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Effect of foliar application of zinc on annual productivity, foliar nutrients, bioactive compounds and oxidative metabolism in pecan

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ABSTRACT

Pecan nut production is quite commonly limited by zinc (Zn) deficiency. Here, we evaluate the response in terms of the concentrations of non-structural carbohydrates, yield components, foliar nutrient levels and oxidative metabolism in young 'Western Schley' pecan nut trees in response to foliar applications of 200 mg · L^{-1} of Zn as one of the following: $ZnSO_4$, Zn-EDTA, ZnO nanoparticles (NPs) or the proprietary product 'nitrazinc' (NZN) (the control). Across two consecutive growing seasons, the spraying of Zn in these various forms helped maintain the foliar concentrations of non-structural carbohydrates, foliar nutrients (total-N, Ca^{2+} and Mg^{2+}) and the kernel percentage of nuts. Likewise, trees sprayed with $ZnSO_4$ maintained the concentrations of Zn in the leaflets across seasons. On the other hand, Zn-EDTA decreased the concentration of chlorophyll and total carotenoids. In general, leaflets treated with $ZnSO_4$, Zn-EDTA and ZnO NPs reduced their oxidative metabolism. Sources of Zn – such as $ZnSO_4$ – are commercially viable alternatives suitable for increasing the performance of some parameters associated with the yield and quality of nuts in pecan. It would be worthwhile to determine the optimal Zn dose rates for the various pecan cultivars in common use and also to increase our understanding of the physiological and biochemical changes associated with foliar Zn applications.

Keywords: *Carya illinoinensis*, guaiacol peroxidase, oxide nanoparticles, 'Western Schley', Zn-EDTA, $ZnSO_4$

Abbreviations: AC, antioxidant capacity; BSA, bovine serum albumin; CAT, catalase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GPx, guaicol peroxidase; H_2O_2, hydrogen peroxide; $Na_2EDTA{\cdot}2H_2O$, ethylene diamine-tetra-acetic acid, disodium salt, dihydrate; NBT, nitroblue tetrazolium; ZnO NPs, zinc oxide nanoparticles; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate (UAN) fertiliser; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase; TFl, total flavonoids; TP, total phenols; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; $ZnSO_4$, zinc sulphate.

INTRODUCTION

The supply of chemical fertilisers to complement the soil's natural fertility is essential in modern agriculture, which is struggling to satisfy the nutritional demands of the world. However, indiscriminate use and/or overuse of fertilisers has also increased agriculture's adverse impacts on ecosystems and environmental quality (Cruz-Crespo et al., 2014; Mazaheri-Tirani et al., 2019). The performance of a chemical fertiliser

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can be reduced by several factors, including the soil characteristics (water content, pH, texture and structure), the fertiliser's solubility and the dose applied (Zulfiqar et al., 2019). Zn is a micronutrient that plays important roles in the activities of oxidoreductases, lyases, isomerases, transferases, hydrolases and ligases (Singh et al., 2018). It is involved in various metabolic processes (photosynthesis; protein and carbohydrate metabolism) and is an integral part of the cell membrane (Küçükyumuk et al., 2014; Nandal and Solanki, 2021). In addition, Zn promotes the synthesis of tryptophan and auxins and plays a role in pollen development and fruit set (Xing et al., 2016; Santis et al., 2019). Because of its low mobility in the soil and plant, foliar supply of Zn is more efficient and also has less environmental impact (Fernández and Brown, 2013).

Among the fruit trees of temperate zones, the pecan tree [*Carya illinoinensis* (Wangenh.) K. Koch] tends to be Zn-deficient (Smith et al., 2021). In particular, Zn suffers limited bioavailability in orchards on calcareous ($CaCO_3$) soils that have alkaline pH values (≥7.5). Moreover, Zn is a determining factor in the occurrence of intravenous chlorosis, impaired growth with short 'rosetted' internodes and small leaflets. Tissue degradation also occurs, leading to interveinal necrosis in leaflets, regressive death in shoots and significant reductions in yield (Heerema et al., 2017). The correction of Zn deficiency in agriculture mainly uses Zn sulphates and nitrates (higher solubility, lower cost). Other Zn compounds are also used but to a lesser extent; these include phosphates, oxides and chelates (Ojeda-Barrios et al., 2009). However, the performance of a foliar Zn spray depends on the characteristics of the spray solution (solubility, pH and coverage), the anatomical structures and age of the leaves, and the environmental factors at the time of spraying (air temperature, relative humidity and wind) (Rossi et al., 2019; Bonomelli et al., 2021).

The chelates are stable molecules that, when sprayed at high concentration, present reduced symptoms of phytotoxicity and can also be combined with humic and carboxylic acids (Doolette et al., 2018). Our earlier studies on foliar Zn applications to 'Western Schley' pecan trees using $ZnNO_3$, zinc ethylene diamine-tetra-acetic acid (Zn-EDTA) or Zn-diethylene triamine-penta-acetic acid (DTPA) (100 mg · L^{-1}) showed significant variations in Zn uptake but without affecting nut yield or quality (Ojeda-Barrios et al., 2009, 2014). Similar results have been reported for the photosynthetic rate in terminal shoots of 'Wichita' pecans with the application of 3.75 mL · L^{-1} urea–ammonium nitrate (UAN), 3.6 g · L^{-1} ZnSO4, 3.6 g · L^{-1} ZnSO4 with 3.75 mL · L^{-1} UAN, 11 mL · L^{-1} Zn-EDTA (Smith et al., 2021). On the other hand, in other cultivars such as 'Western Schley', Naira et al. (2013) report improvements in leaflet growth after applying $ZnSO_4$ (0.5%). Foliar fertilisation is often used to refer to the more general application of mineral nutrients, phytohormones, biostimulants and pesticides to the aerial parts of a plant.

In recent years, a wide range of problems in diverse fields of science and industry have been aided by nanotechnology. Nanomaterials are classified as material particles smaller in size than 100 nm in at least one dimension (Davarpanah et al., 2016). Metal and metal oxide nanoparticles (such as Cu, Ag, Mn, Mo, Zn, Fe, Si and Ti) exhibit different physicochemical characteristics from larger particles and so can be used for the design of new fertilisers with similar or better results compared with their native macro-particulate compounds (Nagula and Ramanjaneyulu, 2020). Compared with conventional fertilisers, nanoparticles (NPs) stand out for their higher specific surface-area ratios, surface-volume ratios and controlled release kinetics (Subbaiah et al., 2016; Zulfiqar et al., 2019). These properties allow much improved uptake efficiencies, so they can be supplied at low concentration but with similar or even better results than those obtained with the doses used in soil-surface fertilisation (Rossi et al., 2019). Thus, the NPs of ZnO have these characteristics, improving its solubility, diffusion rate and availability to the plant.

