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Original article

Effect of different types of branches, growth stages, and rootstocks on mineral nutrients concentration in the leaves of citrus

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Summary

To clarify the difference of leaf mineral nutrients affected by types of branches, growth stages, and rootstocks of navel orange, a field experiment was conducted on nine-year-old 'Newhall' navel orange grafted on trifoliate orange or citrange, which were similar in size and tree vigor. Branches were divided into three types (leaves of spring shoots (LSP), summer shoots (LSU), and autumn shoots (LAU)), and the observation period were divided into four stages (stage I, II, III, and IV). Their leaves were sampled monthly, and mineral nutrient concentrations were measured subsequently. The results showed that the patterns of annual change in the concentrations of leaf nutrients in two rootstocks grafted navel orange were generally similar, but there were significant differences among branch types. For all kinds of branch types, the concentrations of P, K, and Mg in the leaves of two rootstocks grafted navel orange showed decreased tendency with leaf age, and rapidly decrease was observed for P and K concentrations at stage III and early stage IV, but increased at the last sampling point. For Ca concentration in LSP, it was only decreased at the end of stage IV in trifoliate orange-grafted navel orange. In LSP, the concentration of Fe and Mn increased during stage I and II but declined during stage III of navel orange grafted on two rootstocks. Mn concentration was significantly higher in LSP than in LSU and LAU. B concentration in LSP increased during stages I and IV, decreased during stages II and III. Zn concentrations decreased during stage I and increased during stage IV, and they were generally similar in the leaves of all branch types. The concentration of Mo decreased from a maximum just after stage I to a minimum at stage II and then remained steady from stage II to IV in LSP. There were strong positive correlations among P, K, and Mg in all branch types. Principal component analysis revealed that the mineral nutrients in LSP were particularly important than LSU and LAU for navel orange trees. Overall, the results of this study provide new insights for development of precision fertilization in citrus production.

Keywords
citrus, mineral nutrients, nutrition diagnosis, branch type, annual change of concentration, rootstock

Significance of this study

What is already known on this subject?

- Many studies on citrus nutrition have used the foliar nutrient index to characterize annual or seasonal changes of mineral nutrients in the leaves, and the nutritional status of citrus trees. But the accuracy of the foliar nutrient index is affected by sampling period, branch type, etc.

What are the new findings?

- The patterns of annual change in the concentrations of leaf nutrients were significant differences among branch types, and principal component analysis revealed that the mineral nutrients in the leaves of spring shoots were particularly important for navel orange trees.

What is the expected impact on horticulture?

- The results of this study can prove that there were significant differences among branch types of mineral nutrients and the leaves of spring shoots were particularly important. Therefore, branch type should be taken into account when formulating field fertilization strategies for citrus.

Introduction

Mineral nutrients play essential roles in the growth and development of fruit trees and thus affect fruit yield and quality (Martin and Marschner, 1988; Amiri et al., 2008; Liu et al., 2010). Citrus is one of the world's most agriculturally and economically important fruit crops (FAOSTAT, 2021), and the citrus production industry is one of the most important industries in the developing rural economy of southern China. A large number of previous studies have shown that mineral nutrients deficiency or excess occurred frequently on citrus-producing areas. Under mineral nutrients deficiency or excess conditions, the stress resistance was reduced, while the yield and quality of citrus fruit was decreased. Magnesium (Mg) deficiency is the most common nutrient deficiency in citrus production (Huang et al., 2013; Chen et al., 2019). Mg is a secondary essential mineral nutrient, is a component of various structural substances in plants, and plays a critical role in numerous physiological and biochemical processes of plant growth and development. Under low Mg stress, the external quality of lemon fruit changes little, but juice yield, total soluble solid, total acids and vitamin C contents decreased

significantly (Du et al., 2021). Boron (B) deficiency stress is another common physiological disease in citrus production (Wang et al., 2018; Zhou et al., 2020, 2021). Previous studies have shown that B deficiency not only affects the development of citrus roots and leaves, but also leads to the development of 'monkey head fruit' under severe cases conditions (Chen et al., 2012; Wang et al., 2015). Besides, iron (Fe) deficiency (Fu et al., 2017), manganese (Mn) deficiency (Ling et al., 2010; Wang et al., 2018), B deficiency and excess (Wang et al., 2018; Zhou et al., 2020, 2021), Zn deficiency (Ling et al., 2010; Fu et al., 2014; Wang et al., 2018), and Cu deficiency and excess also affected the growth and development, yield and quality of citrus (Ling et al., 2010; Yuan et al., 2018).

Precision fertilization is the best way for addressing the problems associated with deficiencies or excesses in mineral nutrients. However, the development of appropriate fertilization strategies should be based on reliable field data. Appropriate fertilization measures are critically important for the success of citrus cultivation and management (Liu et al., 2020), but symptoms of nutrient deficiency and optimal chemical fertilizer application regimes remain unclear for many fruit growers. Especially, the unscientific and irrational use of chemical fertilizer and the serious pollution caused by it have seriously limited the development and competitiveness of the citrus industry, which is based on cost-saving, high-efficiency, and ecologically safe cultivation technology (Yang et al., 2013; Luckstead and Devadoss, 2021). Another approach is to reduce application amount of chemical fertilizer. Many studies have shown that although the amount of chemical fertilizer applied determines the yield of fruit trees, organic fertilizer is more beneficial to improve fruit quality than chemical fertilizer (Stojanova et al., 2024). In citrus, previous research suggests that the application of organic fertilizer instead of chemical fertilizer, or co-application of organic and chemical fertilizer are effective ways to reduce the application of chemical fertilizer (Lei et al., 2019). Recent studies have shown that the combination of organic and chemical fertilizers for two consecutive years not only reduced 30% of chemical fertilizers application, but also improved citrus yield and soil fertility (Li et al., 2022). In light of the 'Double Reduction' (chemical fertilizer and pesticide) policy of agriculture production in China, the development of scientifically based approaches for chemical fertilizer application that maximize its positive effects while reducing its negative effects has become a major challenge in the fruit production industry. Therefore, understanding of how the nutrient content of the plant varies throughout the season is of primary importance for determining the timing and quantities of nutrients required by fruit tree plants (Liu et al., 2020).

