

Effects of Foliar Application of Various Zinc Fertilizers with Organosilicone on Correcting Citrus Zinc Deficiency

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Additional index words. orange, necrosis, chlorophyll, photosynthesis

Abstract. To compare the effects of various zinc (Zn) foliar fertilizers on correcting citrus Zn deficiency and to explore an effective correcting method, three common Zn fertilizers, Zn sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), Zn chloride (ZnCl_2), and Zn nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], were selected to spray the Zn-deficient citrus leaves, tested at different concentrations, with or without organosilicone surfactant. Zn content, chlorophyll levels, and photosynthesis characteristics of leaves were analyzed. Leaf Zn content was significantly increased with increase of the sprayed Zn concentration of the three Zn fertilizers. However, when the sprayed Zn concentration of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ exceeded $200 \text{ mg} \cdot \text{L}^{-1}$, and Zn concentration of ZnCl_2 or $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ exceeded $100 \text{ mg} \cdot \text{L}^{-1}$, obvious necrotic spots formed on leaves. This necrosis disappeared when 0.025% organosilicone was added to the three Zn fertilizer solutions, even at a Zn concentration of $250 \text{ mg} \cdot \text{L}^{-1}$. Meanwhile, the Zn contents of leaves increased one to four times for these treatments. Furthermore, foliar application of the three Zn fertilizers significantly improved chlorophyll levels and photosynthetic capacity of Zn-deficient leaves. The data of chlorophyll and photosynthesis characteristics indicate that the correcting effect of ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ is better than that of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and could be further improved via supplement of organosilicone. In conclusion, ZnCl_2 or $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ containing $250 \text{ mg} \cdot \text{L}^{-1}$ of Zn and supplemented with 0.025% organosilicone is a safe and effective formulation of Zn foliar fertilizer for correcting citrus Zn deficiency.

Zn is an essential micronutrient for plant growth and development. However, in citrus production, Zn deficiency is one of the most damaging and widespread nutritional disorders in both acidic and alkaline soils (Srivastava and Singh, 2009). Zn analyses have shown that over 70% of citrus orchards in China are low in Zn. Globally, Zn is recognized, next to nitrogen, as the most widely deficient nutrient in citrus, thereby limiting citrus yield and quality (Srivastava and Singh, 2005). Typical symptoms of Zn deficiency are well known as “little leaf,” “mottle leaf,” and “rosetting” that are basically characterized by small, narrow leaves, chlorosis between the veins, and crowding along short stems, respectively (Srivastava

and Singh, 2005). Apart from these effects, Zn deficiency also reduces tree vigor and photosynthetic activity and lowers fruit set, yield, size, and quality, even in its earliest stages (Chapman, 1968; Fu et al., 2014).

Management of Zn deficiency still relies on two conventionally used methods, soil or foliar fertilization. However, soil-applied Zn is less available to plants due to its low mobility and high soil fixation (Chapman, 1968; Razzaq et al., 2013). In addition, due to its much lower labor and time cost, foliar application of Zn fertilizer has almost completely replaced soil application. The positive effects of Zn sprays on nutrient status, yield, and fruit quality have been observed in mandarin (Razzaq et al., 2013; Srivastava and Singh, 2009), orange (Boaretto et al., 2002; Eman et al., 2007; Hanafy Ahmed et al., 2012; Swietlik and Laduke, 1991), and grapefruit (Swietlik, 2002). Foliar application of Zn is now commonly accepted as a relatively effective method for correcting Zn deficiency in citrus production. However, foliar application still has some limitations. First, utilization efficiency of Zn is very low. Based on our statistical data of fertilization amount and leaf Zn content, the leaves absorbed less than 20% of foliar-applied Zn (unpublished data). Second, it is still difficult to facilitate the recovery of the entire leaf to

a green color using foliar application. Recovery of green color is punctiform in the yellow leaf (unpublished data). In addition, necrotic spots often occur on leaves, especially younger ones. This is possibly because the foliar spray often adheres to leaves as droplets. In addition, the Zn poorly transported from the sprayed leaves to the other plant parts, even between the mesophyll cells in one leaf (Boaretto et al., 2002). Boaretto et al. (2002) reported that less than 1% of the applied Zn was transported from sprayed leaves to the other plant organs. As a result, only the locations to which droplets adhered absorbed Zn and increased Zn levels, producing punctiform recovery of green color. Once the sprayed Zn concentration increases to a relatively high value, the locations will very easily accumulate excess Zn, which is poorly transported to other parts of the leaf, finally causing necrotic spots.

In this study, we compared absorption efficiency in citrus leaves of three common Zn fertilizers, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, ZnCl_2 , and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and their correcting effects on citrus Zn deficiency, to find the best utilization efficiency and correcting effect of chemical Zn. In addition, we tested the roles of agricultural organosilicone surfactant in improving the foliar fertilizer absorption efficiency and correcting effect. Organosilicone surfactant is recognized as an adjuvant with super spreading, wetting, and penetration power for lowering the aqueous surface tension (Knoche, 1994; Stevens, 1993), but literature reporting its applications in citrus is scarce. This study provides useful guidance for citrus Zn deficiency management practice.

