Soil-application of Zinc-EDTA Increases Leaf Photosynthesis of Immature ‘Wichita’ Pecan Trees

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ABSTRACT. Zinc deficiency is common in pecan (Carya illinoinensis) grown in alkaline, calcareous soils. Zinc (Zn)-deficient pecan leaves exhibit interveinal chlorosis, decreased leaf thickness, and reduced photosynthetic capacity. Low photosynthesis (Pn) contributes to restricted vegetative growth, flowering, and fruiting of Zn-deficient pecan trees. Our objectives were to measure effects of soil-applied ethylenediaminetetraacetic acid (EDTA)-chelated Zn fertilizer on gas exchange of immature ‘Wichita’ pecan and characterize the relationship between leaf Zn concentration and Pn. The study orchard had alkaline and calcareous soils and was planted in Spring 2011. Zinc was applied throughout each growing season as Zn EDTA through microsprinklers at rates of 0 (Control), 2.2, or 4.4 kg ha⁻¹ Zn. Leaf gas exchange and SPAD were measured on one occasion in the 2012 growing season, four in 2013, and five in 2014. Soil Zn-EDTA applications significantly increased the leaf tissue Zn concentration throughout the study. On all measurement occasions, net Pn was significantly increased by soil-applied Zn EDTA compared with the control, but Pn was not different between the two soil-applied Zn-EDTA treatments. Leaf Pn in midseason did not increase at leaf tissue Zn concentrations above 14–22 mg kg⁻¹. Leaf SPAD consistently followed a similar pattern to Pn. Soil Zn-EDTA application increased leaf stomatal conductance (gs) compared with the Control early through midseason but not after August. Intercellular CO₂ concentration was significantly lower for Zn-EDTA-treated trees than the Control even on dates when there was no significant difference in gs, which suggests that soil application of Zn-EDTA alleviated nonstomatal limitations to Pn caused by Zn deficiency.

The native range for pecan extends through much of the south-central United States and into scattered locations in Mexico. Native pecan groves are found growing in deep, sandy, alluvial soils in river bottoms. The soils that cover most of the native range of this tree species are acidic with pH levels below 6.0, although in the westernmost native range soil pH may exceed 7.0 [U.S. Department of Agriculture (USDA), 2016a; U.S. Geological Survey, 2016]. Since the 1930s, and especially in the last 40 years, improved cultivar pecan orchard plantings in North America have expanded considerably into the semiarid western region, especially in the U.S. states of Texas, New Mexico, and Arizona, and the Mexican states of Chihuahua, Sonora, and Coahuila. Currently, there are at least 100,000 ha of pecan orchards in the semiarid region of North America (Servicio de Información Agroalimentaria y Pesquera, 2014; USDA, 2012). In this region, soil pH generally ranges from 7.0 to 8.5 (USDA, 2016a).

A major challenge with growing pecans in the semiarid region is the widespread occurrence of calcareous and alkaline soils in which micronutrients, particularly Zn, are poorly available for uptake (Fenn et al., 1990; Imtiaz et al., 2006; Marschner, 1993). Zinc deficiency was first identified in pecan and other fruit tree species in the 1930s (Alben et al., 1932a, 1932b; Chandler, 1937). Zinc deficiency in pecan is characterized by shortened internodes (giving rise to shoots with a rosette appearance), severely reduced leaf area, wavy leaf margins, interveinal leaf chlorosis and necrosis, and shoot terminal dieback (Alben et al., 1932a; Heerema, 2013). Along with reduced leaflet area, Ojeda-Barrios et al. (2012) showed that in Zn-deficient pecan, the leaves were thinner (particularly the palisade parenchyma cell layer), had higher stomatal numbers per square millimeter leaf area, and had disorganized spongy parenchyma cell layers.