'Newhall' navel orange is the main variety of citrus in Jiangxi Province (one of the main citrus-producing regions in China). In order to develop its precise fertilization strategy, the basic work is to investigate the nutrient status and annual variation of nutrient elements of citrus trees in orchards. For this purpose, such studies have been conducted by our research team. First, the early and late symptoms of nutrient deficiency and their diurnal changes of photosynthesis have been identified (Zhou et al., 2019). Second, the distribution of mineral nutrients in the different parts of leaves in navel orange has been analyzed for diagnosis the symptoms of nutrient deficiencies or excesses in leaves (Liu et al., 2019). We have also analyzed seasonal changes of mineral nutrients in the fruit of navel orange plants grafted on trifoliate orange and citrange (Liu et al., 2020), as well as the annual variation of micronutrients in 'Newhall' navel orange under different management modes (Zhang et al., 2020). Based on the above research process we found that the leaf mineral nutrient contents of different branch types, growth stages, and rootstocks were significant differences.

They typically have three concentrated periods of shoot growth in the climate of southern Jiangxi: spring, summer, and autumn. Although the growth of spring shoots is not substantial, the number of shoots is high, and this forms the main body for the growth of the shoot. By contrast, the number of summer and autumn shoots is small, but the growth of these shoots is substantial. The evaluation of tree nutrient status, seasonal or annual changes of mineral nutrients, and the diagnosis of nutrient deficiency or toxicity are usually carried out by measuring the content of nutrients in leaves; this approach is more effective than soil nutrient analysis for perennial fruit trees (Jones, 1998). Many studies on citrus nutrition have used the foliar nutrient index to characterize annual or seasonal changes of mineral nutrients in the leaves (Sheng et al., 2009; Li et al., 2019; Zhang et al., 2020), the distribution of mineral nutrients in different parts of leaves (Liu et al., 2019), and the nutritional status of citrus trees (Ling et al., 2010; Yi et al., 2019). However, the accuracy of the foliar nutrient index is affected by sampling period, citrus variety, rootstock type, leaf age, branch type, and the mobility of nutrient elements (Yang et al., 2015). So it is important to make clear of the effects of different types of branches on annual changes in the concentrations of mineral nutrients in the leaves of citrus plants.

The aim of this study was to (1) determine the difference of the main macronutrients (P, K, Ca, and Mg) and micronutrients (Fe, Mn, B, Zn, Cu, and Mo) in the leaves of different types of branches of 'Newhall' navel orange; (2) clarify whether the growth stage and rootstock can affect the uptake dynamics of mineral nutrients in the leaves of navel orange; and (3) determine the correlations among changes of mineral nutrient concentrations in the leaves of different types branches. Overall, the results of this study provide new insights that will aid the development of precision fertilization and citrus production.

Materials and methods

Plant materials and experimental site overview

The experiment was conducted on trees of 'Newhall' navel orange [*Citrus sinensis* (L.) Osb. cv. Newhall] grafted on the rootstock of trifoliate orange [*Poncirus trifoliata* (L.) Raf.] or citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.]. The navel orange trees grafted on the two rootstocks were both 9 years old, were similar in size and tree vigor, and did not show any signs of nutritional disorders.

The experimental site was located in the Germplasm Resource Nursery of citrus belonging to National Navel Orange Engineering Research Center, Ganzhou City, Jiangxi Province. The sites were located in adjacent blocks on a red-yellow loam soil with the following characteristics: organic matter 14.9 ± 0.4 g kg^{-1}, total N 0.79 ± 0.12 g kg^{-1}, available P 24.1 ± 0.6 mg kg^{-1}, available K 135 ± 12 mg kg^{-1}, and pH 5.42 ± 0.25. The study region is characterized by a typical subtropical monsoon climate with an annual average air temperature of 20 ± 2°C and an average annual rainfall of approximately 1,320 ± 85 mm. The trees were spaced 4.5 m and 3 m between and along rows, respectively. Trees were subjected to routine cultural practices suitable for commercial fruit production.

Experimental design and sampling

The experiment began in March 2019, when a large number of 'Newhall' navel orange spring shoots sprouted. Fifty-four plants of 'Newhall' navel orange grafted on two rootstocks with similar in size, tree vigor, and no nutritional disorder were selected, 27 plants of each rootstock. To reduce the intensity of sampling, the selected trees were arranged into 9 groups of 3. Spring shoots with relatively consistent growth situation were selected and labeled, and the same operation was also carried out in summer shoots and autumn shoots. Thirty branches of each branch type were marked with different colored labels in the concentrated leaf development stage. The branches were evenly distributed around the tree. The first sample was taken in May 2019, when the leaves of spring shoots (LSP) were mature. Three groups of each branch type samples were taken at a time, alternately. Similarly, leaves of summer shoots (LSU) and autumn shoots (LAU) were sampled starting in August and November, respectively. Sampling was performed once a month, except in January 2020. The weather was clear at the time of sampling, with no rain or irrigation in the 5 days prior to sampling. All kinds of leaves of different branch types were sampled until May 2020, when the leaves of current spring shoots were fully mature. The total sampling period is 13 months. Fruit development can be divided into stages I, II, and III (Bain, 1958). Based on this report, we divided the observation period (13 months) into four stages: stage I, the young fruit period (from May to June); stage II, the rapid fruit development period (from July to October); stage III, the fruit maturation period (from November to December); and stage IV, the florescence period (March to May of the following year).