Materials and Methods

Plant materials and treatments. On 3 Sept. 2012, 1-year-old ‘Fengwan’ navel orange plants (*Citrus sinensis* Osbeck) grafted on Ziyang xiangcheng (*Citrus junos* Sieb. ex Tanaka) rootstocks that were grown in the orchard of the Citrus Research Institute, Southwest University (Chongqing, China), were selected for spraying with three forms of Zn: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, ZnCl_2 , and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, prepared at different concentrations based on net Zn contents, and supplemented with or without organosilicone surfactant (Table 1). In total, 13 treatments were performed on both sides of the leaves of spring (≈ 7 -month-old), summer (≈ 4 -month-old), and autumn twigs (≈ 1 -month-old). In Chongqing of China, the sprouting time of spring, summer, and autumn twigs is around the beginning of February, May, and August, respectively. For each treatment, three replicates were conducted on 30 plants (10 plants per replicate). After 30 d, the leaves of spring, summer, and autumn twigs were sampled for Zn content analysis.

On 17 Sept. 2013, three 6-year-old ‘Fengwan’ navel orange and three Midknight Valencia orange (*C. sinensis* Osbeck) trees grafted on citrange (*C. sinensis* × *Poncirus trifoliata*) rootstocks which were grown in the orchard of the Citrus Research Institute,

Received for publication 6 Nov. 2015. Accepted for publication 3 Feb. 2016.

This work was financially supported by the National Natural Science Foundation of China (project no. 31301742), the Natural Science Foundation Project of Chongqing (project no. CSTC2013JCYJA80018), the Earmarked Fund for China Agriculture Research System (CARS-27-02A) from Ministry of Agriculture of China, and the Fundamental Research Funds for the Central Universities of China (XDJK2016B005).

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Southwest University (Chongqing, China), were selected as subjects for evaluating the effects of spraying with various Zn treatments on the correction of Zn deficiency. In each tree, nine autumn twigs with Zn-deficient mottled yellow leaves were selected, labeled, and divided into three groups (three twigs per group). For these three groups of twigs in each tree, both sides of the leaves were sprayed with the various Zn fertilizers as shown in Table 2. The spraying concentrations were set based on the measured Zn content data in 2012. The chlorophyll contents and photosynthetic characteristics were analyzed at days 0 and 60 after spraying.

Measurement of Zn content. The sampled leaves were washed with 0.1% mediosilicic detergent, rinsed with distilled water, and

then transferred to 0.2% HCl to soak for 30 s and rinsed again with distilled water. To deactivate enzymes, the leaves were next put in an oven at 105 °C for 30 min, then dried at 75 °C for 2 d. Leaves were powdered, and ≈0.5 g of sample was dissolved in 10 mL of a 5:1 mixture of HNO₃ (65%) and HClO₄ (37%) at 165 °C for 4 h, 185 °C for 2 h, and 206 °C for 2.5 h. The dissolved solution was diluted with distilled water to 50 mL. The concentration of Zn was determined by flame atomic absorption spectrometry (Perkin-Elmer AA800, Shelton, CT).

Measurement of chlorophyll content and photosynthetic characteristics. Chlorophyll contents were determined using a handheld chlorophyll meter (SPAD-502; Konica-Minolta, Japan). For each leaf, six readings were taken at different locations. Net photosynthetic rate

(P_n), stomatal conductance (g_s), substomatal CO₂ concentration (C_i), and transpiration rate (E) were measured at 9:00 AM–12:00 PM under conditions of air velocity 500 mL·min⁻¹, light intensity 1000 μmol·m⁻²·s⁻¹, 380 ± 15 ppm CO₂, and 15 ± 5 °C ambient temperature with an LI-6400 system (LI-COR, Lincoln, NE). Each leaf was analyzed and five transient P_n, g_s, C_i, and E readings were obtained.

Statistical analysis. The data presented herein are shown as mean values ± SE. All data were analyzed using analysis of variance with SPSS statistical software (version 20.0; SPSS Inc., Chicago, IL), and statistical differences were compared based on Fisher's least significant difference tests at the significance level of P < 0.05.

