Does Foliar Zinc Application Boost Leaf Photosynthesis of ‘Wichita’ Pecan Fertigated with Zinc-EDTA?

Cyrus A. Smith, James L. Walworth, and Mary J. Comeau

Department of Environmental Science, University of Arizona, 429 Shantz Building #38, Tucson, AZ 85721

Richard J. Heerema

Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces, NM 88003

Joshua D. Sherman

Arizona Cooperative Extension, University of Arizona, Willcox, AZ 85643

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Abstract. Many growers fertigating their orchards with zinc–ethylenediaminetetraacetic acid (Zn-EDTA) are still using supplemental zinc foliar sprays because of a lack of confidence that soil-applied Zn-EDTA is supplying enough Zn to the trees. A field study was conducted in a pecan orchard located near San Simon, AZ, on 8-year-old ‘Wichita’ trees growing in an alkaline, calcareous Vekol loam soil to evaluate the effectiveness of supplemental foliar Zn sprays. All trees were fertigated with 6.0 kg·ha⁻¹ Zn in the form of Zn-EDTA in 2018 and 11.0 kg·ha⁻¹ Zn in 2019 and did not exhibit visible signs of Zn deficiency. Foliar treatments of 3.75 mL·L⁻¹ urea–ammonium nitrate (UAN), 3.6 g·L⁻¹ zinc sulfate monohydrate (ZnSO₄·H₂O), 3.6 g·L⁻¹ ZnSO₄·H₂O with 3.75 mL·L⁻¹ UAN, 11 mL·L⁻¹ Zn-EDTA, and water alone were applied to individual fruiting shoot terminals of trees on two dates each in 2018 and 2019. Treatments were sprayed directly onto the leaves of the selected terminals. Zn-EDTA was included as a foliar treatment in 2019 only. Leaf photosynthesis was measured to determine the impact of leaf Zn concentrations on plant function. Midday stem water potential (MDSWP) was measured to verify that water stress was not limiting photosynthesis. Both measurements were taken about 2 to 4 weeks after the application of foliar treatments. MDSWP measurements indicated a lack of water stress and therefore no effect on photosynthesis. Leaf samples collected from untreated branches indicated that the average foliar Zn concentration of untreated leaves was 21.3 mg·kg⁻¹ in 2018 and 15.7 mg·kg⁻¹ in 2019. No differences were observed in photosynthesis rates of treated branches. No additional benefit to leaf photosynthetic function or appearance was observed from spraying Zn on foliage of trees fertigated with Zn-EDTA.

Pecans [*Carya illinoensis* (Wangenh.) K. Koch] require more Zn than many crops and can tolerate applications of Zn that would cause toxicity in other plants (Worley, 2002). High-pH calcareous soils are common in the semiarid southwestern United States. In these alkaline soils, Zn binds with hydroxyls and carbonates to form low-solubility compounds, making it less available to plants (Udo et al., 1970). Lack of Zn availability frequently leads to Zn deficiency in desert-grown pecans.

Low concentrations of leaf chlorophyll are one result of Zn deficiency. Hu and Sparks (1991) found that leaf chlorophyll content was

lower in leaves containing less than 14 mg·kg⁻¹ Zn. Zn deficiency can also shorten palisade cells, increase intercellular space, and reduce leaf thickness and surface area (Ojeda-Barrios et al., 2012). Hu and Sparks (1991) noted that stomatal conductance (*g_s*) and net photosynthesis (*P_n*) were reduced concomitantly by low Zn levels. Heerema et al. (2017) determined a threshold leaf Zn concentration between 14 and 22 mg·kg⁻¹, below which *P_n* declined and above which *P_n* did not increase, supporting the findings of Hu and Sparks (1991). Measurements taken in June and July showed leaf Zn concentration thresholds on the upper end of this spectrum, whereas measurements acquired in August showed leaf Zn concentration thresholds on the lower end. Zn concentrations close to or less than 14 mg·kg⁻¹ prevent normal fruit production on the supporting shoot (Hu and Sparks, 1990).

