Nutraceutical Properties of Pecan Kernels Are Affected by Soil Zinc Fertilizer Application

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Abstract. Pecan (*Carya illinoensis*) has high kernel antioxidant activity and unsaturated fatty acid content, which contribute to its nutraceutical properties. In the western United States, where soils are typically alkaline, pecan trees require frequent zinc (Zn) fertilizer applications to maintain normal canopy growth and nut production. Our objective was to investigate the effects of tree Zn fertilization on nutraceutical properties of ‘Wichita’ and ‘Western’ pecan kernels. Trees were fertilized with ethylenediaminetetraacetic acid (EDTA) chelated Zn, which was applied to the soil at one of three seasonal rates for a total of three treatments: 0 (control), 2.2, or 4.4 kg-ha⁻¹ Zn. Nut samples were collected and homogenized for analyses of kernel oil yield, hydrophilic antioxidant capacity, fatty acid profile, and γ -tocopherol content. Although soil Zn treatments did not significantly affect antioxidant capacity of defatted pecan kernels, Zn application had significant positive effects on both total kernel oil yield and γ -tocopherol content compared with the control. In conclusion, soil application of Zn fertilizer may increase the human health-promoting aspects of pecan kernels, a valuable attribute among health-conscious consumers.

Pecan is the only major tree nut crop native to North America (Grauke and Thompson, 2008). Native American tribes consumed and traded pecans long before the arrival of European explorers to the continent, but interest in cultivating pecans as a horticultural crop dates back a little more than 250 years (McHatton, 1957; Wells, 2017). The United States and Mexico remain the world’s top pecan-producing nations, together accounting for more than 90% of current global production, but pecans are also now cultivated commercially in South Africa, Australia, Argentina, Brazil, Peru, and China (Grauke and Thompson, 2008; International Nut and Dried Fruit Council

Foundation, 2019). Average annual inshell production of pecan nuts in the United States was 123 million kg, with Arizona, Georgia, New Mexico, Oklahoma, and Texas as the leading states [U.S. Department of Agriculture (USDA), 2019].

Pecans are higher in oil content than many other tree nuts. Average unroasted pecan kernel total lipid content is 72.0%, compared with 65.2% for english walnuts (*Juglans regia*), 60.8% for hazelnuts (*Corylus avellana*), 49.9% for almonds (*Prunus dulcis*), 46.7% for cashews (*Anacardium occidentale*), and 45.3% for pistachios (*Pistacia vera*) (USDA, 2016). Pecan kernel oil is chemically similar to olive (*Olea europaea*)

oil, consisting mainly of two fatty acids, monounsaturated oleic acid (C18:1) and polyunsaturated linoleic acid (C18:2) (Griel and Kris-Etherton, 2006; USDA, 2016). Linoleic acid (especially certain geometric isomers of conjugated linoleic acid) is often associated with functional foods, and its regular consumption may help prevent a variety of serious chronic human diseases (Koba and Yanagita, 2014; Nagao and Yanagita, 2005).

Pecan kernels are also a high antioxidant capacity food, and among tree nuts, pecans have one of the highest reported total oxygen radical absorbance capacity (ORAC) values [179.4 mmol of trolox equivalents (TE) per gram], total phenolics (20.2 mg of gallic acid equivalents per gram), and total flavonoids (34.0 mg per 100 g) (Bhagwat et al., 2013; Wu et al., 2004). When a chloroform/methanol extraction was used, pecan oil had significantly higher total phenolics than oils from almond, pistachio, pine nut (*Pinus* spp.), hazelnut, and brazil nut (*Bertholletia excelsa*) and had significantly higher antioxidant activity than oil from any of the other tree nut species tested (i.e., the five species listed previously plus walnut) (Miraliakbari and Shahidi, 2008). High antioxidant capacity foods such as pecans have piqued a great deal of interest among researchers, industry marketers, and health-conscious consumers for their potential use as nutraceutical sources to counteract reactive oxygen species’ deleterious effects in the human body, including increased risks for cardiovascular disease, neurological disorders, diabetes, and cancer (Blomhoff et al., 2006; Halliwell, 1996; Lee et al., 2004; Uttara et al., 2009; Violi and Cangemi, 2005; Wu and Cederbaum, 2003).