Till now, research on the effects of ZnO NPs relate mostly to non-woody plants, including peanut (*Arachis hypogea* L.), bean (*Phaseolus vulgaris* L.) and tomato (*Solanum lycopersicum* L.) (Bautista-Díaz et al., 2021; Faizan et al., 2021). However, there are only a few reports for perennial fruit trees, e.g. pomegranate (*Punica granatum* L.) and mango (*Mangifera indica* L.) (Davarpanah et al., 2016; Elsheery et al., 2020). We can find no information on foliar applications of ZnO NPs to pecan trees or of their response in terms of growth, yield and nut quality. The objective of this study was to evaluate any response in the concentrations of non-structural carbohydrates (NSCs), foliar nutrients, oxidative metabolism and yield components in young 'Western Schley' pecan nut trees in response to foliar applications of Zn, including the use of $ZnSO_4$, EDTA and ZnO NPs.

MATERIALS AND METHODS

Study area, plant material and orchard management

The research was carried out during the 2019 and 2020 production cycles on young (7-year-old) pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cv. 'Western Schley' trees, established on native rootstocks, planted at 10 × 10 m spacing (100 trees · ha^{-1}). The orchard is located in Delicias, Chihuahua, Mexico (28º20′46.4″N, 105º34′03.3″W) at an altitude of 1,162 m; here, the mean annual temperature is 27 °C and the mean annual rainfall is 300 mm. The orchard is on a calcareous soil (Xerollic Calciorthid), has an arable layer of 0–35 cm with a pH of 7.8 in a 1:1 v:v soil:water mix, 0.95% organic matter, 20.0% total $CaCO_3$, 1.3 dS · m^{-1} electrical conductivity (EC), 8.8 mg · kg^{-1} NO_3-N and 0.54 mg · kg^{-1} DTPA-extractable Zn. The trees used in this study had not

previously received any Zn treatment. Mineral nutrient supply was by surface application of dry fertiliser (120 N: 100 P_2O_5: 96 K_2O). Standard commercial practices for weed control and irrigation scheduling were followed throughout the experiment.

Four different treatments were applied in a completely randomised design with 10 replications per treatment (40 trees in total). Each tree was one experimental unit. Tree height was 9.0 ± 0.5 m, and trunk girth was 55 ± 10 cm. All the spray solutions used contained 200 mg · L^{-1} Zn (3.06 mM) and included the proprietary product NZN [nitrazinc, containing $Zn(NO_3)_2$ and UAN fertiliser (Smith and Storey, 1979); Tessenderlo K, Inc., Phoenix, AZ, USA]; $ZnSO_4$ ($ZnSO_4 \cdot 7H_2O$ reagent grade); Zn-EDTA (sodium salts and reagent grade $ZnSO_4 \cdot 7H_2O$ were added to make Zn(II)-EDTA); and ZnO NPs. The ZnO was obtained using wet chemistry as wurtzite crystals, of average size 50 nm with no contaminants (purity: 99.7%) and density 5.61 g · cm^{-3} (Figures 1 and 2). In all formulations, 0.1% urea was added as the carrier ion and 100 mg · L^{-1} of Tween® 20 (Thermo Fisher Scientific™, Waltham, MA, USA) was used as the non-ionic surfactant. The pH of the solutions was adjusted to 6.5 with HCl to facilitate foliar uptake in formulations with metallic nutrients (Ojeda-Barrios et al., 2014). The Zn solution was applied between 0600 hr and 0900 hr, using a 25-L motorised backpack fertiliser applicator, six times per season (2019 and 2020) – on 7 April; 6, 16 and 30 May; and 11 and 26 June. In each application, the solution was sprayed to run-off using 17 L per tree (2019) and 20 L per tree (2020).

Sampling of leaflets and nuts

Approximately 50 pairs of leaflets were collected from each tree, from the middle of the canopy, on 20 July (2019 and 2020). This timing was during the growing season and was approximately 215 days after budding, in the water stage of the nut. Leaflets were collected by pooling samples from the four cardinal directions (north, south, east and west) and from both vegetative and fruiting shoots. Collected materials were without obvious mechanical damage, pests or diseases. Nut collection was carried out in the last week of November by mechanical vibration of the trees.

Determination of NSCs

Extraction and quantification of glucose, fructose, sucrose and starch were carried out according to the method described by Ojeda-Barrios et al. (2022).

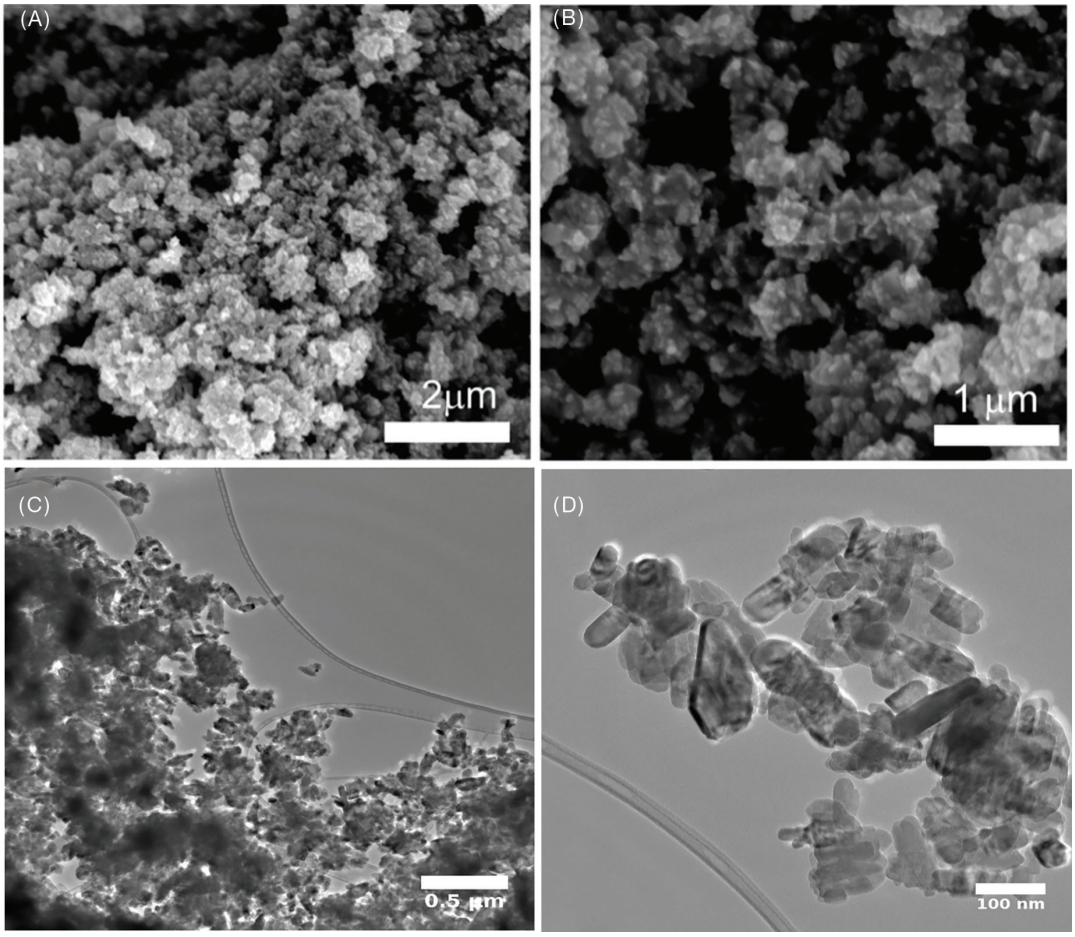

Figure 1. Morphology of the ZnO NP samples determined by scanning (A,B) and transmission (C,D) electron microscopy. ZnO NPs, zinc oxide nanoparticles.

