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RESEARCH ARTICLE

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Interactive effects of organic fertilizer, $CaSO_4$ and amino acid Ca on Fuji apple in Burozem soil in China

Abstract A two-year trial was conducted to test the interactive effects of special-for-apple organic fertilizer, $CaSO_4$, and amino acid Ca on Fuji/Balenghaitang (*Malus robusta* Rehd.) rootstock in Burozem soil in China. Total Ca, exchangeable Ca, and fruit Ca were significantly improved by the soil application of $CaSO_4$ with the highest exchangeable Ca and fruit Ca observed in Fuji apple trees treated at the rate of 3.5 kg $CaSO_4$ per tree. 3.5 kg $CaSO_4$ was divided into two applications, with first application in the first month after full bloom, and the second in the second month after full bloom in summer, progressively to improve fruit Ca concentration. Dividing $CaSO_4$ into two applications in combination with organic fertilizer or amino acid Ca spray, proved to be more effective on improving the fruit Ca concentration, with the highest level of 179.17 mg/kg FW in Fuji apple trees treated with 2 kg $CaSO_4$ applied and amino acid Ca sprayed in the first month after full bloom, 1.5 kg $CaSO_4$ applied in summer, organic fertilizer applied in winter. Organic fertilizer applied in winter alone remarkably improved the fruit Ca concentration, as well as storage Ca in roots and stems, and the best effect was observed in Fuji apple trees which were supplemented organic fertilizer (10 kg) in winter and $CaSO_4$ (1 kg) in the first month after full bloom per tree. The effect on Ca increment became less pronounced when the amount of organic fertilizer reached 15 kg or $CaSO_4$ was more than 3.5 kg per tree. There was a positive correlation between the Ca/Mg ratio in soil and fruit, exchangeable Ca, and fruit Ca concentration, while a negative correlation was observed between the N/Ca ratio in fruit, available P, available K in soil, and fruit Ca concentration. Both N/Ca ratio and Ca/Mg ratio in fruit were affected by different fertilization regime, and bitter pit incidence at the end of storage kept acceptable only if $N/Ca < 17$ and $Ca/Mg \geqslant 7$ at harvest. The commercial fertilization regime turned out to be organic fertilizer in combination with $CaSO_4$ applied in soil in winter, amino acid Ca sprayed in the first month after full bloom, and $CaSO_4$ applied in soil in summer.

Keywords Fuji apple, calcium, fertilization

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1 Introduction

Poverty alleviation and environmental protection are very important issues for many countries. The governments of many countries have introduced the use of synthetic fertilizers containing high nitrogen to increase farm yield and thereby alleviate poverty, especially in their rural sectors. This method has also been implemented in China. In recent years, it has been observed that excessive N is detrimental to apple culture because it promotes excessive shading, interferes with the development of fruit red color, reduces the firmness of fruits (Stiles, 1994) and fruit storage life due to the increase in fruit respiration rate (Faust and Shear, 1972; Lougheed et al., 1979) as well as the development of several physiological disorders resulted from interferences between N and Ca including bitter pit, cork spot, and internal breakdown (Stiles, 1994). Substantial evidence is available that the naturally occurring organic fertilizers provide all nutrition needed by apples, especially those special organic fertilizers solely for apples release nutrients at a slower and more consistent rate, improve the soil structure, and provide increased physical and biological storage mechanisms to soils. It was suggested that the orchards which were supplemented with organic fertilizers could yield fruits with a higher content of total carbohydrate (Schulz and Kopke, 1996).

In China, apple fruits with a production of 24 million tons per year have an important position within the total agricultural production and exportation. Jiaodong Peninsula, the northeast part of Shandong Province, contributes 28% of the apple production to the country's total. However, the dominant soil in this region was Burozem soil, which was known to have less Ca content in nature (Zheng et al., 2005a). So the apple orchards located in Burozem soil were susceptible to the bitter pit and it may be further aggravated when the excessive N was applied during the growth season (Stiles, 1994). Much research has demonstrated that the physiological disorder, especially for the bitter pit, was also exacerbated by bagging, indicated by the decreased Ca concentration in fruits (Scarim et al., 1997). Witney et al. (1991) reported that the apple individual weight and calcium content were decreased by bagging, which maybe the reason why the bitter pit incidence was four times higher in bagged fruit than that of controls. In this case, the overuse of N fertilization and bagging reduced Ca accumulation of Fuji fruits, and made Fuji apples in Jiaodong Peninsula less commercial for marketing issue because of suffering from the bitter pit. Other research demonstrated that foliar spraying is the most effective method for increasing calcium concentration in fruits (Bramlage et al., 1985). Gallerani et al. (1990) found that the incidence of bitter pit in "Delicious" and "Golden Delicious" apples was reduced by 88% and 93%, respectively by spraying Ca solutions at three-week intervals during the summer season. Thus special attention should be paid to the interactive effects of organic fertilizer, Ca fertilizer and Ca spraying that are being introduced to minimize the physiological disorders of apple fruits.

Our previous experiments indicated that Fuji apple accumulated Ca not only in spring, according to which many experiments supplemented Ca in the first month after full bloom (Zhou et al., 2000; Zheng et al., 2005a), but also in summer when fruits grow rapidly (Zheng et al., 2005b). As a result of these studies, Ca supplement should also be adjusted according to the Ca accumulating ability of Fuji trees in different seasons.

The investigations presented here were aiming at determining the patterns of calcium accumulated in fruit and the various organs of Fuji apple trees (root and stem). To assess the effects of Ca fertilizers on the calcium level of the fruits, the special-for-apple organic fertilizer, $CaSO_4$, and amino acid Ca were applied at various developmental stages. These investigations constituted an approach to ascertain the possible involvement of calcium fertilizer in improving Ca concentration and preventing the bitter pit of Fuji fruit.

