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Changes in nutrient concentration and oxidative metabolism in pecan leaflets at different doses of zinc

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Abstract: Zinc deficiency limits pecan nut production. The objective of this study was to evaluate changes in nutrient concentration and oxidative metabolism in pecan leaflets in response to the application at different doses of zinc. The foliar concentration of nutrients, leaflet area, total chlorophyll, dry weight (leaflets and root), superoxide dismutase (SOD), hydrogen peroxide, catalase (CAT), guaiacol peroxidase (GP) and antioxidant capacity were evaluated. Statistical analysis indicates that the application of 200 $\mu\text{mol Zn}^{2+}$ affected the foliar concentration of N-total (24.50 ± 2.51 g/kg), P (10.34 ± 2.53 g/kg), Fe^{2+} (153.33 ± 6.27 mg/kg) and Zn^{2+} (42.00 ± 2.84 mg/kg), showing a greater area of the leaflet, total chlorophyll content and dry weight (leaflets and root). Plants treated with 50 $\mu\text{mol Zn}^{2+}$ showed a higher level of SOD activity (1.38 ± 0.016 units/min/g), GP (5.56 ± 0.229 nmol glutathione/min/g), and the production of hydrogen peroxide, without exceeding the control. On the other hand, Zn treatments caused a significant decrease in CAT activity. Zn is an essential micronutrient for the growth and development of pecan, which promotes the accumulation of other nutrients. Therefore, its absence affects the generation of oxidative stress with the subsequent activation of the antioxidant defense enzyme system.

Keywords: abiotic stress; *Carya illinoensis*; enzymatic activity; nutrient solution; physiological parameters

The fertilisation programs in pecan [*Carya illinoensis* (Wangenh.) K. Koch] usually consider nitrogen (N) and zinc (Zn^{2+}) as the two most important nutrients in the commercial production of this deciduous fruit (Castillo-González et al. 2019). Zinc is a critical element that is required in large quantities for vegetative growth, shoot elongation and quality nut (Huang et al. 2019). In this sense, the supply of Zn^{2+} is mainly carried out *via* foliar and on production trees (Heerema et al. 2017). Its application has been observed to modify the concentration of nutrients, yield and quality of the harvested nut (Ojeda-Barrios et al. 2014); however, the behavior has been inconsistent due to the soil conditions (pH and high carbonate content) and cultivar of pecan tree used (Huang et al. 2019).

The Zn^{2+} deficiency is associated with the appearance of alterations in cell structure, physiological

changes, and biochemical processes (Núñez-Moreno et al. 2018), while the increased function of Zn^{2+} in cells is associated with its ability to form a bond of tetrahedral coordination with the amino acids cysteine, histidine and glutamate (Balafrej et al. 2020). Pecan is a forest species with high sensitivity to Zn^{2+} deficiency, a microelement that is involved in the biosynthesis of chlorophyll, carotenoids and proteins (Kim et al. 2002), that acts as a cofactor of numerous enzymes (dehydrogenases, oxidases, peroxidases and carbonic anhydrase) and plays a significant role in the antioxidant enzyme defense system against oxidative stress (Hounnou et al. 2019).

The removal or inhibition of the activity of reactive oxygen species (ROS) during electron transport is linked to Zn^{2+} in the induction of gene expression of the protein-type antioxidant defense enzyme system

(Feigl et al. 2015). Among these enzymes is guaiacol peroxidase (GP) (EC. 1.11.1.7), whose catalytic function reduces H_2O_2 levels and prevents the plant tissues spreading, superoxide dismutase (SOD) (EC. 1.15.1.1), which stabilises free oxygen, peroxidase (POD) (EC. 1.111.1.7), converts H_2O_2 to water and catalase (CAT) (EC. 1.111.1.6) and removes H_2O_2 (Castillo-González et al. 2019). The application of this micronutrient is carried out *via* foliar, given its low mobility; however, this method implies a high economic and human cost for the farmer (Heerema et al. 2017). At present, studies are required to generate basic information on the behavior of this element applied to the soil by means of a nutrient solution. The objective of this study was to evaluate changes in nutrient concentration and oxidative metabolism in pecan leaflets in response to the application at different doses of zinc.

