Foliar Fertilization with Zinc in Pecan Trees

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Abstract. The objective of this study was to assess the changes in leaflet zinc (Zn), leaf nutritional state, vegetative and physiological parameters, and yield quality in pecan trees sprayed with different Zn compounds. Eight-year-old ‘Western Schley’ pecan trees grafted to native seedlings were treated with ZnNO₃ (100 mg·L⁻¹ Zn), Zn-EDTA (50, 100, and 150 mg·L⁻¹ Zn), and Zn-DTPA (100 mg·L⁻¹ Zn) and compared with the Zn-untreated control. After 3 years of evaluation, the trees with the best appearance were those treated with ZnNO₃ (100 mg·L⁻¹ Zn) and Zn-DTPA (100 mg·L⁻¹ Zn), which showed leaf Zn concentration increases of 73% and 69%, respectively, when compared with the controls. The chlorophyll values of the Zn-treated trees reached 46 SPAD units, equivalent to 43 mg·kg⁻¹ dry weight (DW) of chlorophyll compared with values of 22 mg·kg⁻¹ DW in Zn-deficient leaves. On a leaf area basis, chlorophyll value was 37% lower under Zn deficiency conditions than that of Zn-treated trees. Nut quality was unaffected by the Zn treatments. Data suggest that Zn-DTPA and Zn-NO₃ are good options to carry out foliar Zn fertilization in pecan trees.

Chihuahua is the state that has the greatest production of pecan (*Carya illinoensis*) in Mexico (Servicio de Información Agroalimentaria y Pesquera, 2012). Factors such as an inadequate management of pecan tree orchards demand relatively large amounts of the micronutrient Zn (Smith et al., 1980; Swietlik, 2002; Wood, 2007), and it is estimated that ≈30% of operational costs of pecan orchards is for nitrogen (N) and Zn fertilization (Secretaría de agricultura, ganadería, desarrollo rural, pesca y alimentación, 2008). In fact, Zn deficiency is common in commercial pecan tree orchards, frequently limiting productivity in Zn-poor soils (Fenn et al., 1990; Sparks and Payne, 1982). Some of the consequences of Zn deficiency in pecan include reductions in leaf chlorophyll concentrations and photosynthesis (Hu and Sparks, 1991), impairments in the development of reproductive structures (Hu and Sparks, 1990), decreased carbonic anhydrase

activity in leaves (Snir, 1983), and changes in leaf anatomical structure (Ojeda-Barrios et al., 2012). Pecan orchards affected by Zn deficiency are usually established in soils with low organic matter such as the alkaline, calcareous soils found in the southwestern United States and northern Mexico (Alben and Hammer, 1944; Favela et al., 2000; Núñez-Moreno et al., 2009b).

Management practices for Zn deficiency usually consist of frequent foliar applications using Zn-based products that may include either inorganic Zn salts or Zn chelates (Alben and Hammer, 1944; Favela et al., 2000; Ojeda-Barrios et al., 2009). Among chelates, Zn-EDTA and Zn-DTPA are recommended to use in foliar applications because of their good availability (Gangloff et al., 2006). Based on a limited number of published reports, it appears that Zn foliar sprays are generally effective in stimulating vegetative growth on fruit trees (Favela et al., 2000; Swietlik, 2002; Wadsworth, 1970). Mobility of applied Zn outside the treated area, however, is limited, and ≈89% to 95% of the Zn used in foliar applications to pistachio (*Pistacia vera*) and pea (*Pisum sativum*) was still in the leaf 10 d after foliar treatment (Ferrandon and Chamel, 1988; Zhang and Brown, 1999).

There is still no consensus on what product is most effective to supply Zn-deficient trees. The objectives of the present research

were to evaluate the effectiveness of two Zn chelates, Zn-ethylenediamine tetraacetic acid (Zn-EDTA) and Zn-diethylenetriamine pentaacetic acid (Zn-DTPA) in addition to a commercial product (NZN, nitrazinc) containing the inorganic salt ZnNO₃, to increase leaf Zn in an attempt to improve nut yield and quality in pecan trees as well as to evaluate changes in vegetative and physiological parameters caused by Zn fertilization.