2 Materials and methods

2.1 Plant materials and treatments

The trial was conducted in Penglai City in Shandong Province of China, from September of 2002 until November of 2004. It comprised five trials for the Fuji cultivar. All experiments were located in a commercial orchard with apple growing area of 20 hm^2, in the plow layer of Burozem soil (0–20 cm in depth), with water pH at 6.8, available N of 120.4 mg/kg, available P of 86.55 mg/kg, exchangeable K of 102.3 mg/kg, and 1.22% of organic matter. The trees, grafted on Balenghaitang rootstock (*Malus robusta* Rehd.), were planted in 1994, at a 5.0 m (between rows) × 4.0 m (within a row) spacing, performing 500 trees per hectare. The experimental areas had the same management practices as in the commercial orchard, including the application of fertilizer (12 000 kg/hm^2 chicken manure with fertile soil), insecticides, fungicides, pruning in winter and summer and hand thinning.

To examine the effect of $CaSO_4$ on Fuji fruit Ca in Burozem soil (Group I), $CaSO_4$ was applied to the soil in one month after full bloom in spring at different rates: control (S_0), 0.5 kg/tree ($S_{0.5}$), 1.5 kg/tree ($S_{1.5}$), 2.5 kg/tree ($S_{2.5}$), 3.5 kg/tree ($S_{3.5}$), 4.5 kg/tree ($S_{4.5}$), respectively. Every treatment was located in one single line as a plot with 50 Fuji apple trees in every line. Tested trees were isolated by at least one untreated trees.

A sub-trial was done to examine the interactive effects of $CaSO_4$ and amino acid Ca on Fuji apple in Burozem soil (Group II). Ten Fuji apple trees were randomly selected from every line of Group I to be supplemented with amino acid Ca in the first month after full bloom in spring, coded as $S_{0.5}A$, $S_{1.5}A$, $S_{2.5}A$, $S_{3.5}A$, $S_{4.5}A$, respectively. All spraying applications were performed by a hand sprayer (knapsack) at 20 L per six trees in the morning according to the recommendations and the $CaSO_4$ and amino acid Ca were sprayed for three times at seven-day intervals. Tested trees were isolated by at least one untreated trees.

To investigate the effect of special-for-apple organic fertilizer on total Ca, exchangeable Ca in soil and fruit Ca (Group Ш), special-for-apple organic fertilizer was individually applied at different rates in winter: 5 kg/tree (M_5), 10 kg/tree (M_{10}), 15 kg/tree (M_{15}), respectively. Every treatment was located in one single line as a plot with 80 Fuji apple trees in every line. Tested trees were isolated by at least one untreated trees.

The sub-trial was done to investigate the interactive effects of special-for-apple organic fertilizer and $CaSO_4$ on Fuji apple in Burozem soil (Group IV). $CaSO_4$ was randomly applied in Group Ш in the first month after full bloom in spring at different rates with six replications per treatment, coded as M_5, $M_5S_{0.5}$, $M_5S_{1.5}$, $M_5S_{2.5}$, $M_5S_{3.5}$, $M_5S_{4.5}$; M_{10}, $M_{10}S_1$, $M_{10}S_2$, $M_{10}S_3$, $M_{10}S_4$; M_{15}, $M_{15}S_{0.5}$, $M_{15}S_{1.5}$, $M_{15}S_{2.5}$, $M_{15}S_{3.5}$, respectively. Tested trees were isolated by at least one untreated trees.

In Group V, the same dose of $CaSO_4$ was divided into two applications: with the fist application in the first month after full bloom in spring, and the second in the second month after full bloom in summer. In combination with amino acid Ca spray, $CaSO_4$, and 5 kg special-for-apple organic fertilizer, treatments were set as follows: $S_{2+1.5}$ ($CaSO_4$ 2 kg in spring + 1.5 kg in summer), $S_{0+3.5}M$ ($CaSO_4$ 0 kg in spring + 3.5 kg in summer + organic fertilizer in winter), $S_{0+3.5}A$ ($CaSO_4$ 0 kg in spring + $CaSO_4$ 3.5 kg in summer + amino acid Ca), $S_{0+3.5}AM$ ($CaSO_4$ 0 kg + $CaSO_4$ 3.5 kg in summer + amino acid Ca + organic fertilizer in winter), $S_{2+1.5}M$ ($CaSO_4$ 2 kg in spring + 1.5 kg in summer + organic fertilizer in winter), $S_{2+1.5}A$ ($CaSO_4$ 2 kg in spring + 1.5 kg in summer + amino acid Ca),

$S_{2+1.5}$AM ($CaSO_4$ 2 kg in spring + 1.5 kg in summer + amino acid Ca + organic fertilizer in winter). Tested trees were also isolated by at least one untreated trees.

$CaSO_4$ used in all trials was a byproduct from the compound fertilizer of $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$ made in Haiyang City of Shandong Province, China with 16% of Ca and 14% of S. And an organic fertilizer named "Lü Yuan", one kind of special fertilizer for apple made in Yantai City of Shandong Province, with a Ca content of 9.6%, organic matter of 45.39% ±4.21, an average content of N, P_2O_5, K_2O of 6.12%, and the humic acid higher than 40%, was applied to soil after harvest in the period of 2001−2004.

