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Effects of Spraying Calcium Fertilizer on Photosynthesis, Mineral Content, Sugar–Acid Metabolism and Fruit Quality of Fuji Apples

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Abstract: This study was conducted at the Taidong Base of the Shandong Institute of Pomology, Tai'an City, Shandong Province, China, from May to October 2020. The effects of spraying calcium fertilizer on the photosynthetic characteristics, mineral element content, sugar and acid metabolism, and quality of apples were assessed. Fuji Apple Tianhong.2/SH/Malus robusta (*M. robusta*) was treated with the calcium fertilizers Niucui (Ca $\geq$ 100 g/L, B: 1–10 g/L) and Naipu 9 (Ca: 90 g/L, Mg: 21 g/L, N: 110 g/L) at the young fruit stage (25 May), the expansion stage (5 July), and the coloring stage (25 September). Water was sprayed as the control application. The results revealed that spraying calcium fertilizer increased the chloroplast pigment content and photosynthetic capacity of the apple leaves. Compared with the control, the net photosynthetic rate of apple leaves sprayed with Niucui increased by 4.3–34.6%, and that of leaves sprayed with Naipu 9 increased by 15.0–57.4%. Spraying calcium fertilizer promoted the accumulation of Ca, Mg, and B mineral elements in leaves and fruits while inhibiting the accumulation of Cu. Spraying calcium fertilizer improved the quality of apple fruit. Compared to the control fruit, the single fruit weight, fruit hardness, soluble solid content, and Vitamin C content of fruit sprayed with Niucui and Naipu 9 increased by 6.5 and 12.1%, 3.6 and 16.1%, 6.3 and 12.0%, and 30.5 and 29.4%, respectively. Spraying calcium fertilizer increased the sugar content and decreased the acid content of the apples. Naipu 9 was more effective at increasing fruit sugar content, especially sucrose and sorbitol, while Niucui was more effective at reducing the acid content, especially malic acid. This was observed because Naipu 9 increased the sugar metabolism enzyme activity, while Niucui increased the acid decomposition-related enzyme activity. In conclusion, according to the nutrition quality and flavor of the fruit, Naipu 9 application was determined to be the best fertilizer for the Fuji apple.

Keywords: calcium fertilizer; fruit quality; Fuji; mineral elements; photosynthesis; sugar–acid metabolism

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1. Introduction

Apples are one of the most important fruits in China. Among the apples in the fruit agriculture industry, the planting area of the Fuji apple accounts for more than 70%, with outputs accounting for more than 80% [1]. In production, Fuji apples are mainly cultivated using the bagging method, and the calcium (Ca) deficiency phenomenon is increasingly prominent [2,3]. In addition, extensive management, excessive load, early harvest, etc. [4,5] further aggravate diseases such as bitter pox, water heart disease, and fruit cracking during fruit harvest and storage [6–8]. These plights seriously affect the commodity value of the fruit [9,10].

Applying calcium fertilizer is an effective measure to counteract the aforementioned problems [7,11,12]. Ca is known as "lazy Ca" because its characteristics are not active. If the soil is fertilized, it will be absorbed by the root system and transported to the fruit by the root system, but this takes too long to meet the Ca demand of the fruit during its growth and development. The foliar spraying method helps the fruit absorb calcium more quickly [13,14]. With the advantages of fast nutrient absorption, good fertilizer effect, strong pertinence, and convenient fertilization, it has gradually developed into an important fertilization technique in agricultural production [15–17].

Calcium fertilizer not only improves the photosynthetic and growth rates of plant leaves [18–20], but also significantly improves the fruit quality of some fruit trees [11,21,22], and improves the storage resistance of fruit [23,24]. Recent literature found that the effects of calcium fertilizer on apples are mainly focused on methods aimed at prevention and treatment of physiological diseases through application of exogenous Ca [10–12]. The impacts of exogenous Ca application on fruit quality have been assessed, but few studies exist reporting the changes of sugar and acid accumulation and the mechanisms impacted [25]. This paper analyzes spraying calcium fertilizer on apple trees and its effects on photosynthetic function, mineral element absorption, sugar and acid metabolism, and fruit quality of the Fuji apple. This study also explores the mechanisms of calcium fertilizer in improving apple quality, and provides theoretical bases for the rational application of leaf calcium fertilizer.

2. Materials and Methods

2.1. Basic Information of Test Site

The experiment was conducted in the Taidong base of the Shandong Institute of Pomology in 2020 (36°11′07″ N, 117°06′51″ E) The area belongs to temperate continental semi-humid monsoon climate; in 2020, the annual rainfall was 1035.7 mm, the average temperature was 14.5 °C, and the annual total sunshine was 3247.9 h [26]. The garden is a plain orchard with sandy loam soil and medium cultivation and management levels. The tested variety was an 11-year-old Red Fuji apple (Tianhong.2/SH/*M. robusta*), with north–south directionality and a plant row spacing of 1.5 m × 3 m. The tree shape was a small crown, sparse layer-type. It was healthy, with normal growth and fruit, and conventional fertilization and watering. In late November of last year, sufficient base fertilizer (8325 kg/km^2) was applied, urea (1665 kg/km^2 in late March) was applied before flowering (early April), and N, P, and K compound fertilizers (832.5 kg/km^2) were applied in late June. After base fertilizer and topdressing, water was applied once,, and no tillage technology of intercropping grass (clover) was implemented. Flowers and fruits were thinned manually at flowering and young fruit stages according to the load of 60,000 kg/km^2 (135–150 fruits/tree) [5]. For the experiment, plants with the same tree strength and uniform fruit bearing capacity (about 140 fruits/tree) were selected, and the bagging cultivation mode was used. The conventional pest control mainly includes the control of aphids in late April, the spraying of insecticides and fungicides once before bagging (late May), and the control of fruit rot in early October (after bagging). The fruits were bagged on 15 June and harvested on 27 October.

2.2. Test Design

The two calcium fertilizers tested are commercial foliar fertilizers, commonly known as medium element water-soluble fertilizers: Niucui (USA, Ca ≥ 100 g/L, B: 1–10 g/L) and Naipu 9 (Qingdao, China, Ca: 90 g/L, Mg: 21 g/L, N: 110 g/L). In the text, they are simplified as "Niucui" and "Naipu 9", which were provided by Stoller Agricultural Technology Co., Ltd. (Qingdao, China).

Sprayed water served as the control (simplified as "control"), for a total of three treatment groups: Niucui, Naipu 9, and control. Each treatment group comprised three replicates with five trees in each replicate. The foliar fertilizer was diluted 1250 times and sprayed according to the instructions for the use. Treatments were conducted at the young fruit stage (25 May), the expansion stage (5 July), and the coloring stage (25 September). Spraying occurred before 10:00 a.m., and the spraying amount was based on the appearance of dripping water on the surface of leaves or fruits.

Fifteen trees were labelled and treated for each treatment group. On 24 May, 24 June, 28 July, 28 August, 27 September, and 27 October, the photosynthetic related indexes and leaf pigments of the leaves were measured. On 24 June, 28 July, 28 August, 27 September, and 27 October, the same leaves and fruits used for the photosynthetic determination were collected, flash frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.3. Measuring Methods

2.3.1. Measurements of Net Photosynthetic Rate (P_n) and Other Gas Exchange Parameters of Leaves

Net photosynthetic rate (P_n) and other gas exchange parameters were measured using a Ciras-2 portable photosynthetic measurement system (ppsystems, UK). From 9:00–11:00 a.m., the net photosynthetic rate of mature leaves of fruit stand branches, 0.5 m from the crown to the trunk and 1.2 m high, were measured along with other gas exchange parameters (1000 μmol m^{-2} s^{-1}, 25 °C).