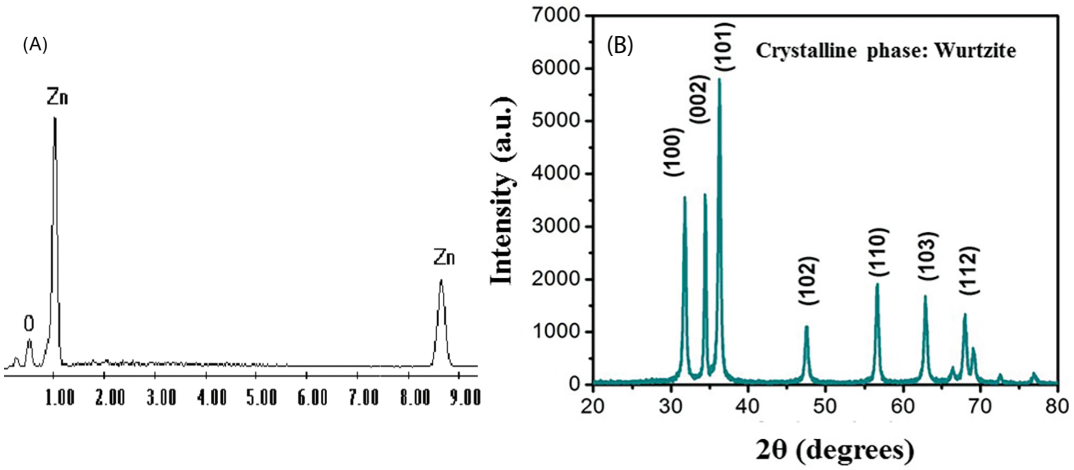

Figure 2. Elemental analysis (chemical composition) by energy dispersive X-ray scattering (A) and crystalline structure by X-ray diffraction (B) of the sample ZnO NPs (Bautista-Díaz et al., 2021). ZnO NPs, zinc oxide nanoparticles.

Briefly, a sample of 1 g of fresh tissue was taken and homogenised twice, first with 5 mL of 95% aqueous ethanol (v:v) and next with 70% aqueous ethanol (v:v). The mixture was centrifuged at 5,500 *g* for 10 min at 4 °C. Next, 0.1 mL of the supernatant was taken, and 3 mL of anthrone solution was added. The mixture was placed in a water bath at 4 °C for 10 min and, after cooling, the absorbance was measured at 650 nm. For the determination of starch, the dry residue of the extraction was taken and incubated in acetate buffer (4.5 M), 0.5% α-glucoamylase (w:v) and water at 37 °C for 48 hr. The results are expressed in milligrams per gram of fresh weight (FW).

Yield components (yield and nut quality)

The performance components were determined according to Mexican Standard NMX-FF-084-SCFI-2009. The weight of the harvested nuts (fruits) was obtained using a Combo-Rhino-122 balance (Rhino®, Mexico City, Mexico) with a sensitivity of 0.1 g. Yield data are expressed in kilograms per tree. For the number of nuts per kilogram, nuts (1 kg) were randomly selected and counted. Next, 300 g of nuts were selected as a sub-sample, and the shells were removed and discarded. The kernels (the edible part) were weighed. Kernel percentage was obtained as the ratio of kernel weight divided by sub-sample weight × 100.

Leaf mineral nutrients

The leaflets were transported for analysis to the Plant Physiology Laboratory at Universidad Autonoma of Chihuahua, Mexico. Extraction and quantification of nutrients were carried out using the method published by Cruz-Álvarez et al. (2020). Briefly, a triple wash was carried out in tap water, then in 4 N HCl and finally in deionised water. Surface moisture was completely removed from the leaflets at room temperature, and the leaflets were then dried in a Heratherm VCA 230® oven (Thermo Scientific, Waltham, USA) at 75 °C for 24 hr. Each sample was homogenised in a Willey R-TE-650/1 mill with a 1 mm mesh (Tecnal, São Paulo, Brazil). The extraction and quantification of total-N and total-P were carried out by the Kjeldhal method (Novatech®, USA and Micro Kjeldahl Labconco®, USA) and by the ammonium metavanadate method (NH_4VO_3) (Thermo Scientific™), respectively. The extraction of K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} was by digestion in 25 mL of a triacid mixture (HNO_3, $HClO_4$ and H_2SO_4; 10:10:25) on a hot plate, under a fume hood. Analyte quantification was carried out using an Analyst 100® atomic absorption spectrophotometer (PerkinElmer®, Waltham, MA, USA). The results are reported as grams per kilogram DW (for macronutrients) and as milligrams per kilogram DW (for micronutrients).

Chlorophyll and total carotenoids

The extraction and quantification of the photosynthetic pigments were carried out according to the method described by Wellburn (1994). Briefly, the samples were placed in flasks with 100 mL of 80% (v:v) acetone. The absorbance values at 665, 653 and 470 nm were recorded using a Lambda 25® ultraviolet (UV)–visible spectrophotometer (PerkinElmer). The results are expressed in milligrams per kilogram FW.

Total phenols and flavonoids

The total phenol (TP) content was determined using the Folin–Ciocalteu method (Waterman and Mole, 1994) with slight modifications. Briefly, 0.5 g of sample was taken and homogenised with 5 mL of 70% (v:v) absolute ethyl alcohol. Next, an aliquot (50 µL) of this solution was taken and mixed with 7.95 mL of distilled water and 500 µL of the Folin–Ciocalteu (2N) reagent, then shaken for 1 min with a digital vortex (Thermo Scientific®) and left to rest for 8 min. Then, 1.5 mL of 20% (w:v) Na_2CO_3 was added, stirred and left to settle for 2 hr in total darkness at room temperature (22 ± 1 °C). Absorbance values

were recorded at 760 nm with a Lambda 25® UV–visible spectrophotometer (PerkinElmer). A standard curve of gallic acid (Sigma Aldrich, St. Louis, MO, USA) was constructed with concentrations of 0, 20, 40, 60, 80 and 100 mg · L^{-1}. The results are expressed in milligrams of gallic acid equivalents (GAE) per gram FW. The total flavonoid (TFl) content was determined using the method of Loizzo et al. (2016) with slight modifications. Briefly, 5 mL of methanol was added to 1 g of sample; this was then homogenised and left at room temperature (22 ± 1 °C) (to evaporate the methanol and dry the extract). Next, 5 mL of distilled water was added to 0.01 g of the dry extract, and this was shaken with a digital vortex (Thermo Scientific®) for 20 min. To 650 µL of this solution, 75 µL of 5% $NaNO_2$ was added, stirred and rested for 6 min. After this, 150 µL of 10% $AlCl_3$ was added, shaken and rested for 4 min. Finally, 500 µL of NaOH (1 M) and 1,150 µL of distilled water were added. The absorbance at 510 nm was recorded. A standard catechin curve (Sigma Aldrich) was constructed with concentrations of 0, 20, 40, 60, 80 and 100 mg · L^{-1}. The results are expressed in milligrams of quercetin equivalents (QE) per gram FW.