At the maturity time, the fruits were harvested and brought to the laboratory immediately after harvest. Ca concentrations in fruits, stems and roots, and other characteristics were analyzed in Groups I, II, Ш and IV. Fuji apples in Group V were stored in a commercial cold station for four months at 2−5°C and 80%−85% of relative humidity (RH), to analyze the total Ca, Mg, N and bitter pit incidence.

2.2 Fruit Ca determination

The fruits collected were thoroughly washed with deionized water in order to remove surface contamination. Each apple was cut longitudinally into four equal wedges and the core tissue was removed. The samples with an amount of 1 000 g for apple flesh from mixed wedges were ground to be homogeneous and digested with a mixture of 15 mL HNO_3 and 3 mL $HClO_4$ in 50 mL beakers. The digested solution was evaporated for dryness, and then the residue was dissolved by concentrated HCl (36%, v/v) after being cooled to room temperature. The solution was filtered through No. 5 Watman filter paper and then transferred to a 50 mL volumetric flask for analysis. The concentration of Ca in aqueous solutions was analyzed by ICPS inductively coupled plasma spectrometer 9000 (JARRLL-ASH, USA).

2.3 Soil characteristic assay

pH is measured using a pH meter with a glass electrode; available N was measured by titrating with H_2SO_4 solution using the boric acid indicator; a sensitive spectrophotometric assay was developed for available P based on their binding with molybdenum-antimony (Li, 2006); available K was extracted by 1 mol/L neutral NH_4Ac for half an hour, and filtered suspension was determined with a flame photometer using a lithium internal standard (Walker and Barber, 1962); organic matter was determined by a simple and fast methodology based on a solvent extraction followed by a ultraviolet (UV) spectrophotometry analysis (Jourdan et al., 2003); exchangeable Ca and Mg were extracted by 1 mol/L neutral NH_4Ac and determined by using inductively coupled plasma spectrometer (ICPS-9000) (JARRLL-ASH, USA), and soil was digested by HF-$HClO_4$ and analyzed for total Ca, total Mg and Na by using ICPS-9000 (JARRLL-ASH, USA).

2.4 Data analysis

A randomized complete block design was adopted with six replications and analysis of variance (ANOVA) of the main effects was obtained using SPSS (Version 10.0). All presented data from effects of Ca fertilization are the means of three fruits per treatment.

3 Results

3.1 Effects of different fertilization regimes on Ca concentration of Fuji apple fruits

The application of the increasing rates of $CaSO_4$ to the soil in the first month after full bloom increased fruit Ca concentration in Fuji apple (Group I in Table 1). In parallel with an increase in $CaSO_4$ rates, the fruit Ca concentration increased, then decreased when the $CaSO_4$ amount was more than 3.5 kg per tree, which was consistent with the exchangeable Ca in soil. The highest fruit Ca concentration was found in Fuji apple trees treated with $CaSO_4$ 3.5 kg/tree, which was 42.85% higher than that of the control trees.

The application of the increasing rates of $CaSO_4$ to the soil in the first month after full bloom in combination with amino acid Ca spraying at the same time significantly improved the

Table 1 Effects of different fertilization regimes on calcium concentration in apple fruit

Group I		Group II		Group IV		Group V	
Treatment	Ca/(mg·kg^{-1}FW)	Treatment	Ca/(mg·kg^{-1}FW)	Treatment	Ca/(mg·kg^{-1}FW)	Treatment	Ca/(mg·kg^{-1}W)
Control	108.02fE	$S_{0.5}A^z$	127.96eE	S_0M^p	110.85eE	$S_{2+1.5}$	166.94eD
$S_{0.5}^y$	116.53eD	$S_{1.5}A$	142.19bB	$S_{0.5}M$	110.95eE	$S_{0+3.5}$ A	160.23fE
$S_{1.5}$	121.24dC	$S_{2.5}A$	164.38aA	$S_{1.5}M$	116.21dD	$S_{0+3.5}M$	173.31cB
$S_{2.5}$	135.61cB	$S_{3.5}A$	136.78cC	$S_{2.5}M$	143.06bB	$S_{0+3.5}$ AM^r	167.42eD
$S_{3.5}$	154.35aA^x	$S_{4.5}A$	130.54dD	$S_{3.5}M$	149.16aA	$S_{2+1.5}A$	177.69bA
$S_{4.5}$	137.96bB	–	–	$S_{4.5}M$	136.59cC	$S_{2+1.5}M$	170.98dC
–	–	–	–	–	–	$S_{2+1.5}AM$	179.17aA

Note: ^{x} means each point represents the mean of three replications; means within a column with the same small letters, capital letters are not significantly different by Duncan's multiple range test at the 5% and 1% levels, respectively; ^{y} means S representing $CaSO_4$ only; ^{z} means SA representing $CaSO_4$ in combination with amino acid Ca; ^{p} means SM representing $CaSO_4$ in combination with organic fertilizer; and ^{r} means SAM representing $CaSO_4$ in combination with amino acid Ca and organic fertilizer.

fruit Ca concentration for Fuji apple (Group II in Table 1). It was proved that $CaSO_4$ (2.5 kg/tree) plus amino acid Ca was the most effective treatment in terms of the increasing fruit Ca concentration than others, which had 21.2% of Ca higher than the treatment of 2.5 kg $CaSO_4$ per tree. The fruit Ca concentration changed in a similar pattern to that of Group I in response to Ca-treatment, which also decreased when the $CaSO_4$ amount applied was more than 3.5 kg/tree per tree.

Compared with Group II, Group IV replaced amino acid Ca with 5 kg organic fertilizer per tree on Fuji apple trees (Group IV in Table 1). It was found that the increment of fruit Ca concentration was less than that in Group II.