For field production, the minimum leaf Zn concentration recommended to avoid loss of yield or nut quality, reduction in vegetative

growth, and visible symptoms of Zn deficiency is usually reported to be at least 40 to 60 mg·kg⁻¹ (Heerema, 2013; Robinson et al., 1997; Smith et al., 2012; Sparks, 1993; Sparks and Payne, 1982). Zn-EDTA applied to the soil through fertigation over a 5-year period at rates of 2.2 and 4.4 kg·ha⁻¹ Zn largely eliminated foliar Zn deficiency symptoms and increased rates of *P_n* (Heerema et al., 2017; Walworth et al., 2017), but these treatments were not sufficient to attain the recommended minimum concentrations. The greatest leaf Zn concentrations obtained during this study were 35 mg·kg⁻¹, but *P_n* showed no significant increase when foliar Zn concentrations exceeded ≈22 mg·kg⁻¹, indicating that *P_n* is not Zn limited beyond this point.

Foliar application of Zn is a common practice to alleviate Zn deficiency. However, managing Zn with foliar applications is costly and time intensive, and foliar-applied Zn is poorly distributed within the plant (Wadsworth, 1970). Although foliar application is effective for increasing leaf tissue Zn concentrations, only a small fraction of applied Zn is actually absorbed. Wadsworth (1970) indicated that only 0.6% to 1.2% of Zn applied to immature pecan leaves was absorbed. In walnuts Brown et al. (1995) found that ≈2% to 4% Zn was absorbed by mature leaves, whereas more than 8% was absorbed by immature leaves. Foliar Zn absorption may be dependent on the form of Zn applied. Frequently used spray materials include ZnSO₄ and zinc nitrate [Zn(NO₃)₂]. In Zn spray-tank mixes that contain nitrate, the nitrate ion aids in the uptake of Zn (Worley, 2002). Urea has been reported to enhance the penetration of nutrients into foliar tissues (Hsu and Ashmead, 1984). Storey (1977) observed that foliar absorption of Zn from either ZnSO₄ or Zn(NO₃)₂ sprays was enhanced by including UAN in the spray mixture. In other crops, a mixture of manganese sulfate, ZnSO₄, and iron sulfate salts were applied to soybean, fava bean, pea, and wheat with and without the addition of 1% urea. In all cases, the addition of urea enhanced the uptake of these metals (El-Fouly et al., 1990).

Ferrandon and Chamel (1988) found that the cuticular sorption of Zn was significantly greater when Zn was applied to the cuticles of tomato leaves in the inorganic forms of ZnSO₄ or zinc chloride than in the organic Zn-EDTA form. Cuticular sorption of Zn-EDTA was ≈5 nM·cm⁻² after 72 h vs. ≈41 nM·cm⁻² for ZnSO₄. In pea plants, Zn uptake rates were ≈1.45 times greater when Zn was applied as ZnSO₄ vs. Zn-EDTA. More of the Zn applied in the form of Zn-EDTA was translocated away from the point of foliar contact than that applied as ZnSO₄ (Ferrandon and Chamel, 1988). Brown et al. (1995) found that walnut tree leaves sprayed with Zn-EDTA did not contain significantly more Zn than leaves sprayed with ZnSO₄. However, Zn concentrations of unsprayed leaves on branches adjacent to those sprayed with Zn-EDTA were

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J.L.W. is the corresponding author. E-mail: walworth@ag.arizona.edu.

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increased significantly compared with the control, whereas leaves on branches adjacent to ZnSO₄ treatments were not, suggesting greater mobility of Zn-EDTA within the tree than ZnSO₄. In pecans, foliar applications of 50, 100, and 150 mg·L⁻¹ Zn-EDTA resulted in an increase in leaf Zn concentrations, chlorophyll content, and leaflet area (Ojeda-Barrios et al., 2014); however, Zn-EDTA sprays generally did not bring foliar Zn concentrations to the desired level of at least 40 to 60 mg·kg⁻¹. They did, however, achieve the 14- to 22-mg·kg⁻¹ concentrations suggested by the data of Heerema et al. (2017).

The identification of adequate minimum pecan foliar Zn concentrations for commercial field-level recommendations is still open to question. Although the results of Heerema et al. (2017) suggest that 14 to 22 mg·kg⁻¹ is adequate to maximize rates of P_n, recommendations for commercial orchards are generally much greater (at least 40 to 60 mg·kg⁻¹) (Heerema, 2013; Robinson et al., 1997; Smith et al., 2012; Sparks, 1993; Sparks and Payne, 1982). In part, these greater mean leaf Zn concentration recommendations are the result of the tree-to-tree variability in leaf Zn that exists within an orchard (Sparks, 1993; Sparks and Payne, 1982). Finally, it is possible that when Zn is applied to foliage, as continues to be the common practice, Zn affects the relationship with P_n in fundamentally different ways than when it is soil applied. In this study, we explore whether foliar Zn applications (in the form of Zn-EDTA, ZnSO₄·H₂O alone or in combination with UAN) will increase leaf P_n of 'Wichita' pecan trees that are already receiving soil-applied Zn-EDTA.