MATERIAL AND METHODS

Experiment site and design. The study was carried out from December 2016 to June 2018 at the Autonomous University of Chihuahua, Mexico. The experiment began with the collection of nuts of the cv. Western Schley from an orchard planted in 1967 (27°40'26.1"N, 105°11'13.3"W), Chihuahua, Mexico. The seeds were scarified with plain water for three days and sown in wooden sandboxes as a substrate, under shady conditions, watered every three days for a period of 54 days (emergence). The seedlings were transplanted into black polyurethane bags of 0.5 m³ capacity filled with sand as a substrate. The supply of nutrients was carried out using the following nutrient solution: 6 mmol NH_4NO_3 , 1.6 mmol K_2HPO_4 , 2.4 mmol K_2SO_4 , 4.0 mmol $CaCl_2 \cdot 2 H_2O$, 1.4 mmol $MgSO_4$, 5 μ mol Fe-EDDHA, 2 μ mol $MnSO_4 \cdot H_2O$, 0.25 μ mol $CuSO_4 \cdot 5H_2O$, 0.3 μ mol $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ and 0.5 μ mol H_3BO_3 . The application of Zn^{2+} was carried out by modifying its concentration in each treatment. The pH of the solution was 5.7 and electrical conductivity 2.7 dS/m. Irrigation was carried out every third day with an applied volume of 1 L per plant.

A completely randomised design consisting of five replications and three seedlings as the experimental unit was established. The treatments consisted of a control (without Zn^{2+}) and three doses of Zn^{2+} : 50, 100 and 200 μ mol. Zinc sulfate ($ZnSO_4 \cdot 7H_2O$) was used as a source with an adjusted pH of 6.0 ± 0.1 . The treatments were applied 106 days after transplantation (June 10, 2017) and concluded at 430 days

(April 30, 2018). The application frequency was every three days on seedlings of 24.85 ± 2 cm height and 2.36 ± 0.5 cm trunk diameter.

Foliar nutrients. Sampling for nutritional analysis was carried out in the first week of May 2018, according to the method described by Ojeda-Barrios et al. (2014). The analysis of the concentration of nutrients was carried out with the method described by Ojeda-Barrios et al. (2016). Each sample was homogenised in a Willey R-TE-650/1 mill with a 1 mm mesh (Tecnal, Piracicaba, Brazil) and placed in hermetic containers until analysis. 1 g of the dry sample was taken, and the concentration of Ca^{2+} , Mg^{2+} , K^+ , Cu^{2+} , Fe^{2+} , Mn^{2+} and Zn^{2+} was determined by triacid digestion (HNO_3 , $HClO_4$ and H_2SO_4) (25 mL of the mixture in a 10:10:25 ratio). Analyte quantifications were performed using an Analyst 100[®] atomic absorption spectrophotometer (PerkinElmer, Waltham, USA), and the P-total was determined with the ammonium vanadate-molybdate method, while the N-total concentration was determined by the micro-Kjeldahl method in separate samples.

Total chlorophyll, leaflet area and dry weight (leaflet and root). The total chlorophyll content was quantified using 80% acetone, and the absorbance readings (665 nm and 653 nm) were performed using a Lambda 25[®] UV-visible spectrophotometer (PerkinElmer, Waltham, USA). The results are reported in μ g/g (Ojeda-Barrios et al. 2009). Leaflet area estimation was performed with the CI-202[®] leaf area meter equipment (Cid Bio-Science., Washington, USA). The samples were placed in a Heratherm VCA 230[®] drying oven (Thermo Scientific, Waltham, USA) for 72 h at 70 °C. Dry weight (g) was obtained with a Scout[®] Pro SP202 portable electronic scale (Ohaus, Parsippany-Troy Hills, USA) with a 0.01 g sensitivity.