In annual fertilization regime, it was observed that the same dose of $CaSO_4$, divided into two applications, with the first application in the first month after full bloom and the second in the second month after full bloom in summer, was more effective on increasing the fruit Ca concentration (Group V in Table 1). The significant increment of fruit Ca concentration in Fuji apple trees treated with the same dose of $CaSO_4$ divided into two applications, in combination with amino acid Ca spraying in the first month after full bloom, was observed. The Fuji apple trees treated with 2 kg $CaSO_4$ and amino acid Ca spraying in the first month after full bloom in combination with 1.5 kg $CaSO_4$ in the second month after full bloom in summer, yielded 177.69 mg/kg FW of fruit Ca, which was 29.9% and 10.9% higher than those treated with the whole dose in the first month after full bloom, or in the second month after full bloom in summer in combination with amino acid Ca spraying in the first month after full bloom, respectively. The same dose divided into two applications, in combination with organic fertilizer in winter was also helpful in improving the fruit Ca concentration of Fuji apple trees, with the highest Ca concentration in trees treated with 2 kg $CaSO_4$ in the first month after full bloom, and 1.5 kg $CaSO_4$ in the second month after full bloom in summer, in combination with amino acid Ca spraying in the first month after full bloom and 5 kg organic fertilizer per tree in winter.

As indicated above, each fertilization regime increased the fruit Ca concentration of Fuji apple trees, however, the organic fertilizer alone, or a low amount of $CaSO_4$ was less effective on the increment of fruit Ca concentration. The application in summer was better than that in the first month after full bloom, when $CaSO_4$ was applied alone. In combination with both 5 kg organic fertilizer per tree in winter, and amino acid Ca spraying in the first month after full bloom, the application had a good effect on increasing fruit Ca concentration, when the same dose of $CaSO_4$ was divided into two applications, with the first application in the first month after full bloom and the second in the second month after full bloom in summer.

3.2 Interactive effects of special organic fertilizer for apple and $CaSO_4$ on soil Ca and fruit Ca level

To investigate the effect of special-for-apple organic fertilizer (Group Ш) on total Ca, exchangeable Ca in soil, and fruit Ca of Fuji apple trees, it was individually applied at different rates in winter (Table 2). There were statistically significant differences between treatments in terms of total Ca, exchangeable Ca in soil and fruit Ca. However, Fuji apple yielded at a somewhat less Ca level in fruits when applied with excessive organic fertilizer, even if the organic fertilizer was made special for apple.

According to the interaction of organic fertilizer and $CaSO_4$ in Fuji apple orchards, the organic fertilizer was effective on improving the Ca concentration regardless of fruits and soils. Concomitant with increasing rates of organic fertilizer to the soil in winter, total Ca level in soil began to increase, whereas the exchangeable Ca concentration in soil and the fruit Ca concentration responded differently. Similar to Fuji trees treated with $CaSO_4$ alone, both the highest exchangeable Ca in soil and the highest fruit Ca were observed in those treated with 5 kg organic fertilizer plus 3.5 kg per tree. The fruit Ca concentration of Fuji apple trees treated with 10 kg organic fertilizer alone turned out to be the highest, statistically significantly higher than that treated with 10 kg organic fertilizer plus different rates of $CaSO_4$. However, there were no progressive differences in the exchangeable Ca among the Fuji apple trees treated with 10 kg organic fertilizer plus different rates of $CaSO_4$ in soil in winter season. Compared with less amounts of organic fertilizer, 15 kg organic fertilizer was still effective on improving the exchangeable Ca, while a remarkable decrease in the exchangeable Ca level was observed in Fuji apple trees treated with 15 kg organic fertilizer in addition to different rates of $CaSO_4$. On the contrary, the fruit Ca was significantly lower

Table 2 Effects of special organic fertilizer for apple in combination with $CaSO_4$ on soil calcium and fruit calcium level

Treatment	Total soil Ca/(g·kg^{-1})	Exch. Ca /(g·kg^{-1})	Ca in fruit /(mg·kg^{-1} FW)	Treatment	Total soil Ca/(g·kg^{-1})	Exch. Ca /(g·kg^{-1})	Ca in fruit /(mg·kg^{-1} FW)	Treatment	Total soil Ca/(g·kg^{-1})	Exch. Ca /(g·kg^{-1})	Ca in fruit /(mg·kg^{-1} FW)
M_5[y]	6.11fF	4.02dDE	110.85eE	M_{10}	6.69eE	4.86a	165.38aA	M_{15}	7.45eE	5.31aA	126.74aA
$M_5S_{0.5}$[p]	6.42eE	4.18cdCD	110.95eE	$M_{10}S_1$	7.82dD	4.89a	161.57bB	$M_{15}S_{0.5}$	7.89dD	5.21bB	124.43bAB
$M_5S_{1.5}$	7.29dD	4.31bcBC	116.21dD	$M_{10}S_2$	8.09cC	4.87a	155.08cC	$M_{15}S_{1.5}$	8.34cC	5.18bB	123.97bAB
$M_5S_{2.5}$	7.42cC	4.56aAB	143.06bB	$M_{10}S_3$	8.59bB	4.88a	151.45dD	$M_{15}S_{2.5}$	8.79bB	5.08cC	123.07bcB
$M_5S_{3.5}$	7.82bB	4.61aA	149.16aA	$M_{10}S_4$	9.21aA	4.91a	142.52eE	$M_{15}S_{3.5}$	9.45aA	4.94dD	121.92cB
$M_5S_{4.5}$	8.64aA[x]	4.41abABC	136.59cC	–	–	–	–	Control	5.41fF	3.81eE	108.02dC

Note: [x] means each point represents the mean of three replications; means within a column with the same small letters, capital letters are not significantly different by a Duncan's multiple range tests at the 5% and 1% levels, respectively; [y] means M representing organic fertilizer; [p] means MS representing $CaSO_4$ in combination with organic fertilizer.

in Fuji apple trees treated with 15 kg organic fertilizer than less amounts of organic fertilizer, and kept unchanged when in combination with different rates of $CaSO_4$.