2.3.2. Measurements of Chlorophyll and Carotenoid Contents

Chlorophyll (Chl) and carotenoid (Car) contents were taken according to methods from Zhao et al. (2002) [27] with some modifications. First, 0.1 g of leaves were extracted in the dark with 10 mL of 80% (*v*/*v*) acetone at 4 °C for 48 h, with shaking several times during this period. Absorbance (A) of the extraction solution was measured at 663, 646, and 470 nm. The concentrations of Chl and Car in the extraction solution were calculated according to Formulas (1)–(3). In the formulas, Ca, Cb, and Cx·c represent the concentrations of Chl a, Chl b, and Car in mg/L.

$$\text{Ca} = 12.21\text{A663} - 2.81\text{A646} \tag{1}$$

$$\text{Cb} = 20.13\text{A646} - 5.03\text{A663} \tag{2}$$

$$\text{Cx}\cdot\text{c} = (1000\text{A470} - 3.27\text{Ca} - 104\text{Cb})/229 \tag{3}$$

After obtaining the concentration of the pigment, the content of each pigment was calculated according to Formula (4) in mg/g.

$$\text{Chloroplast pigment content} = \text{concentration of pigment (c)} \times \text{extract volume} \times \text{dilution times/sample weight (g)} \tag{4}$$

2.3.3. Determination of Mineral Element Content

The extraction and quantification of mineral elements in leaves and fruits refer to Lu et al. [28]. The contents of Ca, Mg, B, Cu, and Zn were determined by inductively coupled plasma emission spectrometer (ICP-AS) (OPTIMA3300DV, Perkin Elmer, Shelton, CT, USA). The argon flow rate was 15 L/min, and the injection volume was 1.5 mL/min.

2.3.4. Determination of Sugar and Acid Content of Fruit

The content of soluble sugar was determined using the anthrone method [29]. Titratable acid content was determined by acid–base neutralization titration [30].

High performance liquid chromatography (HPLC) was used for the determination of sugar and acid components [31,32]. Samples were weighed to 1–2 g, ground under liquid nitrogen conditions, transferred to a centrifuge tube, and mixed with the extraction reagent. Ultrasonic extraction was performed, and the extraction process was repeated three times for each sample. Centrifugal supernatant was recovered at a constant volume, filtered with a filter membrane, and injected into the instrument for analysis.

Chromatographic conditions for the determination of free sugar were as follows: Shimadzu differential detector (RID-20A, Shimadzu Corporation, Kyoto, Japan); acetonitrile: water as mobile phase; 30 °C; amino column (Inertsil NH2, Shimadzu Corporation, Japan); 1 mL/min flow rate for detection; and 20 μL injection volume. Chromatographic conditions for the determination of organic acids were as follows: Shimadzu liquid phase UV detector (SPD-20A, Shimadzu Corporation, Japan); phosphate solution and methanol as mobile phase; C18 chromatographic column (Shimadzu Corporation, Japan) to detect at a certain gradient mobile phase; 30 °C; 0.4 mL/min flow rate; 210 nm wavelength; and 10 μL injection volume. The contents of sugar and acid components were calculated according to the peak area of the sample and the standard curve.

The reagents involved in the above determination are products of Sigma-Aldrich company.(St. Louis, MI, USA)

2.3.5. Determination of Sugar Metabolism-Related Enzyme Activity in Fruit

The enzyme solution was extracted following Lowell et al. [33], with some modifications. Approximately 1.0 g of fruit sample was ground in an ice bath in a pre-cooled mortar. A total of 5 mL of 100 mmol L^{-1} Tris-HCl (pH 7.0) buffer was added for the extraction, containing 5 mmol L^{-1} $MgCl_2$, 2 mmol L^{-1} EDTA-Na2, 2% ethylene glycol, 2% polyvinylpolypyrrolidone (PVPP), 2% bovine serum protein (BSA), and 5 mmol L^{-1} Dithiothreitol (DTT). Solutions were centrifuged at 10,000 rpm for 20 min at 4 °C. A total of 3 mL of supernatant was transferred to a dialysis bag. Dialysis occurred at a low temperature (2–4 °C) with five-fold diluted extraction buffer (PVPP removal) for 15–24 h. The dialyzed enzyme solution was used in the determination of the activities of sucrose phosphate synthase (SPS), sucrose synthase (SS), and acid invertase (AI). SPS and AI activity determination was performed using the methods from Zhu, Komor, and Moore [34]. The sucrose synthase decomposition direction activity (SS-CD) assay was based on Huber's method [35], and the sucrose synthesis direction activity (SS-SD) assay was based on the methods of Lowell et al. [33] and Hubbard et al. [36].

The extraction and activity determination of sorbitol oxidase (SOX) in the enzyme solution followed the methods in Zhang Xian [37]. The extraction and activity determination of NAD^+—sorbitol dehydrogenase (NAD-SDH) in the enzyme solution followed methods in Sun et al. [38] and Park et al. [39].

The above reagents are the products of Beijing Solaibao Technology Co., Ltd. (Beijing, China).

2.3.6. Determination of Enzyme Activity Related to Acid Metabolism in Fruit

The enzyme solution preparation of the acid metabolism-related enzymes malate dehydrogenase (NAD-MDH), malate enzyme (cyME), and phosphoenolpyruvate carboxylation kinase (PEPCK) referred to the methods of Hirai et al. [40]. NAD-MDH, cyME, and PEPCK activities were determined using methods from Hirai and Luo Ancai [40,41].

2.3.7. Determination of Other Fruit Quality Indexes

During the fruit ripening period, according to many years of experience in local production [42] and the technical specifications for apple picking (NY/T 1086-2006) [43], the fruit development period of 170–185 days, hardness of more than 7 kg/cm^2, soluble solids of more than 13%, and starch index of 2–3, two fruits were picked from each tree from five different directions, including east, west, north, south, and middle of the tree crown, at a height of 120 cm, for a total of 10 fruits per tree. Samples from the six treatments were collected separately and transported back to the laboratory in order to determine other fruit quality indicators.

Single fruits were weighed using an electronic platform scale. The vertical and horizontal diameters of fruits were measured with vernier caliper, and the ratio of the vertical to horizontal diameter indicated the fruit shape index. The firmness of fruit was measured using a GY-1 fruit hardness tester. The soluble solid content (SSC) was measured with a WYT hand-held sugar meter. The color of the fruit surface was determined using a CI-410 color difference meter (CI-410, Konica Minolta, Japan). Vitamin C (VC) content was measured through a 2,6-dichloroindophenol sodium titration [44].

Fruit surface coloring index = $\sum$ (number of fruits at all levels × representative grade value)/(total number of fruits × highest value) × 100%. The color grading standard was as follows: Grade 0, 0–5% fruit surface color; Grade 1, 5–25% fruit surface coloring; Grade 2, 25–50% fruit surface coloring; Grade 3, 50–75% fruit surface coloring; Grade 4, 75–100% fruit surface coloring.

Smoothness index = ∑ (number of fruits at all levels × representative grade value)/(total number of fruits × highest value) × 100%. The smoothness index grading standard was as follows: Grade 0, 0–10% smooth fruit surface; Grade 1, 10–30% smooth fruit surface; Grade 2, 30–60% smooth fruit surface; Grade 3, 60–85% smooth fruit surface; Grade 4, 85–100% smooth fruit surface.

2.4. Statistical Analysis

All experiments were conducted in triplicate, and the presented values represent the mean ± standard error (S.E.) of three replicates. The test results were plotted with Sigmaplot 10.0, and the statistical analysis was conducted using the Data Processing System (DPS2000; DataProcessingSystem, Zhejiang University, Zhejiang, China). Differences among the treatments or periods of the same treatment were compared using a Duncan's multiple range test at a 0.05 probability levels ($p \leq 0.05$).

