that the red skin coloration of the fruit skin colour of the Red Delicious apple variety increased with Ca foliar fertilization.

The respiration rate and ethylene production were reduced compared to the control conditions. The respiration rate decreased from 4.1 to 3.5 mL CO_2 kg^{-1} and ethylene production decreased from 3.7 to 3.4 μL C_2H_4 kg^{-1} h^{-1}, as shown in Table 7. In another study, it is reported that the respiration rate and ethylene production in apples correlated negatively with pre-harvest calcium chloride applications (Recasens et al. 2004). These researchers reported that the effect on the respiration rate was stronger than that on ethylene production. Ethylene is important for coordinating the fruit ripening, and calcium may postpone and decrease ripening via reducing the fruit's respiration rate and ethylene production. Flesh firmness of apples increased from 57 to 61 Newton compared to the control conditions, as seen in Table 7, and the varieties were statistically significantly different. While Pink Lady had the highest firmness value (71 N) among the apple varieties, the firmness of the apples of Golden Delicious and Jonabres varieties increased more than in the other varieties. Malakouti et al. (1999) reported that Red Delicious apple firmness at harvest enhanced with $CaCl_2$ applications, which coincides with our results. Fruit flesh firmness is the most important quality parameter that describes the apples from ripening from softening. Some enzymes, such as methylesterase and pectatlyase, decrease intercellular adhesion in the cell wall (Kittemann et al. 2010), while calcium plays an important function in the cell wall's stability.

CONCLUSION

Our results showed that 0.5% $CaCl_2$ enhanced fruit quality parameters, and 1% $CaCl_2$ should be studied in next experiments. Application of 2% Ca may cause foliage burns in apple leaves. Ca foliar sprays of apples can be recommended even when the soil is rich in ca and the Ca concentration in apple tree leaves is optimal. Calcium may not reach the fruit due to insufficient transpiration. Pre-harvest spraying increased quality parameters at harvest and variety-specific effects were significant. Besides varieties, storage conditions should be taken into consideration too.

Calcium was supplied through foliar applications no toxicity symptoms were observed. We may even say that when there was calcium deficiency, ca application resulted in higher quality improvement. For apple growers, foliar application of calcium is needed even when there are no deficiency symptoms. Jonabres and Golden Delicious apple varieties had higher mineral nutrient concentrations generally. Genotypic differences should be taken into consideration while preparing fertilization plans.

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