To investigate the negative effect of excessive fertilizing on fruit Ca, some factors influencing Ca accumulation in soil treated with different amounts of organic fertilizer were analyzed. It was observed that there were no remarkable differences in available N, Na^+, HCO_3^-, SO_4^{-2}, Cl^- (data omitted), while significant increment in available P, K and exchangeable Mg were estimated on Fuji apple orchard treated with the organic fertilizer, especially the 15 kg/tree treatment (Table 3). Surprisingly, no markeble increase in Ca concentration of Fuji apple was observed in 15 kg organic fertilizer group, which was possibly attributed to the interactions between available P, K and Ca in soil.

The univariate analysis showed that there were positive correlations between the exchangeable Ca ($r = 0.77^*$, $P < 0.05$), Ca/Mg in soil, and Ca/Mg and fruit Ca, while negative corrrlations were observed between the available N, P ($r = -0.893^*$, $P < 0.05$), K ($r = 0.822^*$, $P < 0.05$), the exchangeable Mg in soil and the fruit Ca.

3.3 Effect of special organic fertilizer for apple in combination with $CaSO_4$ on Fuji storage quality

Generally, carbohydrate storage in the stem and root during the winter is essential for the development and productivity of deciduous trees, mainly because it is a main source for vegetative and reproductive growth occurring during the spring season (Stephenson et al., 1989). Ca nutrition is similar to carbohydrate storage, and a prerequisite for an annual, high quality apple fruit without physiological disorders is an adequate amount of Ca. As shown in Table 4, applying the organic fertilizer in combination with $CaSO_4$ remarkably improved Ca concentration in roots and stems of Fuji apples, with the best result obtained in the apple trees treated with organic fertilizer 10 kg plus $CaSO_4$ 1 kg per tree, which was 27.54% and 32.46% higher than that of the control trees, respectively.

After harvest, all apples treated with the organic fertilizer and $CaSO_4$ in Group V were stored in a commercial cold station for four months. It was estimated that in the storage period there was an extremely high bitter pit incidence, which rapidly increased as storage time prolonged, although large amounts of diseased Fuji apple fruits were present in the first month (November), and gradually decreased afterward. The bitter pit incidence responded to a low fruit Ca concentration when fruits were harvested, and it was indicated that there was a significantly negative correlation between them ($r = -0.967^*$, $P < 0.05$). Fuji apple trees treated with the organic fertilizer 10 kg plus $CaSO_4$ 1 kg per tree exhibited the lowest bitter pit incidence at the end of storage time, the second lowest was those treated with $CaSO_4$ 3.5 kg/tree, while the highest bitter pit incidence was observed in control trees.

It was observed that there was a significantly positive correlation between N/Ca ratio in fruit and bitter pit incidence ($r = 0.984^*$, $P < 0.05$; Table 4). Statistically significant differences in N/Ca ratio were estimated in November, and the lowest ratio turned out to be in Fuji apple trees treated with 10 kg organic fertilizer plus 1 kg $CaSO_4$ per tree, with 1.8% of bitter pit incidence. Both the control trees and those treated with 15 kg organic fertilizer and 0.5 kg $CaSO_4$ per tree proved to have the highest N/Ca ratio and bitter pit incidence, which were both more than 33.16%, respectively. The N/Ca ratio gradually decreased as storage life prolonged, and the N/Ca ratio of Fuji apple with higher Ca concentration decreased more slowly than that of fruits with less Ca, for example the two treatments of $M_{10}S_1$, $M_{15}S_{0.5}$, whose N/Ca ratio in apple fruits decreased by 47.6% and 59.2%, respectively.

Table 3 Factors influencing fruit Ca accumulation in soil after supplementing organic fertilizers

Treatment	pH	Available N /(mg·kg^{-1})	P_2O_5/(mg·kg^{-1})	K_2O/(mg·kg^{-1})	Organic matter /%	Exchangeable Mg/(g·kg^{-1})	Exchangeable Ca/(g·kg^{-1})	Ca/Mg in Soil	Ca/Mg in Fruit
M_5	7.24aA^x	123.6aA	90.19cC	115.2cC	1.23cC	0.75cC	4.024cC	5.36bB	5.88cC
M_{10}	7.21Bb	125.6aA	105.47bB	136.7bB	1.35bB	0.83bB	4.862bB	5.85aA	10.18aA
M_{15}	7.18cC	127.5aA	138.56aA	164.5aA	1.54aA	0.90aA	5.319aA	5.91aA	7.32bB

Note: ^{x} means each point represents the mean of three replications; means within a column with the same small letters, capital letters are not significantly different by Duncan's multiple range test at the 5% and 1% levels, respectively; ^{y} means M represents organic fertilizer.