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In bearing-age orchards, nut yields are reduced by Zn deficiency through its detrimental effects on pistillate flower production, the number of nuts set and matured, and final individual nut weight (Hu and Sparks, 1990). In addition, there are major reductions in commercially important pecan nut quality parameters such as percentage kernel weight, total in-shell nut volume, and percentage of fruits with dehisced shucks related to increasingly severe Zn deficiency (Hu and Sparks, 1990). In young Zn-deficient pecan orchards, tree survival rates may be reduced (J.L. Walworth and R.J. Heerema, personal observation) and surviving trees are slow to establish and produce nuts (Walworth et al., 2016).

Zinc is an essential enzyme cofactor in a wide range of biochemical pathways in plants (Broadley et al., 2012, 2007; Brown et al., 1993; Vallee and Auld, 1990), so the effects of insufficient Zn on overall pecan tree function are extremely complex. Nevertheless, a substantial part of the reduction in shoot growth and fruit production in Zn-deficient plants may be explained by the negative impacts of Zn deficiency on carbon assimilation. A strong reduction in leaf Pn in response to Zn deficiency has been shown across a broad diversity of plant species (e.g., Amiri et al., 2016; Fu et al., 2015; Mattiello et al., 2015; Tavallali et al., 2009), including pecan (Hu and Sparks, 1991). Zinc has been proposed to have major effects on Pn in a number of different ways, including its essential roles in the function of the carbonic anhydrase enzyme, which assists in movement of CO₂ to the site of the Calvin cycle in the chloroplast stroma, and the Cu–Zn superoxide dismutase enzyme, which is involved in protection of plant cells against oxidative damage by reactive oxygen species (Broadley et al., 2012; Cakmak, 2000; Yruela, 2013). Zinc may have further impacts on Pn through its involvement in the regulation of guard cell function (Cakmak, 2000; Sharma et al., 1995) and photo-assimilate export from leaves via the phloem (Cakmak, 2000).

During the first 2 or 3 months of each growing season, pecan growers in the semiarid region typically make three to eight foliar spray applications of Zn sulfate to maintain adequate Zn in nut-producing trees (Heerema, 2013). Young, rapidly growing trees may be sprayed weekly during the active growing season. When canopy spray coverage is good and not too much time elapses between Zn applications, foliar Zn sprays are generally effective in preventing development of visible foliar Zn deficiency symptoms in pecan, but weather conditions (e.g., wind and precipitation) and conflicting orchard operations (e.g., flood irrigation) often interfere with Zn spray operations. Thus, Zn deficiency remains one of the most important factors limiting pecan orchard nut production and profitability across the North American semiarid growing region.

In the semiarid region, there is strong interest among pecan producers in developing an effective method for managing Zn nutrition through soil fertilizer application and a number of published studies have shown promising options (Fenn et al., 1990; Nuñez-Moreno et al., 2009a, 2009b). We began a long-term experiment in 2011 to evaluate the use of soil-applied EDTA-chelated Zn fertilizer for managing Zn nutrition in an immature ‘Wichita’ pecan orchard grown on a calcareous, alkaline soil. The objectives in the current study were 2-fold: the first experimental objective was to measure how soil-applied Zn-EDTA affects leaf gas exchange of immature ‘Wichita’ pecan and the second was to characterize the relationship between leaf Zn concentration and Pn.

### Materials and Methods

**Study site and Zn treatments.** This study was conducted in a commercial pecan orchard planted in 2011 near San Simon, AZ (lat. 32°15’20.2”N, long. 109°10’29.8”W, elevation 1118 m). The climate is semiarid with an average of <30 cm precipitation annually. A weather station near the orchard site showed total annual precipitation of 17.0, 21.4, and 24.8 cm for 2012, 2013, and 2014, respectively, with well over 50% of the total precipitation falling as rain during the months of July, August, and September in each of those years (University of Arizona, 2016). On average, the region has a frost-free period of ~210 d (USDA, 2016b). The main cultivar in the orchard is Wichita budded on open-pollinated seedling Ideal (synonym Bradley) rootstocks with 25% Western (synonym Western Schley) cultivar pollinizers. ‘Wichita’ is one of the most widely grown cultivars in the semiarid region and is known to be more prone than many other popular pecan cultivars to develop Zn deficiencies when grown in calcareous, alkaline soils (Herrera, 2005). Tree spacing was 6 x 12 m. The soil in the orchard is a Guest silt loam loam (fine, mixed, superactive, calcareous, and thermic Ustertic Torrifuvents) with alkaline pH (USDA, 2011, 2016a). Soil test values at initiation of the study are shown in Table 1.

