ZINC MANAGEMENT AND SALT TOLERANCE OF PECAN IN ARID REGIONS

by

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Abstract

In the alkaline, calcareous soils common to the southwest zinc reacts with hydroxyl and carbonate groups forming compounds of low solubility, reducing its plant availability, and making soil application of zinc oxide (ZnO) or zinc sulfate ($ZnSO_4$) impractical. Therefore, foliar application of zinc in southwestern pecan orchards is common practice. Fertigation with zinc-ethylenediaminetetraacetic acid (Zn-EDTA) is a possible alternative that has shown positive results in alkaline, calcareous soils. However, many growers fertigating their orchards with Zn-EDTA are still using supplemental zinc foliar sprays due to lack of confidence that soil applied Zn-EDTA can supply enough zinc to the trees. We conducted an experiment to determine if the application of foliar zinc sprays to 'Wichita' pecan trees already receiving zinc in the form of Zn-EDTA through fertigation would increase photosynthesis rates. We applied zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$), $ZnSO_4 \cdot H_2O$ + Urea Ammonium Nitrate (UAN), Zn-EDTA, water alone, and water + UAN to seven 'Wichita' pecans growing in alkaline, calcareous soil in San Simon, AZ. Applications were made twice in 2018 and twice in 2019, Zn-EDTA was applied only in 2019. Photosynthesis measurements were taken approximately two to four weeks following each application. Mid-day stem water potential was also measured to verify that water stress was not limiting photosynthesis. Our results showed that photosynthesis rates were not increased by the application of supplemental foliar zinc sprays in trees fertigated with Zn-EDTA with mean leaf zinc concentrations of untreated leaves in the range of 16-21 $mg \cdot kg^{-1}$. We concluded that photosynthesis was not zinc limited and that no additional benefit was conferred with regard to photosynthesis from the application of supplemental foliar zinc sprays.

Another problem for pecan growers in the southwest is high salt content in the soil. Very little experimentation has been conducted to determine pecan response to saline-sodic conditions. To contribute to this research, we performed an experiment with seven rootstock pecan seedlings grown in alkaline, calcareous, saline-sodic soil at the Safford Agricultural Center in Safford, AZ. The seedlings were chosen from different geographic regions. While we only have knowledge of the maternal genetics of the seedlings which were grown from open-pollinated seed, we hypothesized that seedlings with origins in regions with lower precipitation would be more tolerant of the experimental conditions than those from regions with higher precipitation. It was determined that leaf sodium concentration was more strongly correlated with salt injury in the plants than chlorine. Leaf potassium:sodium (K:Na) ratio was strongly correlated with resistance to salt injury, tree growth rates, and vigor. In support of our hypothesis we found the maternal parentage of the most tolerant seedlings in our experiment was 'Elliott', a cultivar with likely Mexican origins (although the 'Elliott' cultivar was a seedling selection from Florida). 'Elliott' generally outperformed the other seedlings in visual observations of resistance to salt injury and overall plant vigor and stood out with the greatest growth each year and cumulatively throughout the study. 'Elliott' had the highest K:Na ratio in 2019, shared the highest K:Na ratio in 2020 with 'VC1-68', was among the seedlings with the lowest leaf sodium concentrations during both years, and had the second lowest mortality of all the seedlings chosen.

Another issue that pecan growers face is tree to tree variability that is reflected in nutrient acquisition within an orchard. It is important to have knowledge of variability so that each tree receives adequate nutrition. The orchard block mean leaf nutrient concentration should be high enough that all individual trees receive adequate nutrition. Practical leaf sampling of orchards requires sampling only a small portion of the trees randomly, and provides a mean value from which it is difficult to determine minimum (or maximum) nutrient concentrations extent in the sampled orchard block. To address this issue, a two-year experiment was conducted in an orchard in San Simon, AZ. The experimental plot consisted of 'Wichita' pecan pollinated with 'Western' (every fourth row). Soil and leaf samples were collected each year. Trunk measurements were made in the dormant seasons. Photosynthesis measurements of the 'Wichita' trees were made in 2019. The data were analyzed to determine magnitude and patterns of variability. Nutrient uptake varied between the cultivars. A lower mean and more variability in leaf zinc concentrations was found among the 'Wichita' trees than 'Western' during both years. We concluded that due to lack of variability sources within the orchard block, as well as finding little difference in row to row average mean leaf zinc concentrations, or in average mean leaf zinc concentrations of tree position within rows in either year, that position in the field was not the primary source of variability in leaf zinc concentration. From our 2019 data set we determined that a mean leaf zinc concentration of approximately 25 $mg \cdot kg^{-1}$ was needed to ensure that no more than 5% of the trees would fall below a target mean leaf zinc concentration (determined from previous research) of 15 $mg \cdot kg^{-1}$. This concentration is significantly lower than many published recommendations. Further, using the leaf nutrient with the highest coefficient of variance (zinc) for 2019 we determined a range of sample sizes and their associated relative margins of error from the true population mean. A sample size of 35 trees with a relative margin of error of 10% from the true population mean at 95% confidence is recommended for practical sampling purposes.