Results

Changes in Zn contents and symptoms of leaves after spraying various concentrations of Zn fertilizers. To compare absorption efficiency in citrus leaves of three types of Zn fertilizers and to determine the critical concentration that causes necrotic spots on leaves, 1-year-old 'Fengwan' navel oranges grafted onto Ziyang xiangcheng rootstocks were selected for spraying with three types of chemical Zn at various concentrations (Table 1). Zn content in leaves of spring, summer, and autumn twigs were all increased in varying degrees after spraying with three types of Zn fertilizers (Table 3). Moreover, Zn content increased with the increase of spraying concentration of Zn fertilizer. As shown in Table 3, when 100 mg·L⁻¹ of Zn from ZnSO₄·7H₂O (treatment 1) was sprayed, Zn contents in the leaves of spring, summer, and autumn twigs were increased by 10.26%, 56.08%, and 82.64%, respectively, compared with those of the control treatment (spraying water). They were all lower than those sprayed 150 mg·L⁻¹ of Zn from ZnSO₄·7H₂O (treatment 3). Among all treatments, the increase of Zn was highest in autumn twigs, followed by summer twigs and then spring twigs. Zn content in leaves sprayed with ZnCl₂ and Zn(NO₃)₂·6H₂O was significantly higher than those sprayed with the same Zn concentration of ZnSO₄·7H₂O,

Table 1. Treatments with various types and concentrations of zinc (Zn) foliar fertilizers.

Treatment	Type of Zn fertilizer	Spraying concn (net content of Zn, mg·L ⁻¹)
1	ZnSO ₄ ·7H ₂ O	50
2	ZnSO ₄ ·7H ₂ O	100
3	ZnSO ₄ ·7H ₂ O	150
4	ZnSO ₄ ·7H ₂ O + 0.025% Si	250
5	ZnCl ₂	50
6	ZnCl ₂	100
7	ZnCl ₂	150
8	ZnCl ₂ + 0.025% Si	250
9	Zn(NO ₃) ₂ ·6H ₂ O	50
10	Zn(NO ₃) ₂ ·6H ₂ O	100
11	Zn(NO ₃) ₂ ·6H ₂ O	150
12	Zn(NO ₃) ₂ ·6H ₂ O + 0.025% Si	250
13	Water (control)	0

Si = organosilicone; ZnSO₄·7H₂O = Zn sulfate heptahydrate; ZnCl₂ = Zn chloride; Zn(NO₃)₂·6H₂O = Zn nitrate hexahydrate.

Table 2. Correction treatments with various zinc (Zn) foliar fertilizers on Zn-deficient citrus trees.

Treatment	Types of Zn fertilizer	Spraying concn (net content of Zn, mg·L ⁻¹)
Group 1	1 Water (control)	0
	2 ZnSO ₄ ·7H ₂ O	200
	3 ZnSO ₄ ·7H ₂ O + 0.025% Si	250
Group 2	1 Water (control)	0
	2 ZnCl ₂	100
	3 ZnCl ₂ + 0.025% Si	250
Group 3	1 Water (control)	0
	2 Zn(NO ₃) ₂ ·6H ₂ O	100
	3 Zn(NO ₃) ₂ ·6H ₂ O + 0.025% Si	250

Si = organosilicone; ZnSO₄·7H₂O = Zn sulfate heptahydrate; ZnCl₂ = Zn chloride; Zn(NO₃)₂·6H₂O = Zn nitrate hexahydrate.

Table 3. Zinc (Zn) contents in leaves of 1-year-old 'Fengwan' navel oranges sprayed with various concentrations of Zn fertilizers for 30 d.

Treatment		Content of Zn (mg·kg ⁻¹)		
		Spring twigs	Summer twigs	Autumn twigs
1	50 mg·L ⁻¹ ZnSO ₄ ·7H ₂ O	25.68 ± 0.25 h	27.14 ± 0.28 h	28.51 ± 0.26 j
2	100 mg·L ⁻¹ ZnSO ₄ ·7H ₂ O	24.61 ± 0.51 h	32.98 ± 0.54 g	34.61 ± 0.64 i
3	150 mg·L ⁻¹ ZnSO ₄ ·7H ₂ O	27.28 ± 0.16 g	37.07 ± 0.32 fg	39.50 ± 0.26 h
4	250 mg·L ⁻¹ ZnSO ₄ ·7H ₂ O + 0.025% Si	52.08 ± 0.66 b	63.22 ± 0.79 e	84.21 ± 0.58 e
5	50 mg·L ⁻¹ ZnCl ₂	29.75 ± 0.54 f	42.09 ± 0.06 f	62.56 ± 1.16 f
6	100 mg·L ⁻¹ ZnCl ₂	37.82 ± 0.59 d	122.05 ± 0.73 b	150.20 ± 5.37 b
7	150 mg·L ⁻¹ ZnCl ₂	46.61 ± 0.46 c	176.35 ± 6.70 a	226.02 ± 10.68 a
8	250 mg·L ⁻¹ ZnCl ₂ + 0.025% Si	54.44 ± 0.37 a	59.54 ± 0.20 e	95.35 ± 1.43 c
9	50 mg·L ⁻¹ Zn(NO ₃) ₂ ·6H ₂ O	29.92 ± 0.41 e	41.52 ± 0.60 f	53.26 ± 0.40 g
10	100 mg·L ⁻¹ Zn(NO ₃) ₂ ·6H ₂ O	32.21 ± 0.39 e	60.00 ± 1.13 e	90.74 ± 0.58 d
11	150 mg·L ⁻¹ Zn(NO ₃) ₂ ·6H ₂ O	36.56 ± 0.39 d	90.16 ± 1.54 c	149.58 ± 0.74 b
12	250 mg·L ⁻¹ Zn(NO ₃) ₂ ·6H ₂ O + 0.025% Si	54.00 ± 0.12 a	71.23 ± 0.43 d	90.74 ± 0.91 d
13	Water (control)	22.32 ± 0.38 i	21.13 ± 0.49 i	18.95 ± 0.61 k