Oxidative metabolism (enzymatic activity and antioxidant capacity)

Superoxide dismutase (SOD; Enzyme Commission [EC] number: 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the method described by Giannopolitis and Ries (1977) and modified by Sánchez et al. (2005). Enzyme activity is reported as units per minute per gram FW, where 1 unit of SOD activity corresponds to the amount of enzyme required to cause a 50% inhibition of NBT reduction as evaluated at 560 nm. The extraction and analysis of total H_2O_2 used a colourimetric method (Sánchez et al., 2000). The results are reported in micromoles of H_2O_2 per minute per gram FW (total peroxides). Last, the extraction and determination of enzymatic activity of the enzymes catalase and guaiacol peroxidase were determined by the method described by Sánchez et al. (2000). The results are expressed as moles of H_2O_2 per minute per gram FW and nanomoles glutathione (GSH) per minute per gram FW, respectively.

Antioxidant capacity (AC) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) method. Aqueous ethanol (80%) was used for sample preparation. The DPPH• assay was carried out in accordance with the procedure followed by Brand-Williams et al. (1995). Briefly, 0.3 mL of extract and 5.7 mL of DPPH were mixed at a concentration of 0.0375 g · L^{-1}. The mixture was kept in the dark for 30 min. The decrease in the quantity of DPPH radical was measured at 515 nm. The results are expressed as percentage inhibition of DPPH.

Statistical analyses

Prior to statistical analysis, the normal distribution of the data was confirmed by the Shapiro–Wilk test ($p \leq 0.05$) (Hanusz and Tarasińska, 2015). Statistical analysis consisted of a general linear model with year and treatment effects (NSCs, yield, nut weight, kernel percentage and foliar nutrient concentration), and the remaining parameters were analysed only by treatment in the year 2020. The comparison of means was carried out with a multiple comparison of means with Tukey's test ($p \leq 0.05$). The data were analysed with the statistical package Statistical Analysis Software (SAS/STAT | SAS), version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Annual productivity (NSCs and yield components)

It is well known that carbohydrates are key molecules in plants since they are part of the plant structure (e.g. cellulose) and are also the primary source of metabolic energy (e.g. starch) (Ojeda-Barrios et al., 2022). Here, the leaflets sprayed with sulphates, chelates and zinc NPs presented only slight numerical differences in the concentrations of NSCs (fructose, glucose, sucrose and starch), so it was not possible to identify a significant effect on NSCs between the two growing seasons examined (Table 1). These results are important, considering the negative impact of alternate bearing (AB) on pecan nut yield and quality (Wood et al., 2003; Rohla et al., 2007). Pecan trees quite commonly present AB; while the intensity of AB can be linked to the concentration of carbohydrates, it can also involve elements of agronomic management, such as irrigation, fertilisation, pruning and crop load. Other more random factors, such as water stress, lack of winter cold, tree age and cultivar, can also be involved (Wood et al., 2003). In Mexico, the main cultivars in commercial production are 'Western Schley', 'Wichita' and, more recently, 'Pawnee'. Previous studies carried out to evaluate the intensity of AB in young and adult pecan trees by Conner and Worley (2000) report intermediate values in 'Wichita' (0.51 and 0.67) and 'Western Schley' (0.65 and 0.68); however, in 'Pawnee', there are no reports of AB, so it can be inferred that this is a promising new cultivar for pecan production.

The dormancy processes (paradormancy, endodormancy and ecodormancy) in temperate fruit trees are linked to various governing influences, including the environment and the genotype (Pertille et al., 2021). Among the internal factors governing dormancy are the concentrations of carbohydrates in leaves and buds (Wood et al., 2003; Rohla et al., 2007). In this study, foliar applications of Zn (NZN, $ZnSO_4$, Zn-EDTA and ZnO NPs) did not affect ($p \geq 0.05$) the concentration of NSCs in the leaflets (Figure 3). Thus, in pecan trees, after nitrogen (N), Zn is the most important nutrient in terms of the number of fertiliser applications required and economic benefit generated by this activity (Heerema et al., 2017). However, for pecan, there is no information on the relationship between foliar applications of Zn and the NSC concentration in the leaflets. It is well known that, for pecan, flower

Table 1. Concentration of NSCs in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles.

Treatments (200 mg · L^{-1})	NSCs (g · kg^{-1})							
	Fructose		Glucose		Sucrose		Starch	
	2019	2020	2019	2020	2019	2020	2019	2020
NZN (control)	37.9 a	34.3 a	41.9 a	38.0 a	41.9 a	43.5 a	38.8 a	40.2 a
$ZnSO_4$	40.7 a	38.5 a	45.0 a	42.6 a	37.5 a	36.7 a	34.6 a	34.5 a
Zn-EDTA	39.3 a	40.3 a	43.5 a	44.6 a	40.8 a	39.4 a	37.5 a	36.6 a
ZnO NPs	39.5 a	38.2 a	43.6 a	42.9 a	36.7 a	39.3 a	35.7 a	39.6 a

Means in the same row followed by the same letter were not significantly different (Tukey's test, $p \leq 0.05$). Data are expressed on a dry weight basis.
NSC, Non-structural carbohydrate; ZnO NPs, zinc oxide nanoparticles; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; $ZnSO_4$, zinc sulphate.