Materials and Methods

Location. The study was conducted during three consecutive growing seasons (2007, 2008, and 2009) in a pecan tree orchard located near the town of Aldam, in the eastern side of the state of Chihuahua, Mexico (lat. 28°50' N, long. 105°53' W, altitude 1262 m), where the climatic and pedological conditions are representative of one of the main pecan-producing regions in the north of the country. The region is arid with 337 mm of annual mean precipitation and a mean annual temperature of 18.6 °C (García, 1973). The orchard was on a calcareous soil (Domino silt loam, Xerollic Calciorthid), has an arable layer of 0 to 35 cm with a pH of 7.2 in 1:1 soil:water, 1.1% organic matter, 30.0% total CaCO₃, 10% active CaCO₃ by the Droineau method (considered high; Duchaufour, 1987), 8.8 mg·kg⁻¹ NO₃⁻, and 0.44 mg·kg⁻¹ DTPA-extractable Zn (Rivera-Ortiz et al., 2003). The orchard consisted of 8-year-old pecan (70 trees/ha). Trees used in the study had not previously received any Zn treatment. The trees were soil-fertilized on 10 Apr. each year with granular fertilizer (120N–183P₂O₅–96K₂O). After fertilization, the soil was immediately plowed and the trees were irrigated. The irrigation system was by gravity feed at ≈20-d intervals with a total application of 120 to 140 mm of water from late March to the end of October as a result of the low occurrence of pests and diseases.

Tree measurements. Trunk circumference was measured 20 cm above ground level once a year with an elastic tape measure, and trunk cross-sectional area (TCSA, in cm²/tree) was used to estimate annual growth (total annual increase). To assess the tree nutritional status, in 2006 leaves were sampled from 30 different trees showing Zn deficiency symptoms and analyzed for mineral concentrations.

Treatments. Six different treatments were applied with a randomized block experimental design and five single-tree replicates per treatment (30 trees in total). The chelating agents used were EDTA and DTPA (sodium salts) and reactive-grade ZnSO₄·7H₂O was added to make the Zn-chelates Zn(II)-EDTA and Zn(II)-DTPA. The solutions were 0 mg·L⁻¹ Zn; 100 mg·L⁻¹ Zn (1.53 mM) as the patented product NZN [nitrazinc, containing Zn(NO₃)₂ and urea-ammonium nitrate fertilizer (Smith and Storey, 1979); Tessenderlo K, Inc., Phoenix, AZ]; 50, 100, and 150 mg·L⁻¹ Zn (0.76, 1.53, and 2.29 mM Zn, respectively) as Zn-EDTA; and 100 mg·L⁻¹ Zn (1.53 mM) as Zn-DTPA. In all formulations, including the control treatment, 0.1% urea was added as

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transporter ion and 100 mg·L⁻¹ Tween 20 (Thermo Scientific, IL) was used as a surfactant. The pH of the solutions was adjusted with sulfuric acid to 6.5, a pH that is thought to facilitate foliar uptake of metal formulations (Fernández et al., 2006). Zinc was applied using a 25-L motorized backpack fertilizer applicator six times per season,

Table 1. Dates of foliar zinc applications during the 3 years of the experiment.^z

Foliar application date		
2007	2008	2009
9 Apr.	11 Apr.	4 Apr.
17 Apr.	17 Apr.	11 Apr.
24 Apr.	26 Apr.	25 Apr.
3 May	3 May	3 May
17 May	17 May	17 May
4 June	6 June	6 June

^zApplications were done on 8-year-old pecan trees grown in Aldama, Chihuahua, Mexico.

starting on 30 Mar., 2 Apr., and 29 Mar. in 2007, 2008, and 2009, respectively (Table 1). In each application, the solution was sprayed to full foliage wetting (≈10, 15, and 18 L/tree in 2007, 2008, and 2009, respectively) between 0700 and 0900 HR. Foliar application was carried out with formulations containing 50, 100, and 150 mg·L⁻¹ Zn (0.76, 1.53, and 2.29 mM Zn, respectively), equivalent (considering a fertilizer formulation volume of 10, 15, and 18 L/tree and application in 2007, 2008, and 2009 and six applications each year) to doses in the ranges from 3.0 to 9.0, from 4.5 to 13.5 and from 5.4 to 16.2 g/tree Zn, respectively. The Zn doses applied were relatively low when compared with other studies (e.g., Smith and Storey, 1979) because of the young age of the trees used in this study.

