Zinc fertilizers for Citrus production: assessing nutrient supply via fertigation or foliar application

Rodrigo Boaretto (rmboaretto@gmail.com)
Instituto Agronômico de Campinas: Instituto Agronomico
Franz Walter Rieger Hippler
Luiz Antônio Junqueira Teixeira
Raíssa Cagnolato Fornari
Jose Antonio Quaggio
Dirceu de Mattos-Jr

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Abstract

Background and Aims

Citrus trees are severely affected by zinc (Zn) deficiency, which impairs plant growth and fruit yield. Zn fertilization is usually recommended in field orchards, and application strategies toward nutrient use efficiency are key for successful crop management.

Methods

Field studies were carried out with sweet orange trees for four growing seasons, testing Zn (nitrate, sulfate or EDTA) applied either via fertigation or foliar spray compared to a control without Zn.

Results

The Zn concentrations in the soil increased with nutrient supply by both application methods. Zn-EDTA via fertigation increased soil nutrient availability compared to the control. Likewise, Zn-nitrate via foliar application also increased soil Zn levels. Regarding the plant, Zn-EDTA via fertigation increased leaf nutrient levels only after the third year of fertilization, whereas Zn-nitrate via foliar spray increased leaf levels in the short term, up to 120 mg kg^{-1}. To a lesser extent, Zn-EDTA or sulfate also increased leaf Zn compared to the control. Accumulated fruit yield was ~ 20% higher in trees with Zn-EDTA via fertigation and ~ 20% higher in trees with Zn-nitrate or sulfate via foliar application compared to the control, with these latter exerting greater responses. Trees supplied with Zn exhibited lower H_2O_2 and higher CAT activity compared to the control, which correlated with a fruit yield increase.

Conclusion

In conclusion, Zn-EDTA via fertigation or nitrate or sulfate via foliar application improved the horticultural performance of trees, supporting the establishment of best nutrient management practices in fruit production.

Introduction

Zinc (Zn) deficiency is widely observed in agricultural crops established in tropical soils because of the low metal content in the soil matrix and high chemical interaction with soil colloids, which decreases nutrient availability to root uptake (Alloway 2009; Arias et al. 2005; Hippler et al. 2015; Noulas et al. 2018). Citrus trees are severely affected by such deficiency, which impairs plant growth and fruit yield (Hippler et al. 2015; Xing et al. 2016). Visual symptoms of Zn deficiency in citrus are characterized by stunted growth of new leaves that exhibit interveinal chlorosis on branches with shortened internodes (rosette-type symptom) (Quaggio et al. 2011).

To prevent or correct Zn deficiency in field orchards, Zn application can be carried out either by soil application or foliar application (Bell and Dell 2008). A fundamental study suggested that soil application of Zn sustains plant nutrient supply for a longer period than foliar application in citrus production (Smith 1967). Despite the demonstration that the supply and recovery of micronutrients from soil depend on fertilizer sources (Hippler et al. 2015; Zekri and Koo 1992), there is a lack of data on the efficacy of Zn-containing fertilizers applied to the soil. Indeed, young citrus trees grown in sandy soil recovered 4% of the Zn applied as sulfate (labeled ^{68}Zn) compared to only 0.12% applied as zinc oxide (Hippler et al. 2015); however, these figures could be even lower in clayey soil (Hippler et al. 2014). Likewise, few studies have evaluated the supply of micronutrients via fertigation in perennial tree crops, comprehensively correlating soil management with fruit yield.

In particular, the nutrient applied via fertigation is delivered to the soil by several split operations throughout the growing season, limited to a reduced volume of soil comprised by the wet bulb, which could prevent adsorption between the metal and soil colloids, allowing increased nutrient availability to roots (Hippler et al. 2014; McBeath and McLaughlin 2014; Mengist et al. 2021). However, limited information about soil Zn application makes room for the unsubstantiated use of foliar sprays as the main strategy for micronutrient fertilization of fruit trees in the tropics. Foliar spraying makes field applications easier to supply plant Zn requirements than soil applications. However, because of the low mobility of Zn in the phloem of citrus trees, frequent leaf sprays are required to meet the nutrient demand for new flushes of growth (Boaretto et al. 2002; Srivastava and Singh 2005).

Leaf sprays with Zn for citrus use 500–1000 mg L^{-1} nutrient solution with water-soluble fertilizers, such as chloride, sulfate or nitrate (Bell and Dell 2008; Quaggio et al. 2011; Srivastava and Singh 2005). Nutrient solution varies according to composition and plant nutritional status, with the lowest concentrations used with the highest salt index fertilizers to avoid leaf burning and not severely deficient trees (Hippler et al. 2018a, b; Sawan et al. 2001). In addition to these soluble salts, chelated fertilizers, such as ethylenediaminetetraacetic acid (EDTA) or sparingly soluble sources, are available for foliar applications (Bell and Dell 2008; Macedo et al. 2021). However, chelated micronutrients are likely not efficiently absorbed by citrus leaves (Boaretto et al. 2002; Sartori et al. 2008; Zhang et al. 2014). The same might be efficient when applied to the soil, mainly via fertigation, which can provide a higher availability of micronutrients to the roots compared to soluble salts. Therefore, expert information is required to support proper micronutrient use in fruit tree crop systems, considering the 4R nutrient stewardship concept (Johnston and Bruulsema 2014).

Zinc is a cofactor of enzymes, such as superoxide dismutase (SOD), a key enzyme in the antioxidant system of plants, scavenging reactive oxygen species (ROS) from cells (Choudhury et al. 2016). SOD is the first enzyme to reduce the superoxide radical ($O_2^{-}\cdot$) to hydrogen peroxide (H_2O_2), which is then eliminated by catalase (CAT) and peroxidases (Choudhury et al. 2016; Gill and Tuteja 2010; Hippler et al.

2016). Moreover, plants well-nourished with Zn should be able to tolerate the deleterious effects of abiotic stress in field conditions (Broadley et al. 2007; Cakmak 2000; Syvertsen and Garcia-Sanchez 2014).

The current global citrus production scenario focuses on increments in fruit yield and quality with balanced nutritional management to achieve sustainability. Therefore, this study evaluated the effects of Zn fertilizer sources and application methods, either via fertigation or foliar application, on the nutritional status, fruit yield, and quality of young-bearing sweet orange trees under field conditions. Furthermore, the enzymatic antioxidant system's potential to alleviate damage to oxidative stress in trees that were well-nourished in Zn was evaluated.

Materials And Methods

Field conditions

Two experiments were carried out in a commercial orchard of young sweet orange trees [*Citrus sinensis* (L.) Osb. cv. 'Pera'] grafted onto Sunki mandarin (*C. reshni* hort. ex Tanaka) for four growing seasons. The orchard was planted in 2008, at 7.0 × 2.9 m (493 trees ha^{-1}), in Colômbia - SP, Brazil (20°19'19.1"S, 48°46'44.1"W; 560 m), in a sandy loam soil (200 g kg^{-1} clay, 40 g kg^{-1} silt and 760 g kg^{-1} sand), pH ($CaCl_2$) 5.1, 68.5 $mmol_c$ dm^{-3} cation exchange capacity (CEC), and exhibiting 23.5 g dm^{-3} organic matter and 5.5 mg dm^{-3} Zn available at the 0 – 20 cm soil depth (DTPA-TEA pH 7.3; Raij et al., 2001). The orchard was fertigated by a single drip line with 0.6 m-spaced drippers and a flow rate of 7.1 L h^{-1}. Irrigation was scheduled every 2 days and the amount of water applied was determined by measuring the evaporation using a Class A pan, the potential, and the crop evapotranspiration, according to Allen et al. (1998). The local climate was classified as Aw according to Köppen, with hot and humid summer and dry winter with an average annual air temperature of 23°C.