Tocopherols have vitamin E activity and are the most abundant antioxidant in tree nuts. Thus, they may play an important role in protecting the body’s cells from lipid peroxidation and free radicals (Alasalvar and Shahidi, 2009; Azzi and Stocker, 2000). Dietary phenolics are divided into six groups (phenolic acids, flavonoids, stilbenes, coumarins, lignans, and tannins) that also contribute significantly to antioxidant capacity in tree nuts (Alasalvar and Shahidi, 2009; He and Liu, 2008; Shahidi and Nacz, 2004; Yang et al., 2009). Hudthagosol et al. (2011) tested plasma concentrations of γ -tocopherol, hydrophilic- and lipophilic-ORAC, oxidized low density lipoprotein, and epigallocatechin-3-gallate. They showed that the antioxidants in pecan kernels were absorbed by human subjects who consumed pecans, and therefore pecans may contribute to postprandial antioxidant defenses.

Biochemical composition of pecan kernels can vary as a function of cultivar, environmental conditions, cropload, and horticultural practices (Heaton et al., 1977; McMeans and Malstrom, 1982; Rudolph et al., 1992; Wakeling et al., 2001), which implies that there could be management techniques for producers to maximize nutraceutical composition in pecan kernels. It would be especially appealing for pecan growers if such production techniques were

also known to have positive impacts on crop yield, other nut quality parameters, or overall tree health. This was recently demonstrated with regard to pecan orchard light management practices where mechanical hedge pruning increased total phenolics content and antioxidant capacity (as ORAC) of pecan kernels (Gong et al., 2020).

Managing tissue levels of the micronutrient zinc (Zn) is one of the most critical practices for maintaining high nut yields and quality in commercial pecan orchards, especially in semiarid and arid production areas where calcareous soils dominate (Heerema, 2013; Walworth and Heerema, 2019). Zinc availability for root uptake is limited due to reactions with soil carbonates and hydroxyl groups in alkaline, calcareous soils resulting in deficiencies that commonly negatively impact an orchard's tree health, fruit production, and commercial performance (Udo et al., 1970; Walworth and Heerema, 2015; Walworth and Pond, 2006; Walworth et al., 2006). Zinc deficiency symptoms in pecan characteristically include shortened internodes ("rosette"), severely reduced leaf area, thinner leaves, wavy leaf margins, interveinal leaf chlorosis and necrosis, and shoot terminal dieback (Alben et al., 1932; Heerema, 2013; Heerema et al., 2017; Hu and Sparks, 1991; Ojeda-Barrios et al., 2012). In immature orchards, pecan tree growth and establishment rates are negatively affected by Zn deficiency (Walworth et al., 2017) and in mature orchards, Zn deficiency reduces pistillate flower production, fruit set, individual nut weight, and percent kernel weight (Hu and Sparks, 1990).

Zinc serves as an essential enzyme cofactor in numerous plant biochemical pathways and is therefore critically involved at many levels of pecan tree growth, development, metabolism, and function, potentially including biosynthesis of the major kernel components with nutraceutical properties (Broadley

et al., 2007; 2012; Brown et al., 1993; Vallee and Auld, 1990). Ashraf et al. (2013) and Huang et al. (2019) demonstrated that foliar applications of ZnSO₄ fertilizer sprays can positively affect oil content of pecan kernels.

Pecan producers in semiarid and arid production areas have long managed orchard Zn nutrition primarily through repeated springtime foliar sprays of Zn fertilizers (such as ZnSO₄). However, soil application of chelated Zn EDTA fertilizer is also an effective strategy for maintaining adequate Zn levels in pecan trees, even in alkaline, calcareous soils (Núñez-Moreno et al., 2009; Walworth et al., 2017) and is now gaining in popularity as a Zn management tool among pecan producers. Zinc acquired by pecan trees through root uptake is distributed more widely in the plant tissues than Zn acquired from foliar fertilizer sprays (Wadsworth, 1970; Walworth et al., 2017) and is therefore expected to impact tree physiology in different ways. Although soil applied Zn EDTA can eliminate Zn deficiency symptoms, orchards managed exclusively by soil Zn fertilizer application usually have lower leaf tissue Zn concentrations than comparable orchards managed with foliar Zn sprays, and it therefore has recently been proposed that leaf tissue critical levels for Zn should be lower for pecan orchards managed with soil applied Zn EDTA than for those managed with foliar sprays (i.e., ≈ 30 mg·kg⁻¹ for soil applied Zn EDTA vs. ≈ 50 mg·kg⁻¹ for foliar applied Zn) (Heerema et al., 2017; Walworth and Heerema, 2019; Walworth et al., 2017).