Materials and Methods

Study site and fertilization treatments.

An experiment was conducted in a commercial pecan orchard near San Simon, AZ (lat. 32°15'20.2"N, long. 109°10'29.8"W; elevation, 1118 m). Soil in this orchard block is Vekol loam (fine, mixed, superactive, thermic Typic Haplargids). The 'Wichita' trees in this experiment, grafted to open-pollinated 'Ideal' rootstocks, were planted in the orchard in 2011. The study had a randomized complete block design. Each tree is considered a block and each treated branch is an experimental unit. The orchard's climate is semiarid and has an average annual precipitation of ≈24 cm (Western Regional Climate Center, n.d.). The orchard is irrigated through a microsprinkler system ≈24 times per year (≈152 cm annually). Nitrogen, phosphorus (P), and potassium (K) were applied through the fertigation system on five

occasions, March through June, at rates of 213, 50.5, and 50.5 kg·ha⁻¹, respectively, in both 2018 and 2019. Sequestar Zn-EDTA (Brandt Inc., Springfield, IL) containing 9% Zn was applied to all trees in the orchard block at a rate of 6.0 kg·ha⁻¹ Zn in 2018 and 11.0 kg·ha⁻¹ Zn in 2019. Trees did not exhibit observable signs of Zn deficiency. In both years, 2.24 kg·ha⁻¹ K and 1.12 kg·ha⁻¹ Ni were applied to the foliage in June (April/May), and 4.48 kg·ha⁻¹ K and 2.24 kg·ha⁻¹ Fe were applied to the foliage in June. Standard commercial weed and insect control measures were conducted by the grower-cooperator. Both varieties in this orchard block exceeded Arizona averages of 1838 and 2129 kg·ha⁻¹ in 2018 and 2019, respectively (U.S. Department of Agriculture, n.d.). In 2018, yields were 2501 kg·ha⁻¹ for 'Western' and 2829 kg·ha⁻¹ for 'Wichita'; in 2019, yields were 3262 kg·ha⁻¹ for 'Western' and 3292 kg·ha⁻¹ for 'Wichita'. Percent kernel was 58.5% for 'Western' in 2018 and 59.4% for 'Wichita', and 61.3% for 'Western' and 66.9% for 'Wichita' in 2019. Average 'Western' nut size was 7.0 g/nut and 'Wichita' nut size was 7.8 g/nut in 2018, and 6.6 g/nut for 'Western' and 7.7 g/nut for 'Wichita' in 2019.

Experimental foliar treatments. Fruiting shoot terminals with full sun exposure were chosen from each of seven trees selected for this study. One of five different foliar spray treatments were applied to the individually selected shoots on each of the seven trees (replicates). The foliar treatments were 1) water control (distilled water alone), 2) UAN control (3.75 mL·L⁻¹ UAN), 3) ZnSO₄ (3.6 g·L⁻¹ ZnSO₄·H₂O (36% Zn), 4) ZnSO₄ plus UAN (3.6 g·L⁻¹ ZnSO₄·H₂O + 3.75 mL·L⁻¹ UAN), and 5) Zn-EDTA (11 mL·L⁻¹ Zn-EDTA, applied in 2019 only).

To prevent spray for one treatment from drifting onto other nearby shoot terminals in the study, a plastic bag with a small hole cut in one corner was closed over each shoot terminal while the spray was being applied. Fertilizer formulations were applied by hand with a spray bottle through the hole until all leaves were thoroughly wetted. The bag was then shaken to ensure all leaf surfaces were coated, and then the bag was removed. Applications were done on 24 May and 25 June 2018, and 24 May and 1 July 2019.