The orchard owner managed all orchard operations except Zn fertilizer applications. Each tree was...
irrigated by one microsprinkler with a wetted area \( \approx 2.5 \text{ m} \) diameter. Trees received \( 2.5 \text{ m} \) irrigation water each season. Weeds in the tree row were controlled with glyphosate (3.5 L ha\(^{-1}\)) four times, carfentrazone (150 mL ha\(^{-1}\)) twice, and glufosinate (3.5 L ha\(^{-1}\)) once per season. Areas between rows were mechanically mowed to suppress weeds. All trees were fertilized uniformly with 16N–3.5P–2.5K–4S injected into the irrigation system at annual application rates of 247, 493, and 740 kg ha\(^{-1}\) in 2012, 2013, and 2014, respectively.

Beginning the year of planting, trees in the study were supplied Zn-EDTA fertilizer (Sequestar 9% Zn Chelate; Monterey Ag Resources, Fresno, CA) by injection through the orchard microsprinkler irrigation system at one of three rates: 0 (Control), 2.2 (Zn1), or 4.4 (Zn2) kg ha\(^{-1}\) Zn per year. In the first 3 years of the study, Zn-EDTA applications were distributed across the whole growing season (on 13, 14, and 16 irrigation dates from Apr. to Oct. in 2011, 2012, and 2013, respectively). In 2014, Zn-EDTA applications were distributed across eight irrigations dates only during the first half of the growing season (April–July). Within each season, soil Zn-EDTA applications were split evenly among application dates. No foliar Zn applications were made. The study was a randomized complete block design (RCBD) with four blocks. Each plot contained at least 15 trees (at least 45 trees per block).

**GAS EXCHANGE MEASUREMENTS AND LEAF TISSUE SAMPLING.** In Summer 2012, four trees were selected per Zn treatment in each block for gas exchange measurements (48 trees total). Within each Zn treatment, trees were selected based on uniformity of general appearance of health and tree size. The same trees were used throughout the study. On one date in 2012 (21 Aug.), four occasions in 2013 (25 June, 6 and 7 Aug., 24 Sept., and 24 Oct.) and five occasions in 2014 (26 May, 26 June, 24 and 25 July, 28 Aug., and 1 Oct.) leaf gas exchange was
measured on a middle leaflet of two sunlight-exposed leaves per tree. The leaves used for gas exchange measurement were visually representative of the overall level of symptom severity of Zn deficiency for the tree. On two measurement occasions, one in Aug. 2013 and another in July 2014, weather conditions did not allow for all of the gas exchange measurements to be made in the same day, so the remaining measurements were made the following morning.

Gas exchange measurements were made with a portable \( P_n \) system (LI-6400XT; LI-COR, Lincoln, NE) equipped with a red/blue light-emitting diode light source (6400-02B; LI-COR) and \( CO_2 \) injector system (6400-01; LI-COR). Chamber photosynthetically active radiation (\( PAR \)) was held at 1700 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). Light saturation of \( P_n \) has been reported for pecan at \( PAR \) 1500 to 1700 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \) (Anderson, 1994; Lombardini et al., 2009). Reference \( CO_2 \) concentration was maintained near the global mean atmospheric concentration at 400 \( \mu \text{mol} \cdot \text{mol}^{-1} \) (U.S. Department of Commerce, 2016). Gas exchange data for each leaf were logged when both \( P_n \) and \( g_S \) had stabilized, typically 30–60 s after the chamber was clamped onto the leaf. Immediately after measuring gas exchange of a leaf, a portable chlorophyll meter (SPAD 502; Konica Minolta, Ramsey, NJ) was used to measure greenness midway between the midrib and margin of the same leaflet. Gas exchange measurements were made between ~0900 and 1300 HR.