To the best of our knowledge, the potential for using Zn nutrition management via soil applied Zn to influence pecan kernel nutraceutical composition has not been previously studied. The objective of the present work was to describe how increasing tree Zn levels with soil application of Zn EDTA fertilizers affects nutraceutical properties of pecan kernels. Information obtained will help in understanding how tree Zn nutrition affects antioxidant activity and biochemical composition in pecan kernels.

Materials and Methods

Study design and sampling. The 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 main cultivar (comprising 75% of the trees) in the orchard was Wichita and the pollinizer cultivar was Western (synonym Western Schley). The rootstocks for both cultivars were open-pollinated Ideal (synonym Bradley) seedlings. Trees were planted on a rectangular pattern, spaced 6 × 12 m. Soil was a Guest silty clay loam (fine, mixed, superactive, calcareous, thermic Ustertic Torrifuvents) with alkaline pH (USDA, 2011, 2020b). The region is semiarid where average annual precipitation is less than 30 cm and frost-free period exceeds 210 d (USDA, 2020a).

Beginning in 2011, trees were supplied with all Zn fertilizer as Zn EDTA (Sequestar 9% Zn Chelate; Monterey Ag Resources, Fresno, CA) injected through the microsprinkler irrigation system at one of three seasonal rates: 4.4, 2.2, or 0 kg·ha⁻¹ Zn (Zn2, Zn1, and control, respectively). During 2011–13, annual Zn EDTA applications were split evenly among at least 13 irrigation dates each year, distributed across the entire growing season (April–October). In 2014 and 2015, Zn EDTA applications were distributed across eight and 10 irrigation dates, respectively, during the first half of each growing season (April–July). Treatment plots each consisted of at least 12 'Wichita' and three 'Western' pecan trees arranged in a randomized complete block design (RCBD) with four blocks. Average midseason leaflet tissue Zn concentrations for 'Wichita' in 2014 were 32, 24, and 14 mg·kg⁻¹ by dry weight, respectively, for Zn2, Zn1, and control. In 2015, average midseason leaflet tissue Zn concentrations for 'Wichita' were 23, 16, and 9 mg·kg⁻¹ for Zn2, Zn1, and control, respectively, and for 'Western' were 25, 18, and 10 mg·kg⁻¹ for Zn2, Zn1, and control, respectively [see Walworth et al. (2017) for methodology details].

On 24 Nov. 2014, when the nuts were fully matured, all of the nuts were hand harvested from all of the 'Wichita' trees within each plot. In 2014, the 'Western' trees had very low nut yields and were not harvested for this nutraceutical study. In 2015, all of the nuts were hand harvested on 19 Nov. from all of the 'Wichita' and 'Western' trees within each plot. With each hand harvest, representative subsamples were collected and stored briefly at 4 °C until shelled. Shelled kernels were stored at -80 °C until extraction.

Sample preparation. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox); fluorescein (sodium salt); 2,2'-Azobis(2-amidinopropane) (AAPH); 2,2-diphenyl-1-picrylhydrazyl (DPPH); (±)- α -tocopherol (vitamin E); and (+)- γ -tocopherol (vitamin E) were purchased from Sigma-Aldrich (St. Louis, MO), and phosphate buffer (pH 7.4) was purchased from Fisher Scientific (Pittsburgh, PA). All the solvents used were of high-performance liquid chromatography (HPLC) grade.

The general process for sample preparation followed the method of Villarreal-Lozoya et al. (2007). Pecan kernels were defatted with hexane using a homogenizer (Model CH-6010; Kinematica, Bohemia, NY). The hexane kernel homogenates were centrifuged at 1500 relative centrifugal force (RCF) for 15 min (Model Centra 7, IEC) and supernatants were collected. The resulting cakes were sequentially defatted two more times, and the resulting hexane supernatants were pooled. A stream of nitrogen was used to remove the hexane solvent and obtain the lipophilic fraction. The cakes were dried under a stream of nitrogen until no detectable difference in mass occurred. The cakes were then gently broken up into a powder. For each cake sample, ≈ 1 g was placed into a 50-mL Falcon tube and immersed in 20 mL of

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acetone:water (7:3, v/v) solution. The Falcon tubes were then placed in an oscillatory shaker (Model 3090; Laboratory-Line Instruments Inc., Melrose Park, IL) and shaken overnight at 260 rpm at 4 °C. Extracts were clarified by centrifugation at 1500 RCF and supernatants were collected, flushed with nitrogen, and stored at -20 °C.