The treatments started when the trees were 3 years old, and the experimental evaluations were performed in the following four years. The treatments consisted of three fertilizer sources containing Zn {sulfate ($ZnSO_4 \cdot 7H_2O$), nitrate [$Zn(NO_3)_2$], or Zn-EDTA ($C_{10}H_{14}ZnN_2O_8$)}, which were applied either via fertigation in the first experiment or foliar application in the second one. A control treatment, common to both experiments, consisted of the nil application of Zn. The experiments were established in a randomized block design with four treatments and five blocks, with one replicate per treatment in each block. Individual plots comprised a line of 16 trees, with 10 central trees taken for the evaluation of treatment effects.

Zinc applications in all treatments (except the control trees) summed up 2.0 kg ha^{-1} yr^{-1} of Zn in the first three years of the study. Due to the increment in tree size during the experiment, the Zn dose was increased to 5.0 kg ha^{-1} yr^{-1} in the fourth year for both experiments (fertigation and foliar application), except for nitrate foliar application. The nitrate source applied via foliar spray was maintained at 2.0 kg ha^{-1} yr^{-1} to avoid plant injury due to excess salt. In the first experiment, Zn sources were applied via fertigation in a total of 20 applications per year between August (late fall) and April of the subsequent year (summer). In the second experiment, the applications were performed in the same period as in the first experiment but with 4–5 foliar sprays per year, according to Quaggio et al. (2022). Orchard fertilization maintenance with nutrients, except Zn, also followed Quaggio et al. (2022).

Zinc concentration and adsorption capacity in the soil

Soil samples were annually collected from the 0–20 cm depth layer and chemically analyzed (Raij et al. 2001). In the first and third year, soil samples were also taken from the 20–40 cm layer.

Adsorption curves for Zn in the soil were performed according to Hippler et al. (2014), based on the Langmuir adsorption isotherm (Bradl 2004). One gram of soil sample (from control treatment) was mixed with 10 mL of 10 mM $CaCl_2$ solution containing 0.5, 2.0, 8.0, 32.0, 128.0 and 512.0 mg L^{-1} $Zn(NO_3)_2$. Samples were shaken for 72 h in a horizontal shaker at 160 rpm (25°C) and then centrifuged for 10 min at 905 × *g*. The supernatant was filtered, and the amount of Zn in the equilibrium solution was quantified with plasma emission spectrometry (ICP-OES, Perkin-Elmer 5100 PC, Norwalk, CT, USA). The soil exhibited a maximum adsorption capacity (b_L) of 1215.1 mg kg^{-1} and a binding energy (K_L) of 0.016 L kg^{-1}.

Zinc concentration in soil and leaves

For both experiments, soil and leaves were sampled every year by the end of the summer (March–April). In the four years of experimental evaluations, soil samples were collected from the 0 – 20 cm soil depth layer. Additionally, in the first and third years, such samples were also collected from the 20 – 40 cm soil depth layer. Soil samples were collected about 30 cm from the irrigation drip lines toward the tree's inter row (Obreza et al. 2008; Quaggio et al. 2011). DTPA-TEA (pH 7.3) was used as the nutrient extractor (Abreu et al. 1998), and the quantification of the Zn available in the soil was performed in an ICP-OES (Perkin-Elmer 5100 PC, Norwalk, CT, USA).

Leaf samples were collected from the intermediate part of the tree from branches that had one terminal fruit (~ 3 cm in diameter) (Mattos Jr et al. 2017). The leaves were washed in distilled water, dried at 55–60°C until constant weight and ground to pass through a 200-mesh sieve. The Zn concentration in the dry mass was determined by nitro-perchloric digestion according to Bataglia et al. (1983) by ICP-OES (Perkin-Elmer 5100 PC, Norwalk, CT, USA).

Zinc concentration in sap extract and flowers

In the last year of the experiment, sap extract from stems and flowers was collected for Zn quantification. The sap extract was obtained as described by Cadahía and Lucena (2005) and adapted by Souza et al. (2012) from stems of new shooting branches collected in the fourth year after treatment application. The samples were composed of 40 stem pieces collected from 10 trees per treatment at half height and around four quadrants of the tree canopies during the morning. Stems were transported on ice and, within 24 h after field sampling, were cut into 1 to 2 cm segments and placed into 250 mL plastic flasks. Ethyl ether (analytical grade) was added until the stems were completely covered by the liquid [the ratio between stems (g) and ethyl ether (mL) was around 1:2.4] and were frozen at − 15°C for 15 d. The samples were then thawed, and the sap extract (formed by a mixture of xylem, phloem and cellular soluble minerals) was separated from the ethyl ether through a separator funnel. The zinc concentration in the sap extract was quantified using an argon plasma spectrometer (ICP-OES, Perkin-Elmer 5100 PC; Norwalk, CT).

Furthermore, fully open flowers were collected at the same moment as the stems and dried until a constant weight, and the Zn concentration was determined according to Bataglia et al. (1983).

Fruit yield and quality

Fruit yield (kg per tree) was evaluated by harvesting fruit from the 10 central plants of the experimental plots. Fruit quality was assessed by sampling 5 oranges per tree, for a total of 50 per plot, to determine fresh weight, mean height and width, juice percentage, acidity, soluble solids (SS) (°Brix), SS/acidity ratio and yield of SS per 40.8 kg per box (Redd et al. 1992).

Hydrogen peroxide content and antioxidant enzyme activity

In the last year, mature leaves (in non-reproductive flushes) of the youngest stems were collected in two periods: (i) before the Zn treatment application started (late fall; August) and (ii) one week after the last application of the Zn sources (late summer; March). The leaf samples were collected between 10 and 11 am in liquid nitrogen and stored at − 80ºC for further measurements.

The H_2O_2 concentration was determined according to Alexieva et al. (2001). Leaf powder was homogenized in 0.1% (w/v) trichloroacetic acid and centrifuged at 5590 × *g* for 15 min at 4°C. The supernatant was mixed with 100 mM potassium phosphate buffer (pH 7.0) and 1.0 M potassium iodide (1:1:4) and incubated at 4°C for 1 h in the dark and for more than 20 min at 25°C. The absorbance of the samples was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve with known H_2O_2 concentrations.

For SOD and CAT activity assays, leaf powder was homogenized in 100 mM potassium phosphate buffer (pH 7.5) with 3 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid and 4% polyvinylpolypirrolidone (w/v) (Andrade et al. 2010). The suspension was centrifuged at 12,100 × *g* for 35 min at 4°C, and the supernatant was stored at − 80°C for further analysis. The total protein concentration was determined according to Bradford (1976) using bovine serum albumin as the standard. SOD activity staining was carried out as described by Hippler et al. (2015) using electrophoresis under non-denaturing conditions in 12% polyacrylamide gel with 75 µg of protein. One unit of bovine liver SOD (Sigma, St. Louis, USA) was used as a positive control of activity. SOD isoforms were distinguished by their sensitivity to inhibition by 2 mM potassium cyanide and 5 mM H_2O_2 (Azevedo et al. 1998). CAT activity was determined according to Kraus et al. (1995), as described by Hippler et al. (2015).

Statistical analysis

Descriptive statistics were applied to the data, and the effects of Zn sources were tested using one-way analysis of variance (ANOVA) for each experiment, followed by Tukey's test ($p < 0.10$) when significance was detected.