Extraction and determination of enzymatic activity. The activity of superoxide dismutase was determined by the method of controlling the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). Total SOD activity was determined in a 5 mL reaction mixture containing 50 mmol HEPES at pH 7.6, 0.1 mmol EDTA, 50 mmol Na_2CO_3 (pH 10), 13 mmol methionine, 0.25% Triton X-100 (p/v), 63 μ mol NBT, 1.3 μ mol riboflavin and an appropriate aliquot of enzyme extract. Enzyme activity was reported as units/min/g, where one unit of SOD activity corresponds to the amount of enzyme required to cause a 50% inhibition of NBT reduction evaluated at 560 nm. The extraction and analysis of total H_2O_2 consisted of the hydroperoxides forming a specific complex with

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titanium (Ti^{4+}), which can be measured by colorimetry at 415 nm. The results are expressed in $\mu\text{mol/g}$, which are the hydroperoxides produced by the reaction (total peroxides). The extraction and analysis of the activity of the enzymes catalase and guaiacol peroxidase were determined by the method described by Sánchez et al. (2000). The results were expressed as $\mu\text{mol H}_2\text{O}_2/\text{min/g}$ and $\text{nmol GSH}/\text{min/g}$, respectively.

Antioxidant activity was evaluated by the method DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. DPPH radical scavenging assay was determined calorimetrically at 515 nm on a spectrophotometer Lambda 25[®] UV-visible (PerkinElmer, Waltham, USA). The results were expressed as % DPPH inhibition.

Statistical analysis. Before analysing the data, tests of variance homogeneity were performed with the Kolmogorov-Smirnov test (Sokal and Rohlf 1995), and when heterogeneity was detected, the data were transformed with \log_{10} . A multivariate analysis of variance and multiple comparisons of means were performed on the data obtained from the evaluated parameters using the Tukey test ($P \leq 0.05$). Subsequently, a Pearson's correlation analysis was performed to determine the degree of association between the concentration of nutrients and the oxidative system, for which a matrix was constructed with the coefficients of each variable analysed. In all cases, SAS statistical analysis software version 9.3 (SAS Institute Inc., North Carolina, USA) was used.

RESULTS AND DISCUSSION

Foliar nutrients. Table 1 shows the data obtained from the concentration of foliar nutrients. The application of $200 \mu\text{mol Zn}^{2+}$ changed the N-total concentration from 19.52 ± 1.35 to $24.50 \pm 2.51 \text{ g/kg}$.

Recent studies have shown that the simultaneous application of N and Zn^{2+} improve root development and the absorption capacity of the micronutrient when the latter is in deficiency conditions (Bouain et al. 2019). One of the possible causes of this behavior may be associated with the ability of nitrogen to cause the change in soil pH and thereby to enhance the bioavailability of Zn^{2+} in the pecan root zone, as indicated by Walworth et al. (2017). On the other hand, Núñez-Moreno et al. (2018), through the application of 118, 236 and 354 kg N/ha for three years in 35-year-old cv. Western Schley pecan trees, a minimal effect on the mineral composition of the leaves, except for Zn^{2+} , B and Cu^{2+} .

The pecan production area in Mexico is characterised by presenting calcareous soils with pH values between 7.0 and 8.6. Those conditions that favour high levels of retention and precipitation of phosphorus (Núñez-Moreno et al. 2018). Among the applied doses of Zn^{2+} , a value of $10.34 \pm 2.53 \text{ g/kg}$ in the foliar concentration of P could be detected with $200 \mu\text{mol Zn}^{2+}$; however, it was statistically equal to the $7.90 \pm 3.05 \text{ g/kg}$ found with the $100 \mu\text{mol}$ treatment. The lack of Zn^{2+} in the plant alters the balance of other nutrients such as Fe^{2+} , P and Cu^{2+} ; consequently, it affects metabolism (Balafrej et al. 2020).

The application of zinc did not affect the foliar concentration of K^+ , Ca^{2+} , Mg^{2+} , Cu^{2+} and Mn^{2+} . Those results that contrast with those obtained by Kim et al. (2002), when these authors were evaluating the hydroponic application of zinc in the cvs. Stuart and Curtis of pecan from seed, they report a modification in the foliar concentration of Ca^{2+} , Mg^{2+} and Cu^{2+} in cv. Stuart, while in cv. Curtis, no changes were observed for these elements, only for Mn^{2+} and sulfur. These authors indicate that

Table 1. The concentration of foliar nutrients in pecan grown at different doses of zinc