Leaf sampling and analysis. Approximately 40 pairs of leaflets were collected five times during the growing season (specific dates and relative phenological stages are

shown in Table 2) from the midcanopy of each tree. Leaflets were collected by pooling samples from the four cardinal directions and from both vegetative and fruiting shoots. Leaflets were then washed in a 0.1% detergent (Mistol; Henkel, Barcelona, Spain) solution, then in running tap water, 1% HCl, and three separate 7-L demineralized water baths, as indicated by Smith and Storey (1979). Leaves were then oven-dried at 55 °C to a constant weight, ground, and stored in airtight containers until analysis. Samples (500 mg) were ashed at 500 °C and dissolved in HNO₃ and HCl following the procedure given by the Association of Official Analytical Chemists (AOAC International and Latimer, 2012). Calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and Zn were determined by flame atomic absorption spectrometry, potassium (K) by flame emission spectroscopy, and phosphorus spectro-photometrically by the molybdate–vanadate method. Nitrogen concentration was determined by the micro-Kjeldahl method in separate samples (Bremner, 1965).

Leaflet area and SPAD chlorophyll indices were determined in all 40 leaflets per tree. SPAD was measured using a SPAD 502 m (Konica Minolta Sensing America Inc., Ramsey, NJ) in the middle part of each leaflet (avoiding major veins). This device assesses chlorophyll levels by comparing the optical density differences at two wavelengths (650 and 940 nm). Average leaflet area was calculated after scanning all leaflets with a flatbed scanner and processing the digital images with image-processing software (Scion Image; Scion Corp., Frederick, MD).

All data were subjected to analysis of variance using a complete randomized block design with six treatments and five replications. Differences among treatment means were found using least significant difference

Table 2. Leaf sampling times and the corresponding phenological stages during the 3 years of the experiment.^z

Leaf sampling times and phenological stages	Yr		
	2007	2008	2009
L1 End of female flower receptivity	10 May (43 DAB)	10 May (43 DAB)	10 May (44 DAB)
L2 Beginning of fruit set	24 May (56 DAB)	24 May (55 DAB)	24 May (57 DAB)
L3 Fruit growth	1 June (65 DAB)	1 June (65 DAB)	1 June (65 DAB)
L4 Brown tip, beginning of water stage in nut	30 June (94 DAB)	30 June (94 DAB)	30 June (94 DAB)
L5 Water stage of the nut	19 July (113 DAB)	16 July (110 DAB)	13 July (107 DAB)

^zApplications were done on 8-year-old ‘Western’ pecan trees grown in Aldama, Chihuahua, Mexico. DAB = days after budbreak, defined as the date when 80% of the tree buds were open.

Table 3. Effects of the zinc (Zn) applications on foliar Zn concentrations (mg·kg⁻¹) during the growing season in ‘Western’ pecan trees grown in Aldama, Chihuahua, Mexico.^z