Lipophilic extract analysis. Oil contents were calculated by dividing the weights of lipophilic extractions by the weights of pecan kernels. Kernel oil (≈ 50 mg) was weighed into a glass vial, followed by adding 1.5 mL of 0.2 N potassium hydroxide (KOH) in methanol. Samples were vortexed for 30 s, placed in a water bath at 50 °C for 30 min, and vortexed every 10 min. The reaction was stopped by adding 0.5 mL of 1 M glacial acetic acid and 1 mL of hexane containing an internal fatty acid methyl ester (FAME) standard (C23:0 at 50 $\mu\text{g}\cdot\text{mL}^{-1}$). An aliquot of the hexane layer was removed and diluted 100-fold into a 2-mL autosampler vial, and 2- μL injections were chromatographed on a J&W DB 23 column 30 m $\times$ 0.25 mm diameter $\times$ 0.25 μm film (Agilent Technologies, Santa Clara, CA) with helium as the carrier gas. The initial oven temperature was 80 °C with a 20 °C per minute ramp up to 220 °C and held for 6 min for a total run time of 13.3 min. Chromatographic signals were matched to a 37 component standard mix from Supelco (Sigma-Aldrich, St. Louis, MO). The C23:0 FAME was used as an internal standard for the quantification of the fatty acids present in the pecan oil.

Antioxidant analysis. Antioxidant capacity was quantified according to the method of Brand-Williams et al. (1995) with modifications. Acetone extracts of the cakes were diluted in methanol, and 13- μL aliquots were pipetted into each well of a 96-well flat-bottom plate (Model 781662; putrGrade Wertheim, Germany). The reaction was started with the addition of 247 μL of 0.05 $\text{g}\cdot\text{L}^{-1}$ DPPH in methanol. Plates were sealed with two layers of parafilm and stored in dark for 24 h. The 96-well flat-bottom plate absorbance values were measured at 515 nm in a microplate spectrophotometer (Model 384PLUS; SPECTRAmax, Molecular Devices, Sunnyvale, CA). Blanks comprised solvent and DPPH reagent. A standard curve was prepared using Trolox as a reference. Antioxidant capacity was expressed as milligrams Trolox equivalents per gram of defatted kernel sample ($\text{TE mg}\cdot\text{g}^{-1}$).

Hydrophilic antioxidant capacity analysis was based on the BioTek ORAC Assay Application Note (Held, 2006). Briefly, fluores-

cein sodium salt (FL) was used as a probe and AAPH as a free radical generator. A set of Trolox dilutions was used as a standard. FL stock solution (FL1) was prepared by dissolving 77.8 mg FL in 50 mL of 75 mM phosphate buffer (pH 7.4) and diluted 1000 times using a volumetric flask (final concentration 4×10^{-3} mM), and wrapped in foil stored at 5 °C. Immediately before use, a second FL solution (FL2) was made by diluting 10 μL of FL1 in 10 mL of 75 mM phosphate buffer (pH 7.4). AAPH (130 mg) was dissolved in 2 mL of 75 mM phosphate buffer (pH 7.4) and made fresh daily. Clear-bottom 96-well black plates (Costar#3631; Corning, Inc., NY) were used for testing and the exterior wells were filled with 300 μL of water to provide large thermal mass. To each of the interior wells, 150 μL of FL2 were added. Aliquots of 25 μL of 75 mM phosphate buffer (pH 7.4), Trolox dilution, or extract were added to the blank, standard, and sample wells, respectively.

The plate was incubated for 30 min in the Multi-Detection Microplate Reader (BioTek Instruments, Inc., Winooski, VT) at 37 °C. Reactions were initiated by the addition of 25 μL of AAPH solution for a final reaction volume of 200 μL . Fluorescence was monitored kinetically using a 485/20 nm excitation filter and 528/20 nm emission filter. Well fluorescence values were collected at 1 min intervals for 1 h.