Results

Zinc concentration in the soil

The Zn concentration in the soil, extracted by DTPA (pH 7.3), increased with nutrient application, either via fertigation or foliar spray (Fig. 1). However, when Zn was applied via fertigation in the first year, the nitrate source already had increased levels of the nutrient in the 0 – 20 cm layer depth, whereas Zn-EDTA increased the same levels in the following years when compared to the control (Fig. 1). Furthermore, Zn-EDTA applied via fertigation caused a higher increment of Zn concentration in the 20 – 40 cm soil layer depth than sulfate and control treatments (Fig. 1). In the second experiment, when Zn was applied via foliar application, the increment of the nutrient concentration in the soil was observed mainly as nitrate after the second year, in the 0 – 20 cm layer, and after the third year in the 20 – 40 cm depth layer (Fig. 1).

Zinc concentration in leaves, sap extract and flowers of citrus trees

Zn application via fertigation increased the nutrient levels in the leaves after three years of application, mainly when applied as EDTA compared to the control, whereas nitrate or sulfate promoted intermediate levels of the nutrient (Fig. 2). The Zn application via foliar spray increased the nutrient level in the leaves up to 120 mg kg^{-1} when applied as nitrate, whereas the control trees exhibited Zn concentrations between 10 and 27 mg kg^{-1} over the four years of evaluation (Fig. 2). In addition, sulfate or EDTA sources also caused increments in Zn concentration when compared to control trees, except for the second year (Fig. 2).

After four years of Zn fertilizer application, whether via fertigation or foliar application, the nutrient concentrations in sap extract (from new twigs) and flowers were highest when the nutrient was applied as EDTA via fertigation or as nitrate or sulfate when applied via foliar spray (Fig. 3).

Fruit yield and quality after the application of Zn fertilizers via fertigation or foliar spray

Zinc via fertigation did not affect fruit yield within individual years of evaluation (Fig. 4), even though accumulated fruit yield (sum of the four years) was higher when trees were fertilized with Zn-EDTA (305 kg per tree) compared to the control trees (262 kg per tree; Fig. 5). In the second experiment, trees with foliar application of nitrate fertilizer exhibited a higher fruit yield in the fourth year compared to those within the EDTA or control treatments (Fig. 4). Moreover, the accumulated fruit yield was higher when Zn was applied either as nitrate or sulfate compared to the control or leaf-sprayed trees with Zn-EDTA

(Fig. 4). Fruit quality did not change for Zn treatments applied either via fertigation or foliar application (data not shown).

Zinc supply alleviates oxidative stress in citrus trees

Before Zn application via fertigation, the trees that received EDTA or sulfate fertilizers exhibited a lower H_2O_2 content in the leaves compared to the control trees. However, no difference in H_2O_2 content was verified in the trees before the application of Zn fertilizers via foliar application (Fig. 5). Considering the same evaluation conducted after Zn fertilizer application, either via foliar spray or fertigation, the trees exhibited lower H_2O_2 content than the control trees (Fig. 5). In addition, when Zn was applied via fertigation as EDTA, the trees displayed lower levels of H_2O_2 in the leaves than those supplied with nitrate fertilizer (Fig. 5).

SOD activity was higher in leaves of control trees before the imposition of the Zn treatments during the fourth year of fertilization in the field, mainly for Cu/Zn-SOD II and III isoforms (Fig. 6). During the same period, trees supplied with Zn-EDTA exhibited higher Mn-SOD activity compared to those supplied with the nitrate source, either when applied via fertigation or foliar application (Fig. 6). However, the trees that had previously received Zn via foliar spray exhibited greater activity of Cu/Zn-SOD II and III isoforms (Fig. 6). After Zn treatment, SOD activity was higher only in trees supplied with the nutrient nitrate or sulfate compared to EDTA or control trees (Fig. 6). Conversely, no differences were observed in CAT activity in the leaves for both experiments before Zn treatment applications (Fig. 7). However, after Zn application, trees fertigated with Zn-EDTA or leaves sprayed with nitrate or sulfate sources exhibited higher CAT activity in both experiments when compared to the control trees (Fig. 7).

Discussion

Citrus are sensitive to Zn deficiency, mainly in tropical regions with acid soils where nutrient availability for root absorption is limited (Mattos Jr et al. 2020; Quaggio et al. 2003; Srivastava and Singh 2005). Currently, in the Brazilian citrus industry, Zn is mostly applied via foliar application during spring and summer, with water-soluble fertilizers, such as nitrate, sulfate or chloride (Quaggio et al. 2011). However, visual symptoms of Zn deficiency are still commonly observed in trees associated with the low mobility of this nutrient in the phloem (Boaretto et al. 2002). Therefore, frequent applications of Zn, reaching each new vegetative flush of the growth of trees, are needed for adequate plant supply.

Moreover, in recent years, the area of irrigated and fertigated orchards has increased in the main citrus production areas worldwide (Fares et al. 2017; Qin et al. 2016). A study using Zn-labeled fertilizer applied to the soil demonstrated that the uptake and redistribution efficiencies for this nutrient varied with soil texture and fertilizer source. In the present work, we studied the effects of different application methods of fertilizer containing Zn on the fruit yield and quality of young citrus trees in the field, assessing plant nutritional status and biochemical parameters related to ROS scavenging.

Nutritional status and fruit yield of trees supplied with Zn fertilizer via fertigation or foliar spray

An increment in Zn concentration in the soil was observed when Zn-containing fertilizers were applied either via fertigation or foliar application (Fig. 1). In addition, the former method of application promoted lower levels of Zn at the soil surface (0 – 20 cm depth) compared to the foliar application (Fig. 1). Despite that the binding energy (K_L; 0.016 L kg^{-1}) for Zn in the soil was lower when compared to either sandy loam soil (0.035 L kg^{-1}; 180 g kg^{-1} of clay) or clay soil (0.032 L kg^{-1}; 640 g kg^{-1} of clay), when cultivated with citrus (Hippler et al. 2015), the maximum adsorption capacity (b_L) of 1215 mg kg^{-1} of the soil in this study was similar when compared to a clay tropical soil (b_L = 1220 mg kg^{-1}; Hippler et al. 2015). Therefore, the high b_L likely limited root absorption and the increment of Zn in the leaves of those trees that received Zn sources via fertigation during the two initial years of the experiment (Fig. 2). Furthermore, for woody trees, such as citrus, Zn absorbed by roots is mainly partitioned into roots and woody parts (trunk and branches), in addition to leaves (Hippler et al. 2015; Sartori et al. 2008).

The enrichment in the Zn content of the soil after foliar spray occurred, primarily by the runoff of the solution sprayed on the leaves to the soil surface, by the washing of the micronutrient adhered to the surface by rain and consequent deposition to the soil or by the cycling of the micronutrients from the leaves dropped off to the ground and then decomposed (Bell and Dell 2008; Deshpande et al. 2017).

Moreover, when the Zn fertilizers were foliar sprayed, Zn concentration in leaves increased in the first year of evaluation up to 110 mg kg^{-1} with Zn-nitrate application (Fig. 2) but caused leaf injuries by salinity in the second year. This phytotoxicity effect caused leaf chlorosis and necrosis of the leaf blade edge and severe leaf fall. Similar phytotoxicity symptoms caused by salt accumulation in leaf tissue were observed with copper nitrate applied via foliar application in young citrus trees in the field (Hippler et al. 2018a) and with copper sulfate in non-bearing trees under greenhouse conditions (Hippler et al. 2018b). For this reason, during the entire course of this study, the Zn dose in the treatment with nitrate applied via foliar spray was maintained at 2.0 kg ha^{-1} year^{-1}, whereas the dose of Zn applied with the other fertilizer sources was twice this amount in the last year. At the lowest Zn dose, nitrate still provided the highest levels of Zn in the leaves, sap extract and flowers (Figs. 2 and 3), followed by the sulfate source.