The data presented here are the mean values ±SE, analyzed by a least significant difference test at significance level P < 0.05. Values followed by the same lowercase letters were not significantly different.

ZnSO₄·7H₂O = Zn sulfate heptahydrate; ZnCl₂ = Zn chloride; Zn(NO₃)₂·6H₂O = Zn nitrate hexahydrate.

leaves sprayed with ZnCl_2 having the highest Zn content (Table 3). These results suggest that the absorption efficiency of citrus leaves to the three Zn fertilizers is highest for ZnCl_2 , followed by $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and then $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. The leaves of autumn twigs showed obvious necrotic spots when $200 \text{ mg}\cdot\text{L}^{-1}$ Zn from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ was sprayed without organosilicone (Fig. 1). These necrotic spots also appeared on leaves sprayed with $100 \text{ mg}\cdot\text{L}^{-1}$ Zn from ZnCl_2 or $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and became more serious when the concentration was increased to $150 \text{ mg}\cdot\text{L}^{-1}$ (Fig. 1). In the leaves of summer twigs, no necrotic spots occurred after application of $200 \text{ mg}\cdot\text{L}^{-1}$ Zn from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ without organosilicone, while application of $150 \text{ mg}\cdot\text{L}^{-1}$ Zn from ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ caused obvious necrotic spots. In the leaves of spring twigs, no necrotic spots were observed in any treatment. Interestingly, when 0.025% organosilicone was added in the three Zn fertilizers, none of the leaves of autumn, summer, or spring twigs showed necrotic spots, even after spraying with $250 \text{ mg}\cdot\text{L}^{-1}$ of Zn (Fig. 1). Furthermore, the Zn contents in these leaves increased 1- to 4-fold

compared with control treatments (Table 3). As shown in Table 3, Zn contents in spring-flush leaves sprayed with 100 or $150 \text{ mg}\cdot\text{L}^{-1}$ Zn from ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ without organosilicone, as well as in summer-flush leaves sprayed with $100 \text{ mg}\cdot\text{L}^{-1}$ Zn from $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ without organosilicone, were lower than those in corresponding leaves sprayed with $250 \text{ mg}\cdot\text{L}^{-1}$ Zn from ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ containing organosilicone. However, we also found that the Zn contents in summer- and autumn-flush leaves sprayed with $250 \text{ mg}\cdot\text{L}^{-1}$ Zn from ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ containing organosilicone were even lower than those in corresponding leaves sprayed with $100 \text{ mg}\cdot\text{L}^{-1}$ or $150 \text{ mg}\cdot\text{L}^{-1}$ of corresponding Zn fertilizer without organosilicone (Table 3). This is because leaves of the latter treatment absorbed and accumulated excess Zn at local regions to induce necrotic spots, and this excess Zn was poorly transported to other parts of the plant, as mentioned above. Thus, as high Zn content in necrotic spots were not removed and washed away, they increased the whole-leaf determination results of Zn. Taken together, the results indicate that although foliar

application of high-concentration Zn fertilizers could maximally increase Zn levels in leaves, this easily resulted in necrotic spots in young leaves; the highest Zn concentrations that could be safely applied were $200 \text{ mg}\cdot\text{L}^{-1}$ for $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $100 \text{ mg}\cdot\text{L}^{-1}$ for ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; however, when 0.025% organosilicone was supplemented, the applied Zn concentration of the three Zn fertilizers could be increased to $250 \text{ mg}\cdot\text{L}^{-1}$ with no necrotic spots being formed.

Changes of chlorophyll levels in Zn-deficient leaves after spraying different Zn fertilizers. As shown above, the threshold Zn concentrations causing necrotic spots were $200 \text{ mg}\cdot\text{L}^{-1}$ for $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $100 \text{ mg}\cdot\text{L}^{-1}$ for ZnCl_2 and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. It means that applicable Zn concentration should not exceed above threshold for three Zn fertilizers. Based on this result, correcting effects were compared among the three Zn fertilizers supplemented without organosilicone (spraying threshold concentrations of Zn) or with 0.025% organosilicone (spraying $250 \text{ mg}\cdot\text{L}^{-1}$ of Zn) under conditions that do not cause necrotic spots while also maximally increasing Zn levels, to explore optimal solution for correcting Zn deficiency. Three Zn fertilizers were tested in three groups, respectively. Each group received three treatments, as shown in Table 2.