Yr	Treatment	Leaf sampling time				
		L1	L2	L3	L4	L5
2007	Control, 0 mM Zn	5.6 c	7.4 c	7.5 c	5.5 c	5.7 c
	1.53 mM ZnNO ₃	7.3 b	8.0 abc	9.0 a	7.7 b	9.9 b
	0.76 mM Zn-EDTA	8.3 ab	8.2 ab	8.0 bc	6.5 bc	10.1 a
	1.53 mM Zn-EDTA	7.7 b	7.7 bc	7.6 bc	7.9 b	13.3 a
	2.29 mM Zn-EDTA	7.7 b	8.4 ab	8.4 ab	10.1 a	9.8 b
	1.53 mM Zn-DTPA	9.2 a	8.5 a	7.9 bc	11.5 a	10.1 ab
2008	Control, 0 mM Zn	17.2 c	14.8 c	12.1 b	13.5 b	14.0 b
	1.53 mM ZnNO ₃	41.4 ab	22.9 bc	28.1 b	20.0 ab	24.4 ab
	0.76 mM Zn-EDTA	26.0 bc	28.1 a	32.2 b	19.2 ab	22.8 ab
	1.53 mM Zn-EDTA	29.4 bc	23.8 bc	21.6 b	20.8 ab	20.0 b
	2.29 mM Zn-EDTA	29.3 bc	24.4 bc	19.1 b	20.1 ab	21.7 b
	1.53 mM Zn-DTPA	55.1 a	58.1 a	46.0 a	29.6 a	36.1 a
2009	Control, 0 mM Zn	13.1 c	16.5 c	13.3 c	20.1 b	20.6 b
	1.53 mM ZnNO ₃	38.4 a	39.2 a	34.2 a	44.5 a	35.7 a
	0.76 mM Zn-EDTA	31.3 ab	27.8 abc	30.9 ab	38.7 a	31.7 ab
	1.53 mM Zn-EDTA	25.9 abc	34.0 ab	24.3 b	45.9 a	28.5 ab
	2.29 mM Zn-EDTA	18.7 bc	25.0 bc	21.4 bc	35.2 a	26.8 ab
	1.53 mM Zn-DTPA	30.0 ab	24.1 bc	21.6 bc	35.1 a	34.9 a

^zDates of treatments and tree developmental stages at sampling are indicated in Tables 1 and 2, respectively. In each year, treatment means within leaflet sampling time followed by different letters are statistically different according to the least significant difference test at 95%. Zinc foliar applications were carried out with 0.76, 1.53, and 2.29 mM Zn (50, 100, and 150 mg·L⁻¹ Zn), equivalent (considering a fertilizer formulation volume of 10, 15, and 18 L/tree in 2007, 2008, and 2009) to doses in the ranges from 3.0 to 9.0, from 4.5 to 13.5 and from 5.4 to 16.2 g/tree of Zn, respectively.

Table 4. Effect of zinc (Zn) foliar applications on leaf mineral composition (at the L5 stage, water stage of the nut) in pecan trees grown in Aldama, Chihuahua, Mexico.

Yr	Treatment	Macronutrients (g·kg ⁻¹)				Micronutrients (mg·kg ⁻¹)		
		Nitrogen	Potassium	Calcium	Magnesium	Iron	Copper	Manganese
2007	Control, 0 µM Zn	2.20 c ^z	1.08 b	0.35 b	0.64 c	40.0 b	2.4 c	78.7 b
	1.53 mM ZnNO ₃	2.48 bc	1.69 ab	0.70 a	1.26 a	41.1 b	5.6 a	78.9 b
	0.76 mM Zn-EDTA	2.89 a	1.82 ab	0.57 a	1.27 a	41.7 b	5.2 ab	78.8 b
	1.53 mM Zn-EDTA	2.54 b	1.86 a	0.54 ab	1.30 a	54.0 a	4.2 b	119.8 b
	2.29 mM Zn-EDTA	2.68 ab	1.74 ab	0.65 a	0.98 b	54.6 a	5.2 ab	185.8 a
	1.53 mM Zn-DTPA	2.44 bc	1.57 b	0.70 a	1.16 ab	49.2 ab	4.9 ab	121.7 b
2008	Control, 0 µM Zn	2.48 c	0.88 c	1.06 c	0.61 b	55.3 b	3.9 c	130.6 c
	1.53 mM ZnNO ₃	2.68 ab	1.06 ab	1.18 b	0.66 ab	74.4 a	5.0 b	192.3 b
	0.76 mM Zn-EDTA	2.74 a	1.01 b	1.18 ab	0.67 a	73.4 a	6.1 a	170.3 bc
	1.53 mM Zn-EDTA	2.72 a	1.12 a	1.25 ab	0.67 a	78.0 a	5.5 ab	271.5 a
	2.29 mM Zn-EDTA	2.67 ab	1.10 a	1.25 a	0.69 a	77.1 a	5.8 ab	189.3 bc
	1.53 mM Zn-DTPA	2.61 b	1.05 ab	1.20 ab	0.67 a	74.4 a	5.1 b	178.6 bc
2009	Control, 0 µM Zn	2.39 b	1.00 b	1.17 b	0.68 c	45.1 c	4.3 b	143.8 b
	1.53 mM ZnNO ₃	2.57 a	1.16 a	1.31 a	0.74 ab	70.3 ab	5.9 a	217.4 a
	0.76 mM Zn-EDTA	2.53 ab	1.12 a	1.36 a	0.76 a	73.0 a	5.6 a	217.6 a
	1.53 mM Zn-EDTA	2.65 a	1.12 a	1.36 a	0.73 ab	70.3 ab	6.0a	240.6 a
	2.29 mM Zn-EDTA	2.56 ab	1.11 a	1.35 a	0.73 b	60.9 b	6.2 a	189.9 ab
	1.53 mM Zn-DTPA	2.61 a	1.15 a	1.35 a	0.73 ab	61.2 b	5.9 a	226.0 a