For every sampling, one leaf was sampled from each branch of 3 rotating groups in each rootstock-scion combination. Which means all 30 leaves samples taken from one tree at a time as one biological replicate, 3 biological replicates for each combination. The leaves were transferred to the laboratory soon after they were collected in plastic bags; they were then dried at 105°C for 30 min and then to constant weight in an oven at 75°C. After they were milled through a 0.5-mm stainless steel screen, and stored in an air-tight glass container for subsequent analysis.

Determination of mineral nutrients

Concentrations of the main macronutrients (P, K, Mg, and Ca) and micronutrients (Fe, Mn, B, Zn, Cu, and Mo) in the leaf of different branch types were determined following the method of Zhou (2019). Briefly, 0.50 g of each dried sample was coked in an electric furnace until no black smoke appears, then they were dry-ashed in a muffle oven at 520°C for 6 h. The resulting white ash was then dissolved in 10 mL of 5% HNO_3 to determine the concentrations of macronutrients and micronutrients. Suitable dilutions were conducted to determine the concentrations of various minerals by ICP-MS (Agilent 7900, U.S.A.). The annual variation trends and difference of mineral nutrients average content were analyzed. Furthermore, correlations between elements were determined, and the relationship between the nutritional status of leaves and the development of flowers and fruits was examined.

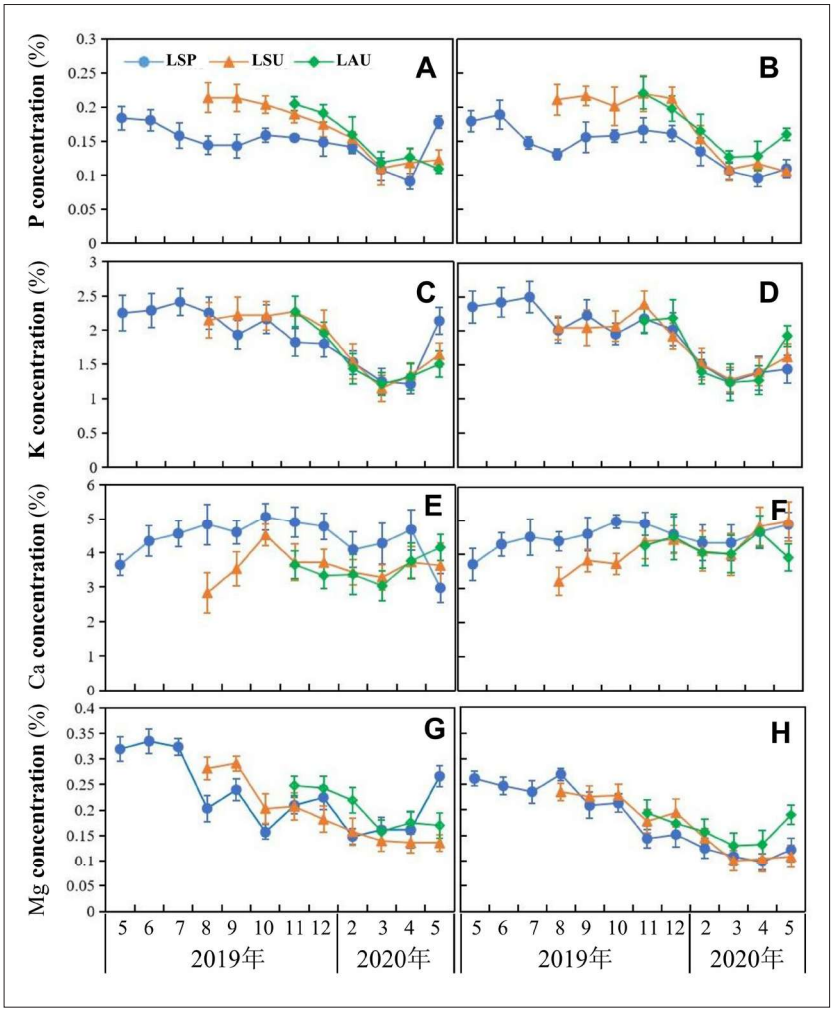


Figure 1. Annual changes in macronutrient concentrations in the leaves of different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange. A, C, E, and G: 'Newhall' navel orange plants grafted on trifoliate orange; B, D, F, and H: 'Newhall' navel orange plants grafted on citrange. LSP: leaves of spring shoots; LSU: leaves of summer shoots; LAU: leaves of autumn shoots.

Statistical analysis

The values were presented as means ± SE of three trees (three plants in each group and three replicates). Analysis of variance (ANOVA) was conducted using SAS, and the significance of differences between groups was determined using Duncan's multiple range test; the threshold for statistical significance was $P<0.05$ (SAS 8.1, SAS Institute Inc., Cary, NC, U.S.A.). Principal component analysis (PCA) was conducted to analyze the variation in macronutrients and micronutrients in the leaves of different types of branches. The first two principal components were used to draw the score and loading plots.