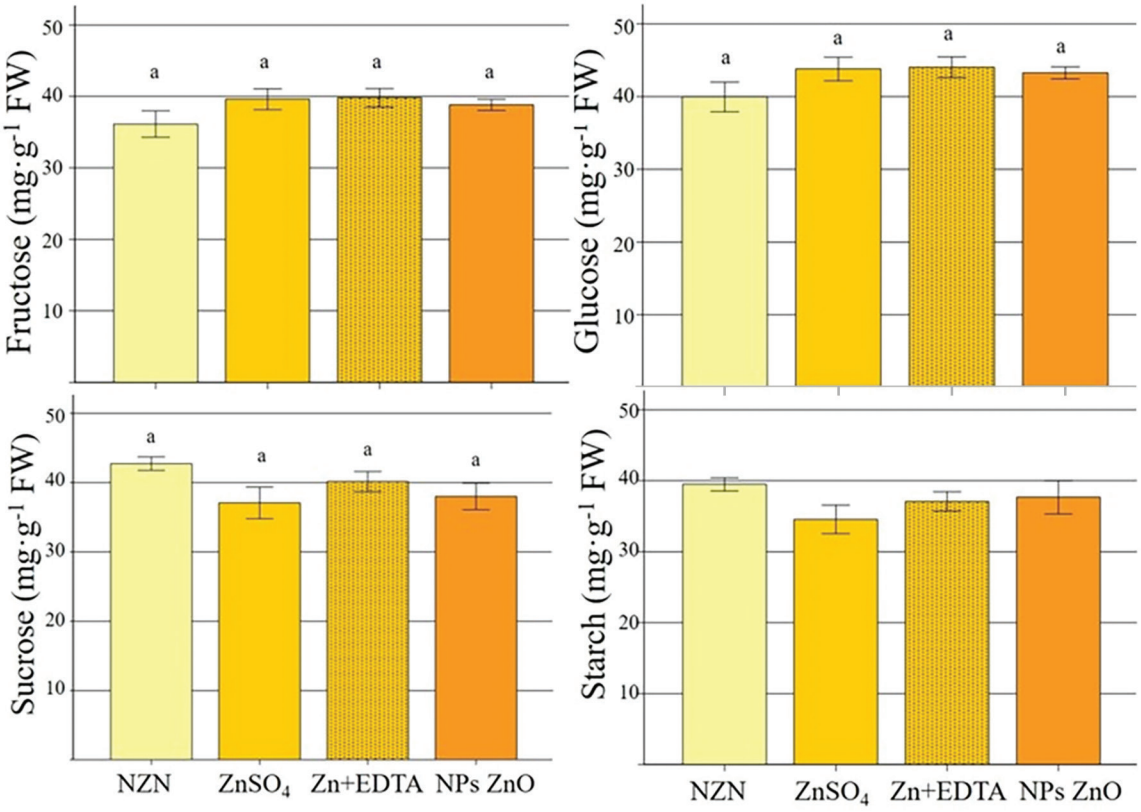

Figure 3. NSCs in 'Western Schley' pecan leaflets sprayed with sulphates, chelates and zinc nanoparticles. The data are means of treatments in 2019 and 2020. Bars with the same letter are not significantly different (Tukey's test, $p \leq 0.05$). Error bars represent standard deviations ($n = 10$). NSC, Non-structural carbohydrate; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; ZnO NPs, zinc oxide nanoparticles; $ZnSO_4$, zinc sulphate.

development depends on reserve carbohydrates and the concentration of nutrients, including that of Zn (Tsuchida et al., 2015; Breen et al., 2018).

On the other hand, we did find variation ($p \leq 0.05$) between years with respect to yield and nut weight (grams per kilogram), but not for kernel percentage (Table 2). This behaviour indicates the occurrence of AB and the null effect of Zn applications. However, the kernel percentage was maintained across the two seasons, which is an aspect of high economic importance to farmers and is highly appreciated by marketers and consumers of this nut. The values of these parameters are similar to those reported by Castillo-González et al. (2019) in young trees (≈12 years old) of 'Western Schley' grown under Zn-sufficiency conditions. According to the NMX-FF-084-SCFI-2009 (NOM-2009) for improved nuts, with the

Table 2. Yield, nut weight and kernel percentage in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles.

Treatments (200 mg · L^{-1})	Nut quality					
	Yield (kg per tree)		Nut weight (g · kg^{-1} FW)		Kernel (%)	
	2019	2020	2019	2020	2019	2020
NZN (control)	9.9 a	15.6 b	156.1 a	166.1 b	52.2 a	53.0 a
$ZnSO_4$	9.1 a	14.1 b	153.4 a	167.4 b	60.4 a	58.0 a
Zn-EDTA	8.7 a	14.9 b	152.5 a	164.5 b	54.3 a	54.3 a
ZnO NPs	9.9 a	14.5 b	158.6 a	166.6 b	54.9 a	54.5 a

Means in the same column followed by the same letters were not significantly different (Tukey's test, $p \leq 0.05$).
ZnO NPs, zinc oxide nanoparticles; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; $ZnSO_4$, zinc sulphate.

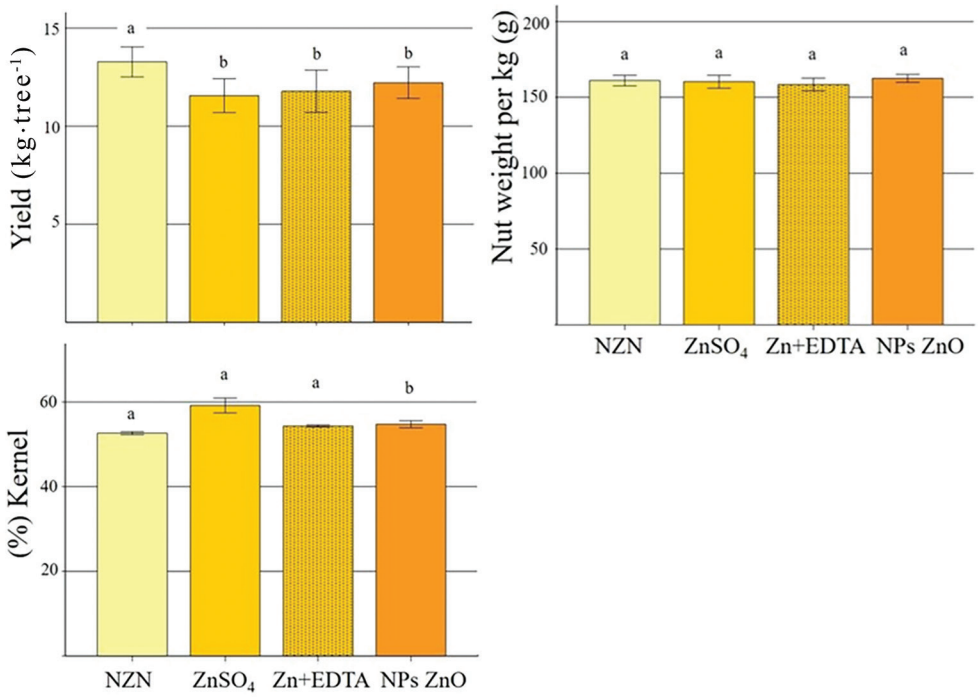

Figure 4. Yield, nut weight and kernel percentage in 'Western Schley' pecan trees with the application of sulphates, chelates and zinc nanoparticles. The data are means of treatments in 2019 and 2020. Bars with the same letter are not significantly different (Tukey's test, $p \leq 0.05$). Error bars represent standard deviations ($n = 10$). NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; ZnO NPs, zinc oxide nanoparticles; $ZnSO_4$, zinc sulphate.

exception of treatment with $ZnSO_4$ (Figure 4), the nuts harvested in this experiment correspond to Quality II, i.e. 140–170 nuts · kg^{-1} and 58% kernel weight.