^zIn each year, treatment means within each column followed by different letters are statistically different according to the least significant difference test at 95%.

test (at 95% confidence) with SAS software (SAS Institute Inc., Cary, NC).

Results and Discussion

Zinc concentration in leaves sampled from the trees showing deficiency symptoms was 7.5 mg·kg⁻¹ Zn, which is considered to be within the deficiency range in pecan trees (Medina, 2004). Leaflets of Zn-deficient trees exhibited “rosette”-like Zn deficiency symptoms, typical for leaf Zn levels below 11 mg·kg⁻¹ Zn (Ojeda-Barrios et al., 2012; Walworth and Pond, 2006).

In the first leaf sampling of 2007, when Zn-treated trees had already received four foliar applications, the average Zn concentration in the Zn-treated trees was 8.0 mg·kg⁻¹ Zn, i.e., 43% higher than that in the untreated control (5.6 mg·kg⁻¹ Zn; Table 3). However, all leaf Zn concentrations recorded during 2007 (both in Zn-treated and untreated trees) still indicated a severe Zn deficiency, with values being always less than 14 mg·kg⁻¹ Zn. These low leaf Zn concentrations were similar to those reported in pecan trees by previous studies conducted in the same area (Núñez-Moreno et al., 2009b; Ojeda-Barrios et al., 2009, 2012). The optimal critical levels of Zn in pecan trees have been established in the range of 20 to 60 mg·kg⁻¹ Zn (Favela et al., 2000; Núñez-Moreno et al., 2009a; Ojeda-Barrios et al., 2012). In the last sampling of 2007 (19 July), the average Zn concentration in the treated trees was 10.6 mg·kg⁻¹ Zn, i.e., 87% higher than that in the untreated controls (5.7 mg·kg⁻¹ Zn).

In 2008 and 2009, the leaf Zn concentrations of the untreated control trees were higher than those found in 2007 but still lower than the deficiency threshold with values 17.2 or less and 20.6 mg·kg⁻¹ or less Zn in 2008 and 2009, respectively (Table 3). In the last samplings of 2008 and 2009 (mid-July), the average Zn concentrations in the treated trees

Table 5. Effect of zinc (Zn) applications on trunk cross-sectional area, leaflet area, and leaflet SPAD index in pecan trees grown in Aldama, Chihuahua, Mexico.^z

	2007	2008	2009	Total increase (%) 2007–09
<i>Trunk cross-sectional area (cm²/tree)</i>				
Control, 0 µM Zn	159.0 a ^y	202.7 a	250.7 a	91.7
1.53 mM ZnNO ₃	124.4 a	164.0 a	216.5 a	92.1
0.76 mM Zn-EDTA	145.2 a	198.7 a	251.6 a	106.4
1.53 mM Zn-EDTA	129.5 a	192.4 a	245.4 a	116.0
2.29 mM Zn-EDTA	138.5 a	188.6 a	247.2 a	108.6
1.53 mM Zn-DTPA	139.9 a	190.1 a	241.9 a	102.1
<i>Leaflet area (cm²)</i>				
Control, 0 µM Zn	20.9 d	18.0 b	16.5 b	18.5
1.53 mM ZnNO ₃	27.8 bc	24.3 a	28.8 a	27.0
0.76 mM Zn-EDTA	25.2 c	26.7 a	29.5 a	27.1
1.53 mM Zn-EDTA	31.0 ab	24.4 a	25.6 a	27.0
2.29 mM Zn-EDTA	30.0 ab	28.0 a	27.3 a	28.4
1.53 mM Zn-DTPA	34.1 a	26.9 a	27.3 a	29.4
<i>Leaflet SPAD units</i>				
Control, 0 µM Zn	35.7 c	39.8 b	41.4 b	39.0
1.53 mM ZnNO ₃	40.9 b	45.9 b	47.2 a	44.7
0.76 mM Zn-EDTA	42.6 ab	49.3 ab	47.0 a	46.3
1.53 mM Zn-EDTA	44.8 a	46.3 b	47.8 a	46.3
2.29 mM Zn-EDTA	42.0 ab	46.6 b	47.3 a	45.3
1.53 mM Zn-DTPA	40.6 b	50.0 a	47.4 a	46.0