Treatment (μmol)	Macronutrient (g/kg)					Micronutrient (mg/kg)			
	N-total	P	K^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	Cu^{2+}	Mn^{2+}	Zn^{2+}
Control	19.52 $\pm 1.35^b$	1.83 $\pm 0.63^c$	8.70 $\pm 2.28^a$	27.30 $\pm 2.30^a$	2.90 $\pm 0.45^a$	104.07 $\pm 14.20^c$	5.67 $\pm 0.54^a$	197.67 $\pm 19.28^a$	17.21 $\pm 0.30^d$
50	20.32 $\pm 1.44^b$	4.30 $\pm 3.59^{bc}$	9.00 $\pm 1.00^a$	28.00 $\pm 1.49^a$	3.10 $\pm 0.46^a$	119.33 $\pm 21.56^{bc}$	5.83 $\pm 0.57^a$	204.354 $\pm 9.8^a$	25.26 $\pm 0.86^b$
100	20.60 $\pm 0.25^b$	7.90 $\pm 3.05^{ab}$	10.80 $\pm 1.68^a$	29.80 $\pm 4.80^a$	3.20 $\pm 0.70^a$	150.00 $\pm 19.16^{ab}$	5.99 $\pm 0.70^a$	212.20 $\pm 47.37^a$	32.03 $\pm 2.11^c$
200	24.50 $\pm 2.51^a$	10.34 $\pm 2.53^a$	11.12 $\pm 0.74^a$	33.60 $\pm 5.53^a$	3.2 $\pm 0.20^a$	153.33 $\pm 6.27^a$	6.35 $\pm 1.18^a$	236.51 $\pm 7.82^a$	42.00 $\pm 2.84^a$

Mean values \pm standard deviation ($n = 5$); values with the same letters within each column do not differ statistically ($P \leq 0.05$; Tukey's test)

the differences can be associated with the ability of each genotype to absorb and translocate the different elements to the leaves. Pond et al. (2006) report ranges of low, normal and high concentration of foliar nutrients in cv. Western Schley pecan trees grown in Arizona, USA. Based on this statement, the following concentrations detected in this study for K^+ ranging 8.70 ± 2.28 – 11.12 ± 0.74 g/kg resulted normal, Ca^{2+} (27.30 ± 2.30 – 33.60 ± 5.53 g/kg) (high), Mg^{2+} (2.90 ± 0.45 – 3.2 ± 0.20 g/kg) (low), Cu^{2+} (5.67 ± 0.54 – 6.35 ± 1.18 mg/kg) (normal) and Mn^{2+} (197.67 ± 19.28 – 236.51 ± 7.82 mg/kg) (normal), variations that could be associated with the heterogeneity induced by the seed propagation and the natural ability of the root system of pecan seedlings to absorb nutrients and their subsequent transport to the leaves (Kim et al. 2002).

Fe^{2+} showed higher concentration in seedling leaflets treated with 100 and 200 $\mu\text{mol Zn}^{2+}$ at 150.00 ± 19.16 and 153.33 ± 6.27 mg/kg, respectively. The concentration of this micronutrient is in the normal range (50–250 mg/kg) for this crop, according to Heerema (2013); however, according to Pond et al. (2006), this concentration is in the high range (50–118 mg/kg). Unlike other micronutrients such as Zn^{2+} , Fe^{2+} deficiencies are closely related to soil drainage and texture (Heerema 2013). In this regard, Hounnou et al. (2019), when applying various products and concentrations of $\mu\text{mol Zn}^{2+}$ in cv. Pawnee pecan, they report significant variation in the concentration of Fe^{2+} in leaves and inconsistency throughout the years of evaluation.

Among the peculiarities of the pecan tree is its high requirement for Zn^{2+} during the sprouts-growing stage (Hounnou et al. 2019). In this study, the foliar concentration of Zn^{2+} among treatments fluctuated between 17.21 ± 0.30 and 42.00 ± 2.84 mg/kg, where the highest value was observed with the application of 200 $\mu\text{mol Zn}^{2+}$. In the commercial cultivar Western Schley pecan, leaf concentration values between