3. Results

3.1. Effects of Spraying Calcium Fertilizer on the Qualities of Apple Fruits

Applying calcium fertilizer significantly increased the single fruit weight ($p < 0.05$) (Table 1). Compared with the control, the single fruit weight of Niucui and Naipu 9 treated apples increased by 6.5 and 12.1%, respectively. Naipu 9 increased the fruit shape index, while Niucui had no significant effect on the fruit shape index. Both calcium treatments significantly increased the coloring index. Niucui had no significant effect on the finish index, while Naipu 9 significantly increased the finish index. Spraying calcium fertilizer decreased the a * value. Naipu 9 increased the b * value, but Niucui exhibited no significant effect on it.

Table 1. Effects of spraying calcium fertilizer on appearance quality of apple fruit.

Treatment	Single Fruit Weight (g)	Fruit Shape Index	Coloring Index (%)	Finish Index (%)	Chromatic Aberration		
					L *	a *	b *
Niucui	200.1 ± 21.5 ab	0.862 ± 0.0029 b	81.25 ± 2.37 a	73.33 ± 3.25 b	49.30 ± 3.55 b	27.62 ± 3.75 ab	12.62 ± 2.03 b
Naipu 9	206.8 ± 33.1 a	0.891 ± 0.032 a	83.57 ± 2.66 a	82.81 ± 3.33 a	52.94 ±4.52 a	25.65 ± 2.98 b	14.95 ± 1.33 a
control	184.1 ± 20.1 b	0.854 ± 0.041 b	75.01 ± 4.80 b	70.00 ± 3.78 b	49.07 ± 3.99 b	29.38 ± 4.82 a	12.91 ± 2.44 b

Note: L * indicates brightness; the larger the value, the greater the brightness and the better the finish; a * and b * are chromaticity indicators. The larger the positive value of a *, the heavier the red, and the larger the negative value, the heavier the green; the larger the positive value of b *, the heavier the yellow, and the larger the negative value, the heavier the blue. Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

Calcium fertilizer application significantly improved the fruit firmness, SSC, soluble sugar content and sugar–acid ratio (Table 2). Compared with the control, the firmness of Niucui and Naipu 9 apples were increased by 3.6 and 16.1%, respectively, and the SSC increased by 6.3 and 12.0%, respectively. For Niucui and Naipu 9 treated apples, the soluble sugar content increased by 6.3 and 12.0%, respectively, and the sugar–acid ratio increased by 6.3 and 12.0%, respectively. For Niucui and Naipu 9 treated apples, the VC increased by 30.5 and 29.4%, respectively. Spraying calcium fertilizer significantly decreased the titratable acid content of fruit, and the titratable acid of Niucui and Naipu 9 treated apples was decreased by 30.4 and 6.0%, respectively.

Table 2. Effects of spraying calcium fertilizer on the internal quality of apple fruit.

Treatment	Firmness (kg/cm^2)	SSC (%)	Soluble Sugar Content (%)	Titratable Acid Content (%)	Sugar–Acid Ratio	VC Content (mg/100 g)
Niucui	7.43 ± 0.41 b	14.4 ± 0.9 b	12.66 ± 0.56 b	0.231 ± 0.009 c	55.04 ± 4.81 a	8.17 ± 0.558 a
Naipu 9	8.29 ± 0.49 a	15.9 ± 0.8 a	13.29 ± 0.51 a	0.312 ± 0.006 b	42.87 ± 5.13 b	8.10 ± 0.712 a
control	7.19 ± 0.52 b	13.8 ± 0.9 b	11.02 ± 0.30 c	0.332 ± 0.007 a	33.39 ± 4.99 c	6.26 ± 0.495 b

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

3.2. Effects of Spraying Calcium Fertilizer on Sugar and Acid Accumulation of 'Fuji' Apple Fruit

The soluble sugar content showed a continuous increasing trend (Figure 1a). Compared to the control, spraying calcium fertilizer significantly increased the soluble sugar content of apples, and the Naipu 9 treatment effected a higher increase than did the Niucui application. The titratable acid content showed a decrease–increase–decrease trend (Figure 1b). Spraying calcium fertilizer increased the titratable acid content of the fruit from 24 June and decreased the titratable acid content of fruit from 27 September and 27 October. From September–October, Niucui application significantly decreased the titratable acid content of fruit.

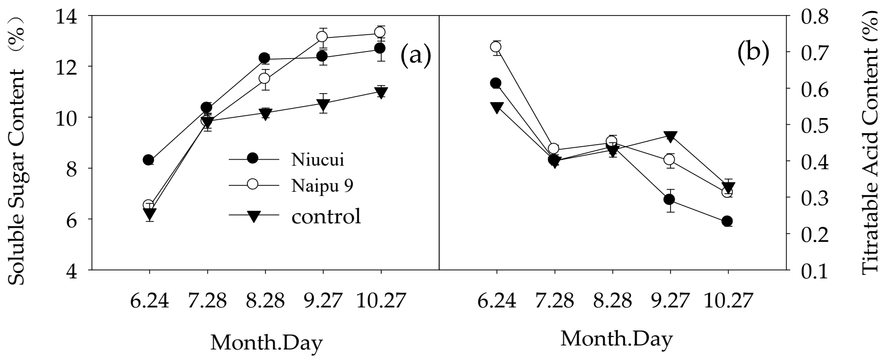

Figure 1. Effects of spraying calcium fertilizer on soluble sugar content (**a**) and titratable acid content (**b**) of apple fruit.

Calcium fertilizer application had no significant effect on the fructose, sucrose, or sorbitol fruit contents from June–August, but it significantly increased the contents of the three in September and October (Figure 2). Spraying calcium fertilizer significantly increased the glucose content from June–October. There were no significant differences in fructose and glucose content during the mature period between fruits treated with either Niucui or Naipu 9. Naipu 9 increased the fruit sucrose and sorbitol content significantly more than Niucui.

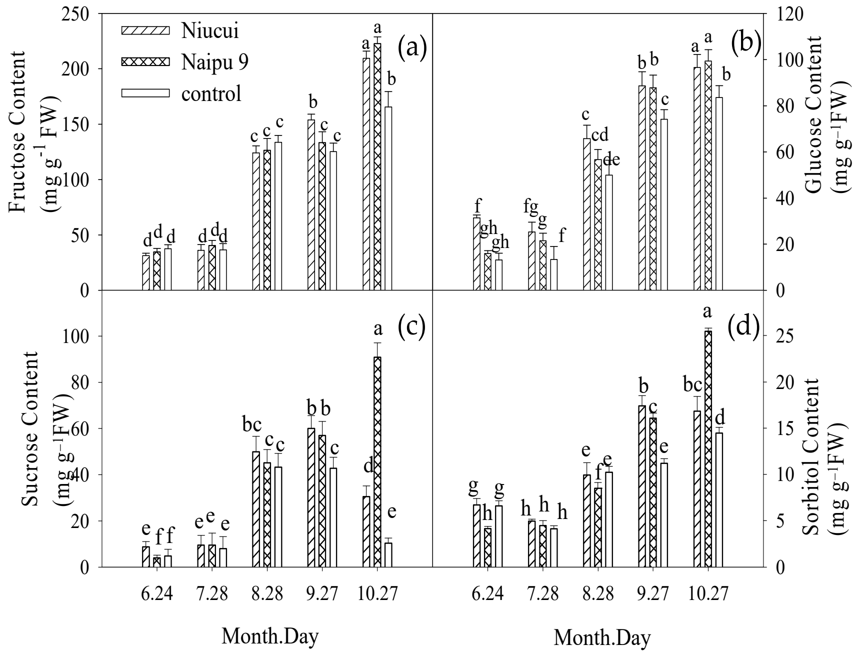

Figure 2. Effects of spraying calcium fertilizer on the contents of (**a**) fructose, (**b**) glucose, (**c**) sucrose, and (**d**) sorbitol in apple fruit. The values are mean ± S.E. of three replicates. Bars represent S.E. Bars with the same letter were not significantly different at $p < 0.05$.