Tocopherol analysis. Tocopherol content was determined as explained by Mendoza et al. (2003) and Villarreal-Lozoya et al. (2007). Two milliliters of methanol were added to ≈ 0.5 g pecan oil and vortexed for 1 min. Five hundred μL of hexane were added and vortexed again. Samples were then centrifuged at 13,000 rpm for 30 min and 1 mL of the top layer was sampled. α -tocopherol and γ -tocopherol standard solutions were made from 500 ppm to 0.98 ppm by stepwise dilution with methanol. Methanol was used as a blank. Twenty microliters of each sample were injected into the HPLC (Waters 2695 Separations Module with Waters 996 Photodiode Array Detector; Waters Corporation, Milford, MA), which was equipped with a C18 column (5 μm particle size, 4.6 $\times$ 250 mm) and a guard column of the same chemistry. An isocratic flow of 1 $\text{mL}\cdot\text{min}^{-1}$ of HPLC grade methanol was used as the mobile phase. Peak spectra were collected between 250 and 400 nm. Chromatograms were extracted at 295 nm and used for identification and quantification.

Statistical analyses. The data were processed employing SAS software version 9.4 (SAS Institute Inc., 2016). The data were

analyzed as a RCBD with repeated measures with fixed block effects. Tree group (Levels: 'Wichita' 2014, 'Wichita' 2015, and 'Western' 2015) was the repeated factor and an unstructured covariance with the experimental unit as the subject was fitted to account for correlations among the three measurements. When the treatment (tree group) main effect was significant but the tree group $\times$ treatment interaction was not, then means of each treatment (tree group) were estimated and compared with least squares means (LSMEANS). When the interaction was significant, slices were used to compare treatments within each tree group; treatment means were compared only within tree groups with significant slices. Statistical significance level was 0.1.

Results and Discussion

Kernel oil. Across Zn treatments, average total oil content of 'Wichita' kernels was ($\text{mean} \pm \text{SE}$) $70.3 \pm 0.5\%$ in 2014 and $73.6 \pm 0.3\%$ in 2015 (Table 1). In 2015 'Western' kernels it was $74.5 \pm 0.8\%$ across Zn treatments (Table 1). These are in close agreement with previously published data for pecan (Bhagwat et al., 2013; Venkatachalam et al., 2007).

Kernel total oil contents exhibited an increasing trend with increasing Zn (treatment main effect $P = 0.0433$; Table 2). The Zn1 treatment mean kernel oil content was estimated to be $2.45 \pm 0.98\%$ points ($P = 0.0471$) higher than that of the untreated control while the Zn2 treatment mean was $3.11 \pm 0.98\%$ points ($P = 0.0196$) higher than that of the control. Assuming the 28 g serving size for pecan used by the USDA National Nutrient Database (USDA, 2016), a consumer eating pecan kernels would ingest ≈ 0.9 g more oil per serving if the nuts came from trees grown under Zn2 treatment than if they were grown under the untreated control conditions.

Our data showing an increase in total oil content in pecan kernels due to Zn fertilization had some parallels with previous results from pecan, as well as from other oil seed and nut crops. Ashraf et al. (2013) reported an increase in 'Western' pecan kernels from 56.1% oil for an untreated control to 62.6% with two early season foliar ZnSO_4 sprays and to 67.3% with two foliar ZnSO_4 + urea sprays. Interestingly, in that study, they showed effects of foliar Zn sprays on kernel oil content even though the leaf tissue Zn concentration of the control trees was 190.5 ppm, well above the widely accepted recommended minimum threshold for leaf tissue Zn

Table 1. Pecan kernel content of lipophilic components that varied significantly by cultivar or year.

Tree group		Fatty acids ²				
Yr	Cultivar	Oil content (% w/w)	SFA (%)	MUFA (%)	PUFA (%)	γ -tocopherol ($\mu\text{g}\cdot\text{g}^{-1}$)
2014	Wichita	70.3 b ³	14.3 b	71.2 a	14.5 b	85.9 a
2015	Wichita	73.6 a	16.7 ab	56.6 b	26.7 a	55.1 b
2015	Western	74.5 a	16.4 a	53.2 c	30.4 a	56.6 b

²SFA= saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. Data are means given as a percentage of total fatty acids detected as their respective fatty acid methyl esters.

³Within a column, estimates sharing the same letter do not differ at $P \leq 0.10$.

Table 2. Pecan kernel content of lipophilic components that varied significantly among soil applied zinc treatments (0, 2.2 and 4.4 kg·ha⁻¹ Zn for control, Zn1, and Zn2, respectively) across tree group (cultivar-year).

Treatment	Oil content (% w/w)	MUFA ^z (%)	γ-Tocopherol (μg·g ⁻¹)
Control	70.9 b ^y	59.4 b	59.2 b
Zn1	73.4 a	61.7 a	71.1 a
Zn2	74.0 a	59.8 b	67.2 a

^zMUFA = monounsaturated fatty acids given as percentage of total fatty acids detected as their respective fatty acid methyl esters.