Physiological water status of one tree in each plot was measured as midday stem water potential [MDSWP (Shackel et al., 1997)] between \( \approx 1300 \) and 1500 HR on each gas exchange measurement date. One shaded leaf in the lower, interior portions of each tree canopy was placed in a sealed reflective bag for at least 20 min before leaf water potential was measured with a Scholander pressure chamber (PMS Instrument Co., Albany, OR). Except in Oct. 2013, average MDSWPs throughout the study ranged from \(-0.3 \) to \(-0.6 \) MPa (data not presented), indicating that the trees were not water stressed on those dates (Othman et al., 2014). On 24 Oct. 2013, the average MDSWP was \(-1.2 \) MPa, which indicates that on that date the trees were under physiological water stress severe enough to negatively affect \( P_n \) (Othman et al., 2014).

In 2012, leaflets for tissue nutrient analyses were collected on only one date (14 Aug.) and samples were pooled across all ‘Wichita’ trees in each plot. In 2013, separate leaf tissue samples were collected for Zn analysis from each of the trees used for gas exchange measurements on the August and September measurement dates and, in 2014, tissue samples were similarly collected on all of the measurement dates. With each tissue sampling in 2013 and 2014, 16 leaflets were collected per tree according to the method recommended by the University of Arizona and New Mexico State University Extension Services (Heerema, 2013; Walworth et al., 2006). Leaflet samples were first washed in a water bath with a phosphorus-free detergent (0.05% detergent), followed by rinses in deionized water and a rinse in dilute hydrochloric acid (1% HCl), and finally one additional rinse with ultrapure water. Leaflets were spun to remove excess water, dried in an oven for 3 days at 65 °C, and mechanically ground to 20-mesh particle size.

Tissue samples were dry ashed by placing 0.5-g subsamples into 30-mL porcelain crucibles, ramping the temperature to 500 °C over 3.5 h and maintaining the temperature for 5.5 h (Jones and Case, 1990). Cooled ash was dissolved in 10 mL of 2.0 N HCl, allowed to sit for several hours, diluted to 50 mL, and allowed to sit overnight. Zinc concentration was analyzed with an atomic absorption spectrophotometer (3100; PerkinElmer, Waltham, MA) at a wavelength of 213.86 nm.

**Statistics.** For each response variable [\( P_n \), SPAD, \( g_S \), transpiration, intercellular \( CO_2 \) concentration (C\text{s}), and tissue Zn concentration], analysis was conducted on plot averages. The 2012 data were analyzed as an RCBD with fixed blocks. Data for 2013 and 2014 were analyzed separately as RCBDs with repeated measures. Block, Zn treatment, date, block x date
Following a preliminary analysis to assess whether fitting a common trend was adequate, for each summer date (Aug. 2013, June 2014, July 2014, and Aug. 2014) and for the response variables $P_n$ and SPAD, a nonlinear broken lines model with a line segment and plateau (Robbins et al., 2006) was fit with leaf tissue Zn concentration as the explanatory variable. Only observations with leaf tissue Zn concentrations 50 mg·kg$^{-1}$ or lower were used. Fitted models are presented graphically with the 95% confidence interval (CI) breakpoint estimate. The preliminary analysis combined data from the four summer dates and fitted two mixed nonlinear models. Both models accounted for random tree effects within year and included a line segment-plateau trend in the fixed portion. The first model fit separate broken lines to each date and the second model fit a single broken line to all four dates. The models were compared using likelihood ratio tests which suggested the need to fit separate trends to the four dates ($P \leq 0.05$). The broken lines models were fit using PROC NLMIXED software (SAS version 9.3).