Zinc has limited mobility in citrus phloem (Boaretto et al. 2002), and thus, when Zn is supplied by spraying on leaves, foliar application of Zn is recommended for each new growth flush (Quaggio et al. 2022). Despite the limited distribution of Zn in the plants, in the fourth year of experimentation for both experiments, increases in Zn concentrations were observed in the sap extract and flowers of tissues developed before the application of Zn treatments in this season. These results showed that even though the mobility of Zn in the plant phloem is limited, in trees with greater reserves of the nutrient, the amount of Zn mobilized to the new plant tissues was greater, and this amount of mobilized Zn represented an increase of 40 to 60% in the Zn concentration for the new tissues, demonstrating the importance of building the Zn reserve in the soil–plant system (Fig. 3).

Productivity per area can be estimated by fruit production per plant and the number of trees per hectare. The average productivity levels showed that the orchard was managed with a good technological level. In the first two harvests, when the trees were between three and four years old, the average productivity was close to 25 t ha^{-1} of fruit, and in the third and fourth harvests, the productivity was higher than 45 t ha^{-1}. The average difference in productivity between the first two crops for the next few years was because the plants almost reached the adult stage, with 5 to 6 years after planting. For both experiments, no annual differences in fruit yield were observed due to Zn fertilization over the four years of evaluation, except for the fourth year of production in the foliar experiment. However, for both experiments, fruit yield gains were verified when the accumulated production was evaluated over the four years (Fig. 4).

In the fertigation experiment, trees supplied with Zn-EDTA via fertigation exhibited a 18.5% higher accumulated fruit yield than the control trees, and trees supplied with Zn sulfate or nitrate showed intermediate productivity (Fig. 4). A chelate refers to metallic nutrient ions that are encircled by an organic molecule, usually called a ligand. Chelated fertilizers have been developed to reduce the interaction of a nutrient with the soil complex, protecting a metallic element from oxidation and immobilization (Havlin et al. 2005). In this case, when Zn was complexed with a ligand such as EDTA and supplied by fertigation, the micronutrient remained in the soil in an available form for a longer period, enhanced nutrient uptake and use efficiency, and consequently improved fruit production.

In contrast to the fertigation experiment, when Zn was applied via foliar applications, the accumulated fruit yield was higher either when Zn was applied as a nitrate (17.3%) or sulfate (14.6%) source compared to the control trees and the Zn-EDTA treatment (Fig. 4). In general, synthetic chelates are much larger and have higher points of deliquescence than the inorganic mineral salts commonly used as active ingredient carriers (Fernández 2013), justifying the results observed in our study, which showed lower efficiency of micronutrient utilization when Zn was applied to leaves in a chelated form when compared to Zn sources in the form of soluble salts. In apple orchards, comparing different foliar sources of Zn, higher phytoavailability and increased concentrations of Zn in leaves for Zn nitrate were also observed when compared to other chelated sources (Peryea, 2006).

Antioxidant enzyme system before and after Zn fertilizer supply in citrus trees

Differences in leaf Zn concentration observed for the tested methods of nutrient application (fertigation < foliar application) could also be explained by the determination of Zn adhered to the leaf cuticle of sprayed trees, which do not play a role in biochemical plant functions but are accounted for by the analytical method (Boaretto et al. 2003). In this study, a biochemical approach was used to determine how Zn treatments affect plant metabolism related to oxidative stress in citrus trees. Once SOD isoforms are classified by the metal-cofactor (Cu/Zn-SOD, Mn-SOD and Fe-SOD), the activity of this enzyme can be assessed as a biochemical marker for plant nutritional status for micronutrients (Cakmak 2000; Hippler et al. 2015), as well as for oxidative stress levels of plants exposed to biotic and abiotic stress conditions (Choudhury et al. 2016; Pérez-Clemente et al. 2015; Syvertsen and Garcia-Sanches 2014).

Before imposing the Zn treatment application in the fourth year, trees fertigated with Zn, mainly as EDTA or sulfate sources, exhibited lower levels of H_2O_2 in the leaves than the control (Fig. 5). In this case, trees fertigated with EDTA also exhibited an increment in Mn-SOD activity in the same period (Fig. 6), although no difference was observed for CAT activity (Fig. 7). Peroxidase enzymes and antioxidant compounds, such as ascorbate and glutathione, play a role on H_2O_2 scavenging in these trees (Aravind et al. 2004). However, before Zn application via foliar spray, no differences were observed in H_2O_2 content or SOD or CAT activities (Figs. 5, 6 and 7). However, oxidative stress was reduced (H_2O_2 content; Fig. 5) in the trees after the Zn treatment supply, as shown by the increment of SOD and CAT activities (Figs. 6 and 7).

In the field, citrus trees are frequently prone to environmental stress conditions. Within the site of this study (North of the São Paulo State), long dry periods with high air temperatures commonly occur throughout the year, causing severe flower and fruitlet drop, resulting in fruit production losses, as in many related areas. In this case, the balanced nutritional status of plants with Zn supply could alleviate the effects of abiotic stress, as verified by the positive relationship between the increment of CAT activity and accumulated fruit yield (Fig. 8), mainly when trees were supplied with Zn-EDTA via fertigation or with nitrate or sulfate via foliar applications (Figs. 4 and 7). This is in line with the demonstrated role of Zn in alleviating plant drought stress by Zn-mediated increase in the photosynthesis pigment and active oxygen scavenging substances and reduction in lipid peroxidation (Ma et al. 2017).

Conclusion

The adequate supply of Zn in citrus orchards cultivated in tropical soils, where the availability of nutrients is low for the roots, provides significant productivity increments in the field, in addition to promoting the activity of the antioxidant enzyme system by increasing the activity of SOD and catalase, correlated with fruit production. Moreover, the best strategy for applying this nutrient, whether via soil by fertigation or foliar spraying on trees, depends on the source of fertilizer used. Zn-EDTA is efficient for use in fertigation because it reduces the specific adsorption of nutrients with soil colloids in relation to soluble salts, such as nitrate and sulfate. However, foliar Zn absorption is enhanced with the use of water-soluble salts in relation to Zn-EDTA, and the increase in foliar Zn levels is greater when applied in the form of nitrate in relation to sulfate, although at equal concentrations in the spray solution, Zn nitrate can damage leaves and defoliate plants due to its high salt index compared to sulfate. Therefore, our research results support the proper use of micronutrients in fruit tree cultivation systems as a key to implementing the 4R nutrient stewardship concept for sustainable agricultural production.

Declarations

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Author Contributions

All authors contributed to the study conception, design and/or conduction. Material preparation, data collection and analysis were performed by [RM Boaretto], [LAJ Teixeira], [JA Quaggio], [FWR Hippler], [RC Fornari] and [D Mattos Jr]. The first draft of the manuscript was written by [RM Boaretto], [FWR Hippler] and [D Mattos Jr] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing Interests

The authors have no relevant financial or non-financial interest to disclose.