^yWithin a column, estimates sharing the same letter do not differ at $P \leq 0.10$.

concentration in foliar treated trees of ≈ 50 ppm (Heerema, 2013; Pond et al., 2006; Sparks, 1993; Wells and Harrison, 2017). Huang et al. (2019) showed that a single early-season application of a 0.4% ZnSO₄ solution, but not a 1.6% ZnSO₄ solution, significantly increased total oil content of mature ‘Western’ pecan kernels from 64.7% (for a purified water sprayed control) to 70.2% when it was sprayed only on the leaves adjacent to the developing fruit or 74.1% when it was sprayed only on the shuck of the developing fruit.

For sunflower (*Helianthus annuus*), Mirzapour and Khoshgoftar (2006) showed an increase in seed oil content with soil applications of 10 or 20 kg·ha⁻¹ Zn (as ZnSO₄) compared with an untreated control. However, soil Zn application rates higher than 20 kg·ha⁻¹ reduced sunflower seed oil content and foliar Zn sprays had little or no effect on oil content. Rose et al. (1981) reported that foliar sprays of Zn fertilizer increased oil content in soybean (*Glycine max*), but responses were different among the four varieties tested. Soil application of ZnSO₄ fertilizer to mature ‘Tombul’ hazelnut trees growing in a moderately acidic soil (soil pH 5.7) significantly increased kernel oil concentration even at application rates as low as 0.2 kg·ha⁻¹ Zn, but higher Zn application rates did not give further increases in kernel oil concentration (Özenç and Özenç, 2015).

As shown in Table 2, kernels harvested from trees given the Zn1 treatment had a significantly higher proportion of monounsaturated fatty acids (MUFAs) than either Zn2 and control (treatment main effect $P = 0.0947$), but Zn2 was not different from the control. In our experiment, there was no significant difference for saturated fatty acids (SFAs) proportion among the soil Zn EDTA treatments and control (data not presented), which is in agreement with the results of Huang et al. (2019), who applied ZnSO₄ sprays to ‘Western’ pecan leaves and shucks. We furthermore found no statistically significant difference for polyunsaturated fatty acids (PUFAs) proportion among the soil Zn EDTA treatments and control (data not presented). The overall levels of total unsaturated fatty acids (MUFAs plus PUFAs) occupied $\approx 86\%$ of total FAMES in the 2014 ‘Wichita’, 83% in 2015 ‘Wichita’, and 84% in 2015 ‘Western’ (Table 1). MUFAs were predominant over PUFAs in pecan kernel samples and were found to comprise $71.2 \pm 0.4\%$, $56.6 \pm 1.0\%$, and $53.2 \pm 0.7\%$ of total FAMES for 2014 ‘Wichita’, 2015 ‘Wichita’, and 2015 ‘Western’, respectively (Table 1).

In our study, the MUFA oleic acid (C18:1) was the most abundant fatty acid overall. Linoleic acid (C18:2) was the predominant PUFA followed by linolenic acid (C18:3) (data not shown). Fatty acid composition of pecan oil extracts from the current study were generally similar to those previously reported in the literature (Malik et al., 2009; Venkatachalam et al., 2007). As a source of MUFAs and PUFAs pecan is comparable with peanut (*Arachis hypogaea*), which contains 13.6% of SFAs, 52.8% of MUFAs, and 33.6% of PUFAs (USDA, 2016). The MUFA content in the pecans from this study is comparable with almond, which contains 8.0%, 66.2%, and 25.8% of SFAs, MUFAs and PUFAs, respectively (USDA, 2016).

Concerning PUFAs, omega-3 fatty acids in the diet are essential for normal human growth and development. Furthermore, they may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, and other inflammatory and autoimmune diseases. In our data, omega-3 fatty acids represented a relatively minor constituent of pecan oil, being mainly comprised of alpha-linolenic acid (ALA; C18:3n3) (data not presented).