Introduction

The United States and Mexico are the world's top pecan (*Carya Illinoinensis* (Wang.) K. Koch) producing countries. Pecans are a relatively small, but important agricultural commodity in the United States, with production in 2018 totaling 221 million pounds with a value of $423 million (National Agricultural Statistical Services, 2019). Pecans are grown in native groves and in orchards of improved cultivars in many southern states, including Alabama, Arizona, Arkansas, California, Florida, Georgia, Kansas, Louisiana, Missouri, Mississippi, New Mexico, North Carolina, South Carolina, and Texas. In the eastern United States, the soils are generally acidic whereas in the west, most soils are alkaline. This difference in soil conditions requires crop management that is tailored to the demands of the location. Fertilizer formulations, rates, and methods of application as well as irrigation practices vary depending on the particular soil characteristics and growing environments.

Literature Review

Importance of Zinc in Pecan

Pecans require relatively high concentrations of zinc, especially during the short period of shoot elongation (Sparks, 1993). They can also tolerate applications of zinc that would cause toxicity in many other plants (Worley, 2002). Hafeez et al. (2013) noted that zinc activates enzymes that play a role in many important plant functions including carbohydrate metabolism, protein synthesis, maintenance of the integrity of cell membranes, regulation of auxin synthesis, and pollen synthesis. Zinc is important in the regulation of photosynthate transfer from the chloroplast to the cytoplast, an essential phase in the movement of photosynthates to high demand sinks such as developing fruit (Hounnou et al., 2019).

Zinc has also been reported to aid in tolerance to saline soil conditions. Saline and sodic soil conditions create oxidative stress and zinc promotes the synthesis and activity of anti-oxidative enzymes (Cakmak, 2000; Weisany et al., 2012). Zinc has been shown to have a positive correlation with increased K:Na ratios in higher plants. Saxena and Rewari (1990) found that the addition of 5 $mg \cdot kg^{-1}$ of zinc applied as $ZnSO_4 \cdot 7H_2O$ to a washed river sand medium decreased sodium uptake by 70% and increased potassium uptake by 10% in chickpea (*Cicer arietinum L.*) seedlings growing in saline sand. In an experiment with barley (*Hordeum vulgare L. cv Herta*) grown hydroponically, Norvell and Welsh (1993) reported that addition of zinc as Zn-HEDTA applied to the nutrient solution resulted in retention of sodium in the root and decreased sodium in the shoot. A higher K:Na ratio may be related to plant salinity tolerance (Munns, 2002; Wakeel 2013). This may be of particular importance in the southwestern United States where both zinc deficiency and saline and sodic soil conditions are common.

Zinc Deficiency

Zinc deficiency in pecan trees is a major problem throughout the United States and is particularly pronounced in the southwestern part of the country due to the presence of high pH, calcareous soils. Under these conditions zinc reacts with soil hydroxyl and carbonate groups forming compounds of low solubility, reducing availability to plants (Udo et al., 1970). Based on the findings of several researchers, Camp (1945) determined that a critical point for decrease in available zinc is between a pH of 5.5 and 6.5. Water-extractable soil zinc increased from 12 to

394 mg·kg^{-1} as soil pH was lowered from 8.0 to 4.0 in a calcareous south Texas soil (Fenn et al., 1990).

Visual symptoms of zinc deficiency include wavy leaf margins, interveinal chlorosis, necrosis, and reduced shoot length. “Rosette disease” is a term used to refer to reduced shoot internode length that results from severe zinc deficiency that can cause tightly clustered branches to appear as a rosette. Rosetting was first reported in pecan trees growing in Sacaton, Arizona in 1911 according to Finch and Kinnison (1934).