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Figures

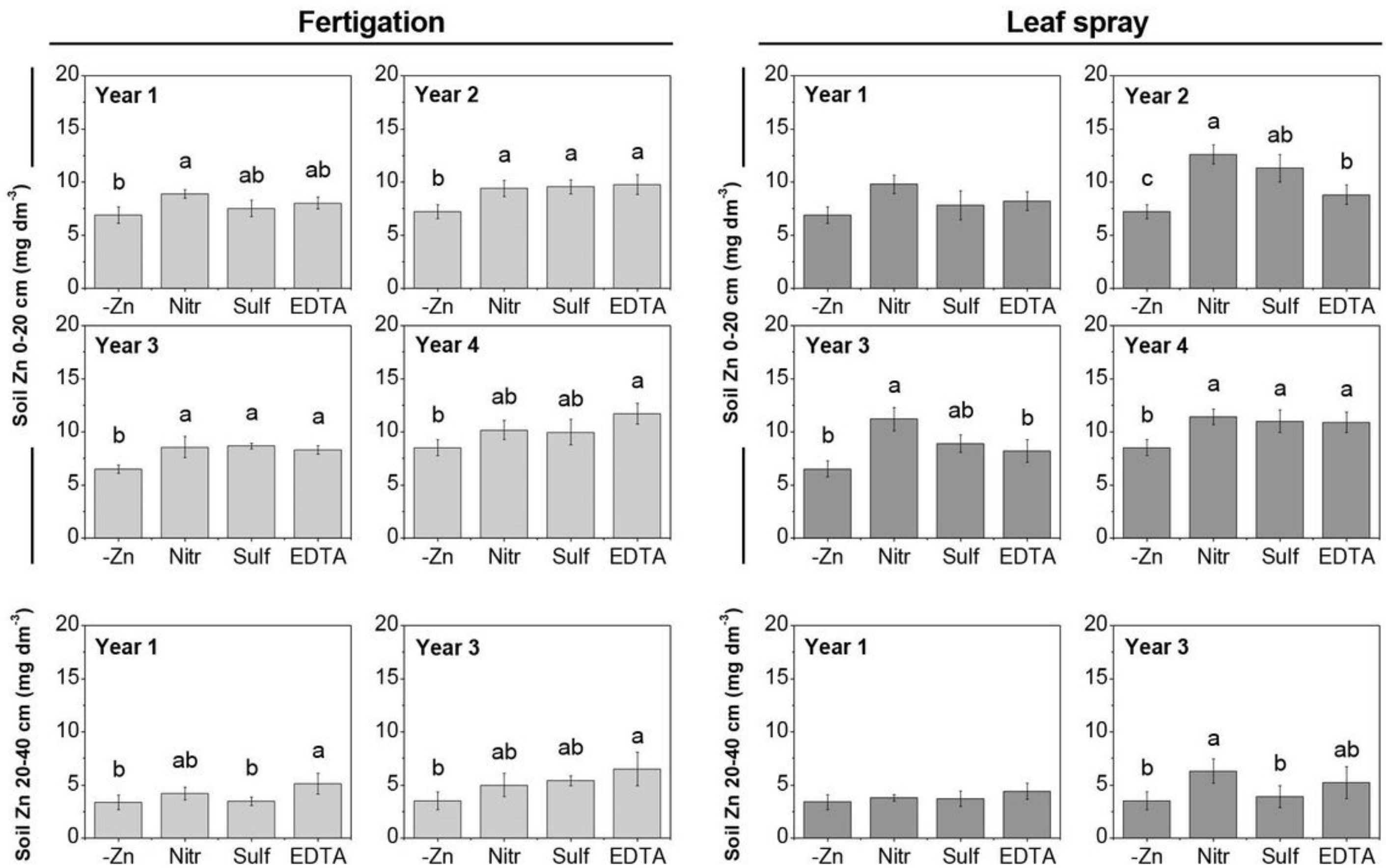

Figure 1

Soil level of zinc (Zn) in the 0–20 and 20–40 cm depth layers over four years of application of different Zn fertilizer sources via fertigation or foliar spray in 'Pera' sweet orange trees in a field orchard. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – zinc-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test (p<0.10).

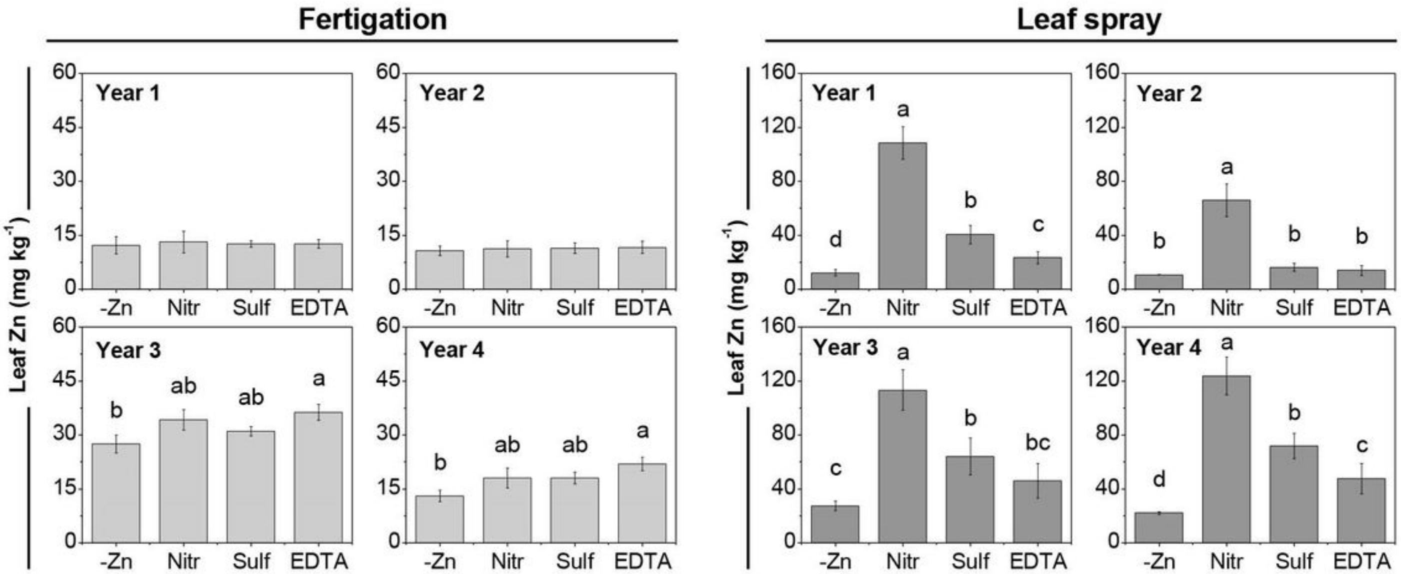

Figure 2

Concentration of zinc (Zn) in leaves of ‘Pera’ sweet orange trees over four years of application of different Zn fertilizer sources via fertigation or foliar sprays. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – zinc-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test ($p<0.10$).