Results

Concentrations of macronutrients

The patterns of annual changes in the concentrations of macronutrients in trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar. As shown in Figure 1, the concentrations of P, K, and Mg in the leaves of both trifoliate orange-grafted and citrange-grafted 'Newhall' navel orange showed decreased tendency with leaf age, and only increased slightly at the last sampling point. At the end of stage III and early stage IV, the P and K concentrations decreased rapidly in all branch types. There were no significant changes in the Ca concentration in LSP, and only decreased

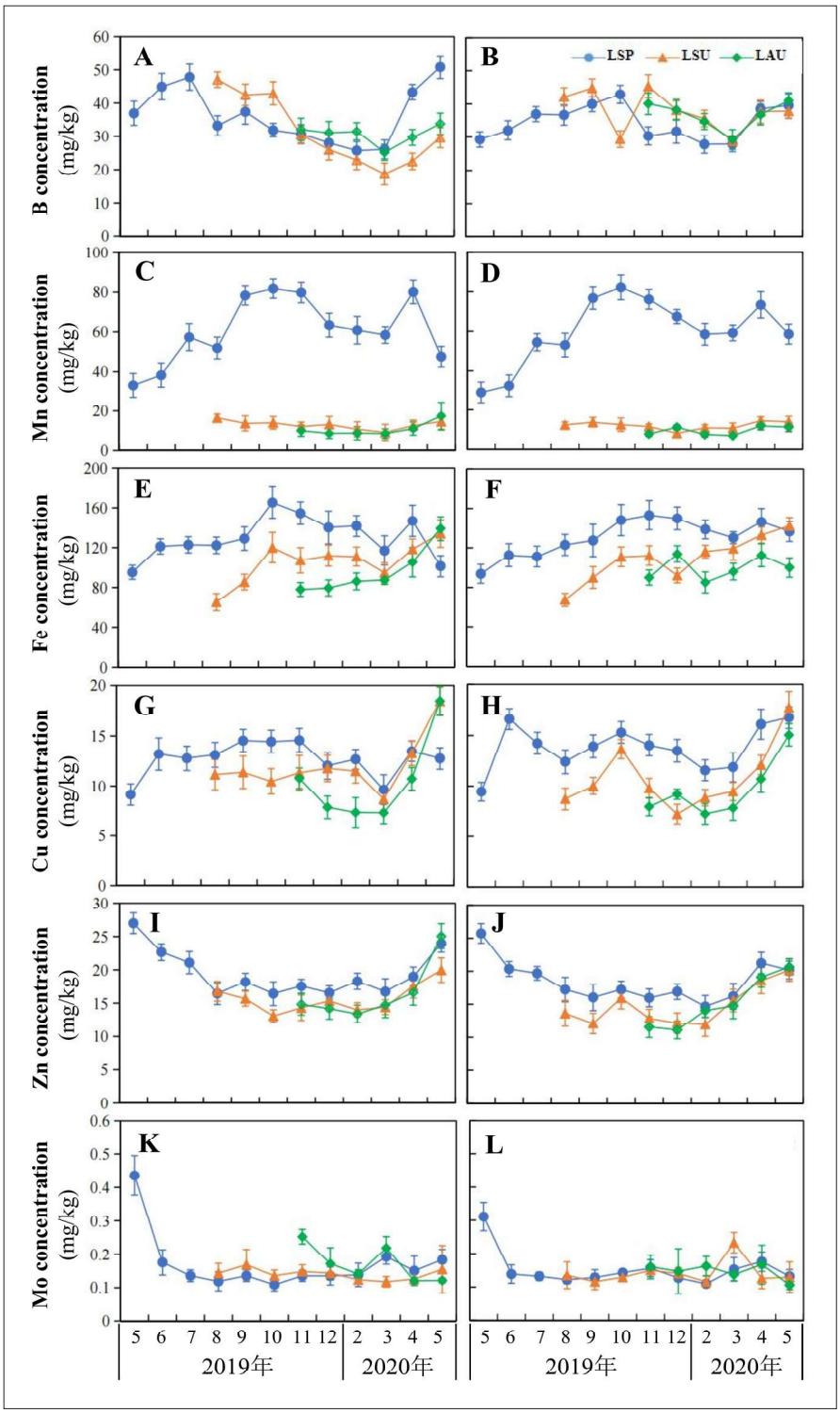


Figure 2. Annual changes in micronutrient concentrations in the leaves of different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange. A, C, E, G, I, and K: 'Newhall' navel orange plants grafted on trifoliate orange; B, D, F, H, J, and L: 'Newhall' navel orange plants grafted on citrange. LSP: leaves of spring shoots; LSU: leaves of summer shoots; LAU: leaves of autumn shoots.

at the end of stage IV in trifoliate orange-grafted navel orange. Interestingly, the Ca concentration increased during stage II and decreased during stage III in LSU. The Mg concentrations, it should be noted, decreased significantly during stage II in LSP, but in LSU and LAU, the Mg concentrations showed continuously decreasing trend.