The content of individual organic acids initially generally decreased, followed by an increase and another decrease (Figure 3). Applying calcium fertilizer promoted the malic acid content of apple fruits in the early stage. Niucui significantly decreased ($p < 0.05$) the malic acid content of apple fruits in the color and mature stages, while Naipu 9 slightly decreased the malic acid content of apple fruits during the color and mature stages ($p < 0.05$) (Figure 3a). Niucui had almost no effect on the citric and tartaric acid contents, while Naipu 9 significantly increased the citric acid content and decreased the tartaric acid content (Figure 3b,d). Niucui treatment reduced the accumulation of succinic acid. Naipu 9 application had no significant effects on the fruit succinic acid content from 24 June–27 September, but it increased fruit succinic acid by 27 October (Figure 3c). Overall, calcium fertilizer application slightly decreased the fruit malic and tartaric acid contents, but it significantly increased the accumulation of citric and succinic acids.

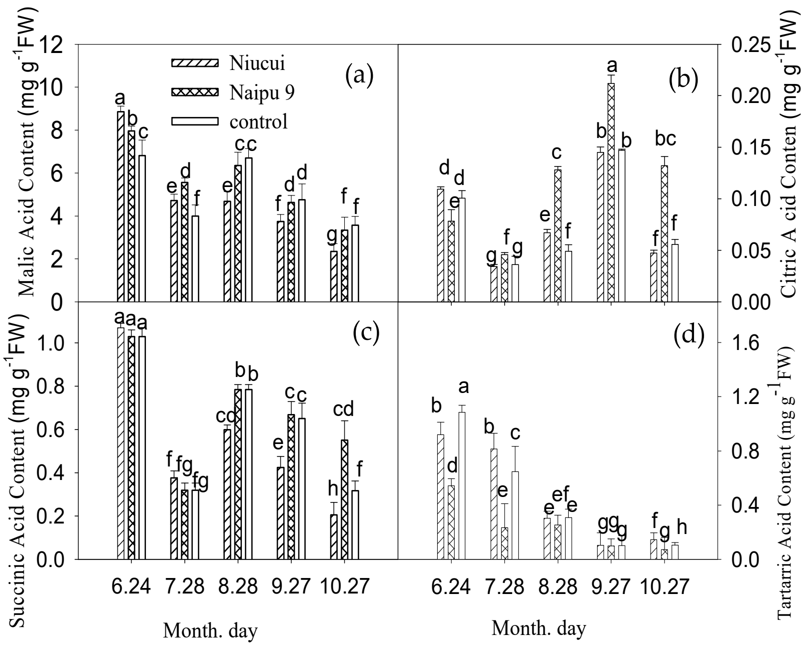

Figure 3. Effects of spraying calcium fertilizer on (**a**) malic acid, (**b**) citric acid, (**c**) succinic acid, and (**d**) tartaric acid contents in apple fruit. The values represent mean ± S.E. of three replicates. Bars indicate S.E. Bars with the same letter were not significantly different at $p < 0.05$.

3.3. Effects of Spraying Calcium Fertilizer on Mineral Element Content of Apple Leaves and Fruits

3.3.1. Effects of Spraying Calcium Fertilizer on Mineral Element Content of Apple Leaves

The effects of spraying calcium fertilizer on the mineral element content in leaves was measured and displayed in Table 3. From June–October, the Ca content of apple leaves showed an increase–decrease–increase trend, with the lowest value observed in August, which was related to the lower transpiration rate (as shown in Table 8) in August and the lower Ca absorption. The appropriate Ca content in apple leaves is 1–2%, and the calcium content in leaves after spraying calcium fertilizer was basically in the high value range (>2%) [45]. Spraying calcium fertilizer significantly increased the content of Ca in leaves. Spraying Niucui and Naipu 9 increased the content of Ca in leaves at maturity (10–27) by 33.2 and 4.9%, respectively.

The Mg content in leaves showed a decrease–increase–decrease trend from June–October (Table 3). Spraying Naipu 9 significantly increased the Mg content in leaves, while those sprayed with Niucui had a significantly higher content than those in the control group in August. There was no significant difference between calcium fertilized and control leaves at the other dates.

Table 3. Effects of spraying calcium fertilizer on mineral element content of apple leaves.

Element	Treatment	Month.Day				
		6.24	7.28	8.28	9.27	10.27
Ca (%)	Niucui	2.64 ± 0.14 a	3.55 ± 0.18 a	1.68 ± 0.07 a	2.54 ± 0.14 a	2.73 ± 0.05 a
	Naipu 9	2.30 ± 0.08 b	2.62 ± 0.14 b	1.53 ± 0.06 ab	2.12 ± 0.08 b	2.15 ± 0.13 b
	control	1.61 ± 0.04 c	2.28 ± 0.08 c	1.32 ± 0.04 c	1.94 ± 0.10 c	2.05 ± 0.07 bc
Mg (g/kg)	Niucui	0.28 ± 0.01 b	0.17 ± 0.02 b	1.18 ± 0.02 a	2.34 ± 0.12 ab	2.28 ± 0.10 ab
	Naipu 9	0.37 ± 0.01 a	0.32 ± 0.01 a	1.19 ± 0.02 a	2.46 ± 0.07 a	2.39 ± 0.05 a
	control	0.28 ± 0.11 b	0.17 ± 0.05 b	1.14 ± 0.06 b	2.25 ± 0.13 b	2.19 ± 0.11 b
B (mg/kg)	Niucui	103.59 ± 8.02 a	128.95 ± 8.46 a	64.82 ± 3.64 a	96.70 ± 5.89 a	93.16 ± 0.52 a
	Naipu 9	54.53 ± 2.98 b	80.31 ± 4.56 b	59.56 ± 5.44 b	65.86 ± 6.44 b	72.51 ± 1.23 b
	control	34.41 ± 2.56 c	57.29 ± 6.78 c	38.28 ± 0.88 c	61.23 ± 2.84 c	63.92 ± 2.54 c
Cu (mg/kg)	Niucui	32.40 ± 0.46 a	18.65 ± 1.43 a	22.87 ± 2.41 a	15.42 ± 1.88 b	12.87 ± 3.06 ab
	Naipu 9	28.99 ± 1.84 b	16.46 ± 0.94 b	22.93 ± 1.46 a	14.06 ± 2.42 c	10.18 ± 1.82 c
	control	23.94 ± 3.06 c	10.05 ± 0.46 c	21.02 ± 2.04 a	16.25 ± 0.86 a	13.13 ± 0.68 a
Zn (mg/kg)	Niucui	27.00 ± 3.28 b	66.73 ± 6.62 a	72.60 ± 4.05 a	55.96 ± 3.42 a	23.75 ± 1.08 b
	Naipu 9	38.59 ± 0.44 a	30.58 ± 2.89 b	35.36 ± 3.04 bc	36.01 ± 2.66 c	23.36 ± 1.48 b
	control	38.71 ± 2.78 a	33.51 ± 0.98 b	38.94 ± 4.06 b	51.00 ± 5.64 ab	35.51 ± 2.35 a

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

From June–October, the B content in leaves exhibited an increase–decrease–increase trend. Applying calcium fertilizer significantly increased the B content in leaves. Spraying Niucui and Naipu 9 increased the content of Ca in mature leaves by 45.7 and 13.4%, respectively.

From June–October, the Cu content in leaves showed a decreasing–increasing–decreasing trend. Spraying calcium fertilizer increased the Cu content in leaves in June and July, but had no effect on the Cu content in August. Calcium fertilizer application decreased the Cu content in leaves in September and October.