Zinc deficiency results in many adverse effects within the pecan tree. These include low leaf chlorophyll concentration (Ojeda-Barrios et al., 2012) which affects photosynthesis, and consequently many other facets of pecan health. Leaf zinc concentrations below 14-22 mg·kg^{-1} have been reported to result in a reduction of carbon assimilation rates in pecan (Heerema et al., 2017; Hu and Sparks, 1991). Zinc deficiency has deleterious effects on production of pistillate and staminate inflorescences, thereby reducing the number of nuts set and matured, delaying shuck dehiscence, and reducing the final weight of nuts (Hu and Sparks, 1990). The use of microscopy has shown that leaves of zinc deficient trees have shorter palisade cells, more intercellular spaces, and reduced leaf thickness and surface area compared to leaves with adequate zinc nutrition (Ojeda-Barrios et al., 2012). Zinc deficiency influences membrane integrity, increasing root exudation of organic materials, resulting in an increased possibility of pathogens (Cakmak and Marchner, 1988). Wheat (Triticum aestivum L.) plants with zinc deficiency have been shown to be more susceptible to soil borne pathogens (Sparrow and Graham, 1988).

The discovery that rosette was a symptom of zinc deficiency was made in 1932 (Finch, 1932; Alben et al., 1932). Researchers had been injecting iron salts into dormant trees, applying iron salts to the soil, making foliar applications of iron salts to rosetted trees, and dipping affected branches in iron salt solutions with no results. In a few trials positive results were obtained when containers made from galvanized iron were used. It was found that the iron salts used in the containers contained significant amounts of zinc. Following this discovery, experiments were conducted comparing treatments in which branches with rosette symptoms were dipped in solutions containing $ZnSO_4$, ZnCl, or iron sulfate salts. The zinc treatments eliminated rosette symptoms whereas iron treatments did not (Alben et al., 1932). Since this discovery, management techniques have been developed to minimize zinc deficiency, particularly in non-native areas of the southwest United States with high pH, calcareous soils. Pecans growing in orchards in native pecan areas typically experience more acidic soils, and are less likely to experience severe zinc deficiency, although zinc deficiency occurs even in acidic pecan-growing areas (Wood, 2007).

Leaf Zinc Concentrations

The minimum acceptable concentration of zinc in pecan leaves is frequently cited as greater than 40 to 60 $mg \cdot kg^{-1}$. Sparks (1993) determined by modeling data of four measured indices (nut yield, percent of trees without visible deficiency symptoms, vegetative growth, and trees without deficiency symptoms plus nut yield) gathered from both published and unpublished sources that the responses of all indices measured were similar. His analysis indicated that a leaf zinc concentration of greater than approximately 50 $mg \cdot kg^{-1}$ was needed to avoid visible deficiency symptoms or reduction in nut yield or vegetative growth. In a six year experiment in Byron, Georgia, Sparks and Payne (1982) tested the reliability of a commonly recommended sufficiency range of 50-100 mg·kg^{-1} leaf zinc concentration for pecan and found that the mean leaf zinc concentration needed to be maintained at 50 mg·kg^{-1} for all trees to be completely free of visible symptoms of zinc deficiency. Sufficiency ranges in Oklahoma for native groves and both low and high input cultivar orchards were determined to be 60-150 mg·kg^{-1} (Smith et al., 2012). O'barr et al. (1978) indicated that an adequate range of foliar leaf zinc for pecan in the state of Louisiana was 50-150 mg·kg^{-1}. Heerema (2013) classified leaf zinc concentrations of 50 to 100 mg·kg^{-1} as "adequate" for pecans growing in New Mexico. Storey et al. (1971) recommended a leaf zinc threshold value of 60 mg·kg^{-1} for peak production of pecan in Texas and a critical level of 40 mg·kg^{-1} was identified by Worley et al. (1972) for Georgia pecans. "Normal" leaf zinc concentrations from high yielding trees growing in commercial pecan orchards in Arizona were reported to be 86 to 256 mg·kg^{-1}, although the surveyed trees received foliar zinc sprays which may have elevated assayed foliar zinc concentrations (Pond et al., 2006). Differences in sufficiency levels from state to state may not be true differences of tree need, but a reflection of the trees' response to wide ranges of conditions associated with different geographic regions (Smith et al., 2012).

In many cases no visual symptoms are observable in trees with leaf zinc concentrations below 50 mg·kg^{-1} (Nunez-Moreno et al., 2009). Kim et al. (2002) reported no visual symptoms in pecans with zinc concentrations as low as 11.2 mg·kg^{-1}. Similarly, Hu and Sparks (1990) reported leaves with zinc concentrations of approximately 14 mg·kg^{-1} to be normal and free of visual deficiency symptoms. Ojeda-Barrios et al. (2014) found that sampled trees showing deficiency symptoms had leaf zinc concentrations of 7.5 mg·kg^{-1}. Walworth et al. (2017) found trees with leaf zinc concentrations of 16-35 mg·kg^{-1} in an orchard fertigated with Zn-EDTA to be largely free of visual zinc deficiency symptoms. Because individual tree leaf zinc concentrations vary in an orchard and perhaps the concentration at which visual deficiency symptoms occur in various cultivars, an orchard block mean leaf zinc concentration needs to be maintained that will account for variability within the orchard.