Concentration of micronutrients

The B concentration in LSP increased with leaf age during stages I and IV of 'Newhall' navel orange grafted on both trifoliate orange and citrange rootstock. There was a significant decline of B concentration in LSP during stage II in trifoliate orange-grafted navel orange but not in citrange-grafted navel orange. In LSU and LAU, the B concentration decreased during stages II and III and increased during stage IV, with the exception of October in citrange-grafted navel orange. The concentrations of micronutrients in LSP, LSU, and LAU of trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar, except Mn in LSP. The concentration of Mn was significantly higher in LSP than in LSU and LAU. In LSP, the concentration of Fe and Mn increased during stage I and II but declined during stage III of navel orange grafted on two rootstocks. There was no significant change for Mn in LSU and LAU. Fe concentration in LSU increased during stages II and IV. The concentration of Cu reached its lowest point at the end of stage III and early stage IV in the leaves of all branch types. The patterns of annual changes in the concentrations of Zn and Mo in trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar. Zn concentrations decreased during stage I and increased during stage IV, and they were generally similar in the leaves of all branch types. The concentration of Mo decreased from a maximum just after stage I to a minimum at stage II and then remained steady from stage II to IV in LSP.

Correlation analysis of annual changes in mineral nutrients

The correlation analysis of annual changes of mineral nutrients showed that there were strong positive correlations among P, K, and Mg in all branch types of 'Newhall' navel orange grafted on both trifoliate orange and citrange rootstock. The correlations between Ca and other mineral nutrients varied among branch types (Figure 2). There was no significant correlation between B and other mineral nutrients in LSP, but there was a significant positive correlation of B with P, K, Mg, and Mn in LSU and a significant positive correlation between B and Ca in LAU of 'Newhall' navel orange grafted on both trifoliate orange and citrange rootstock. The leaf Zn content was significantly negatively correlated with Ca, Fe, and Mn in LSP, not significantly correlated with Ca in LSU, and negatively correlated with Ca in LAU. There was a strong positive correlation between the concentration of Fe and Mn in LSP and LAU, but not in LSU (Figure 3).

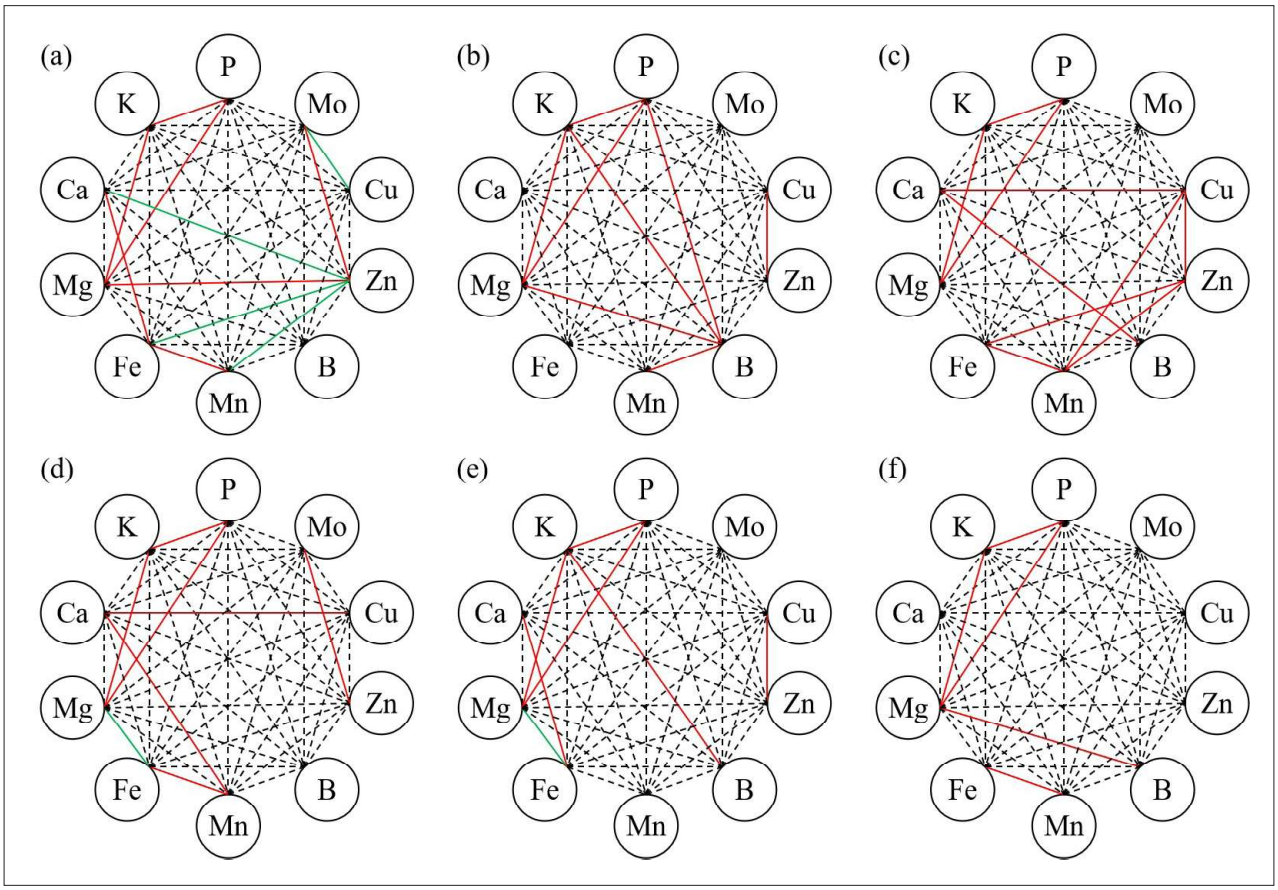


Figure 3. Correlations between elements in the leaves of different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange. (a), (b), and (c): Leaves in the spring shoots, summer shoots, and autumn shoots of 'Newhall' navel orange plants grafted on trifoliate orange; (d), (e), and (f): Leaves in the spring shoots, summer shoots, and autumn shoots of 'Newhall' navel orange plants grafted on citrange. Red solid lines refer to significant positive correlations between elements ($P < 0.001$), green solid lines refer to significant negative correlations between elements, and black dotted lines refer to non-significant correlations between elements.