After 60-d treatment, all control leaves showed more seriously mottled yellowing than before treatment (day 0), while most of the leaves sprayed with the three fertilizers, especially those supplemented with 0.025% organosilicone, turned green, except for a small number of leaves that still showed slight yellow coloring (Fig. 2). Determination of chlorophyll showed that after 60 d of treatments, chlorophyll content was significantly decreased in control leaves and increased in the three fertilizer-sprayed leaves (Table 4). Chlorophyll content was highest in the treatment supplemented with organosilicone, followed by the treatment supplemented without organosilicone and then in the control for all three Zn fertilizers. Among these, the highest increment of chlorophyll content was the leaves treated with $250 \text{ mg}\cdot\text{L}^{-1}$ of Zn from $\text{ZnCl}_2 + 0.025\%$ organosilicone, followed by $250 \text{ mg}\cdot\text{L}^{-1}$ of Zn from $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} + 0.025\%$ organosilicone.

Changes of photosynthetic characteristics in Zn-deficient leaves after spraying different Zn fertilizers. As shown in Fig. 3, the leaves sprayed with the three Zn fertilizers had significantly higher P_n than controls after 60 d of treatment. Moreover, the P_n in the treatments supplemented with organosilicone were significantly higher than those without organosilicone. There was no significant difference in g_s among the treatments of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and control, and the treatments of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} +$ organosilicone, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and control, while the treatments of $\text{ZnCl}_2 +$ organosilicone, ZnCl_2 , and control showed significant differences in comparison with each other. Similar variation was also found in E (Fig. 3). For the C_i , the treatments of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} +$ organosilicone,

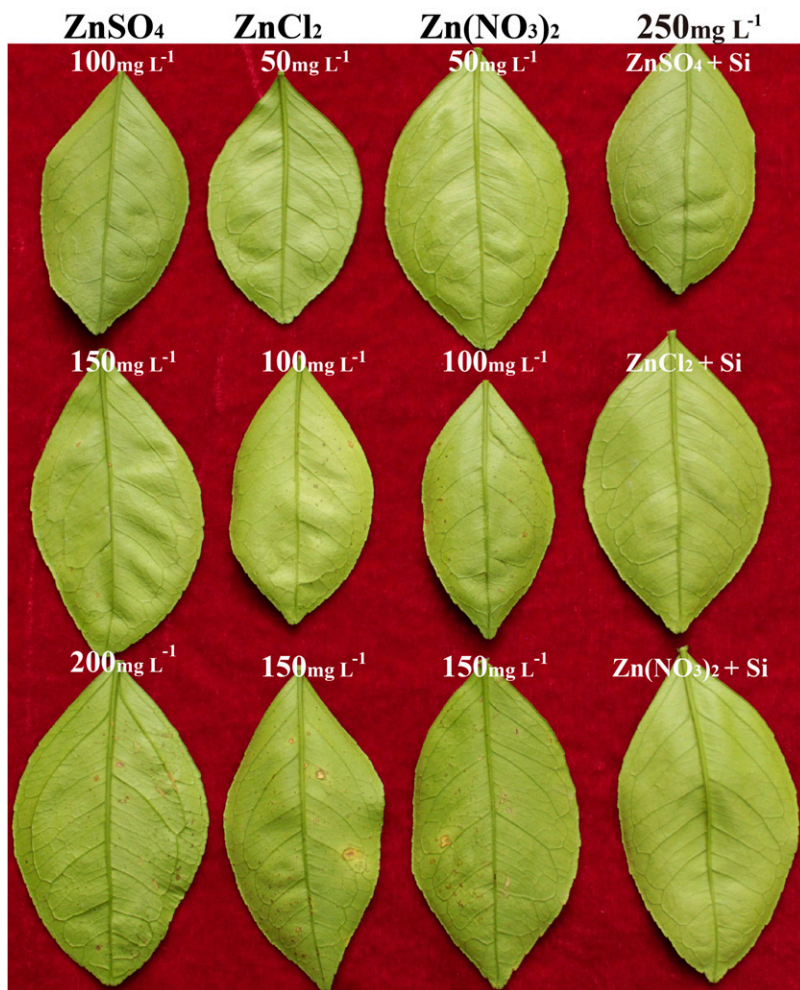


Fig. 1. Leaf symptoms after spraying with zinc (Zn) fertilizers of various concentrations. Leaves of autumn twigs of 1-year-old 'Fengwan' navel oranges sprayed with treatments of: 50–200 $\text{mg}\cdot\text{L}^{-1}$ of Zn from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and ZnCl_2 , and $250 \text{ mg}\cdot\text{L}^{-1}$ of Zn from $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and ZnCl_2 supplemented with 0.025% organosilicone (Si) for 30 d.