The changing trends of Zn content in leaves of all treatments was not consistent. The trend of Zn content in leaves increased and then decreased with Niucui treatment, and the trend of Zn content in leaves decreased–increased–decreased with Naipu 9 and control treatments. The Zn content in leaves from June–August increased with Niucui application, but the effects on Zn content in September were not significant. The Zn content in leaves from October was lower. Spraying Naipu 9 reduced the Zn content in leaves.

3.3.2. Effects of Spraying Calcium Fertilizer on Mineral Element Content of Apple Fruits

The effects of spraying calcium fertilizer on fruit mineral element content are shown in Table 4. With the extension of the growth period of apples, the Ca content in the fruit generally demonstrated a decreasing–increasing trend, and was stabilized from August–October. Spraying calcium fertilizer significantly increased the Ca content of fruit. From May–October, Niucui application increased Ca content in fruit by 50.6, 63.9, 49.0, 17.5, and 27.9%, respectively. Treatment with Naipu 9 increased fruit Ca content by 28.9, 39.5, 8.2, 10.5, and 13.1%, respectively. Niucui application had a more significant effect on Ca content in fruit than did the Naipu 9 treatment.

The total Mg content in fruit showed an increasing–decreasing trend. Spraying Niucui decreased the fruit Mg content during the expansion period (June–August) but had no significant effect during the color and maturity periods (September and October). Spraying Naipu 9 significantly increased the content of Mg during the entire fruit development period (June–October).

The B content in fruit showed a decreasing–increasing–decreasing trend. B content during coloring and mature stages (9.27 and 10.27, respectively) was lower than that in the swelling stage (June and August). Overall, spraying calcium fertilizer increased the B content of the fruit. From June–October, Niucui application increased fruit B content by 168.4, 201.06, 71.4, 87.4, and 46.2%, respectively, and spraying Naipu 9 increased it by 114.4, 131.5, 28.6, 12.5, and 28.4%, respectively.

Table 4. Effect of spraying calcium fertilizer on mineral element content of apple fruits.

Element	Treatment	Month.Day				
		6.24	7.28	8.28	9.27	10.27
Ca (g/kg)	Niucui	1.99 ± 0.08 a	1.95 ± 0.04 a	0.73 ± 0.02 a	0.67 ± 0.04 a	0.78 ± 0.04 a
	Naipu 9	1.70 ± 0.06 b	1.66 ± 0.03 bb	0.53 ± 0.03 b	0.63 ± 0.06 ab	0.69 ± 0.02 b
	control	1.32 ± 0.04 c	1.19 ± 0.04 c	0.49 ± 0.01 bc	0.57 ± 0.01 b	0.61 ± 0.03 b
Mg (g/kg)	Niucui	0.07 ± 0.01 c	0.11 ± 0.01 b	0.65 ± 0.01 c	1.54 ± 0.06 b	1.07 ± 0.03 b
	Naipu 9	0.17 ± 0.01 a	0.18 ± 0.02 a	0.86 ± 0.03 a	1.93 ± 0.05 a	1.57 ± 0.08 a
	control	0.11 ± 0.02 b	0.13 ± 0.01 b	0.77 ± 0.04 b	1.54 ± 0.01 b	1.01 ± 0.02 b
B (mg/kg)	Niucui	120.80 ± 10.02 a	97.69 ± 5.64 a	105.11± 11.05 a	90.62 ± 8.46 a	62.45 ± 3.56 a
	Naipu 9	96.49 ± 6.84 b	74.98 ± 5.66 b	78.83 ± 5.89 b	54.40 ± 3.64 b	54.85 ± 0.85 b
	control	45.01 ± 2.46 c	32.39 ± 1.08 c	61.32 ± 8.42 c	48.35 ± 2.64 c	42.73 ± 0.76 c
Cu (mg/kg)	Niucui	17.37 ± 0.49 c	14.25 ± 0.68 b	11.90 ± 0.22 b	12.61 ± 0.34 b	10.16 ± 0.18 b
	Naipu 9	24.16 ± 0.68 ab	18.40 ± 0.10 a	12.03 ± 0.28 ab	15.61 ± 0.42 a	10.43 ± 0.23 b
	control	26.11 ± 1.20 a	19.32 ± 1.12 a	13.12 ± 0.64 a	16.66 ± 0.52 a	13.20 ± 0.84 a
Zn (mg/kg)	Niucui	81.89 ± 4.66 a	53.07 ± 5.46 a	25.75 ± 2.44 a	20.97 ± 2.13 b	16.86 ± 0.84 b
	Naipu 9	23.18 ± 1.08 b	21.38 ± 2.34 b	24.61 ± 3.46 a	30.38 ± 1.64 a	20.80 ± 2.42 a
	control	25.15 ± 0.88 b	22.29 ± 1.22 b	25.55 ± 4.02 a	21.75 ± 1.84 b	12.66 ± 1.45 c

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

The Cu content in the fruit showed a decreasing trend. Spraying calcium fertilizer reduced the accumulation of Cu in the fruit. From June–October, Niucui application decreased it by 33.5, 26.2, 9.3, 24.3, and 23.0%, respectively, and spraying Naipu 9 decreased it by 7.5, 4.8, 8.3, 6.3, and 21.0%, respectively.

The Zn content of fruit showed a decreasing–increasing–decreasing trend. The Zn content of fruit in June, July, and October were increased when sprayed with Niucui, but Niucui application had no effects on the fruit Zn content in August or September. Spraying Naipu 9 decreased the Zn content in fruits from June–August, and increased the Zn content in fruits from September–October. Both Niucui and Naipu 9 increased the Zn content of fruit at maturity (10.27).

3.4. Effects of Spraying Calcium Fertilizer on Chlorophyll (Chl) and Carotenoid (Car) Contents in Apple Leaves

The effects of spraying calcium fertilizer on the Chl and Car contents of leaves are displayed in Table 5. Before calcium fertilizer application (24 May), there were no significant differences in Chl and Car content of leaves of trees in any treatment group ($p < 0.05$). The Chl content of leaves sprayed with calcium fertilizer from June–October was significantly higher than that of control ($p < 0.05$). The effects of spraying Naipu 9 were better than those of spraying Niucui, especially in August and September. The Chl content of leaves sprayed with Naipu 9 was also significantly higher than those treated with Niucui. During the testing period, the changes in Car content were consistent with those in Chl content.

3.5. Effects of Spraying Calcium Fertilizer on Net Photosynthetic Rate (P_n) of Apple Leaves

The effects of spraying calcium fertilizer on the net photosynthetic rates (Pn) of the apple leaves are shown in Table 6. From May–October, the Pn of each treatment showed a trend of increase–decrease–increase. Among these results, the highest values for the Niucui and control groups were in July (20.6 μmol m^{-2} s^{-1}, 18.7 μmol m^{-2} s^{-1}, respectively), and the highest value from the Naipu 9 group was in June (23.4 μmol m^{-2} s^{-1}). There were no significant differences ($p < 0.05$) in the Pn of leaves among the treatment groups before calcium fertilizer application (24 May), and the average Pn was approximately 19.0 μmol m^{-2} s^{-1}. From June–October, the Pn of the leaves sprayed with calcium fertilizer was significantly higher than that of the control ($p < 0.05$). Compared with the control, the Pn of the leaves treated with Niucui increased by 4.3, 10.2, 0.0, 27.0, and 34.6%, respectively, and that of the leaves treated with Naipu 9 increased by 25.8, 15.0, 36.5, 57.4, and 45.7%, respectively.

Table 5. Effects of spraying calcium fertilizer on chloroplast pigment content of apple leaves.