Leaf samples and P_n measurements. Gas exchange measurements were acquired on middle leaflets from nonterminal, sun-lit leaves on each treated shoot in June and July 2018 and 2019, about 2 to 4 weeks after spray applications. A portable P_n system (LI-6800; LI-COR, Lincoln, NE) equipped with a red/blue light source (Li-6800-02) was used. Photosynthetically active radiation (PAR) in the chamber was

maintained at 1700 μmol·m⁻²·s⁻¹. Light saturation of P_n for pecan is reached between a PAR level of 1500 to 1700 μmol·m⁻²·s⁻¹ (Anderson, 1994; Lombardini et al., 2009). The reference carbon dioxide (CO₂) concentration was kept at 400 μmol·mol⁻¹, near the global mean atmospheric concentration (U.S. Department of Commerce, 2020). After P_n and g_s stabilized (typically between 30–60 s after the chamber was clamped onto the leaf), gas exchange data were logged for each leaf. Gas exchange measurements were taken between 0900 and 1300 HR.

Following the technique described by Fulton et al. (2014), MDSWP was measured for each tree on the same dates as the gas exchange measurements were acquired. Sealed reflective bags were placed over a shaded leaf in the lower interior part of the tree and equilibrated for ≈30 min. The leaf was then cut from the tree and the water potential was measured immediately using a Scholander pressure chamber (PMS Instrument Co., Albany, OR).

About 30 leaflets were collected from untreated fruiting shoots on the opposite side of each tree from the treated branches on 25 June 2018 and 7 June 2019 to determine background Zn concentrations. On 18 June 2018, 20 leaflets were also collected from the treated shoots. All leaflets were washed in a P-free detergent, and then rinsed in deionized water, followed by a 1% hydrochloric acid bath and a final rinse in deionized water. The leaflets were spun dry and placed in an oven for 48 h at 65 °C. They were then ground using a cyclone mill (UDY Cyclone Sample Mill, Belt Drive, model 3010-030, 120 Volt ac; UDY Corporation, Fort Collins, CO).

A 0.5-g aliquot of ground leaf tissue was ashed at 500 °C for 5.5 h, dissolved in 10 mL 2.0 N hydrochloride, and diluted to 50 mL. Concentrations of Zn were analyzed with an atomic absorption spectrometer (model 3100; PerkinElmer, Waltham, MA) at a wavelength of 213.9 nm.

JMP Pro 15 software (SAS Institute, Cary, NC) was used to perform analysis of variance. Connecting letters reports to show differences in the means for all data in figure and table form were obtained using each pair Student's *t* tests. JMP software was also used to perform linear regressions to determine the relationship between MDSWP and P_n. An alpha value of 0.05 was used in all statistical tests.

Results and Discussion

Average background leaf Zn concentrations of untreated leaves (i.e., those collected from the opposite side of the tree from

Table 1. Leaf zinc (Zn) concentrations averaged from all individually sampled pecan trees for each of four treatments taken July 18, 2018, 23 d after treatment application, and leaf Zn concentrations averaged from untreated samples taken from the backside of each tree on 25 June 2018. Values that do not share the same letter are statistically different.

Leaf samples	Untreated	Water	Water + UAN	ZnSO ₄ ·H ₂ O	ZnSO ₄ ·H ₂ O + UAN
Average Zn concentration (mg·kg ⁻¹)	21.3 c	21.1 c	22.5 c	126.8 b	151.5 a

UAN = urea-ammonium nitrate; ZnSO₄·H₂O = zinc sulfate monohydrate.

the sprayed branches) in our study were 21.3 mg·kg⁻¹ in 2018 and 15.7 mg·kg⁻¹ in 2019. In 2018, leaf tissue Zn concentrations from shoots treated with water alone (21.1 mg·kg⁻¹) or water + UAN (22.5 mg·kg⁻¹) were not statistically different from that of untreated leaves sampled from the opposite side of the canopy (21.3 mg·kg⁻¹) (Table 1). The leaf Zn concentrations from shoots treated with Zn-containing sprays were significantly greater than those of shoots treated with water alone or water + UAN. The addition of UAN to ZnSO₄·H₂O treatments increased leaf Zn concentrations significantly relative to ZnSO₄·H₂O alone, confirming the findings of Storey (1977). Comparable measurements were not made in 2019.

P_n measured on two dates each in 2018 and 2019 is shown in Fig. 1. No significant differences were noted. There was no significant difference in g_s on any of the measurement dates (data not shown). The overall average g_s from all four measurement dates was 0.210 mol·m⁻²·s⁻¹. Only one significant difference was found in levels of intercellular CO₂; the water + UAN treatment had significantly lower levels than the other treatments on 18 July 2018 (Table 2).