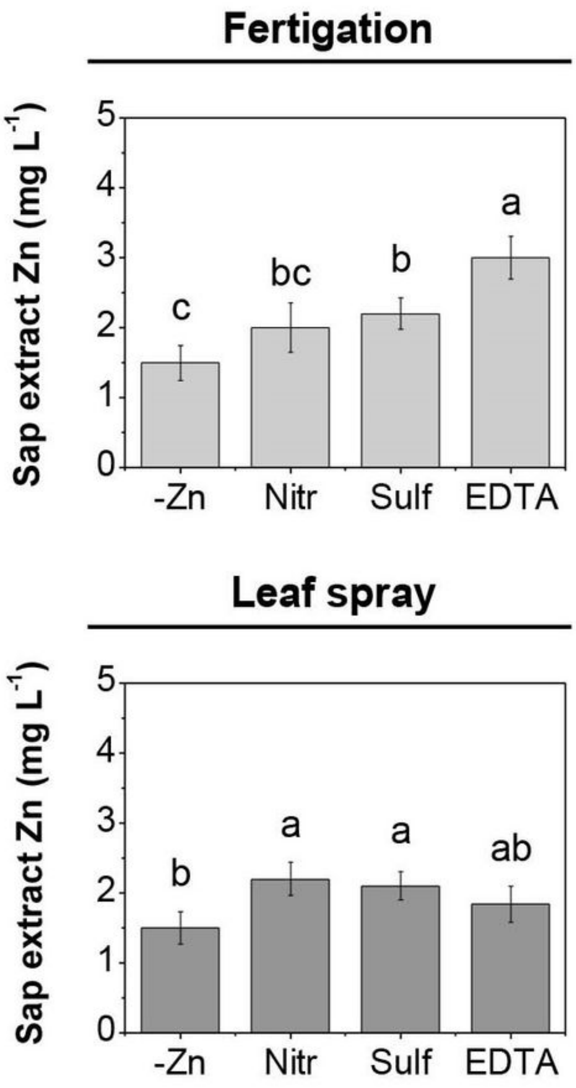

Figure 3

Concentration of zinc (Zn) in sap extract of young stems and flowers of ‘Pera’ sweet orange trees in the fourth year after application of different Zn fertilizer sources via fertigation or foliar spray. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – zinc-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test (p<0.10).

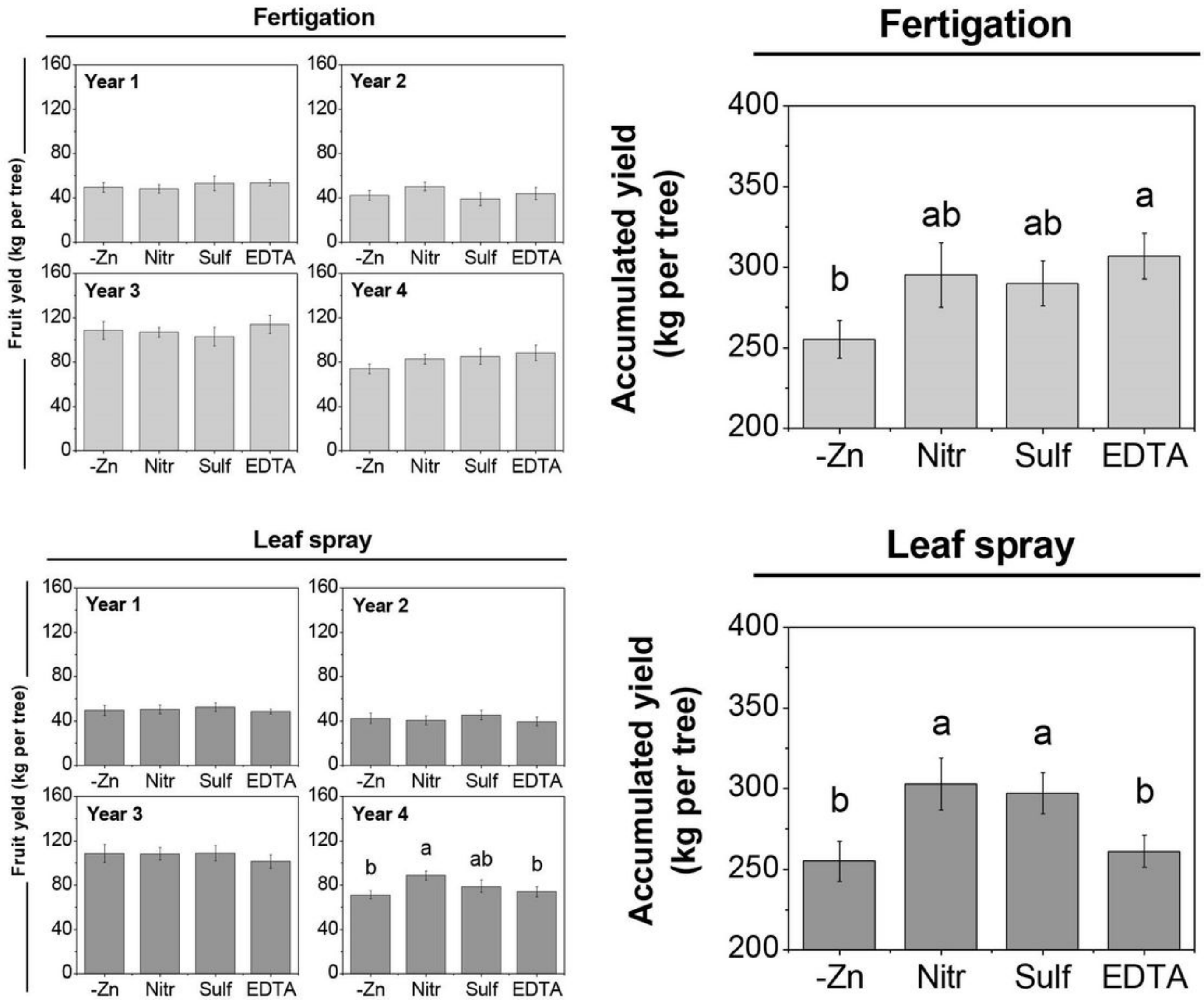

Figure 4

Fruit yield of ‘Pera’ sweet orange trees over four years of application of different fertilizer sources of zinc (Zn) via fertigation or foliar spray. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – zinc-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test (p<0.10).