Results and Discussion

The leaf tissue Zn concentrations by dry weight were significantly different between the untreated Control and the Zn-EDTA-treated trees on all of the tissue sampling dates in 2013 and 2014 (Fig. 1). Generally, average leaf Zn concentrations of the trees receiving the lower soil Zn-EDTA application rate were about double that of the Control and leaf Zn concentrations of the trees receiving the higher soil Zn-EDTA application rate were about triple that of the Control. In 2013, the treatment $\times$ date interaction was not significant and leaf Zn concentrations averaged across the two sampling dates were ($\pm$SE) 9.6 $\pm$ 0.8 mg·kg$^{-1}$ for the Control, 18.8 $\pm$ 2.4 mg·kg$^{-1}$ for the Zn1 treatment, and 31.2 $\pm$ 4.4 mg·kg$^{-1}$ for the Zn2 treatment. Although pairs of Zn treatment averages differed in the preplanned analysis, when fitting separate variances to each treatment group, the difference between the Zn2 average and Zn1 average across the two sampling dates in 2013 was just short of significant [12.5 $\pm$ 5.0 mg·kg$^{-1}$ ($P = 0.059$)]. The interaction was not significant in 2014 and across the five sampling dates in 2014, average leaf tissue Zn concentration was lower for the untreated Control (11.4 $\pm$ 0.6 mg·kg$^{-1}$), than for the Zn1 treatment (22.6 $\pm$ 2.6 mg·kg$^{-1}$) or the Zn2 treatment (31.3 $\pm$ 5.3 mg·kg$^{-1}$) (Fig. 1). Tissue Zn concentrations for the Control, Zn1, and Zn2 in 2012 were 9.6, 18.6, and 25.4 mg·kg$^{-1}$, respectively (data not presented). On every tissue sampling date during the 3 years of the study, the average leaf concentrations of even the Zn2 treatment were well below the current extension recommendations of 50–100 mg·kg$^{-1}$ (Heerema, 2013).

The positive influence of soil applied Zn-EDTA and consequent increase in leaf Zn on leaf net $P_n$ of immature
higher leaf area index. the larger tree size (Walworth et al., 2016) and, presumably, expected to be greatly magnified in the Zn-treated trees by at the leaf level on total tree-wide carbon assimilation is higher for trees in both soil Zn treatments than for trees in the Control on every measurement date except 1 Oct. 2014 (Fig. 2). Leaf $P_n$ of the two soil Zn-EDTA treatments were not significantly different from each other on any measurement date during the three seasons of the study (Fig. 2). Across dates, the low and high soil Zn-EDTA treatments increased $P_n$ over the Control by 17.1% and 25.1%, respectively, in 2012; by 35.3% and 40.5%, respectively, in 2013; and by 62.7% and 65.8%, respectively, in 2014. Much of the positive response in growth, overall tree health, nut production, and kernel quality of soil Zn-EDTA-treated ‘Wichita’ pecan trees (Walworth et al., 2016; Wang, 2016) could likely be accounted for by the higher leaf-level carbon assimilation rates measured in the current study. Furthermore, the impacts of such increases in $P_n$ at the leaf level on total tree-wide carbon assimilation is expected to be greatly magnified in the Zn-treated trees by the larger tree size (Walworth et al., 2016) and, presumably, higher leaf area index.

Hu and Sparks (1991) showed that leaf $P_n$ of ≈60-year-old ‘Stuart’ pecan trees in mid-August increased with increasing leaf tissue Zn concentration and leaf $P_n$ was approaching, but had not yet reached, maximum levels at 14.3 mg kg$^{-1}$ Zn (leaves with tissue Zn concentration >14.3 mg kg$^{-1}$ were not included in that study). In our experiment, when leaf $P_n$ was modeled as a function of leaf tissue Zn concentration (by dry weight) using a broken-lines regression model with a line segment-plateau form, the breakpoint leaf tissue Zn concentrations, above which $P_n$ no longer increased with increasing Zn, were 14.4 mg kg$^{-1}$ (95% CI = 10.6–18.2 mg kg$^{-1}$) and 15.2 mg kg$^{-1}$ (95% CI = 11.9–18.4 mg kg$^{-1}$) on the 7 Aug. 2013 and 28 Aug. 2014 measurement occasions, respectively (Fig. 3). In 2014, the breakpoints were slightly higher on the 24 June (20.2 mg kg$^{-1}$ (95% CI = 16.5–24.0 mg kg$^{-1}$) and 25 July (21.7 mg kg$^{-1}$ (95% CI = 14.9–28.4 mg kg$^{-1}$)) measurement occasions than the 28 Aug. occasion (Fig. 3). That these breakpoints are in such close agreement with the findings of the earlier work of Hu and Sparks (1991) is interesting especially in light of the individual tree canopy (multiple leaf) tissue sampling approach used in our study vs. the individual shoot sampling approach used in their study [see discussion of the subject of sampling level for tissue nutrient analyses in pecan orchards in Hu and Sparks (1990) and Hu and Sparks (1991)].