Zn(NO₃)₂·6H₂O + organosilicone, ZnCl₂ + organosilicone, and ZnCl₂ were significantly lower than the control, and no difference was observed in other treatments. These results show that application of the three Zn fertilizers obviously increased P_n and decreased C_i, respectively, in Zn-deficient citrus leaves, especially for those supplemented with 0.025% organosilicone.

Discussion

As a main control strategy of citrus Zn deficiency, foliar fertilization still presents some problems, including low-utilization efficiency, punctiform recovery of green color, and production of necrotic spots. Knowledge about the differences in absorption and

utilization efficiency, correcting effect, and applicable concentration remains scarce for commonly used Zn fertilizers such as ZnSO₄·7H₂O, ZnCl₂, and Zn(NO₃)₂·6H₂O. In this study, we found that ZnCl₂ and Zn(NO₃)₂·6H₂O were better absorbed by leaves than ZnSO₄·7H₂O. Moreover, the young leaves had significantly higher absorption efficiency than old leaves, as supported by the higher Zn content in the leaves of autumn twigs vs. summer and spring twigs (Table 3). This result also supports the previous viewpoint that application of Zn fertilizers on new-growth leaves is more effective than on mature and old leaves (Chapman, 1968; Srivastava and Singh, 2004). However, we also found that when the applied Zn concentration exceeded 200 mg·L⁻¹ for

ZnSO₄·7H₂O and 100 mg·L⁻¹ for ZnCl₂ and Zn(NO₃)₂·6H₂O, obvious necrotic spots appeared on the leaves. This result seems contradictory to the usual concentration in citrus field practice of 500–1000 mg·L⁻¹ of ZnSO₄·7H₂O (≈114–228 mg·L⁻¹ of Zn) or Zn(NO₃)₂·6H₂O (≈110–220 mg·L⁻¹ of Zn) used in foliar application for controlling citrus Zn deficiency without necrosis occurring on leaves. It is possible because more Zn solutions were sprayed on both sides of the leaves in our experiment, and the locations of leaves to which droplets adhered accumulated excess Zn, which could not be transported to other plant parts (Boaretto et al., 2002; Srivastava and Singh, 2005). In addition, the high-temperature and low-humidity conditions in September of Chongqing of China may have further facilitated formation of necrotic spots. Interestingly, we demonstrated that supplementation of organosilicone could effectively reduce the production of necrotic spots. Since the 1970s, organosilicone, such as Silwet L-77, has been reported as a useful adjuvant in an agricultural context owing to its properties of super spreading, wetting, and penetration power (Knoche, 1994; Stevens, 1993). However, in citrus production, organosilicone has been rarely applied and reported on before now. Our results provide positive information for the improvement of citrus foliar fertilization.

It is well known that Zn deficiency lowers chlorophyll levels and photosynthetic activity of plants (Cakmak, 2000; Chen et al., 2008; Fu et al., 2014; Hu and Sparks, 1991). In this study, the similar result was found in untreated Zn-deficient leaves (Table 4). This is possible because Zn deficiency resulted in deformation of chloroplast structure and limitation of photosynthetic enzymes, such as carbonic anhydrase, fructose-1,6-bisphosphase, and ribulose 1,5-bisphosphate carboxylase/oxygenase (Chen et al., 2008; Sasaki et al., 1998). In addition, Zn deficiency could also decrease the intercellular CO₂ concentration and g_s, resulting in reduction of photosynthesis (Sharma et al., 1995). Application of the three Zn fertilizers on Zn-deficient leaves significantly increased their chlorophyll levels and photosynthetic activities, especially when organosilicone was supplemented. Moreover, better recovery of green and higher chlorophyll levels and photosynthetic activities were observed on ZnCl₂- and Zn(NO₃)₂·6H₂O-treated leaves,



Fig. 2. Recovery of zinc (Zn)-deficient leaves after spraying with various Zn fertilizers. Zn-deficient leaves of 6-year-old ‘Fengwan’ navel orange autumn twigs sprayed with (A) water (control), 100 mg·L⁻¹ of Zn from ZnCl₂, and 250 mg·L⁻¹ of Zn from ZnCl₂ with 0.025% organosilicone (Si), (B) 100 mg·L⁻¹ of Zn from Zn(NO₃)₂·6H₂O and 250 mg·L⁻¹ of Zn from Zn(NO₃)₂·6H₂O with 0.025% of Si, and (C) 200 mg·L⁻¹ of Zn from ZnSO₄·7H₂O and 250 mg·L⁻¹ of Zn from ZnSO₄·7H₂O with 0.025% of Si for 60 d. Similar results were determined in Midnight Valencia orange (data not shown).