Month.Day	Chloroplast Pigment	Chloroplast Pigment Content (mg/g)		
		Niucui	Naipu 9	Control
5.24	Chl	6.61 ± 0.38 a	6.57 ± 0.37 a	6.60 ± 0.28 a
	Car	2.53 ± 0.05 a	2.44 ± 0.03 ab	2.51 ± 0.11 a
6.24	Chl	7.06 ± 0.18 a	7.12 ± 0.12 a	6.98 ± 0.29 b
	Car	2.63 ± 0.11 ab	2.74 ± 0.02 a	2.53 ± 0.09 bc
7.28	Chl	6.71 ± 0.09 a	6.75 ± 0.05 a	6.05 ± 0.11 b
	Car	1.62 ± 0.06 b	1.89 ± 0.03 a	1.53 ± 0.03 c
8.28	Chl	4.91 ± 0.09 b	5.55 ± 0.05 a	4.85 ± 0.11 b
	Car	0.35 ± 0.06 b	0.42 ± 0.03 a	0.33 ± 0.03 b
9.27	Chl	3.62 ± 0.09 b	3.89 ± 0.05 a	3.56 ± 0.03 c
	Car	0.24 ± 0.05 ab	0.25 ± 0.01 a	0.23 ± 0.04 b
10.27	Chl	2.27 ± 0.01 ab	2.37 ± 0.15 a	2.05 ± 0.12 c
	Car	0.29 ± 0.03 b	0.31 ± 0.05 a	0.24 ± 0.04 c

Note: Different lowercase letters after peer data indicate that the difference between different treatments in the same period is significant, at the level of $p = 0.05$.

Table 6. Effects of spraying calcium fertilizer on net photosynthetic rate (P_n) of apple leaves.

Month.Day	P_n (μmol m^{-2} s^{-1})		
	Niucui	Naipu 9	Control
5.24	18.9 ± 1.9 a	19.3 ± 1.4 a	19.0 ± 0.4 a
6.24	19.4 ± 3.6 b	23.4 ± 1.7 a	18.6 ± 3.8 b
7.28	20.6 ± 2.3 a	21.5 ± 1.1 a	18.7 ± 1.8 b
8.28	13.7 ± 0.6 b	18.7 ± 3.5 a	13.7 ± 0.2 b
9.27	14.6 ± 0.9 b	18.1 ± 0.5 a	11.5 ± 1.2 c
10.27	17.1 ± 0.8 a	18.5 ± 0.8 a	12.7 ± 1.8 b

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

3.6. Effects of Spraying Calcium Fertilizer on Other Photosynthetic Parameters of Apple Leaves

Spraying calcium fertilizer affected the stomatal conductance (Gs) of leaves (Table 7). During the growth period, the Gs of each treatment showed an increasing and then decreasing trend, and there were no significant differences in Gs of leaves from each treatment group before fertilizer application (5.24) ($p < 0.05$). From June–October, the Gs of leaves treated with calcium fertilizer was significantly higher than that of those sprayed with water. The Gs of the leaves from the Niucui treatment increased by 8.4, 3.4, 85.3, and 9.7%, respectively, and that of the Naipu 9 treated leaves increased by 41.3, 39.0, 133.8, 31.8, and 19.5%, respectively. This is consistent with the changes in leaf P_n during the same period. It is speculated that spraying Naipu 9 is beneficial in maintaining higher stomatal conductance of leaves, and in providing sufficient C to maintain higher carbon assimilation capacity.

The effects of spraying calcium fertilizer on the leaf transpiration rate (Tr) is shown in Table 8. The changes in Tr were consistent with Gs. There were no significant differences in Tr among treatment groups before fertilizer application (24 May) ($p < 0.05$). From June–October, the Tr of leaves sprayed with calcium fertilizer was higher than that of the control, and the Tr of leaves treated with Naipu 9 was higher than that of those treated with Niucui.

Table 7. Effects of spraying calcium fertilizer on stomatal conductance (Gs) of apple leaves.

Month.Day	Gs (mmol m^{-2} s^{-1})		
	Niucui	Naipu 9	Control
5.24	257.0 ± 5.0 a	257 ± 10.2 a	260 ± 6.9 a
6.24	266.7 ± 39.2 b	347.8 ± 62.7 a	246.1 ± 31.3 b
7.28	261.7 ± 32.3 b	351.7 ± 56.8 a	253.0 ± 47.1 b
8.28	180.7 ± 16.2 ab	228.0 ± 15.6 a	97.5 ± 18.2 b
9.27	114.4 ± 19.1 ab	120.7 ± 10.0 a	91.6 ± 13.0 b
10.27	98.5 ± 11.5 ab	107.3 ± 6.5 a	89.8 ± 12.3 b

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant at the level of $p = 0.05$.

Table 8. Effects of spraying calcium fertilizer on transpiration rate (Tr) of apple leaves.

Month.Day	Tr (mmol m^{-2} s^{-1})		
	Niucui	Naipu 9	Control
5.24	5.3 ± 0.3 a	5.1 ± 0.1 a	5.5 ± 0.8 a
6.24	4.5 ± 0.9 b	6.2 ± 0.6 a	4.4 ± 1.0 b
7.28	4.3 ± 0.5 b	5.3 ± 0.9 a	4.0 ± 0.3 b
8.28	4.2 ± 0.5 b	5.2 ± 0.3 a	2.4 ± 0.3 c
9.27	3.2 ± 0.4 ab	3.3 ± 0.8 a	2.5 ± 0.3 b
10.27	1.9 ± 0.4 a	2.1 ± 0.3 a	1.8 ± 0.2 a

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

The results of intercellular CO_2 concentration (Ci) of leaves are shown in Table 9. Before treatment (24 May), there were no significant differences in Ci of leaves from each treatment group. After June (excluding July), the Ci of the calcium fertilizer treatment groups was significantly lower than that of the control group, which coincided with the increases in P_n observed in leaves treated with calcium fertilizer. Meanwhile, the Ci of leaves treated with Naipu 9 coincided with the highest P_n during the same period.

Table 9. Effects of spraying calcium fertilizer on intercellular CO_2 concentration (Ci) of apple leaves.

Month.Day	Ci (µmol mol^{-1})		
	Niucui	Naipu 9	Control
5.24	210.7 ± 16.2 a	205.7 ± 7.6 a	208.5 ± 18.6 a
6.24	200.0 ± 16.7 b	201.0 ± 7.9 b	221.6 ± 12.8 a
7.28	186.7 ± 9.3 a	193.1 ± 18.2 a	189.6 ± 14.3 a
8.28	181.5 ± 7.8 b	165.0 ± 12.3 b	230.0 ± 12.2 a
9.27	143.9 ± 8.5 b	117.8 ± 5.4 c	182.0 ± 10.5 a
10.27	190.6 ± 10.5 a	123.0 ± 6.4 b	192.8 ± 12.2 a

Note: Different lowercase letters after the same column of data indicate that the difference between treatments is significant, at the level of $p = 0.05$.

3.7. Effects of Spraying Calcium Fertilizer on the Activities of Major Enzymes Related to Sugar and Acid Metabolism in 'Fuji' Apple Fruit

3.7.1. Effects of Spraying Calcium Fertilizer on the Activities of Major Enzymes Related to Sugar Metabolism in 'Fuji' Apple Fruit

The activities of enzymes related to sucrose and sorbitol metabolism were measured, and the results are shown in Figure 4. From June–October, the overall SPS activity increased, and spraying calcium fertilizer increased SPS activity. Applying Naipu9 increased the activity more than treatment with Niucui did (Figure 4a). The SS−SD activity was consistent with that of SPS. Spraying calcium fertilizer significantly increased the activity of SS−SD, and treatment with Naipu 9 increased the activity more than application of Niucui did (Figure 4b).