Average mineral nutrient concentrations and principal component analysis

As shown in Table 1, there were significant differences in the average annual concentrations of mineral nutrients in the leaves of different types of branches in both trifoliate orange-grafted and citrange-grafted 'Newhall' navel orange. For macronutrient, the P average concentration was significantly lower in LSP than in LSU, especially in LAU of citrange-grafted navel orange. The average K concentration was significantly higher in LSP than in LAU, but there was no significant difference in the K concentration between LSP and LSU. In contrast to LSP, the average Ca concentration in LSU and LAU was significantly reduced, except in LAU of citrange-grafted navel orange. The average Mg concentration was significantly higher in the leaves of the same type of branch in trifoliate orange-grafted than in citrange-grafted navel orange, and the highest concentration of Mg was observed in LSP. The average annual Fe, Mn, Zn, and Cu concentrations were significantly lower in LSP than in LSU and LAU, and their patterns in both rootstocks of 'Newhall' navel orange were similar, with the exception of Cu in LSU. In contrast to other micronutrients, the B concentration was significantly higher in LSP than in LSU and LAU of trifoliate orange-grafted navel orange, but no significant differences were observed in citrange-grafted navel orange. The Mo concentration was significantly lower in LSU than in LSP and LAU of trifoliate orange-grafted navel orange, but not in citrange-grafted navel orange.

PCA revealed that the cumulative contribution rates of the first principal component (PC1) and the second principal component (PC2) were 69.0% and 67.9%, respectively. PC1 explained 39.0% and 36.9% of the variation in the total

Table 1. Mineral nutrient concentrations in the leaves of different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange.

	'Newhall' navel orange grafted on trifoliate orange			'Newhall' navel orange grafted on citrange		
	Leaf of spring shoot	Leaf of summer shoot	Leaf of autumn shoot	Leaf of spring shoot	Leaf of summer shoot	Leaf of autumn shoot
Macronutrient (%, DW)						
P	0.15 ± 0.01 b	0.17 ± 0.01 a	0.15 ± 0.02 ab	0.15 ± 0.01 b	0.17 ± 0.02 a	0.17 ± 0.01 a
K	1.92 ± 0.11 a	1.84 ± 0.13 ab	1.61 ± 0.15 b	1.94 ± 0.12 a	1.81 ± 0.12 ab	1.70 ± 0.16 b
Ca	4.39 ± 0.16 a	3.60 ± 0.14 c	3.55 ± 0.15 c	4.51 ± 0.10 a	4.15 ± 0.17 b	4.23 ± 0.11 ab
Mg	0.23 ± 0.02 a	0.19 ± 0.02 b	0.20 ± 0.02 ab	0.18 ± 0.02 b	0.17 ± 0.02 bc	0.16 ± 0.01 c
Micronutrient (mg kg^{-1}, DW)						
Fe	129.8 ± 5.8 a	105.3 ± 6.5 bc	95.9 ± 8.7 c	130.9 ± 5.1 a	109.2 ± 7.2 b	99.5 ± 4.4 bc
Mn	60.91 ± 4.59 a	13.06 ± 0.67 b	10.89 ± 1.25 b	60.08 ± 4.62 a	12.11 ± 0.61 b	9.54 ± 0.82 b
B	36.58 ± 2.43 a	31.41 ± 3.24 b	30.56 ± 1.10 b	34.44 ± 1.44 ab	37.76 ± 1.87 a	36.70 ± 1.58 a
Zn	19.48 ± 0.95 a	15.67 ± 0.67 b	16.46 ± 1.61 b	18.45 ± 0.85 a	14.78 ± 0.95 b	15.21 ± 1.46 b
Cu	12.69 ± 0.48 a	11.99 ± 0.85 ab	10.41 ± 1.59 b	13.81 ± 0.61 a	10.83 ± 1.01 b	9.66 ± 1.09 b
Mu	0.17 ± 0.02 a	0.14 ± 0.01 b	0.17 ± 0.02 a	0.15 ± 0.02 b	0.14 ± 0.01 b	0.15 ± 0.01 b

DW, Dry weight. Data are presented as mean ± SE. Samples from five plants were collected for each replicate. Different small letters behind the mean values indicate significant differences ($p<0.05$) between different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange.