Foliar nutrients

The data generated with the Zn spray and the plants' responses to the foliar nutrient application between growing seasons are shown in Table 3. Previous studies have shown the nutritional effects of N and Zn^{2+} on the establishment and commercial production of pecan nuts (Cruz-Álvarez et al., 2020; Smith et al., 2021). Nitrogen is an essential nutrient in the accumulation of biomass, including for the development of shoots and leaflets. Its major roles in trees include its association with chlorophyll biosynthesis and the production of sugars by photosynthesis (Moran-Duran et al., 2020). Similar foliar N concentrations are reported by Pond et al. (2006) when determining the levels of foliar nutrients in 'Western Schley' pecan from high-yield commercial orchards established in the USA (Arizona, New Mexico and Georgia) (25.5, 24.7 and 27.2 g · kg^{-1}) and in Sonora (Mexico) (24.8 g · kg^{-1}). The variations between the values are probably associated with the physicochemical properties of the soil and the agronomic managements between growing seasons. On the other hand, in our study, the Zn concentrations fluctuated between 35.81 mg · kg^{-1} and 48.46 mg · kg^{-1}, wherein NZN and $ZnSO_4$ showed the best results. These results can be considered within

Table 3. Nutrient concentrations in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles.

Treatments (200 mg · L^{-1})	Concentration (g · kg^{-1})									
	Total-N		Total-P		K^+		Ca^{2+}		Mg^{2+}	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
NZN (control)	27.9 a	23.9 b	2.7 a	2.0 b	19.7 a	17.6 a	33.66 a	28.32 b	4.8 a	4.1 b
$ZnSO_4$	26.3 a	27.3 a	2.1 a	1.7 b	19.24 a	20.3 a	31.62 a	33.10 a	3.9 a	4.3 a
Zn-EDTA	24.7 a	25.4 a	2.7 a	1.8 b	17.0 a	22.2 b	31.10 a	32.14 a	3.8 a	4.4 a
ZnO NPs	24.3 a	25.9 a	2.5 a	1.9 b	21.4 a	19.4 a	30.30 a	31.14 a	4.3 a	4.1 a
	Concentration (mg · kg^{-1})									
	Fe^{2+}		Cu^{2+}		Mn^{2+}		Zn^{2+}			
NZN (control)	139.4 a	181.9 b	6.3 a	5.8 b	231.0 a	212.4 b	48.5 a	45.3 a		
$ZnSO_4$	154.9 a	191.6 b	6.4 a	6.2 a	236.8 a	233.8 a	39.6 a	40.4 a		
Zn-EDTA	160.8 a	194.5 a	6.5 a	6.3 a	242.4 a	236.0 a	35.8 a	38.0 b		
ZnO NPs	118.3 a	179.4 b	6.2 a	6.6 b	227.8 a	241.0 b	39.3 a	35.8 b		

Means in the same row followed by the same letters were not significantly different (Tukey's test, $p \leq 0.05$).
ZnO NPs, zinc oxide nanoparticles; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; $ZnSO_4$, zinc sulphate.

Table 4. Oxidative metabolism and AC in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles.

Treatments (200 mg · L^{-1})	SOD	H_2O_2	CAT	GPx	AC
NZN (control)	1.4 a	0.4 a	3.5 a	3.2 b	83.1 a
$ZnSO_4$	1.3 ab	0.3 b	2.3 b	5.6 a	80.6 a
Zn-EDTA	1.2 c	0.4 b	2.3 b	5.2 a	76.9 b
ZnO NPs	1.3 b	0.3 b	2.4 b	5.3 a	82.6 a

Means in the same column followed by the same letters were not significantly different (Tukey's test, $p \leq 0.05$).
AC, antioxidant capacity (% of DPPH inhibition); CAT, catalase (nmol GSH · min^{-1} · g^{-1}); DPPH, 2,2-diphenyl-1-picrylhydrazyl; GPx, guaiacol peroxidase (µmol GSH · min^{-1} · g^{-1}); H_2O_2, hydrogen peroxide (µmol · g^{-1}); ZnO NPs, zinc oxide nanoparticles; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; SOD, superoxide dismutase (units · min^{-1} · g^{-1}); Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; $ZnSO_4$, zinc sulphate.

the sufficiency range (30–100 mg · kg^{-1}), as reported by Walworth and Heerema (2019). In contrast, Pond et al. (2006) report a foliar Zn sufficiency concentration of 54.6 mg · kg^{-1}; however, our trees did not show visible deficiency symptoms (internode shortening, chlorosis and interveinal necrosis).

In most established pecan orchards, soil P is a relatively immobile element, and banding has been used to rapidly ameliorate its deficiency (Smith, 2009). In our study, the Zn spray did not maintain the concentration of P in the leaflets between the years evaluated. Similar results were reported by Naira et al. (2013) with foliar applications of 0.5% Zn ($ZnSO_4$), urea + $ZnSO_4$ (0.5%+0.5%), boric acid + $ZnSO_4$ (0.1% + 0.5%) and the combination of these in 'Western Schley' nuts, with values of 1.6, 1.8 and 2.2 mg · kg^{-1}, respectively. In pecan nuts, the information generated for this element with foliar applications of Zn is scarce. However, Zn remained at sufficiency levels for pecan, according to Ojeda-Barrios et al. (2012), with values between 1.05 mg · kg^{-1} and 1.67 mg · kg^{-1}. On the other hand, with the exception of treatment with Zn-EDTA, no changes in K^+ levels were observed. However, in this experiment, no significant changes were observed in kernel percentage, a characteristic associated with the optimal supply of Zn in pecan nut. The results are consistent with those published for 'Western Schley' (Ojeda-Barrios et al., 2014) with foliar application of Zn-EDTA (0.76, 1.53 and 2.29 mM). In contrast, lower concentrations of 8.4, 9.1, 8.6 and 11.1 mg · kg^{-1} were reported by Naira et al. (2013) after spraying with $ZnSO_4$, urea + $ZnSO_4$, boric acid + $ZnSO_4$ and $ZnSO_4$ + amino acids, respectively.