^zTrunk cross-sectional area was measured at the beginning of the season and the rest of parameters at the M5 stage (water stage of the nut).

^yTreatment means within each column followed by a different letter are statistically different according to the least significant difference test at 95%.

were 25.0 and 31.5 mg·kg⁻¹ Zn, i.e., 79% and 53% higher than those in the untreated controls (14.0 and 20.6 mg·kg⁻¹ Zn in 2008 and 2009, respectively). At the final July 2009 sampling, both 1.5 mM ZnNO₃ and Zn-DTPA had induced the largest increase (73% and 69%, respectively) in leaflet Zn concentration when compared with the controls. However, no significant differences in leaf Zn concentrations among the five Zn treatments were found at this stage (Table 3). Favela et al. (2000) also reported that Zn applications increased Zn concentrations by 67% over the control values in 22-year-old pecan trees.

Leaf concentration of most macro- and micronutrients were affected by Zn foliar fertilization (Table 4). Large changes in macronutrient concentrations in the Zn-treated trees (considering the averages of the five Zn treatments) occurred in the first year with increases of 18%, 60%, 81%, and 87% over the control values for N, K, Ca, and Mg, respectively. In the second and third years of the experiment, concentration changes also occurred but they were generally more moderate, in the range from 8% to 34%, when compared with the control values. In the case of the micronutrients Fe and Mn, average concentration increases in the treated trees

were also found when compared with the controls in the 3 years of the study (from 20% to 49% for Fe and from 49% to 53% for Mn). In the case of Cu, a large increase (109%) was found the first year, but the increase was more moderate (38% to 41%) in 2008 and 2009. Overall, mean values of foliar concentrations of N and K were within the sufficiency interval; those of Mg and Mn were high and those of Ca, Fe, and Cu were relatively low [according to the reference values reported in Medina (2004)].

The foliar applications of Zn during the 3 years evaluated showed no significant effects on TCSA (Table 5). Increases in TCSA were found in mandarin trees (*Citrus reticulata*) after application of foliar Zn (Srivastava and Singh, 2009) and also in 'Wichita' pecan trees after Zn soil fertilization (Núñez-Moreno et al., 2009b).

There was a good correlation between the SPAD readings and chlorophyll concentration at the L5 stage, water stage of the nut (Fig. 1). All Zn treatments increased total leaf area when compared with the untreated controls in the 3 years of the experiment. Average increases were $\approx 42\%$, 45% , and 68% in 2007, 2008, and 2009, respectively (Table 5). There was a significant correlation between leaflet area and leaflet Zn concentration at L5 stage, water stage of the nut, in each of the 3 years of the experiment (Fig. 2). However, the leaflet area was similar in all years, whereas the Zn concentration ranges increased progressively (Fig. 2).

Zinc treatments generally led to an increase in leaflet SPAD values, which was significant in all treatments in 2007 and 2009 and only in leaflets treated with Zn-DTPA in 2008 (Table 5). Average increases in chlorophyll concentration after Zn fertilization were 18%, 20%, and 14% in 2007, 2008, and 2009, respectively. The SPAD values found in this study were slightly higher than what previously found in pecan tree in June in the same area (Ojeda-Barrios et al., 2012) but similar to those reported for 'Wichita' pecan trees in the southeast United States (Núñez-Moreno et al., 2009b). There was also a significant correlation between SPAD readings and foliar Zn concentration at the L5 stage, water stage of the nut, in each of the 3 years of the experiment (Fig. 3).