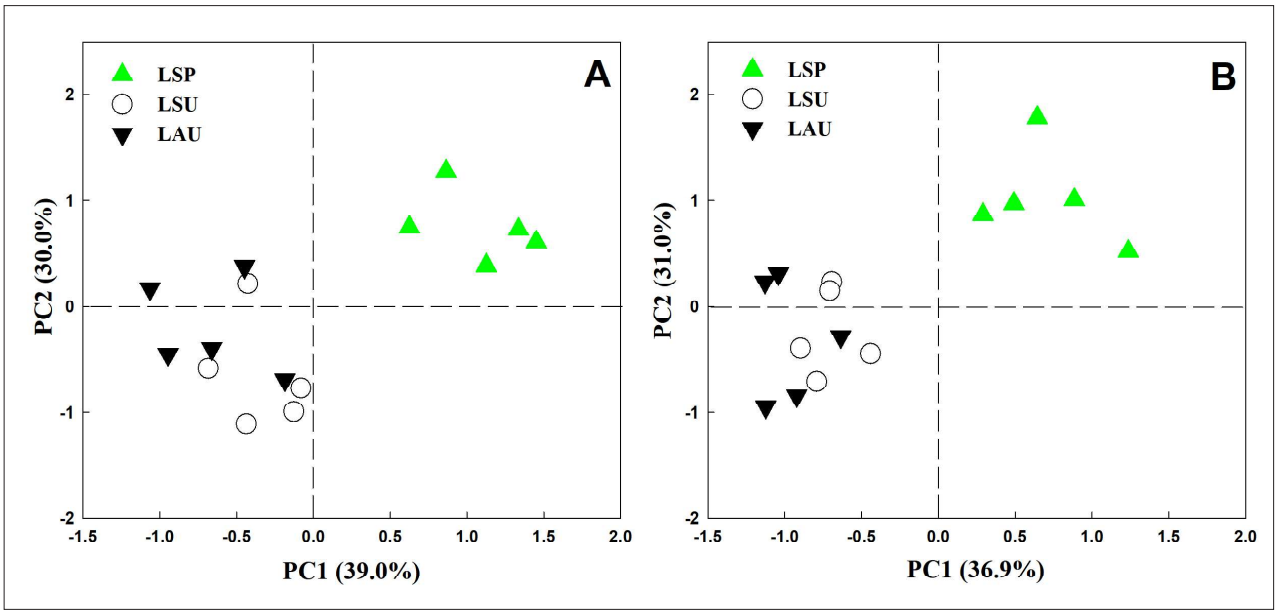


Figure 4. Principal component analysis of 10 elements in the leaves of different type of branches of 'Newhall' navel orange plants grafted on trifoliate orange (A) and citrange (B). LSP: leaves of spring shoots; LSU: leaves of summer shoots; LAU: leaves of autumn shoots.

data and clearly distinguished between LSP and LSU and between LSP and LAU, respectively. PC2 accounted for 30.0% and 31.0% of the variation in the total data and failed to distinguish the 10 elements in the leaves of different types of branches of 'Newhall' navel orange plants grafted on trifoliate orange and citrange (Figure 4). This results indicated that the content of the 10 elements in LSP was the main factor in both rootstocks of 'Newhall' navel orange, but the content of the 10 elements was similar in LSU and LAU.

Discussion

Sampling method affects nutrition diagnosis result of fruit trees

Determining the concentrations of nutrient elements in the leaves is the most direct and effective way to clarify the nutritional status of citrus trees, diagnose nutritional deficiencies or toxicity, and then form the field fertilization plan and establish the industry or local standards for nutritional diagnoses of citrus plants (Yang et al., 2013). However, due to the mobility of nutrient elements and differences between sampling methods, there were significant differences in the analysis results (Yang et al., 2015), and this conclusion has been validated by a previous study in apple (Liu et al., 2014). For example, leaves are sampled at a single point in time using the single-stage sampling method, and dynamic changes in the nutrient elements of leaves are not considered. This sampling method has great limitations and instability, resulting in great differences in results, and can't accurately reflect the element concentrations of leaves, so it is not authoritative representative (Bould, 2010; Radrizzani et al., 2011). As another example, leaves are sampled in a single type of branch using the single-branch type sampling method; however, we observed significant differences in the concentrations of nutrients in the leaves of different types of branches (Figures 1 and 2). Therefore, leaf age, branch type, and seasonal variation of nutrient elements in citrus leaves should be considered when sampling. Such considerations can not only provide new insights into the abundance of nutrients in leaves varying in age or at different periods but also enhance our understanding of the metabolism of mineral nutrients in citrus leaves. The optimal fertilization scheme for citrus should be established based on this information. In this study, the leaves were sampled from three different types of branches (LSP, LSU, and LAU), which are the main three types of branches in 'Newhall' navel orange. Sampling was conducted from the LSP development period to the same period of the following year; this sampling scheme provides more complete information on patterns of variation in plant nutrients. As the results show that the patterns of annual changes in the concentrations of leaf macro- and micronutrients in trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar; however, there were significant differences among different types of branches, as well asin the average annual concentrations of mineral nutrients (Figures 1 and 2; Table 1).

Branch type affects the annual changes in leaf mineral nutrient concentrations

Dynamic changes in the mineral nutrient concentration in citrus leaves during the annual cycle and life cycle are affected by many factors, such as the mobility of the nutrient elements, rootstock type, flower and fruit development, leaf age, and branch type (Yang et al., 2015). Element mobility affects annual changes in mineral nutrient concentrations in citrus leaves. Generally, the concentration of movable elements decreases gradually and the concentration of unmovable elements increases with the age of plant tissues and organs (Hänsch and Mendel, 2009). Previous studies have shown that the mobility and reusability of P, K, Mg, Mn, B, Cu, and are higher than that of Zn, Ca, and Fe in navel orange plants (Yang et al., 2015). The concentrations of P, K, Mg, Mn, B, Cu, and Mo declined with leaf age, but not observed for Zn, Ca, and Fe. Similarly, the concentrations of P, K, and Mg in the leaves of trifoliate orange-grafted and citrange-grafted 'Newhall' navel orange showed decreased patterns of annual change with leaf age and only increased slightly at the last observation point (Figure 1). It is worth noting that the pattern of annual change for B concentration was declined significantly during stages II and III, but increased during stages I and IV in LSP (Figure 2A). The concentration of Mo decreased from a maximum just after stage I to a minimum at stage II, and then remained stable during stages II to IV in LSP (Figures 2K and L). These results indicated that the concentrations of B and Mo, despite being removable and reusable mineral nutrients, do not always decline with leaf growth. In sum, the concentrations of removable and reusable mineral nutrients in different stages were obviously different. Therefore, the mobility and reusability of mineral nutrients are important considerations when formulating a suitable fertilization strategies.