Over the 2 years evaluated, Zn application did not affect the concentrations of Ca^{2+} (30.4–33.7 mg · kg^{-1}) or Mg^{2+} (3.8–4.8 mg · kg^{-1}), which – according to Pond et al. (2006) – are in the high and normal ranges, respectively. The leaflets did not present any necrosis related to high calcium concentrations. Mg^{2+} deficiency has been reported in acid and alkaline soils (Sparks, 1996). The soil characteristics were as follows: pH value: 7.8 and $CaCO_3$ content: 20%. These can interfere with the high concentration of Ca in the soil and can also affect Mg, as indicated by Walworth and Heerema (2019). Among the micronutrients, the concentrations of Fe^{2+}, Cu^{2+} or Mn^{2+} were not affected by Zn-EDTA; however, the levels of Cu and Mn were similar to those observed with $ZnSO_4$. Similar behaviour was observed for Cu and Mn in a previous study carried out by Ojeda-Barrios et al. (2014) with Zn-EDTA sprays (0.76, 1.53 and 2.29 mM) over 3 years with young trees (≈8 years old) of 'Western Schley'.

In general, Zn applications improve nut yield in pecan, but they have also been shown to increase the

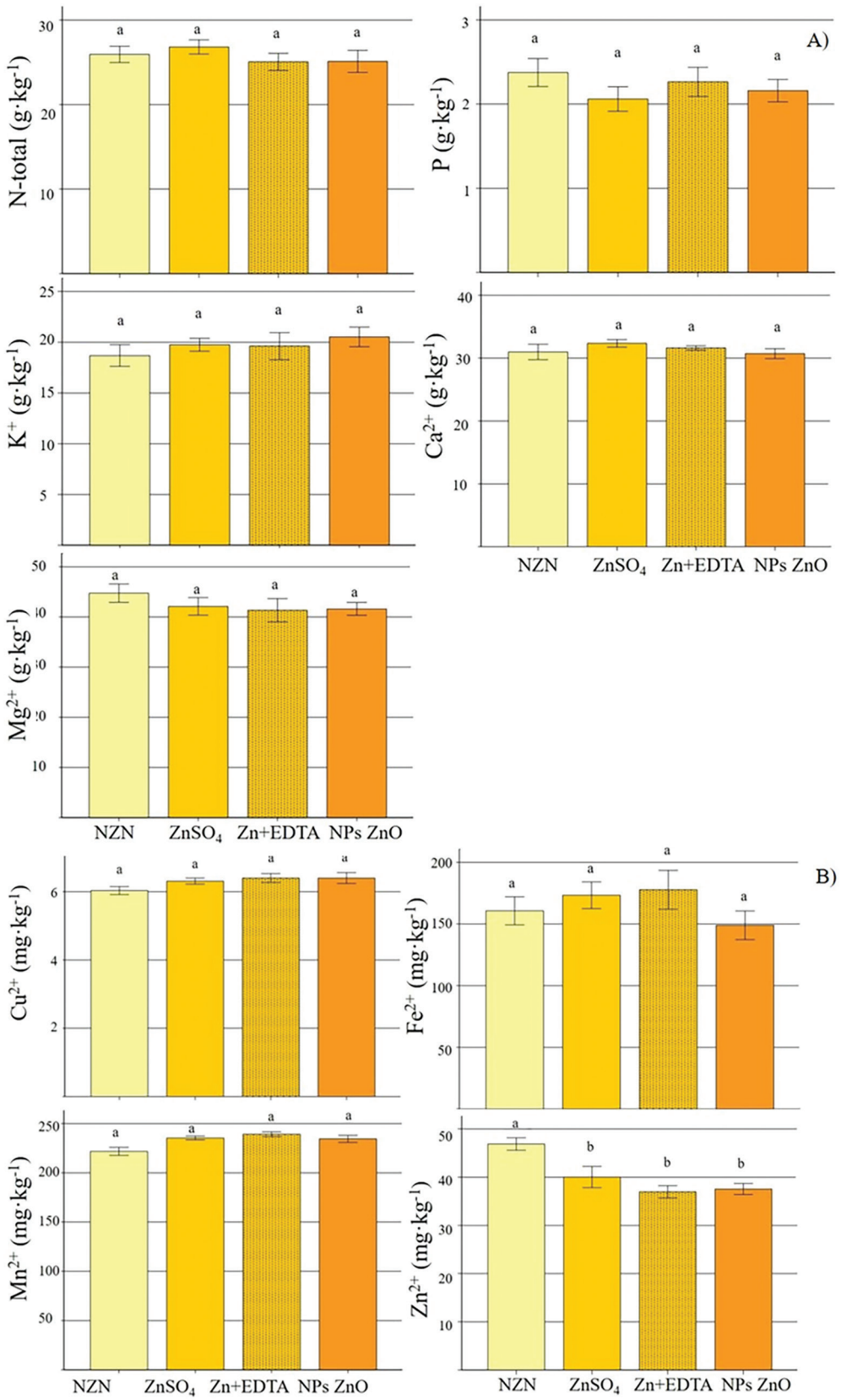

Figure 5. (A) Micronutrient concentrations in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles. Bars with the same letter are not significantly different (Tukey's test, $p \leq 0.05$). Error bars represent standard deviations ($n = 10$). NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; ZnO NPs, zinc oxide nanoparticles; $ZnSO_4$, zinc sulphate. (B) Micronutrient concentration in 'Western Schley' pecan trees with the application of sulphates, chelates and zinc nanoparticles. Bars with the same letter are not significantly different (Tukey's test, $p \leq 0.05$). Error bars represent standard deviations ($n = 10$). NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; ZnO NPs, zinc oxide nanoparticles; $ZnSO_4$, zinc sulphate.

occurrence of AB (Castillo-González et al., 2019). In our study, Zn applications (NZN, $ZnSO_4$, Zn-EDTA and ZnO NPs) maintained the concentrations of the essential elements with the exception of Zn^{2+}, whereby they did not exceed the control values (Figures 5A and 5B). A similar behaviour was observed by Naira et al. (2013) with foliar $ZnSO_4$ sprays, with maximum values of Zn between 195 mg · kg^{-1} and 294 mg · kg^{-1}, i.e. under toxic conditions according to Walworth and Heerema (2019). In this sense, cultivated plants can vary their nutrient composition and show optimum growth and development (Cruz-Crespo et al., 2014). Most pecan orchards are established in arid or semi-arid regions, and growers have managed Zn supply through multiple foliar Zn sprays, mainly $ZnSO_4$ and Zn-EDTA, with Zn-EDTA proving more efficient in alkaline soils (Walworth et al., 2017). Other authors such as Smith and Storey (1979) point out that $Zn(NO_3)_2$ sprays show better Zn absorption than $ZnSO_4$ for similar doses. In general, the other Zn sources, including NPs, show a similar behaviour. Results can be associated with the relatively high dose and number of applications made. However, foliar application of ZnO NPs at concentrations between 0.1 mg · L^{-1} and 150 mg · L^{-1} have been shown to improve the nutritional status of numerous crops including mango (*M. indica* L.) (Elsheery et al., 2020), pomegranate (*P. granatum* L.) (Davarpanah et al., 2016), coffee (*Coffea arabica* L.) (Rossi et al., 2019) and common bean (*P. vulgaris* L.) (Bautista-Díaz et al., 2021).