0 and 85 mg/kg Zn^{2+} are considered deficient by Pond et al. (2006). However, Heerema et al. (2017) and Castillo-González et al. (2019) point out that Zn^{2+} deficiency in leaflets occurs when the concentration of the micronutrient is less than 20 mg/kg and that the range of sufficiency is between 20 and 50 mg/kg. For its part, Kim et al. (2002) indicates that between 50 and 200 mg/kg can be considered normal for pecan since its normal growth and optimum quality of the pecan are guaranteed. The variation among the concentration values of Zn^{2+} leaf to define the range of pecan sufficiency could be determined by the pH and carbonate content of the soil (Walworth et al. 2017). In addition, it would be necessary to consider the unique characteristics of rusticity of the seedlings roots of the pecan, its absorption and translocation of this micronutrient under low availability conditions in the areas of natural growth (Kim et al. 2002, Bouain et al. 2019). On the other hand, Feigl et al. (2015), using the application of 50, 150 and 300 mmol $ZnSO_4$ in *Brassica juncea* L. and *Brassica napus* L., report a significant increase in Zn^{2+} in plants treated with high doses of this micronutrient.

The pecan seedlings showed statistical differences in relation to the area of the leaflet, total chlorophyll and dry weight (leaflets and root) (Table 2), where the highest dose showed the best result; however, the value of the leaflet area was observed similar to that obtained with the application of 100 $\mu\text{mol Zn}^{2+}$. In this sense, the control and the lowest doses showed visual symptoms due to Zn^{2+} deficiencies, reflected in the reduction of the area of the leaflets, as well as the dry weight of the leaflets and root (basic structures for the absorption and translocation of nutrients). Castillo-González et al. (2019) conducted an experiment on cv. Western Schley pecan trees and found a relationship between Zn^{2+} deficiency and the decreased leaf area, with a negative impact on the biosynthesis of tryptophan, which is a precursor of auxins and responsible for division cellular.

Table 2. Leaflet area, total chlorophyll, and dry weight (leaflets and root) of pecan grown at different doses of zinc

Treatment (μmol)	Leaflet area (cm^2)	Total chlorophyll ($\mu\text{g/g}$)	Dry weight (g)	
			leaflets	root
Control	10.02 ± 1.48^c	40.60 ± 0.66^c	6.66 ± 0.51^c	13.33 ± 1.02^d
50	16.12 ± 2.61^b	41.50 ± 2.50^c	10.68 ± 0.59^b	18.32 ± 1.56^b
100	20.30 ± 1.31^{ab}	44.40 ± 2.29^b	11.66 ± 0.95^b	23.33 ± 1.62^c
200	23.84 ± 3.78^a	45.80 ± 1.81^a	13.54 ± 0.87^a	26.64 ± 1.68^a

Mean values \pm standard deviation ($n = 5$); values with the same letters within each column do not differ statistically ($P \leq 0.05$; Tukey's test)

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Net photosynthesis and biomass accumulation in plants are highly correlated with leaf area and chlorophyll concentration (Núñez-Moreno et al. 2018), in addition to the N concentration and micronutrients. Heerema et al. (2017) found that in pecan, the application of Zn^{2+} through fertigation, young leaves increased their photosynthetic activity. Similar behavior is reported by Prasad et al. (2012) in cv. K-134 peanut leaves (*Arachis hypogaea*) when performing foliar applications of ZnO and $ZnSO_4$ nanoparticles.

In similar experiments using mandarin orange (*Citrus reticulata* Blanco), Subba et al. (2014) require an application of 10, 15 and 20 mmol zinc to report an increase in the plant's growth using 10 mmol; however, the growth stopped when applied higher doses, which is a behavior linked to the ability of zinc to modify the stability and normal functions of the cell membrane (García-López et al. 2019). Among the fruit trees most sensitive to Zn^{2+} deficiency, there is the pecan (Balafrej et al. 2020). There are few or no quantitative data relating this nutrient to vegetative growth (Pond et al. 2006), in addition to the fact that pecan shows a wide variability regarding its Zn^{2+} absorption and translocation organs (Huang et al. 2019).