In walnut, a species closely related to pecan, Verardo et al. (2013) showed an increase of linoleic acid and decrease of linolenic acid content with high amounts of nitrogen fertilization, but there exist few investigations characterizing Zn fertilization effects on omega-3 fatty acids content in tree nuts. Huang et al. (2019) showed a statistically significant reduction in the proportion of linolenic acid in ‘Western’ pecan kernels with application of 0.4% ZnSO₄ sprays to leaves or shucks (1.1% linolenic acid) compared with a purified water-treated control (1.4% linolenic acid) but saw no effect on linolenic acid from application of 1.6% ZnSO₄ sprays. In our study, significant effects from soil applied Zn on omega-3 fatty acids were not detected ($P > 0.10$; data not presented). However, tree groups differed ($P = 0.0025$) with ‘Wichita’ in 2014 having a higher percentage of fatty acids as omega-3 fatty acids than either ‘Wichita’ in 2015 or ‘Western’ in 2015 ($1.45 \pm 0.06\%$ of total FAMES vs. $1.15 \pm 0.11\%$ and $1.01 \pm 0.04\%$, respectively).

Tocopherols. Tocopherols are known antioxidants (Huang et al., 2005; Jiang et al., 2001), and γ-tocopherol is the most abundant tocopherol in pecan kernels. For ‘Wichita’ kernels, γ-tocopherol contents were $85.9 \pm 3.6 \mu\text{g}\cdot\text{g}^{-1}$ in 2014 and $55.1 \pm 2.2 \mu\text{g}\cdot\text{g}^{-1}$ in 2015; for ‘Western’ kernels in 2015, γ-to-

copherol content was $56.6 \pm 3.0 \mu\text{g}\cdot\text{g}^{-1}$ (Table 1). A significant treatment main effect ($P = 0.0525$) was detected with the γ-tocopherol content for the Zn1 treatment $11.90 \pm 3.83 \mu\text{g}\cdot\text{g}^{-1}$ ($P = 0.0209$) higher than for the untreated control (Table 2). The γ-tocopherol content for Zn2 treatment was estimated to be $7.95 \pm 3.83 \mu\text{g}\cdot\text{g}^{-1}$ ($P = 0.0832$) higher than for the untreated control (Table 2). The γ-tocopherol results were similar to those previously reported by Wyatt et al. (1998) and Villarreal-Lozoya et al. (2007). In this study, we did not detect any α-tocopherol in the pecan kernels (data not shown). Interestingly, ‘Wichita’ kernels in the first season contained a higher proportion of tocopherol with respect to total oil content than in the second season (Table 1).

Previous studies have reported that most people in the United States failed to meet the current recommendations for vitamin E intake of 15 mg·d⁻¹ for most life stage groups (Maras et al., 2004; Monsen, 2000). Unlike α-tocopherol, γ-tocopherol could possess anti-inflammatory properties through inhibition of cyclooxygenase activity (Jiang et al., 2001). Furthermore, studies indicated that γ-tocopherol is a more effective trap for lipophilic electrophiles than α-tocopherol (Jiang et al., 2001). Thus γ-tocopherol may contribute to human health via a separate mechanism from that of α-tocopherol. On the basis of the research conducted by Hudthagosol et al. (2011), the concentration of γ-tocopherol in human plasma doubled 8 h after ingestion of pecan test meals. The higher the concentration of γ-tocopherol in the pecan nuts, the more γ-tocopherol accumulates in plasma after consumption, presumably increasing the health benefit.

Hydrophilic antioxidant capacity. Whereas for ORAC, the treatment × tree group interaction was significant ($P = 0.0199$), no slices comparing treatments within tree groups were significant (Fig. 1). According to the DPPH assay, antioxidant capacity was significantly lower for defatted ‘Wichita’ kernels in 2014 than for either ‘Wichita’ in 2015 ($P < 0.0001$) or ‘Western’ ($P = 0.0001$) kernels in 2015, and the average antioxidant capacity was significantly higher for defatted ‘Wichita’ kernels in 2015 than for ‘Western’ kernels in 2015 ($P = 0.0105$; data not presented). Average antioxidant capacities for defatted ‘Wichita’ kernels were 37.9 ± 0.8 and $74.6 \pm 0.9 \text{ TE mg}\cdot\text{g}^{-1}$ in 2014 and 2015, respectively, and for defatted ‘Western’ kernels DPPH values were $63.2 \pm 3.4 \text{ TE mg}\cdot\text{g}^{-1}$ in 2015. However, neither the Zn treatment main effect nor the

Fig. 1. Antioxidant capacity (AC) tested by oxygen radical absorption capacity (ORAC) assay of pecan kernels with different Zn treatments. (A) 2014 'Wichita' pecan kernels. (B) 2015 'Wichita' pecan kernels. (C) 2015 'Western' pecan kernels. Error bars represent SE ($n = 4$). X-axis shows soil applied Zn ethylenediaminetetraacetic acid treatments, Zn2, Zn1, and control representing 4 kg·ha⁻¹ Zn, 2 kg·ha⁻¹ Zn, and no Zn fertilizer, respectively. Y-axis represents antioxidant capacity with micromoles trolox equivalent per milligram kernel (TE $\mu\text{mol} \cdot \text{mg}^{-1}$) as unit. Within cultivar and year, means accompanied by the same letter are not significantly different ($\alpha = 0.1$).