Table 4 Factors influencing incidence of apple bitter bit disease during fruit storage

Treatment	Root Ca /(g·kg^{-1}FW)	Stem Ca /(g·kg^{-1}FW)	Fruit Ca /(mg·kg^{-1}FW)	N/Ca		Ca/Mg		Apple bitter bit percentage /%		
				Nov.	Jan.	Nov.	Jan.	Nov.	Jan.	Feb.
Control	5.596dD	13.831dD	108.02	33.86	10.31	5.33	8.39	18.7	27.9	30.2
$S_{3.5}{}^y$	6.467bB	16.916bB	154.305	17.55	8.23	6.45	10.67	4.2	6.1	6.3
$M_5S_{3.5}{}^z$	6.475bB	15.921cC	149.165	18.74	9.26	5.79	9.95	6.7	9.4	10.6
$M_{10}S_1$	7.137aA^x	18.319aA	161.576	16.93	8.87	6.79	11.31	1.8	4.1	4.6
$M_{15}S_{0.5}$	6.091cC	13.903eE	124.436	32.89	13.42	5.45	9.01	16.1	21.7	22.8

Note: ^{x} means each point represents the mean of three replications; means within a column with the same small letters, capital letters are not significantly different by Duncan's multiple range test at the 5% and 1% levels, respectively; ^{y} means S representing $CaSO_4$; ^{Z} means MS representing $CaSO_4$ in combination with organic fertilizer.

Supplementary $CaSO_4$ alone, or in combination with organic fertilizer significantly increased the Ca/Mg ratio in Fuji apple fruit, with a better result in $CaSO_4$ 3.5 kg/tree treatment, or $CaSO_4$ 1.0 kg plus organic fertilizer 10 kg per tree treatment, with an unacceptable result observed in $CaSO_4$ 0.5 kg plus organic fertilizer 15 kg per tree. Fuji apple fruit which had a higher Ca/Mg ratio definitely had lower bitter pit incidence.

4 Discussion

Environmental factors such as soil types, nutrient levels and weather patterns influenced the uptake and distribution of calcium (Bartz et al., 1992). It was known that total Ca and exchangeable Ca content in soil varied with soil types and soil characteristics (Yang et al., 1998; Zheng et al., 2005a) and monitoring soil Ca availability is fundamental for apple production. The lowest total Ca and exchangeable Ca contents were observed in Burozem soil (Zheng et al., 2005a), which was dominant in the northeast part of Shandong Province, a main apple production zone in China, so apple trees in many orchards over there were susceptible to Ca deficiency when the trees are old and the fruit load is too high. In our experiment, the application of $CaSO_4$ and organic fertilizer with a high content of Ca which was special for apple significantly improved the total Ca content in soil, however, there was no linear relationship between the exchangeable Ca content and the increasing rates of $CaSO_4$ to the soil. In some cases, large doses of Ca fertilizer application such as $CaSO_4$ 4.5 kg/tree or organic fertilizer 15 kg/tree were less effective on the increment of fruit Ca concentration than small doses such as $CaSO_4$ 3.5 kg/tree or organic fertilizer 10 kg/tree, which seems to be due to the interactions, precipitation between Ca and N, P, K, S and other ions, because the increasing ionic strength could reduce the activity of calcium (Grattan and Grieve, 1992).

Calcium is an essential element involved in cell division, elongation and fruit growth (Ehret and Ho, 1986), in addition to having a positive effect on fruit quality criteria such as storage ability and firmness (Bangerth, 1979). Disturbances in calcium nutrition may result in the appearance of characteristic symptoms in fruits of various species, such as bitter pit in apple fruits (Shear, 1975). It was well known that Ca deficiency in soil could result in a less Ca concentration in fruits (Zheng et al., 2005a), but was not the only reason. Nowadays extremely high N/Ca caused by excessive N fertilizer application is thought to be one of the important reasons for bitter pit incidence (Shu, 1997; Yang et al., 1998; Zhou et al., 2000; Casero et al., 2004). Not only a significantly negative correlation between N content and Ca content, but also a significantly positive correlation between N content and P content, K content and Mg content can be observed in fruit (Casero et al., 2004). To our knowledge, apple trees in many orchards were more susceptible to bitter pit as increasing rates of N/Ca in the fruit. This agrees with what has been suggested by Faust and Shear (1972) who considered that fruits with N/Ca <10 would not exhibit the symptoms of bitter pit, while they could not be stored commercially because of serious deterioration of bitter pit when N/Ca>30. Zhou et al. (2000) insisted that Ca spraying could decrease the N/Ca ratio in Fuji apple to 5, and bitter pit incidence to 8.8%. In our experiment, the bitter pit incidence at the end of storage remained acceptable ($<5\%$) when the N/Ca ratio was less than 17, while it would be higher than 10% when the N/Ca ratio was >18. It was consistent with the findings by Faust and Shear (1972) and Zhou et al. (2000). However, forthcoming efforts about N/Ca and bitter pit incidence should also take cultivars and testing environmental characteristics into consideration, such as processing and storage conditions. It was also found that a higher bitter pit incidence and N/Ca ratio happened in our experiment, when the excessive special-for-apple organic fertilizer was applied, although the increased total Ca content and the increased exchangeable Ca were observed in the orchard containing a high amount of N in Burozem soil.