Oxidative metabolism. Zn^{2+} is part of the antioxidant non-enzymatic system and a stabilising micronutrient for the effects of oxidative, peroxidative stress, loss of integrity and cellular permeability (Subba et al. 2014). In this study, the SOD activity was higher in control with 1.38 ± 0.016 units/min/g, which was statistically similar to that observed in seedlings treated with 50 μmol Zn^{2+} (1.19 ± 0.019 units/min/g). Furthermore, the production of hydrogen peroxide (H_2O_2) also showed a behavior similar to the previous enzyme (Table 3). Results that are similar to those from Burman et al. (2013), who evaluated foliar applications of zinc nanoparticles (ZnO and $ZnSO_4$) (1.5 and 10 ppm, in both cases) in chickpea seedlings (*Cicer arietinum* L.) cv. HC-1 report higher SOD activity in the treatments

without zinc applications. Under normal conditions, SOD removes ROS through the transfer of electrons in redox reactions. The pecan tree is a zinc-deficient plant (Heerema et al. 2013), which could be one of the conditions that favoured an increase in ROS production, and thus a higher SOD activity was required, as indicated by Subba et al. (2014).

The application of Zn^{2+} through the irrigation system affected the catalysis and dismutation of H_2O_2 (water and oxygen) as part of the activity of the catalase enzyme (CAT), whose values fluctuated between 2.06 ± 0.040 and 2.22 ± 0.024 μmol H_2O_2 /min/g. In contrast to the control, its value was 3.54 ± 0.002 μmol H_2O_2 /min/g. In this sense, Castillo-González et al. (2019) described that when evaluating pecan leaflets with Zn^{2+} deficiency: light (20 mg/kg), moderate (9–11 mg/kg) and severe (≤ 9 mg/kg) report a direct relationship between the level deficiency and hydrogen peroxide production, which could explain the significant increase in CAT and SOD activity in this study. On the other hand, García-López et al. (2019) point out that the application of metallic elements such as Zn^{2+} can induce stress and the generation of ROS. However, this micronutrient is vital for the activation and gene expression of various mechanisms of inhibition and removal of free radicals, where its efficiency depends on their level of absorption and transport (Burman et al. 2013).

In this study, the application of 50 μmol of zinc showed the maximum reduction in H_2O_2 levels through the activity of the guaiacol peroxidase (GP) enzyme (5.56 ± 0.229 nmol GSH/min/g); however, the application was statistically similar to that observed in the control seedlings. The zones of adaptation and commercial production of pecan are characterised by having calcareous soils (Heerema 2013), where Zn^{2+} is not available (precipitated or as part of poorly soluble compounds) and the considerable reduction in yield of pecan, even with very slight deficiencies (Kim et al. 2002), so the

Table 3. Oxidative metabolism and antioxidant capacity in pecan seedlings grown at different doses of zinc

Treatment (μmol)	SOD (units/min/g)	H_2O_2 ($\mu\text{mol/g}$)	CAT (μmol H_2O_2 /min/g)	GP (nmol GSH/min/g)	AC (% DPPH inhibition)
Control	1.38 ± 0.016^a	0.45 ± 0.007^a	3.54 ± 0.002^a	5.96 ± 0.109^a	44.67 ± 0.078^d
50	1.19 ± 0.019^a	0.34 ± 0.0065^{ab}	2.22 ± 0.024^b	5.56 ± 0.229^a	52.50 ± 0.068^c
100	1.00 ± 0.027^b	0.26 ± 0.043^b	2.20 ± 0.018^b	3.30 ± 0.306^b	66.35 ± 0.039^b
200	1.00 ± 0.033^b	0.21 ± 0.017^b	2.06 ± 0.040^c	2.54 ± 0.329^c	73.56 ± 0.062^a

Mean values \pm standard deviation ($n = 5$); values with the same letters within each column do not differ statistically ($P \leq 0.05$; Tukey's test). SOD – superoxide dismutase; H_2O_2 – hydrogen peroxide; CAT – catalase; GP – guaiacol peroxidase; GSH – glutathione; AC – antioxidant capacity; DPPH – 2,2-diphenyl-1-picrylhydrazyl