At leaf tissue Zn concentrations below the breakpoints, segmented line regression analyses showed increases in $P_n$ of 0.62 (7 Aug. 2013), 0.89 (24 June 2014), 0.69 (25 July 2014), and 1.25 (28 Aug. 2014) μmol·m$^{-2}$·s$^{-1}$ for every increase of 1 mg·kg$^{-1}$ Zn (Fig. 3). Plateau leaf $P_n$ values above the breakpoints for the 7 Aug. 2013, 25 July 2014, and 28 Aug. 2014 measurement occasions were similar to one another, ranging from 16.1 to 16.6 μmol·m$^{-2}$·s$^{-1}$, but the plateau $P_n$ value on the 24 June 2014 measurement occasion was 18.8 μmol·m$^{-2}$·s$^{-1}$ (Fig. 3). Although the mean $P_n$ values at optimal Zn (i.e., the maximum estimated $P_n$ values) in our study were slightly lower than Hu and Sparks (1991) reported for pecan (16–19 μmol·m$^{-2}$·s$^{-1}$ in the present study vs. >20 μmol·m$^{-2}$·s$^{-1}$ in their study), the overall relationships between leaf Zn and $P_n$ were similar. Our data and those of Hu and Sparks (1991) show that maximum leaf $P_n$ for pecan may be achieved at leaf tissue Zn concentrations much lower than the extension recommended range for Zn of 50–100 μmol·m$^{-2}$·s$^{-1}$ (Heerema, 2013).

The SPAD (leaf greenness) index of trees in both soil Zn treatments was significantly higher than the Control on every measurement date during the three seasons of the study (Fig. 4). The Zn soil treatment by date interaction for SPAD was significant in 2014, but was not significant in 2013. Low leaf SPAD in the Control indicated the onset of interveinal chlorosis, which is widely accepted as part of the syndrome of visible symptoms associated with Zn deficiency in plants and a loss of chlorophyll from the leaf photosynthetic tissues (Broadley et al., 2012). Trees in the Zn2 treatment had significantly higher leaf SPAD than the trees in the Zn1

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Fig. 5. The relationship of leaf SPAD to leaf Zn concentration (by dry weight) in ‘Wichita’ pecan trees on 7 Aug. 2013 ($r^2 = 0.47$), 24 June 2014 ($r^2 = 0.44$), 25 July 2014 ($r^2 = 0.39$), and 28 Aug. 2014 ($r^2 = 0.29$) measurement occasions. The solid vertical line represents the breakpoint leaf Zn tissue concentration below which leaf photosynthesis begins to decline. Dashed vertical lines represent the 95% confidence interval for the breakpoint.
midseason measurement occasions (Fig. 5). Were similar (ranging from SPAD 39 to 41) on all four of the mean leaf SPAD values above the Zn concentration breakpoint August measurement occasions in 2013 and 2014 (Fig. 5). Treatment (SPAD 34.8 vs. 32.3) on 26 May 2014, but leaf SPAD values of trees in the two soil Zn treatments were not significantly different from each other on any of the other measurement occasions throughout the study (Fig. 4). Consistent with our leaf SPAD results, strong direct relationships between Zn nutrition and leaf content of chlorophyll or SPAD have been previously shown in diverse plant species including pecan (Fu et al., 2015; Hu and Sparks, 1991; Mukhopadhyay et al., 2013; Ojeda-Barrios et al., 2012; Tavallali et al., 2009).