Zn treatment $\times$ tree group interaction were significant ($P > 0.10$; data not shown).

Six pecan cultivars—Desirable, Kanza, Kiowa, Nacono, Pawnee, and Shawnee—

were evaluated for kernel antioxidant capacity by Villarreal-Lozoya et al. (2007). In that study, DPPH values ranged from 81 to 135 TE $\text{mg} \cdot \text{g}^{-1}$ defatted kernel, whereas ORAC

values ranged from 372 to 817 TE $\mu\text{mol} \cdot \text{g}^{-1}$ defatted kernel. Both DPPH and ORAC values were higher than our results.

With the ORAC method, antioxidants react with or neutralize free radicals generated in the assay and the antioxidant capacity is the reflection of the ability to inhibit the peroxy radical from oxidation (Blomhoff et al., 2006; Ou et al., 2001; Prior et al., 2005). However, the DPPH assay is based on an electron transfer reaction (Huang et al., 2005), where the odd electron-containing nitrogen atom within the DPPH molecule is reduced. These basic differences between the ORAC and DPPH techniques may help explain differences in results for these techniques in our experiments.

Along with its importance for plant growth and development (Broadley et al., 2012), Zn has been noted to have a critical role in defense against reactive oxygen species (Cakmak, 2000). In pecan, Zn fertilizer application and higher leaf tissue Zn concentrations up to ≈ 14 –22 $\text{mg} \cdot \text{kg}^{-1}$ (by dry weight) are associated with increasing chlorophyll content and photosynthesis rate (Heerema et al., 2017; Hu and Sparks, 1991), which would likely support the biosynthesis of antioxidants and an increase in antioxidant activity. Studies have shown that foliar or soil application of Zn improved the total phenolic content and increased the antioxidant enzyme activity in other crop species, including wheat (*Triticum aestivum*; Ma et al., 2017), grape (*Vitis vinifera*; Song et al., 2015), and pistachio (Tavallali et al., 2010).

There are reports of other mineral nutrients also affecting the antioxidant content in assorted horticultural crops (Anttonen et al., 2006; Jeppsson, 2000; Wold and Opstad, 2007). Alizadeh et al. (2010) described how fertilization with a complete fertilizer increased antioxidant activity in summer savory (*Satureja hortensis*) by DPPH analysis. Verardo et al. (2013) demonstrated that N fertilization had a significant negative effect on the phenolic compounds in walnut kernel samples.

Conclusion

Fertilizer application and plant mineral nutrition studies rarely consider effects on human health-promoting components (Bagheri et al., 2014; Maggio et al., 2008), but in this study, we analyzed the effect of soil Zn fertilizer applications on pecan kernel nutraceutical properties, including oil yield, fatty acids profile, γ -tocopherol content, and hydrophilic antioxidant capacities. Although not a primary objective of this study, it is interesting to note that for most biochemical observations, tree group (cultivar-year) differences were detected. In most cases, the 2014 'Wichita' group differed from the 2015 groups, suggesting that year-to-year variability might be greater than the differences between the two cultivars in this study. Even for variables where the treatment main effect was significant, magnitudes of differences among tree groups exceeded differences among the treatment groups.

Assuming the standard serving size of kernels, the maximum hydrophilic ORAC antioxidant capacities found in this study would be equivalent to about 5516 μmol of TE per serving, which is about the same as the recommended daily intake (Wu et al., 2004). Thus, a single serving of pecans as a regular part of a diet may meet all of an individual's daily antioxidant requirements. But these requirements could vary considering an individual's situation.

Soil applied Zn-EDTA treatments on pecan trees increased kernel oil yield and γ -tocopherol contents. In conclusion, soil application of Zn fertilizer may positively affect human health-promoting aspects of pecan kernels, which would be valuable to pecan distributors for product promotion amongst health-conscious consumers. Our data also highlight that further research is needed into how and to what extent nutraceuticals vary in mature trees with biennial fluctuation in crop load.

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