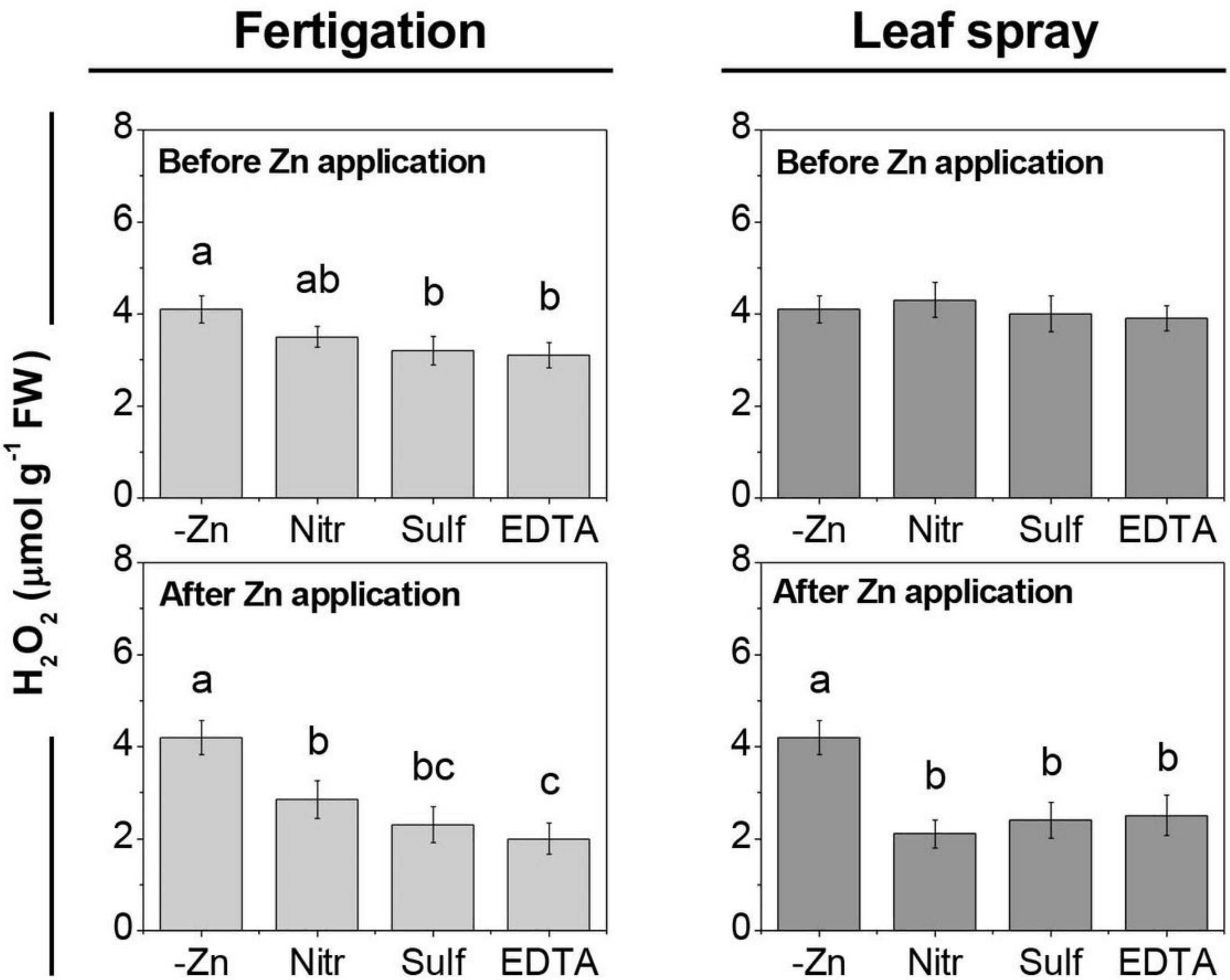

Figure 5

Concentration of hydrogen peroxide (H_2O_2) in leaves of 'Pera' sweet orange trees, in the fourth year, before (late fall – August) and after (late summer – March) application of different zinc (Zn) fertilizer sources via fertigation or foliar spray. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – zinc-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test (p<0.10).

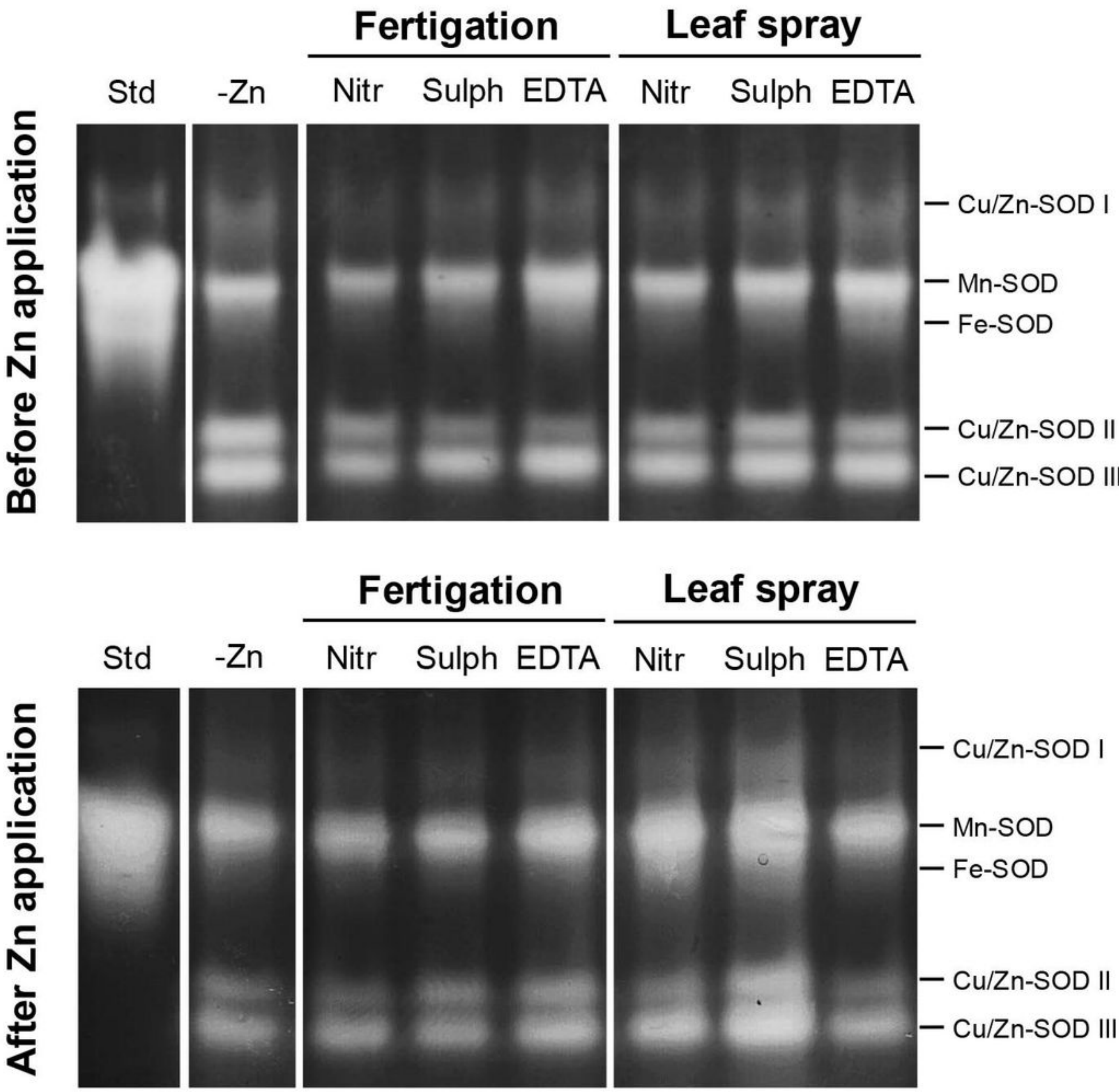

Figure 6

Activity in polyacrylamide gel electrophoresis (PAGE 12%) of superoxide dismutase (SOD) activity in leaves of ‘Pera’ sweet orange trees, in the fourth year, before (late fall - August) and after (late summer - March) application of different zinc (Zn) fertilizer sources via fertigation or foliar spray. Legend: Std = Bovine SOD standard; -Zn – Control trees without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – Zn-EDTA.