Oxidative metabolism

Plants under abiotic stress activate a defence system that includes antioxidant enzymes (peroxidases, SOD, catalase and ascorbate peroxidase) and antioxidant compounds (phenols, flavonoids, GSH and carotenoids, among others), which can inhibit or reduce the production and effects of reactive oxygen species (ROSs) (Teran-Erazo et al., 2019). These ROSs are associated with oxidative damage and cell death, and in pecan nut trees, they can influence AB by reducing growth and productivity (Ojeda-Barrios et al., 2022). Our study explored the behaviour of some antioxidant enzymes in the leaflets, including SOD, CAT and guaicol peroxidase (GPx) (Table 4). Zinc is a micronutrient that has stabilising properties and protective effects against oxidative and peroxidative damage, loss of integrity and alteration of membrane permeability (Subba et al., 2014). In general, Zn-EDTA spraying significantly reduced the activities of SOD, CAT and H_2O_2 production. However, $ZnSO_4$ and ZnO NPs showed similar values for CAT and H_2O_2. These results are likely linked to the activity of Zn as a cofactor for several enzymes, including dehydrogenases, oxidases and peroxidases (Balafrej et al., 2020).

Applications of 200 mg · L^{-1} Zn increased the activity of GPx (5.19–5.64 nmol GSH · min^{-1} · g^{-1}), which is associated with a reduction in peroxide production. An increase in GPx activity has been reported with edaphic applications of $Zn(NO_3)_2 \cdot 6H_2O$ (150, 300 and 600 μM) in *Chenopodium murale* L. (Zoufan et al., 2018). The role of Zn in the peroxidation of cell membrane lipids and the increase in activity of several antioxidant enzymes caused by generation of O^-_2 and H_2O_2, including GPx, have been reported (García-López et al., 2019). Similarly, this behaviour may be caused by the maintenance of the balance in the O^-_2 and H_2O_2 contents under Zn-stress conditions (Ibrahim and Ramadan, 2015).

Under abiotic stress, plant cells can increase their AC to minimise the negative effects of oxidative stress on physiological and biochemical processes (Davarpanah et al., 2016). In our study, the values of AC in the leaflets fluctuated between 76.9% and 83.1% of DPPH inhibition, the application of Zn-EDTA standing out among the treatments (Table 4). None of our trees showed symptoms of Zn deficiency, as was also corroborated by the sufficiency range measurements of their foliar Zn. However, in pecan tree, there is little information on Zn spraying and changes in the AC. Subba et al. (2014) indicate that Zn plays important roles in non-enzymatic antioxidant systems and is a micronutrient that helps stabilise the effects of oxidative and peroxidative stress, loss of cell integrity and permeability.

One of the most reliable indicators of stress in plants is associated with the synthesis and accumulation of photosynthetic pigments, where Zn actively participates in their biosynthesis (Subba et al., 2014). The concentrations of the photosynthetic pigments, chlorophyll and carotenoids, are presented in Table 5. With Zn-EDTA, decreased concentration of chlorophyll and total carotenoids was determined, while with $ZnSO_4$ and ZnO NPs, the concentration of chlorophyll and total carotenoids was reduced. Foliar Zn uptake depends on the Zn source. $ZnSO_4$ and $Zn(NO_3)_2$ are most common in pecan. Most of the studies on foliar application of ZnO NPs are for vegetables and cereals; there is little information for perennial plants, such as vines and fruit trees, especially not for pecan trees. Previous studies were conducted on the spraying of ZnO NPs as follows: 150 mg · L^{-1} in the common bean (*P. vulgaris* L.) (Bautista-Díaz et al., 2021); 50 mg · L^{-1} in broad bean (*Vicia faba* L.) (Ghidan et al., 2020); and 0.10 mg · mL^{-1} in carrot (*Daucus carota* L.) (Siddiqui et al., 2019). These experiments showed significant effects on the concentrations of chlorophyll and carotenoids.

Among the bioactive compounds that we evaluated in pecan leaflets, TP and TFl did not show significant variation due to Zn application (Table 5). This lack of response may be associated with the metabolic role of Zn, including synthesis of proteins, regulation of carbohydrate metabolism and the activation of some antioxidant enzymes that act against oxidative and peroxidative cell damage (Elsheery et al., 2020). In potato leaves (*Solanum tuberosum* L.), for 0.4% $ZnSO_4$ sprays before and after flowering, Korkmaz et al. (2018) found that there was a direct relationship between TP concentration and the increase in the Zn dose, but there was no effect of application time.

Table 5. Concentration of photosynthetic pigments and phenolic compounds in 'Western Schley' pecan tree leaflets sprayed with sulphates, chelates and zinc nanoparticles.

Treatments (200 mg · L^{-1})	TChl (μg · g^{-1})	TC (μg · g^{-1})	TFl (mg GAE · g^{-1})	TP (mg QE · g^{-1})
NZN (control)	45.1 a	14.7 a	55.7 a	18.4 a
$ZnSO_4$	45.5 a	10.5 b	53.7 a	17.1 a
Zn-EDTA	41.7 b	10.7 b	61.6 a	20.0 a
ZnO NPs	40.7 b	12.7 ab	63.6 a	22.0 a

Means in the same column followed by the same letters were not significantly different (Tukey's test, $p \leq 0.05$). Data are on the basis of FW. FW, fresh weight; GAE, gallic acid equivalents; NZN, nitrazinc, containing $Zn(NO_3)_2$ and urea–ammonium nitrate fertiliser; QE, quercetin equivalents; TC, total carotenoids; TChl, total chlorophyll; TFl, total flavonoids; TP, total phenols; Zn-EDTA, zinc ethylene diamine-tetra-acetic acid; ZnO NPs, zinc oxide nanoparticles; $ZnSO_4$, zinc sulphate.

CONCLUSIONS

For the growing seasons analysed, foliar sprays of $ZnSO_4$, Zn-EDTA and ZnO NPs helped maintain the foliar concentrations of NSCs, nutrients (total-N, Ca and Mg) and kernel percentages of the harvested nuts. Trees sprayed with $ZnSO_4$ better maintained leaflet Zn concentration. It would be worthwhile to determine the optimal Zn dose rates for the various pecan cultivars in common use and also to increase our understanding of the physiological and biochemical changes associated with foliar Zn applications. On the other hand, Zn-EDTA spraying significantly reduced the activities of SOD, CAT and H_2O_2 production. However, $ZnSO_4$ and ZnO NPs showed similar values for CAT and H_2O_2. In general, these results are valuable, considering the negative effects of AB in pecan.

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AUTHOR CONTRIBUTIONS

D.L.O.-B., O.C.-A., E.S.-C. and J.P.C.-L. designed the experiments and performed analytical measurements. D.L.O.-B. and J.P.C.-L. were involved in the collection and statistical analysis of data. D.L.O.-B. and O.C.-A. were involved in the writing and review of this paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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