A lack of visual zinc deficiency may not accurately indicate whether the trees' physiology is affected in ways that may reduce growth, fruiting, nut quality, and/or yield. Zinc deficiency without visible symptoms is referred to as "hidden hunger" (Heerema, 2013). Low zinc concentrations reduce rates of photosynthesis, thereby affecting overall plant health (Heerema et al., 2017; Hu and Sparks, 1991). At leaf zinc concentrations below 14-22 mg·kg^{-1} photosynthesis has been shown to be inhibited by a lack of zinc (Heerema et al., 2017; Hu and Sparks, 1991), well below the generally recommended leaf zinc concentration of greater than 40 to 60 mg·kg^{-1} noted above. Because of tree-to-tree variability in an orchard these higher recommendations are made to ensure that all trees in an orchard maintain adequate mean leaf zinc concentrations. Monitoring leaf zinc concentrations with regular leaf sampling, even in the absence of visible symptoms, is important so that appropriate measures can be taken to maintain sufficient leaf zinc concentrations, however, leaf zinc concentrations necessary in pecan to maintain optimal health remain unresolved. A deeper discussion of this topic appears later in this review.

Zinc Nutrient Relationships

Zinc nutrition can affect concentrations of other nutrients within the pecan tree (Kim et al., 2002). Some studies have shown that foliar zinc and manganese concentrations are interrelated. In trees with low concentrations of zinc, foliar manganese concentrations may be elevated (Kim et al., 2002; Hounnou et al., 2019). In contrast, Wood (2007) observed an increase in both foliar manganese and zinc levels with increasing amounts of soil banded zinc sulfate.

Kim et al. (2002) noted that tree responses to varying zinc levels were dependent upon tree cultivar. ‘Stuart’ seedstocks grown in hydroponic culture exhibited elevated foliar phosphorus, calcium, magnesium, sulfur, boron, and iron concentrations when zinc was withheld. On the other hand, ‘Curtis’ pecans grown under the same conditions exhibited only elevated foliar manganese concentrations.

Worley and Mullinix (1993) compared the mean leaf concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, zinc, manganese, iron, and copper among 40 pecan cultivars over a 3-year period. Although there were inconsistent differences in the concentration of many of the elements, no significant difference was found in leaf zinc concentrations among any of the cultivars. In a separate study conducted in Texas, Sparks and Madden (1977), also found no significant difference in leaf zinc concentrations among many of the same pecan cultivars.

A relationship between phosphorous and zinc has been noted in other crops (Fageria, 2001; Sumner and Farina, 1986). Saeed (1977) reported that zinc deficiency has been found in beans (*Phaseolus vulgaris L.*), soybeans (*Glycine max [L.] Merr.*), and citrus in soils that are treated with recurrent or large applications of phosphorus, and according to Kubota and Allaway (1972), plants grown in soils formed from phosphatic rock have a tendency to be zinc deficient. Dwivedi et al. (1975) found that applying high levels of phosphorus to maize (Zea mays L. *var. ganga-5*) resulted in zinc deficiency and reduction in yield. Cakmak and Marschner (1986) observed that zinc deficient cotton plants (Gossypium hirsutum L. cv. Deltapine 15/21) took up more phosphorus, resulting in high phosphorus concentrations in the leaves. Zinc sulfate ($ZnSO_4$) plus di-ammonium phosphate ($[NH_4]_2HPO_4$) applications were more effective at increasing both growth and yield than the sole application of $ZnSO_4$ to maize crops (*Zea mays L.*) grown in an alkaline calcareous soil with low zinc phytoavailability (Imran et al., 2016). Sparks (1988) found that the leaves of 11-year-old ‘GraBohls’ pecans contained reduced levels of zinc as a result of the soil application of 2.2 kg per tree of phosphorous on two of three sampling dates. However, no difference was seen in 7-year-old ‘Mahan’ pecan treated with the same rate of soil applied phosphorus for two consecutive years. These two cultivars were growing on different soil types, which may have been a contributing factor to the difference in results.