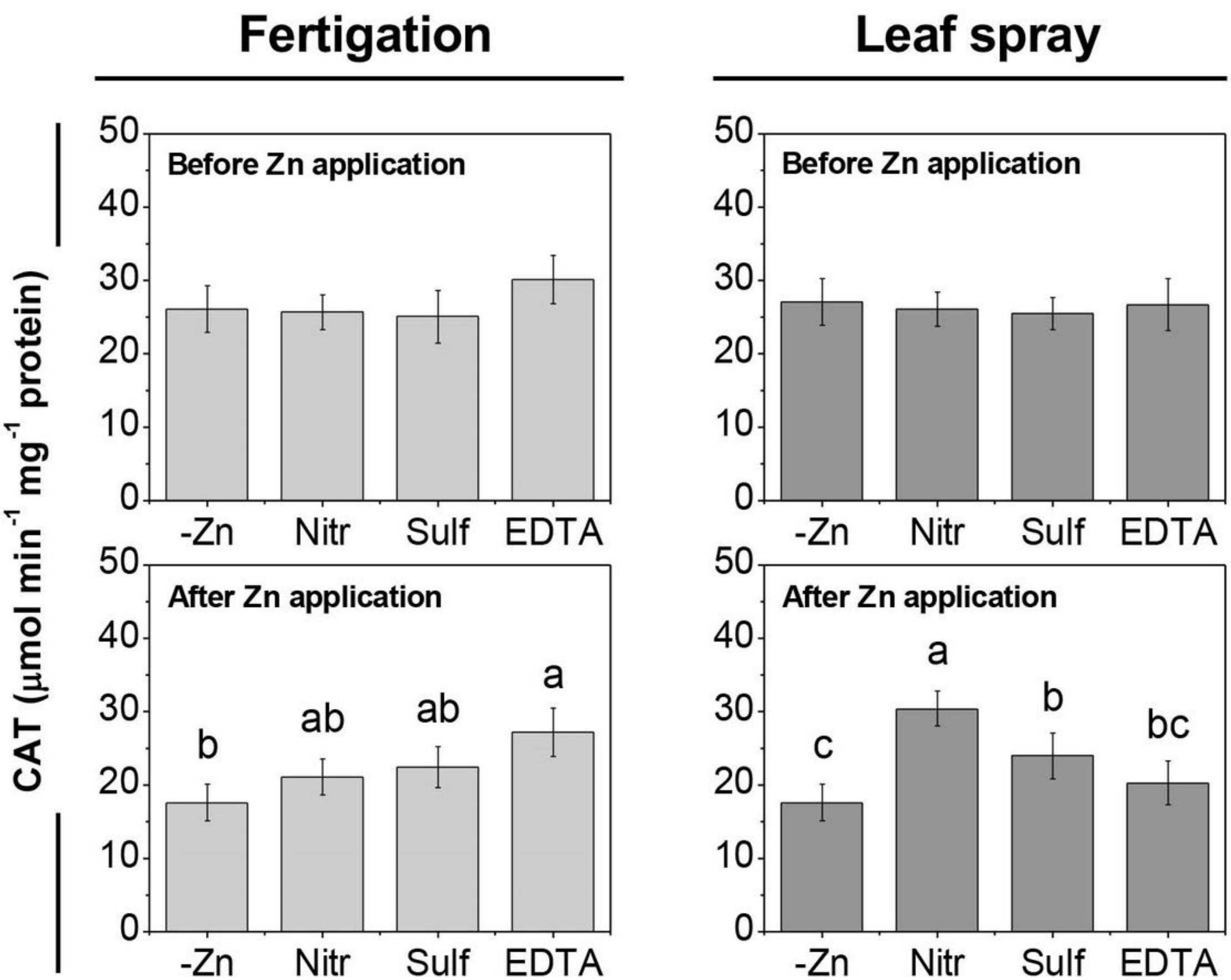

Figure 7

Catalase (CAT) activity in leaves of 'Pera' sweet orange trees, in the fourth year, before (late fall - August) and after (late summer - March) application of different zinc (Zn) fertilizer sources via fertigation or foliar spray. Legend: -Zn – Control plants without Zn application; Nitr – zinc nitrate; Sulf – zinc sulfate; EDTA – Zn-EDTA. The vertical lines represent the standard error of the mean (n=5). Different letters are significantly different according to the Tukey test (p<0.10).

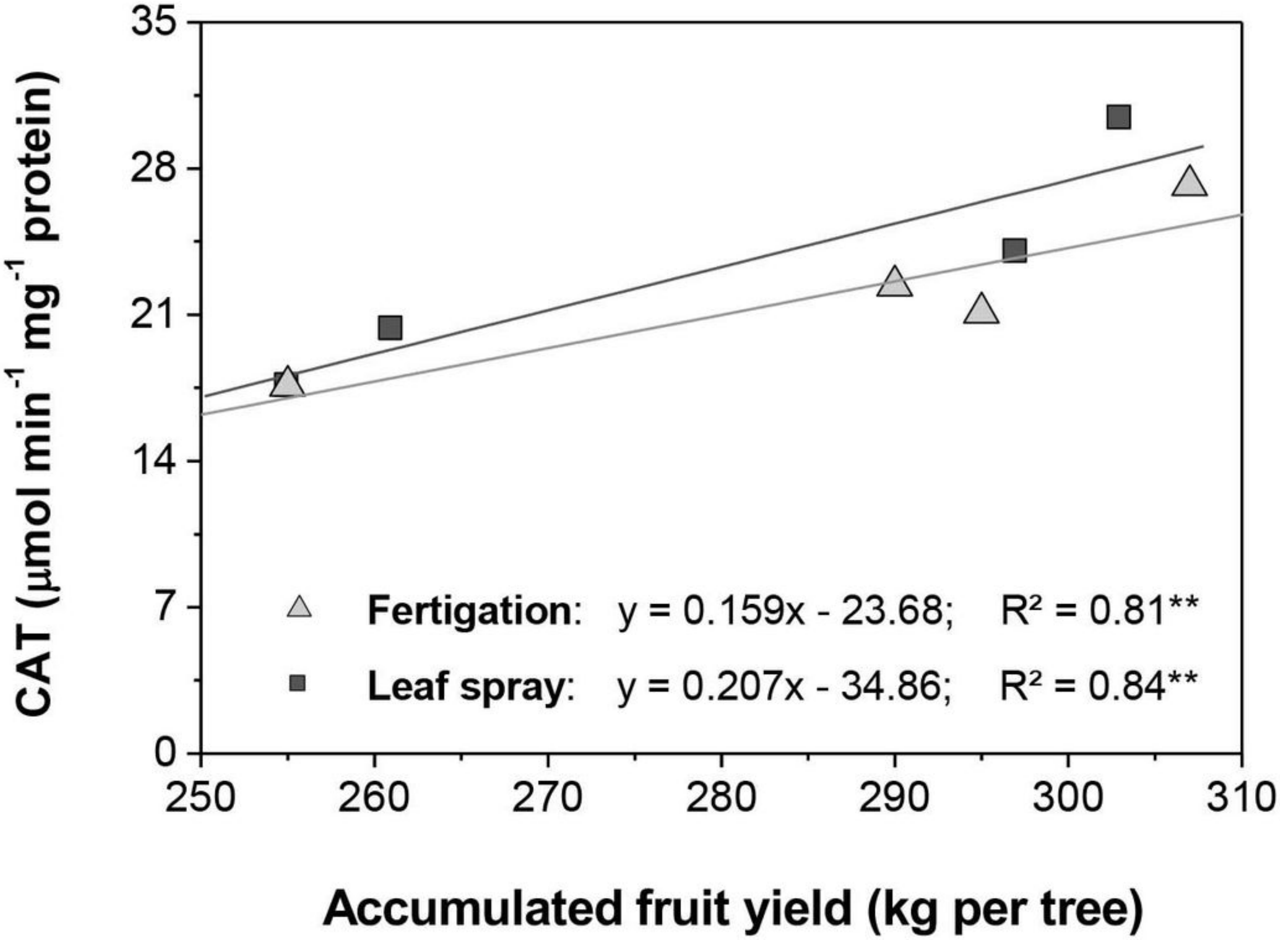

Figure 8

Catalase (CAT) activity in the leaves and accumulated fruit yield of 'Pera' sweet orange trees after application of different zinc (Zn) fertilizer sources via fertigation or foliar spray. Legend: ** signifficant (p<0.01).