Table 4. Chlorophyll contents in zinc (Zn)-deficient leaves of 6-year-old ‘Fengwan’ navel oranges sprayed with three types of Zn fertilizers at 0 or 60 d.

Treatments		Content of chlorophyll (SPAD)		Increment of chlorophyll (SPAD _{60 d} –SPAD _{0 d})
		Before treatment (0 d)	After treatment (60 d)	
Group 1	1	43.12 ± 2.30 c	34.36 ± 1.11 d	-8.76
	2	42.04 ± 1.14 c	51.02 ± 1.40 b	8.98
	3	46.48 ± 1.06 bc	60.94 ± 2.40 a	14.46
Group 2	1	43.06 ± 1.35 c	34.08 ± 3.88 d	-8.98
	2	41.80 ± 1.40 c	52.84 ± 1.81 b	11.04
	3	35.26 ± 2.15 d	66.12 ± 1.83 a	30.86
Group 3	1	48.48 ± 0.86 c	41.72 ± 1.20 d	-6.76
	2	42.24 ± 1.95 d	54.10 ± 1.46 b	11.86
	3	49.32 ± 1.41 c	68.18 ± 1.92 a	18.86

The data presented here are mean values ±SE, analyzed by a least significant difference test at a significance level of *P* < 0.05. Values followed by the same lowercase letters were not significantly different. Similar results were determined in Midnight Valencia orange (data not shown).

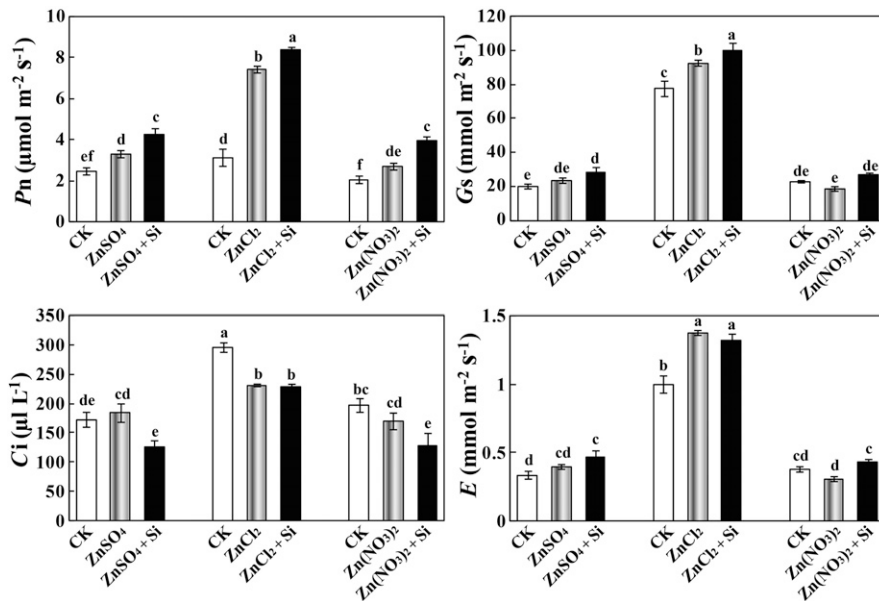


Fig. 3. Photosynthetic characteristics of zinc (Zn)-deficient leaves after spraying with various Zn fertilizers. The net photosynthetic rate (P_n), stomatal conductance (g_s), substomatal CO_2 concentration (C_i), and transpiration rate (E) were measured on Zn-deficient autumn twig leaves of 6-year-old 'Fengwan' navel oranges, which were sprayed with water (control), 100 $mg\cdot L^{-1}$ of Zn from $ZnCl_2$, and 250 $mg\cdot L^{-1}$ of Zn from $ZnCl_2$ with 0.025% organosilicone (Si), 100 $mg\cdot L^{-1}$ of Zn from $Zn(NO_3)_2\cdot 6H_2O$, and 250 $mg\cdot L^{-1}$ of Zn from $Zn(NO_3)_2\cdot 6H_2O$ with 0.025% of Si, 200 $mg\cdot L^{-1}$ of Zn from $ZnSO_4\cdot 7H_2O$, and 250 $mg\cdot L^{-1}$ of Zn from $ZnSO_4\cdot 7H_2O$ with 0.025% of Si for 60 d. The data presented here are the mean values \pm SE, which were analyzed based on a least significant difference test at the significance level of $P < 0.05$. The same letters indicates no significant difference between groups. Similar results were determined in Midknight Valencia orange (data not shown).

suggesting that $ZnCl_2$ and $Zn(NO_3)_2\cdot 6H_2O$ had a more effective correcting effect than $ZnSO_4\cdot 7H_2O$.