Flower and fruit development affect annual changes of mineral nutrient concentrations in citrus leaves. Our previous studies have shown that the quantity of each element accumulated increases steadily throughout growth and peaks by harvest in both grafted plants, with the exception of Fe, Mn, and Zn (Liu et al., 2020). In this study, P, K, Mg, B, Zn, and Mo concentrations decreased markedly during stage II in LSP and LSU (Figures 1 and 2). Stage II is the period of rapid growth for navel orange fruit, as well as the period of maximum accumulation of mineral nutrients in fruit. During this stage, the mineral nutrients are transferred from leaves to fruit, which results in a decrease in leaf nutrient concentrations. At the end of stage II and early stage III, the accumulation rate of nutrient elements in fruit decreased markedly, and the concentrations of nutrient elements in leaves remained relatively stable. These results are consistent with the findings of previous studies of other citrus varieties (Sheng et al., 2009). Another period during which the concentrations of mineral nutrients declined was stage IV, especially from February to March, which corresponds to the period when navel orange flowers develop. During this stage, the concentrations of P, K, Mg, Mn, B, Mn, and Cu declined significantly in the leaves of all types of branches (Figures 1 and 2). These findings indicate that the distribution and transfer of nutrients were affected most strongly by flower development.

The type of rootstock affects annual changes in the concentration of mineral nutrients in citrus leaves. Rootstock plays an important role in promoting nutrients absorption and improving stress tolerance for citrus plants (Murkute et al., 2006; Zhou et al., 2014; Fu et al., 2017). Previous studies have shown that seasonal and annual changes in the concentrations of nutrients in leaves, roots, and fruits significantly differ when the same citrus cultivars are grafted on different rootstocks (Liu et al., 2020). In our work, trifoliate orange-grafted and citrange-grafted 'Newhall' navel orange were used to study annual changes of mineral nutrient concentrations in leaves. As widely used citrus rootstocks, the ability of nutrient absorption and utilization is different between trifo-

liate orange and citrange (Zhou et al., 2014). As shown in Figures 1 and 2, the patterns of annual changes in the concentrations of nutrients in leaves of trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar, but differences were observed for some nutrients. For example, there was a significant decrease in the B concentration in LSP during stage II of trifoliate orange-grafted navel orange, but not for citrange-grafted navel orange (Figures 2A and B). Many previous studies have demonstrated that the difference between trifoliate orange and citrange on B absorption and utilization (Zhou et al., 2015), so the difference of annual changes of mineral nutrient concentrations in leaves may be explained by this conclusion.

It is very important that branch type also affects the patterns of annual changes of mineral nutrient concentrations in citrus leaves. In this study, the Mn concentration in LSP was markedly higher than that in LSU and LAU at all sampling points (Figures 2C and D; Table 1). Similarly, the Ca and Fe concentrations in LSP were significant higher than that in LSU and LAU during stages II and III (Figures 2E and F). Moreover, as shown in Table 1, there were significant differences in the average annual concentrations of mineral nutrients in the leaves of different types of branches in both trifoliate orange-grafted and citrange-grafted 'Newhall' navel orange. Although branch type affects the concentrations of mineral nutrients in citrus leaves, no differences were observed in annual changes, with the exception of Ca, B, and Mn. Our findings were consistent with previous studies on apple (Liu et al., 2014).

Overall, the characteristics of mineral nutrient, rootstock type, flower and fruit development stage, leaf age, and branch type should be taken into account when formulating field fertilization strategies for navel orange. For example, P, K, and Mg should be applied 10–15 days before stage IV using quick-acting fertilizers combined with organic fertilizers. Soil Ca content is rarely low because occasional applications of lime (calcium carbonate, $CaCO_3$) are used to control soil acidity, and it is absorbed and utilized at a low rate by citrus plant (Zekri and Obreza, 2013, 2015). thus, Ca foliar fertilizer should be applied to fields before stage III. Furthermore, it has proved that one of the main reasons why a large number of shoots spring up during the summer in citrus plants was because of the application of excess fertilizer. Therefore, in order to prevent competition for nutrients between summer shoots and young fruit, and decrease the occurrence of disease, an appropriate amount of fertilizer should be applied during the spring (Huang et al., 2011; Wang et al., 2018).

Conclusions

In conclusion, the patterns of annual changes in the concentrations of macro- and micronutrients in the leaves of trifoliate orange-grafted and citrange-grafted navel orange trees were generally similar, but there were significant differences among branch types and in annual average concentrations of mineral nutrients. Furthermore, there were strong positive correlations among P, K, and Mg in all branch types of 'Newhall' navel orange grafted on both trifoliate orange and citrange rootstock. PCA revealed that the mineral nutrients in LSP were particularly important than LSU and LAU for navel orange trees, and those in LSU and LAU were similar. Overall, mineral nutrient identity, rootstock type, flower and fruit development stage, leaf age, and branch type should be taken into account when formulating field fertilization strategies for 'Newhall' navel orange.

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Conflict of interests

The authors declare that they have no competing interests.

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