Among other minerals, Mg is one of the very important factors that are related to bitter pit in fruits (Martin et al., 1960; Burmeister and Dilley, 1994; Wang et al., 2001), and it was observed that the Mg^{2+} concentration in the outer flesh of diseased fruits was significantly higher than that of non-diseased fruits (Wang et al., 2001). According to the increment of bitter pit incidence by dipping apple fruits in solutions containing Mg^{2+}, it was indicated that there was a negative correlation between Mg and Ca, that is to say, a low Ca/Mg ratio inhibited the Ca accumulation in fruits (Burmeister and Dilley, 1994; Liu et al., 1998; Wang et al., 2001; Bian et al., 2002). It was suggested that the exchangeable Ca/Mg ratio should be at least higher than 6 if a commercial yield with high quality fruits free from bitter pit was going to be obtained according to the research of fertilizer effect on Fuji apple using the method recommended by Agro Service International Inc. (ASI) (Bian et al., 2002). Although the exchangeable Ca/Mg in the Burozem soil having different fertilizing treatments was less than 6 in our experiment, it was suggested that the exchangeable Ca/Mg should be more than 8 in case of Ca deficiency, because the exchangeable Ca/Mg ratio in other soils rich in Ca was more than 8 (Zheng et al., 2005a), and Fuji is a cultivar which needs much Ca nutrition during growth developments. Fuji fruits had a very low bitter pit incidence when Ca/Mg>7, and N/Ca<17, so it was thought that the bitter pit incidence and Ca/Mg, N/Ca in Fuji fruits were closely correlated, and therefore, the Ca/Mg and N/Ca criteria over predicting bitter pit incidence needed to be defined in future work. There were some other findings in our experiment, for example, besides N and Mg, the available P, K also had a remarkably negative effect on Ca accumulation, which gave a better perception of fertilizing balance.

Measuremental method and timing of Ca fertilizer will help answer our questions as to how effective a particular treatment is for increasing Ca concentration in the tree and when to apply it (Wojcik, 2001a). Many researchers reported that spring is the most effective season for increasing Ca content in leaves and fruits (Zhou et al., 2000). According to this theory, Ca spraying in the first month after full bloom should be put to use in main apple production areas and no more Ca fertilizers are used in summer. In our experiment, summer application of Ca in soil effectively increased fruit Ca concentration, and the summer application was thought to be better than that in the first month after full bloom when the same doses were applied, maybe the increase in Ca concentration was related with the strong absorptive capacity of Fuji apple root in summer. It was consistent with a previous finding that Fuji apple has a climactic absorption of Ca in summer, which is even stronger than that in the first month after full bloom (Zheng et al., 2005b). Much research has proved that frequent application of calcium chloride ($CaCl_2$) after full bloom may increase fruit concentration (Wojcik, 2001b; Bian et al., 2002; Kadir, 2004), and more than six applications of calcium chloride on Jonathan apple trees improved the fruit quality at harvest, including an increase in fruit weight, size, appearance, skin redness and reduction of physiological disorders, such as bitter pit (Kadir, 2004). Other researchers suggested that injecting citric acid plus IAA into stems before full bloom or harvest was more effective than other treatments in improving the fruit Ca concentration (Bian et al., 2002). However, each of the current methods, as improving Ca level, also has disadvantages. For example, foliar Ca spray: 1) the response is only temporary; 2) only very low doses can be applied; and 3) there are limitations due to foliar toxicity. The disadvantages of injection method are that the technique is critical to correct application, repeated applications may cause coalesces and overdoses may cause a severe damage to the cambium and xylem. Thus multiple fertilization formula using many fertilizing methods should be put to practical use in control of physiological disorders. In our experiment, Fuji trees treated with $CaSO_4$ 2 kg in the first month after full bloom in spring + $CaSO_4$ 1.5 kg in summer + amino acid Ca foliar spray + organic fertilizer, exhibited the highest Ca concentration, which was thought to be the best treatment for improving Ca concentration, if it was taken into consideration that the organic fertilizer not only affected the soil physicochemical characteristics, but also improved the store carbohydrates in the trees. It was recommended that the commercial annual fertilization formula was organic fertilizer + $CaSO_4$ in soil in winter or next spring + foliar Ca in spring + $CaSO_4$ in soil in summer, which could combine winter and spring applications to one time for reducing labor cost.

5 Conclusion

Foliar Ca spray in one month after full bloom in combination with applying $CaSO_4$ 3.5 kg into soil in summer can significantly increase the fruit Ca concentration. Applying $CaSO_4$ in summer is more effective than that in spring. The significant increment of fruit Ca concentration in Fuji apple trees treated with the same dose $CaSO_4$ divided into two applications, with the first application in the first month after full bloom and the second in summer, in combination with the amino acid Ca spray in spring, or the organic fertilizer application in winter was observed. The highest Ca concentration was observed in trees treated with 2 kg $CaSO_4$ in spring, 1.5 kg $CaSO_4$ in summer, in combination with amino acid Ca spray in spring and 5 kg organic fertilizer per tree in winter.

The addition of the special organic fertilizer for apples significantly increased Ca concentration in fruits, roots and stems. The best result was estimated in "Fuji" apple trees treated with organic fertilizer 10 kg plus 1 kg $CaSO_4$ per tree.

There are positive correlations between fruit Ca and exchangeable Ca, total Ca in soil, Ca/Mg in fruits, and negative correlations between fruit Ca and available P, K in soil, N/Ca ration in fruits. A bitter pit incidence at the end of storage keeps acceptable when $N/Ca < 17$ and $Ca/Mg > 7$ at harvest time.