In conclusion, foliar application of $ZnCl_2$, $Zn(NO_3)_2\cdot 6H_2O$, and $ZnSO_4\cdot 7H_2O$ could correct citrus Zn deficiency to varying degrees, as supported by the increased Zn content, chlorophyll levels, and photosynthetic activities, and $ZnCl_2$ and $Zn(NO_3)_2\cdot 6H_2O$ were more effective than $ZnSO_4\cdot 7H_2O$. However, the applied Zn concentration should not exceed 100 $mg\cdot L^{-1}$ for $ZnCl_2$ and $Zn(NO_3)_2\cdot 6H_2O$ and 200 $mg\cdot L^{-1}$ for $ZnSO_4\cdot 7H_2O$. Supplementation of organosilicone raised the threshold concentration of three Zn fertilizers to 250 $mg\cdot L^{-1}$ and averted the production of necrotic spots, which further improved the absorption efficiency and correcting effect. Based on these results, $ZnCl_2$ or $Zn(NO_3)_2\cdot 6H_2O$ containing 250 $mg\cdot L^{-1}$ of Zn and supplemented with

0.025% organosilicone could be recommended as a safe and effective spray formation for correcting Zn deficiency in citrus production.

Literature Cited

- Boaretto, A.E., R.M. Boaretto, T. Muraoka, V.F. Nascimento Filho, C.S. Tiritan, and F.A.A. Mourao Filho. 2002. Foliar micronutrient application effects on citrus fruit yield, soil and leaf Zn concentrations and ^{65}Zn mobilization within the plant. *Acta Hort.* 594:203–209.
- Cakmak, I. 2000. Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol.* 146:185–205.
- Chapman, H.D. 1968. The mineral nutrition of citrus [M], p. 127–289. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). *The citrus industry*. Vol. 2. Univ. California Div. Agr. Sci., Berkeley, CA.
- Chen, W., X. Yang, Z. He, Y. Feng, and F. Hu. 2008. Differential changes in photosynthetic

capacity, 77 K chlorophyll fluorescence and chloroplast ultrastructure between Zn-efficient and Zn-inefficient rice genotypes (*Oryza sativa*) under low zinc stress. *Physiol. Plant.* 132:89–101.

- Eman, A.A., A. El-Moneim, M.M.M. Abd El-Migeed, A. Omayma, and M.M. Ismail. 2007. GA3 and zinc sprays for improving yield and fruit quality of Washington navel orange trees grown under sandy soil conditions. *Res. J. Agr. Biol. Sci.* 3:498–503.
- Fu, X.Z., L.Z. Peng, F. Xing, L.L. Ling, C.P. Chun, C.L. Jiang, and L. Cao. 2014. Zinc deficiency in citrus: Current studies and future perspectives (in Chinese). *J. Fruit Sci.* 31:132–139.
- Hanafy Ahmed, A.H., M.K. Khalil, A.M. Abd El-Rahman, and A.M. Nadia. 2012. Effect of zinc, tryptophan and indole acetic acid on growth, yield and chemical composition of valencia orange trees. *J. Appl. Sci. Res.* 8:901–914.
- Hu, H. and D. Sparks. 1991. Zinc deficiency inhibits chlorophyll synthesis and gas exchange in 'Stuart' pecan. *HortScience* 26:267–268.
- Knoche, M. 1994. Organosilicone surfactant performance in agricultural spray application: A review. *Weed Res.* 34:221–239.
- Razzaq, K., A.S. Khan, A.U. Malik, M. Shahid, and S. Ullah. 2013. Foliar application of zinc influences the leaf mineral status, vegetative and reproductive growth, yield and fruit quality of 'Kinnow' mandarin. *J. Plant Nutr.* 36:1479–1495.
- Sasaki, H., T. Hirose, Y. Watanabe, and R. Ohsugi. 1998. Carbonic anhydrase activity and CO_2 -transfer resistance in Zn-deficient rice leaves. *Plant Physiol.* 118:929–934.
- Sharma, P.N., A. Tripathi, and S.S. Bisht. 1995. Zinc requirement for stomatal opening in cauliflower. *Plant Physiol.* 107:751–756.
- Srivastava, A.K. and S. Singh. 2004. Zinc nutrition and citrus decline: A review. *Agr. Rev.* 25:173–188.
- Srivastava, A.K. and S. Singh. 2005. Zinc nutrition, a global concern for sustainable citrus production. *J. Sustain. Agr.* 25:5–42.
- Srivastava, A.K. and S. Singh. 2009. Zinc nutrition in 'Nagpur' mandarin on Haplustert. *J. Plant Nutr.* 32:1065–1081.
- Stevens, P.J.G. 1993. Organosilicone surfactants as adjuvants for agrochemicals. *Pestic. Sci.* 38:103–122.
- Swietlik, D. and J.V. Laduke. 1991. Productivity, growth, and leaf mineral composition of orange and grapefruit trees foliar-sprayed with zinc and manganese. *J. Plant Nutr.* 14:129–142.
- Swietlik, D. 2002. Zinc nutrition of fruit trees by foliar sprays. *Acta Hort.* 594:123–129.