Leaf SPAD, also modeled as a function of leaf tissue Zn concentration using a broken-lines regression model with a line segment-plateau form, showed breakpoints at leaf tissue Zn concentration of 16.3 mg kg⁻¹ (95% CI = 12.5–20.0 mg kg⁻¹) by dry weight on 7 Aug. 2013 and 14.1 mg kg⁻¹ (95% CI = 11.4–16.8 mg kg⁻¹) by dry weight on 28 Aug. 2014 (Fig. 5). As leaf tissue Zn concentration fell below the breakpoints, leaf SPAD fell at a rate of 0.88 SPAD units for every 1 mg kg⁻¹ Zn decline in leaf tissue Zn concentration on the 7 Aug. 2013 measurement occasion and 1.4 mg kg⁻¹ on 28 Aug. 2014. As with Pₚ, the breakpoint was higher on the 24 June 2014 [28.3 mg kg⁻¹ (95% CI = 19.3–37.4 mg kg⁻¹)] and 25 July 2014 [23.4 mg kg⁻¹ (95% CI = 16.6–30.1 mg kg⁻¹)] measurement occasions than on the August measurement occasions in 2013 and 2014 (Fig. 5). Mean leaf SPAD values above the Zn concentration breakpoint were similar (ranging from SPAD 39 to 41) on all four of the midseason measurement occasions (Fig. 5).

As expected, transpiration measured at the leaf level followed the same pattern as gₛ (data not presented). Along with larger tree canopy size associated with Zn applications (Walworth et al., 2016), increased gₛ and transpiration, could have implications for pecan orchard water use, irrigation scheduling, and water use efficiency as has been shown for other crops. For example, water use by Zn-sufficient chickpea (Cicer arietinum) plants was higher than that of Zn-deficient plants, but water use efficiency also increased (Khan et al., 2004). And, in cauliflower (Brassica oleracea var. botrytis) grown in sand culture, a significant increase in leaf water potential accompanied higher tissue Zn concentration, Pₚₛ, gₛ, and transpiration rates when Zn fertilizer was supplied at “normal” vs. “deficient” rates (Sharma et al., 1994). In our study, acceptable plant water status (as MDSWP) was usually observed across Zn treatments, but across measurement occasions, the percent increase in transpiration with the soil Zn-treated trees compared with Control trees was far less than that of Pₚₛ (about one-half to two-thirds that for Pₚₛ in 2013 and 2014, respectively). Although it will require further investigation to confirm, this suggests that water use efficiency of ‘Wichita’ pecan improved with soil Zn-EDTA applications.

Intercellular CO₂ concentration was not significantly different among the Control and two soil Zn treatments on 21 Aug. 2012 and the early season (May–June) measurement occasions in 2013 and 2014 (Fig. 7). On the other six midlate season
measurement occasions in 2013 and 2014, \( C_i \) was significantly higher for leaves of Control trees than for leaves in the two soil Zn treatments (Fig. 7). The Zn treatment main effect was significant but the Zn treatment by date interaction for \( C_i \) was not significant in either 2013 or 2014. This indicates an increase in Calvin cycle CO₂ demand relative to the CO₂ supply rate from the atmosphere through the stomata of the leaves of Zn-sufficient pecan trees compared with that of Zn-deficient trees. Nonetheless, \( C_i \) was not significantly different between the two Zn treatments on any of the measurement dates (Fig. 7) or averaged across dates.

In summary, Zn-EDTA application via fertigation in this alkaline, calcareous soil increased leaf Zn concentration and \( P_n \) of immature ‘Wichita’ pecan trees with optimal \( P_n \) in midseason (June–August) occurring at leaf Zn concentrations as low as 14–22 mg·kg⁻¹. Zinc-EDTA applications appear to increase \( P_n \) through alleviating nonstomatal limitations, including low tissue chlorophyll levels, but direct effects on \( g_s \) in early and midseason are also possible.

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