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References

Bangerth F (1979). Calcium-related disorders of plants. Annual Review of Phytopathology, 17: 97–122

Bartz J, Locascio S, Weingartner D (1992). Calcium and potassium fertilization of potatoes grown in North Florida. Effect on the bacterial soft rot potential in the tubers. American Potato Journal, 69: 39–50

Bian S F, Sun J J, Zhang J G, Liu Y F (2002). Study on the effects of citrate calcium on the quality and calcium concentrations of "Red Fuji" apples. Journal of Agricultural University of Hebei, 25(3): 37–40 (in Chinese)

Bramlage W, Drake M, Weis S (1985). Comparisons of calcium chloride, calcium phosphate, and a calcium chelate as foliar sprays for "McIntosh" apple trees. Journal of the American Society for Horticultural Science, 110: 786–789

Burmeister D M, Dilley D R (1994). Correlation of bitter pit on northern spy apples with bitter pit like symptoms induced by Mg^{2+} salt infiltration. Postharvest Biology and Technology, 4(4): 301–308

Casero T, Benawides A, Puy J, Recasens I (2004). Relationships between leaf and fruit nutrients and fruit quality attributes in Golden Smoothee apples using multivariate regression techniques. Journal of Plant Nutrition, 27(2): 313–324

Ehret L D, Ho C L (1986). Translocation of calcium in relation to tomato fruit growth. Annals of Botany, 58(5): 679–688

Faust M, Shear C B (1972). The effect of calcium on respiration of apples. Journal of American Society Horticultural Science, 97: 437–439

Gallerani G, Pratella G, Bertolini P, Marchi A (1990). Lack of relationship between total calcium of apple fruit and a calcium deficiency related disorder (Bitter pit): A four year report. Acta Horticulturae, 274: 141–148

Grattan S, Grieve C (1992). Mineral element acquisition and growth response of plants grown in saline environments. Agriculture, Ecosystems, and Environment, 38: 275–300

Jourdan B, Ollivier J, Thomas O (2003). Determination of organic matter in grinding sludges by UV spectrophotometry. Environmental Technology, 24(5): 597–603

Kadir S A (2004). Fruit quality at harvest of "Jonathan" apple treated with foliarly-applied calcium chloride. Journal of Plant Nutrition, 27(11): 1991–2006

Li Y (2006). Some questions in determining total phosphorus by ammonium molybdate spectrophotometric method. Instrumentation Analysis Monitoring, 4: 40–41 (in Chinese)

Liu G D, Yang L, Song G H, Quan W J, Lu G J (1998). The effect of macronutrients Ca, Mg and S on apple. Journal of Shandong Agricultural Science, 5: 32–34 (in Chinese)

Lougheed E C, Murr D P, Miller S R (1979). Effects of calcium and daminozide on ethylene production and softening of apple fruits. Cellular and Molecular Life Sciences, 35(1): 43–44

Martin D, Lewis T L, Cerny J (1960). Bitter pit in the apple variety Cleopatra in Tasmania in relation to calcium and magnesium. Australian Journal of Agricultural Research, 11(5): 742–749

Scarim A L, Arnush M R, Hill J, Marshall C A, Baldwin A L, McDaniel M, Corbett J A, Hofman P J, Smith L G, Joyce D C, Johnson G I, Meiburg G F (1997). Bagging of mango (*Mangifera indica* cv. "Keitt") fruit influences fruit quality and mineral composition. Postharvest Biology and Technology, 12: 83–91

Schulz D G, Kopke U (1996). Effects of intensified mineral and organic fertilizer and biodynamic preparations on yield, quality and shelf life of potatoes. In: Raupp J, ed. Main Effects of Various Organic and Mineral Fertilization on Soil Organic Matter Turnover and Plant Growth. Darmstadt: the Institute for Biodynamic Research, 5: 42–47

Shear C B (1975). Calcium-related disorders of fruits and vegetables. Hortscience, 10: 361–365

Shu H R (1997). The Pomologic Cultivation Physiology. Beijing: Chinese Agricultural Press, 94–99 (in Chinese)

Stephenson R A, Rasmussen T S, Gallagher E C (1989). Timing of nitrogen application to macadamias 2. Storage carbohydrates. Australian Journal of Experimental Agriculture, 29(4): 575–579

Stiles W C (1994). Nitrogen management in the orchard. In: Peterson A B, Stevens R G, eds. Tree Fruit Nutrition. Yakima, WA: Good Fruit Grower, 41–50

Walker J M, Barber S A (1962). Absorption of potassium and rubidium from the soil by corn roots. Plant and Soil, 17(2): 243–259

Wang L J, Jiang W B, He Q F, Fan H B (2001). Studies on the relationship of the development of bitter pit in apple fruits with the contents of calcium and magnesium and the activities of antioxidant enzymes. Acta Horticulturae Sinica, 28(3): 200–205 (in Chinese)

Witney G W, Kushad M M, Barden J A (1991). Induction of bitter pit in apple. Journal of American Society of Horticultural Science, 47: 173–176

Wojcik P (2001a). Effect of calcium chloride sprays at different water volumes on "Szampion" apple calcium concentration. Journal of Plant Nutrition, 24(4&5): 639–650

Wojcik P (2001b). "Jonagold" apple fruit quality as influenced by fall sprays with calcium chloride at high rates. Journal of Plant Nutrition, 24(12): 1925–1936

Yang L, Liu G D, Song G H, Quan W H, Lu G J, Wan L B (1998). Exchangeable calcium content in soil and distribution in Shandong Province. Journal of Shandong Agricultural Science, 4: 17–21 (in Chinese)

Zheng W W, Li R C, Zhao S X, Qin D, Zhai H (2005a). Apple calcium content in relation to calcium levels in soil types cultivated. Deciduous Fruits, 37(3): 1–3 (in Chinese)

Zheng W W, Zhai H, Xu Y H, Zhang J, Wang X F (2005b). Analysis of the changes of calcium content of several apple cultivars in developing season. Scientia Agricultura Sinica, 38(11): 2296–2300 (in Chinese)

Zhou W, Li S T, Lin B, Zhang X S, Lin J X, Xie Z X (2000). The physiological influence of spray Ca on apple. Soil and Fertilizer Sciences in China, 6: 25–28 (in Chinese)