correction must be done using foliar applications due to the inability of this crop to transport it from the roots to the aerial part (Ojeda-Barrios et al. 2014). However, the excessive application can cause oxidative stress due to the ability of Zn^{2+} to bind to the oxygen, nitrogen, and sulfur atoms of the functional groups in various biomolecules and cause enzymatic inactivation (Feigl et al. 2015). The presence and generation of ROS as the superoxide anion ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), peroxy ($ROO\cdot$), hydrogen peroxide (H_2O_2), among others, can cause serious damage to proteins, lipids and DNA by the generation of oxidative stress (Blasco et al. 2015). It is important to point out that all organisms, including plants, have various antioxidant mechanisms (either enzymatic or non-enzymatic) to remove, reduce or inhibit the negative effect of these reactive species (oxygen and nitrogen) (Feigl et al. 2015). In this study, the leaflets of the pecan seedlings showed AC values that fluctuated between 44.67 ± 0.078 and $73.56 \pm 0.062\%$ DPPH inhibition, where the 200 mmol treatment was the most outstanding. Blasco et al. (2015) in *Brassica rapa* ssp. *trilocularis* seedlings subjected to foliar application of low (0.05 μmol) and high (500 μmol) zinc doses report that high doses showed an increase in antioxidant activity due to the activity of oxidative enzymes such as SOD and ascorbate peroxidase. However, the activity of SOD, in addition to CAT and GP, was greater in conditions of deficiency. Tewari et al. (2008) report that in cv. Kanva-2 mulberry plants (*Morus alba* L.) that deficiency and excess conditions cause an accumulation of H_2O_2 and lipid peroxidation, which generates oxidative stress that limits the growth and normal development of the plant, where the activity of antioxidant enzymes (SOD, CAT and GP) is not sufficient. Previous studies point out the pecan as

a zinc-deficient plant (Ojeda-Barrios et al. 2014, Castillo-González et al. 2019); as mentioned previously, this micronutrient plays an important role in the resistance gene expression to oxidative stress. Therefore, it must be maintained in sufficient concentrations to increase its efficiency (García-López et al. 2019). Variation of these conditions can generate strong oxidative stress and limit the effectiveness of the antioxidant enzyme system (Blasco et al. 2015), which could explain low values of antioxidant capacity in control and 50 μmol treatment.

On the other hand, among the factors associated with the generation of oxidative stress is the application of nutrients, given the role that these elements play in metabolic processes. There is evidence that after N, Zn^{2+} is the most important nutrient in pecan production (Núñez-Moreno et al. 2009), and its deficiency affects the growth and development of branches, leaves, flowers and fruits with the subsequent decrease in yield and quality of the harvested nut (Walworth et al. 2017). As expected, an inverse relationship was shown between the concentration of foliar nutrients and SOD, H_2O_2 , CAT and GP (Table 4), given that among the various functions that nutrients have, the control and development of the metabolic process are found relevant (respiration and photosynthesis) (García-López et al. 2019) and the attenuation of the oxidative stress generated. As noted above, Zn^{2+} is a nutrient associated with protein synthesis, carbohydrate metabolism and gene expression related to the regulation of oxidative stress (Rivera-Espejel et al. 2019, Balafrej et al. 2020). In this study, among the nutritional elements with the highest correlation coefficient are Fe^{2+} and Zn^{2+} , closely associated with plant homeostasis, that is,

Table 4. Pearson's correlation coefficients for foliar nutrient concentration and oxidative metabolism in pecan grown at different doses of zinc

	SOD (units/min/g)	H_2O_2 ($\mu mol/g$)	CAT ($\mu mol H_2O_2/min/g$)	GP (nmol GSH/min/g)
N-total	-0.54*	-0.63**	-0.48*	-0.63**
P	-0.61**	-0.56**	-0.56**	-0.64**
K ⁺	-0.55*	-0.60**	-0.43	-0.62**
Ca ²⁺	-0.39	-0.51*	-0.36	-0.50*
Mg ²⁺	-0.21	-0.21	-0.27	-0.20
Fe ²⁺	-0.79**	-0.76**	-0.63**	-0.75**
Cu ²⁺	-0.22	-0.26	-0.24	-0.31
Mn ²⁺	-0.21	-0.24	-0.23	-0.32
Zn ²⁺	-0.89**	-0.93**	-0.80**	-0.90**

SOD – superoxide dismutase; H_2O_2 – hydrogen peroxide; CAT – catalase; GP – guaiacol peroxidase; * $P < 0.05$; ** $P < 0.01$

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elements that by their nature are linked as cofactors in electron transport along with redox processes and structural part of various proteins and enzymes, including metalloenzymes (Huang et al. 2019). Heerema (2013) point out that micronutrients reduce plant growth and development, but their most important impact is found in the generation of oxidative stress due to the increased activity of the superoxide radical.

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