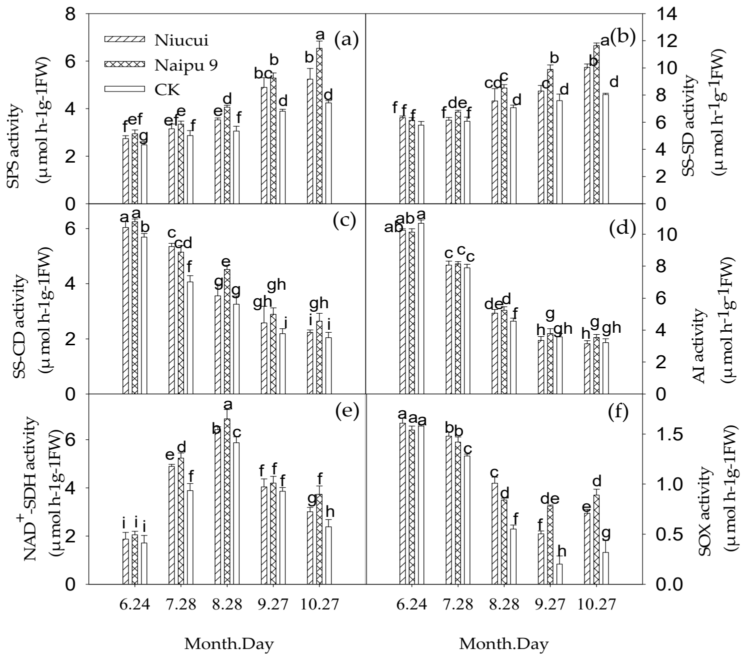

Figure 4. Effects of spraying calcium fertilizer on (**a**) SPS activity, (**b**) SS−SD activity, (**c**) SS−CD activity, (**d**) AI activity, (**e**) NAD−SDH activity, and (**f**) SOX activity of apple fruit. The values represent mean ± S.E. of three replicates. Bars indicate S.E. Bars with the same letter were not significantly different, at $p < 0.05$.

Overall, SS-SC activity exhibited a decreasing trend, however, calcium fertilizer application significantly increased its activity, with greater effects observed with Naipu 9 treatment (Figure 4c). AI activity decreased over time, and calcium fertilizer had no significant effects on the activity (Figure 4d). The NAD−SDH activity increased initially, followed by a decrease. The use of calcium fertilizer increased its activity, especially during the mature period (27 October) (Figure 4e). The SOX activity demonstrated a decreasing−increasing trend, and calcium fertilizer application significantly increased the activity in the later stages of fruit development (Figure 4f).

Spraying calcium fertilizer significantly increased the activities of key enzymes of sugar metabolism, especially SPS, SS−SC, and SOX, and the effects of Naipu 9 application were more significant than those of Niucui treatment.

3.7.2. Effects of Spraying Calcium Fertilizer on the Activity of Main Enzymes Related to Acid Metabolism in 'Fuji' Apple Fruit

NAD−MDH had a decreasing–increasing–decreasing trend with a small range. Spraying Niucui on the apples reduced NAD−MDH activity at maturity, but there were no significant differences in NAD−MDH activities between Naipu 9 treatment and the control treatment at maturity (Figure 5a). PCPE displayed an increasing–decreasing trend. Spraying Niucui calcium fertilizer reduced the PCPE enzyme activity of mature fruits, while Naipu 9 increased the PCPE activity of mature fruits (Figure 5b). PEPCK activity exhibited an upward trend. Spraying Niucui and Naipu 9 calcium fertilizers significantly increased the PEPCK activity of fruit at the later stages of development, and Naipu 9 had a greater effect (Figure 5c). Spraying Niucui and Naipu 9 calcium fertilizers significantly increased the cyME activity, and the cyME activity of the fruit treated with Niucui was higher than that of fruit treated with Naipu 9 (Figure 5d).

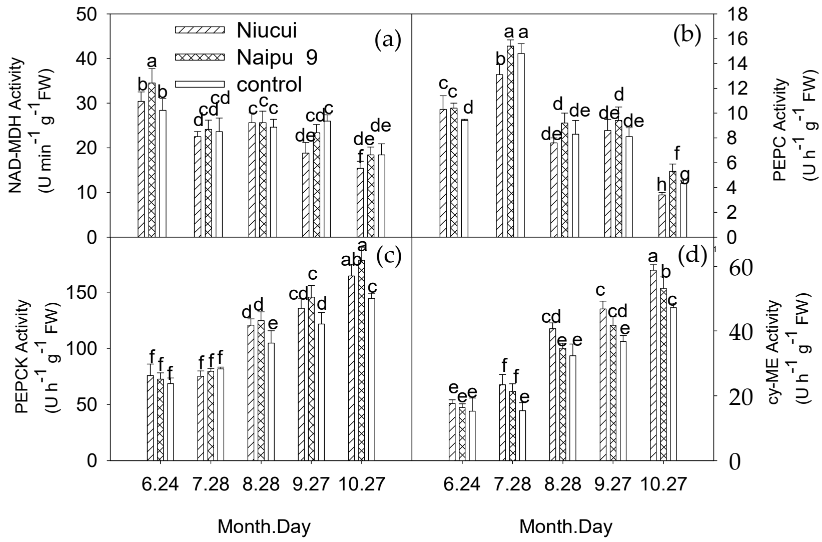

Figure 5. Effects of spraying calcium fertilizer on (**a**) NAD-MDH activity, (**b**) PEPC activity, (**c**) PEPCK activity, and (**d**) cyME activity of apple fruit. The values represent mean ± S.E. of three replicates. Bars indicate S.E. Bars with the same letter were not significantly different at $p < 0.05$.

4. Discussion

4.1. Spraying Calcium Fertilizer to Improve Fruit Quality

Calcium fertilizer treatments significantly increased the single fruit weight, and there was no significant difference between Niucui and Naipu 9 treatments. In this study, the load of fruit trees is consistent, that is, calcium fertilizer treatments significantly increased the yield (Table 1), and this is consistent with the research results on grape [46].

Calcium fertilizer treatments improved the fruit firmness, especially the Naipu 9 treatment (Table 1). This may be related to the increase in calcium pectinate in fruit by calcium fertilizer treatment. The reason for the decrease in fruit hardness is that calcium pectinate in fruit is broken down. The decomposition of calcium pectinate is mainly related to the increase in pectin methylesterase and polygalacturonase activities. Some studies have shown that different forms of calcium treatment can inhibit the activities of these two enzymes [47,48]. The increase in fruit firmness hindered the entry of harmful substances, which was beneficial to improving the storage quality of fruits. In this experiment, the rate of bitter pox and black spot in fruits treated by Niucui and Naipu 9 were 0% and 0% and 0% and 2%, while the rates in the control group were 4% and 8%, respectively.

Calcium fertilizer treatments significantly increased the soluble sugar content, and increased the taste, or sugar–acid ratio (Table 1), which is consistent with the measurement results of sugar acid components (Figures 2 and 3). The increase in the sugar–acid ratio of Niucui treated apples is related to the decrease in total acid accumulation, while the increase in sugar–acid ratio of Naipu 9 treated apples is attributed to the increase of soluble sugar accumulation. Overall, this relates to the differences of effects on sugar acid metabolism (Figures 4 and 5). Calcium fertilizer significantly increased the VC content, which is an important regulatory substance for fruit adaptation to strong light and preventing photooxidation damage (sunburn), and this played an important role in defense against photooxidation [49,50]. It was speculated that calcium fertilizer affects the VC anabolic pathway of fruit, and the effect of Niucui was greater than that of Naipu 9. The mechanism warrants further research.

The rainfall of the test site in 2020 was 1035.7 mm, which was higher than that of the normal year, so the SSC was generally low. However, in calcium fertilizer treatment, especially Naipu 9 treatment, the SSC was still at a high level, the same as that in previous years (more than 15%) (Table 1).