In conclusion, in the present 3-year study, all the foliar Zn treatments generally resulted in significant increases in leaflet Zn concentrations, leaflet area, and leaf chlorophyll concentration in pecan trees. The concentration of other nutrients was also affected. However, the sustained Zn input (six treatments per year during 3 years) was unable to change the TCSA (Table 5), nut yield, and nut quality parameters, including percent kernel and size (results not shown). Although no whole-tree data are available regarding the Zn requirement in pecan, a recent study has shown that mature trees of a different fruit tree species, peach (*Prunus persica* L.), need ≈ 1 g/year of Zn, which takes into account Zn losses and Zn immobilized in permanent structures (El Jendoubi et al., 2013). Results

Fig. 1. Correlation between SPAD readings and chlorophyll concentrations at the L5 stage, water stage of the nut, in 2009 measured by extracting leaflets with 100% acetone. $y = 0.276x + 34.972$, $R^2 = 0.9162$, $P < 0.001$ ($n = 58$).

Fig. 2. Correlation between leaflet area and leaflet zinc (Zn) concentration at the L5 stage, water stage of the nut, during the 3 years of the experiment. Solid line (2007): $y = -0.157x^2 + 4.289x + 1.912$, $R^2 = 0.53$, $P < 0.001$ ($n = 30$); dashed line (2008): $y = -0.035x^2 + 2.133x - 4.186$, $R^2 = 0.660$, $P < 0.001$ ($n = 30$); dotted line (2009): $y = -0.067x^2 + 4.460x - 45.492$, $R^2 = 0.748$, $P < 0.001$ ($n = 30$).

show that leaf Zn was generally increased with tree age, even in the $0\text{-}\mu\text{M}$ Zn control treatment (Table 3), and also that SPAD values and leaf areas were similar among years under different total leaf Zn concentrations (Figs. 2 and 3, respectively). The reason for these findings is still not known, but it is likely to reside in changes in the allocation of Zn in the leaf tissues over time. Very little is still known about Zn fluxes between foliage and permanent tree structures in spring (and fall) in this species, but one can hypothesize that in these trees, part of the Zn rapidly remobilized in spring from permanent tree structures can be stored in leaves in pools that are not physiologically functional (e.g., in the apoplast, cell wall, or vacuole). Because Zn is an element that could exert a certain degree of toxicity, it is likely that when a sudden flush of Zn occurs, homeostasis mechanisms aimed to control Zn concentrations in the cytoplasm could exist. The fact that Zn in

leaves appears to be located mainly in the palisade and spongy parenchyma of mesophyll cells (Ojeda-Barrios et al., 2012) supports that the vacuole may be a candidate for Zn storage. Further studies are needed to answer this question. Results found in this study indicate that the effectiveness of Zn foliar applications (with total Zn doses up to 16 g Zn/tree in the 3-year period) is quite low, probably associated with a limited translocation to the leaf mesophyll, and supports the need for further research to understand and improve the efficiency of Zn fertilizers. Because in the present study the maximum Zn concentration was 2.29 mM , further experiments should envisage using higher Zn concentrations such as those used in older trees (up to 13 to 16 mM) by Smith and Storey (1979). According to Smith et al. (1979) ZnNO_3 is the best option to fertilize with Zn; however, our study suggests that there is no particular Zn chemical form among those

Fig. 3. Correlation between leaflet SPAD readings and leaflet zinc (Zn) concentrations at the L5 stage, water stage of the nut, during the 3 years of the experiment. Solid line (2007): $y = -0.063x^2 + 2.335x + 24.515$, $R^2 = 0.84$, $P < 0.001$ ($n = 30$); dashed line (2008): $y = -0.027x^2 + 1.805x + 20.454$, $R^2 = 0.802$, $P < 0.001$ ($n = 30$); dotted line (2009): $y = -0.056x^2 + 3.536x - 9.608$, $R^2 = 0.794$, $P < 0.001$ ($n = 30$).

tested (ZnNO₃, Zn-EDTA, and Zn-DTPA) that provides significant advantages for pecan nut growers, indicating that product cost could be a decisive factor.

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