The observation that application of additional Zn, either with or without UAN, did not increase rates of P_n on any of the measurement dates suggests that P_n rates were not limited by lack of Zn on trees where Zn fertilizers were applied exclusively to the soil. Maximum P_n rates in southwestern ‘Wichita’ pecans has been reported to be between 16 and 18 μmol·m⁻²·s⁻¹ (Heerema et al., 2017), whereas rates in our study ranged from 12.2 to 17.1 μmol·m⁻²·s⁻¹, slightly less than typical maximum rates. Both Heerema et al., (2017) and Hu and Sparks (1991) found that maximum P_n was observed in pecans when leaf Zn concentrations were at least 14 to 22 mg·kg⁻¹. The level of Zn in untreated branches in 2018 was 21.3 mg·kg⁻¹, high enough that additional Zn was not expected to elicit a response based on the findings of Heerema et al. (2017) and Hu and Sparks (1991), but well below other published standards of at least 40 to 60 mg·kg⁻¹. In 2019, the average leaf Zn concentration was 15.7 mg·kg⁻¹, within a range in which P_n could potentially be limited by inadequate foliar Zn. Our data indicate that rates of P_n were not limited by the concentrations of foliar Zn and that no benefit with respect to P_n was observed from foliar Zn sprays. However, further research is needed to determine whether these foliar Zn concentrations have an impact on other horticulturally relevant vegetative and reproductive tree functions that were not measured in this study.

Many different kinds of environmental stresses can limit rates of P_n in pecans, including those related to insufficient nutrients, sunlight, and water (e.g., Heerema et al., 2014; Lombardini et al., 2009; Othman et al., 2014). Othman et al. (2014) found that water potentials less than -0.9 MPa reduced pecan

Foliar Treatments and Photosynthesis Rates in 2018 and 2019

Fig 1. Photosynthesis rates of sprayed shoots in 2018 and 2019. The mean photosynthesis rate for treatments from all seven pecan trees (replicates) on each of four measurement dates are compared. Treatments that do not share the same letter within the same sampling date are significantly different. EDTA, ethylenediaminetetraacetic acid; UAN, urea–ammonium nitrate; Zn, zinc.

Table 2. Intercellular carbon dioxide concentrations (measured in micromoles per mole) on all four measurement dates.

Treatment	22 July 2018	18 July 2018	7 June 2019	18 July 2019
Water	236.71 a	264.48 a	237.18 a	264.72 a
Water + urea–ammonium nitrate	224.86 a	245.66 b	244.24 a	265.15 a
Zinc sulfate	231.43 a	267.59 a	241.92 a	264.30 a
Zinc + urea–ammonium nitrate	233.14 a	263.30 ab	239.93 a	261.09 a
Zinc-ethylenediaminetetraacetic acid	—	—	246.45 a	261.27 a

Values in each column that do not share the same letter are statistically different.

Fig. 2. Relationship between midday stem water potential (MDSWP) and photosynthesis rates for all seven pecan trees (replicates) in the experiment. *P* values of 0.076 on 22 June 2018, 0.979 on 18 July 2018, 0.280 on 7 June 2019, and 0.292 on 18 July 2019 were obtained. With an alpha value of 0.05, none of these relationships were significant.

P_n . Our values were close to or greater than this level on all measurement dates. P_n averaged across all treatments for each tree was plotted vs. MDSWP and linear regressions performed on data from each measurement date to evaluate the effect of MDSWP on P_n (Fig. 2). Statistical analysis showed no indication that P_n corresponded to MDSWP. Although combining the data indicates a nonsignificant weak trend ($r^2 = 0.0415$) toward declining P_n with increasing water stress, we conclude that water stress was probably not an important factor in our study.

Conclusion

The measured rates of P_n suggest that leaf tissue Zn concentrations did not limit P_n in our study. MDSWP measurements indicate that water stress did not limit P_n . We conclude that supplemental foliar Zn sprays did not confer any additional benefit to leaf photosynthesis of pecan trees in Zn-EDTA-fertigated orchards with foliar Zn concentrations in the 16 to 21-mg·kg⁻¹ range, because P_n was not Zn limited under these conditions. However, sprays applied earlier in the growing season may provide some benefit not exhibited by the trees in our study. Our research further questions the validity of greater minimum acceptable foliar Zn concentrations for Zn-EDTA-fertigated trees. It is recognized that foliar Zn concentrations vary from tree to tree in an orchard block and that a greater average Zn concentration is likely needed to ensure that most, or all, trees exceed this range. In addition, further testing is needed to determine whether there are any other horticulturally important benefits from Zn sprays in these orchards.

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