4.2. Spraying Calcium Fertilizer to Increase Sugar and Reduce Acid

Spraying calcium fertilizer is performed in order to increase sugar and reduce acid, which is one of the reasons for improving the taste (**Sugar–acid Ratio**) (Table 2) [22].

By measuring the contents of fructose, glucose, sorbitol, and sucrose in the fruit as well as the activities of enzymes related to sorbitol and sucrose metabolism, it was found that there were differences among the three treatments, with the sugar content and enzyme activities of Naipu 9 treated fruits being the highest. Among the four soluble sugars, sucrose and sorbitol contents were most affected by calcium fertilizers, and the activities of enzymes related to sucrose and sorbitol metabolism were significantly different among the treatments. Particularly, the sugar metabolism of the fruit was more vigorous in Naipu 9 treated apples, which could be related to the higher Mg content of this treatment [51,52].

By measuring the organic acid content of the apples, it was found that Niucui significantly reduced the malic acid content, but Naipu 9 had little effect on it, which is attributed to the fact that Niucui had a higher effect on reducing NAD−MDH activity and increasing cyME activity than Naipu 9 did (Figure 5). Naipu 9 significantly increased the contents of citric and succinic acids and decreased the tartaric acid content, while Niucui increased the tartaric acid content. There were no significant differences between citric and succinic acids and the control group apples. It is speculated that B is conducive to tartaric acid accumulation in fruit, while Mg is conducive to citric acid and succinic acid accumulation, which requires further research.

4.3. Spraying Calcium Fertilizer to Improve the Photosynthetic Capacity of Apple Leaves

Photosynthesis is the basis of plant yield. Ca is a beneficial element for plant growth and development, which is closely related to photosynthetic metabolism [53]. Wang [54] found that the P_n, Gs, Ci, and Tr of peanut (Arachis hypogaea) leaves increased with increasing Ca application; Lu et al. [55] found that Ca3000 significantly increased the Gs, Ci, and Tr of sugarcane seedlings.

In this study, it was found that spraying calcium fertilizer had a significant effect on the photosynthetic parameters of apple leaves. Both Niucui and Naipu 9 applications significantly increased the P_n, Gs, and Tr of apple leaves during different developmental periods (Tables 5–7), which is consistent with previous studies [54,55], but they exhibited different effects on the Ci of apple leaves. In this study, the use of calcium fertilizer reduced the Ci. The analysis revealed that the primary explanation for this observation was related to the inconsistency of varieties and calcium fertilizer used. Secondly, it is believed that the period of this study, May–October, was a high−temperature and high−light−intensity environment. The control leaves may have non−stomatal restriction, leading to carbon assimilation obstruction. However, spraying calcium fertilizer can improve the carboxylation efficiency of leaves in this environment, which is conducive to carbon assimilation, thus reducing the Ci. It is speculated that this is related to the improvement of plant stress resistance by Ca [56–58].

It can also be seen from Tables 5–7 that the effect of Naipu 9 on improving photosynthetic capacity was higher than that of Niucui. This is related to the high content of Mg in the leaves treated with Naipu 9 (Table 3). Mg is a constituent element of chlorophyll. If there exists a magnesium deficiency, plant chlorophyll synthesis is affected [59]. The Mg content of the leaves treated with Naipu 9 was higher, which was more conducive to improved photosynthesis. This is a potential reason why Naipu 9 improves the photosynthetic function of the leaves more than Niucui.

4.4. Spraying Calcium Fertilizer Affects the Absorption and Accumulation of Mineral Nutrients in Apples

Calcium not only affects the photosynthetic physiology of plants, but it also affects the absorption and transportation of mineral elements by plants. There are synergistic and antagonistic effects between the absorption of different elements [60–62], and the effects of calcium fertilizers Niucui and Naipu 9 on the accumulation of mineral elements in apple leaves and fruits were not the same (Tables 3 and 4).

This study revealed that spraying calcium fertilizer increased the content of Ca and Mg in leaves and fruits (Tables 3 and 4). The Ca content in the leaves reached a high level (>2%), and the Mg content reached a normal level in the late stage of fruit development (0.22–0.35%) [45]. The effect on Ca and Mg content of fruits was consistent with that of leaves, which is consistent with results from Wang [54]. This resulted from the effective absorption of calcium fertilizer and was related to the improvement of photosynthetic capacity of leaves by the calcium fertilizers, especially Tr (Tables 6–8). Transpiration pulling is conducive to absorption and transportation of mineral elements. Concurrently, calcium fertilizer increased the B content in leaves and fruits, as both B and Ca are transported by exosomes.

The appropriate B content in apple leaves is 20–60 g/kg, and the B content in leaves after spraying calcium fertilizer was basically in the high value range (60–120 g/kg) [45]; this is intended to improve the yield and quality. The absorption of B and Ca by leaves and fruits is mutually promoting, which is consistent with the experimental results of Bian et al. [63].

The research of Li et al. [64] and Yao et al. [65] showed that, within a certain range of organic calcium dosage, Mg content was negatively correlated with Ca dosage, which may be due to the antagonistic effect between Ca and Mg. After spraying calcium fertilizer on plants, the absorption of Mg by trees was inhibited to a certain extent. In this study, it was found that spraying Naipu 9 promoted Mg absorption by leaves, likely because Naipu 9 contains a certain amount of Mg, and the Ca content of Naipu 9 is lower than that of Niucui. At the same time, it is also attributed to the promotion effect of nitrogen on Mg absorption.

The appropriate Cu content in apple leaves is 5–15 g/kg [45]; from June–August, the Cu content was significantly higher than 15 g/kg. Spraying calcium fertilizer reduced the Cu content in leaves and fruits. This is beneficial to reduce potential Cu toxicity. This may be because there is an antagonistic relationship between Ca and Cu, and these results verify that antagonism.

During this study, the Zn content in leaves was at a normal value (15–80 g/kg [45]). The effects of Niucui and Naipu 9 on the Zn content of leaves and fruits were different. Niucui increased the Zn content of leaves, while Naipu 9 decreased the Zn content of leaves. At the same time, Niucui increased the Zn content of fruits in the early stage, and Naipu 9 increased the Zn content of fruits in the later stage. It was speculated that the difference in action was related to Ca and the other elemental contents, and also related to the action pathway and mechanism of calcium fertilizers.

The content of mineral elements in fruit is closely related to fruit quality [66]; the N, P, Ca, and Mg in the fruit play a leading role in the main quality indexes of the fruit, such as total sugar, total acid, firmness, and soluble solids. The trace elements Zn, Fe, and B also play an important role use [67]. The effect of Ca on minerals may be one of the reasons for improving fruit quality by spraying calcium fertilizer. In addition, the effects of Ca on the contents of other such as N, P, and Fe maybe involved in it. This requires further study.

5. Conclusions

In general, applying calcium fertilizer improved the quality of apple fruits, increased VC content, increased fruit firmness, increased sugar content, and decreased acid content. Niucui was more conducive to the reduction of acid, while Naipu 9 was more conducive to increasing sugar content. Naipu 9 increased the activities of key enzymes in sugar metabolism, while Niucui increased the activities of enzymes related to acid decomposition. Spraying calcium fertilizer improved the photosynthetic function of apple leaves, increased the yield, increased the Ca, Mg, and B contents in apple leaves and fruits, and decreased the Cu content. For the Fuji apple, the effects of spraying calcium fertilizers containing Mg and N were more desirable than spraying calcium fertilizers containing B.

Author Contributions: G.W. and J.W. conceived and designed the experiments; G.W. performed most of the experiments, analyzed the data, and compiled the original manuscript; X.H. and R.C.

assisted with the photosynthesis experiments; X.H., R.C. and X.X. assisted with the sugar and acid metabolism experiments; X.H., R.C. and X.X. participated in the analysis of the apple quality; X.X. contributed suggestions and discussion. All authors have read and agreed to the published version of the manuscript.

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