<u>Foliar Zinc Treatments</u>

Spraying zinc onto the tree foliage is the standard commercial method for supplying zinc to pecans. This method is used both for the rapid correction and continued maintenance of zinc deficiency in pecan. However, sprayed zinc is very immobile and translocation from the treated leaf is limited, so leaves that are not contacted directly by the spray do not benefit from foliar fertilization (Wadsworth, 1970). Therefore, new foliage must be treated with repeated sprays

(Swietlik, 2002). The actual proportion of zinc absorbed by the leaf is small, with only 0.6 to 1.2% of zinc applied to immature pecan leaves actually absorbed (Wadsworth, 1970).

Although $ZnSO_4$ is most commonly applied to pecan foliage, several other formulations are available. In a comparison of foliar fertilizers, Zn-EDTA (Zn-ethylenediamine tetra-acetic acid), Zn-DTPA (zinc-diethylenetriaminepenta-acetic acid), and $Zn(NO_3)_2$ were applied six times per season to 'Western' pecans grafted to native seedlings growing in a calcareous Domino silt loam soil over a three-year period (Ojeda-Barrios et al., 2014). Zn-EDTA was applied with formulations containing 50, 100, and 150 mg·L^{-1} of zinc. Zinc-DTPA, and $Zn(NO_3)_2$ formulations contained 100 mg·L^{-1} of zinc. All zinc spray treatments resulted in increased foliar zinc concentrations (Table 1.1), leaflet area, and chlorophyll content, but nut yield and nut quality were not affected. No clear advantage to any spray formulation was observed.

Table 1.1. Foliar zinc concentrations (mg·kg^{-1}) in pecan trees sprayed with Zn-EDTA, $Zn(N0_3)_2$, or Zn-DTPA (Ojeda-Barrios et al., 2014).

Treatment	**2007**	**2008**	**2009**
Control	5.7 c	14.0 b	20.6 b
50 mg·L^{-1} Zn-EDTA	10.1 a	22.8 ab	31.7 ab
100 mg·L^{-1} Zn-EDTA	13.3 a	20.0 b	28.5 ab
150 mg·L^{-1} Zn-EDTA	9.8 b	21.7 b	26.8 ab
100 mg·L^{-1} $Zn(N0_3)_2$	9.9 b	24.4 ab	35.7 a
100 mg·L^{-1} Zn-DTPA	10.1 ab	36.1 a	34.9 a

Alben (1962) applied Zn-EDTA (14.2% zinc) at material concentrations of 50, 120, 240, and 360 g/100 L of water and $ZnSO_4$ (36% zinc) at 240 g/100 L of water foliarly to zinc deficient 'Stuart' and 'Schley' pecans growing in an east Texas orchard. He found that the percent of recovery from rosette in the trees treated with Zn-EDTA increased with increasing spray concentration. Zn-EDTA was more effective when applied to ‘Stuart’ pecan at equal concentrations but $ZnSO_4$ gave better results when applied to ‘Schley’ trees. Zn-EDTA treatments at 360 g/100 L of water resulted in the greatest percent of recovery in both cultivars (90% in ‘Stuart’ and 82% in ‘Schley’).

In a greenhouse experiment, Pisani et al. (2020) compared the effectiveness of $ZnSO_4$ alone with $ZnSO_4$ plus a nanoparticle carrier when foliarly applied to two-year-old ‘Zinner’ and ‘Byrd’ pecan scions grafted to ‘Elliott’ seedling rootstock. A nanoparticle adjuvant and a surfactant were added to the spray mixtures. No significant differences were found in chlorophyll content or leaf zinc concentrations. Inconsistent results were observed in net carbon assimilation rates, transpiration rates, and stomatal conductance between the two cultivars. Nano-technology is relatively new and there are few applied studies evaluating the efficacy of this approach.

Adjuvants are often added to foliar sprays to enhance the penetration of nutrients, herbicides, fungicides, or pesticides. Adjuvants take the form of surfactants, oils, or nitrogen-compounds (Hock, 2018). Urea alone and UAN (urea ammonium nitrate) have been used successfully as adjuvants in foliar zinc fertilizer formulations. Hsu and Ashmead (1984) reported that urea penetrates plant leaves with a velocity higher than simple diffusion and that UAN enhances iron penetration into foliar tissues of corn (*Zea mays L.*). Worley (2002) indicated that nitrate included in spray mixtures enhances zinc uptake. Smith et al. (1979) found that application of zinc sulfate-urea-surfactant solutions resulted in significantly greater increase in leaf zinc concentrations than the application of zinc sulfate alone or chelated zinc (Oxyplex-Zn and Zn-EDTA).

In an experiment conducted on 15-year-old pecan trees, treatments of $ZnSO_4$ (86.3 grams of zinc per 100 liters of water) and $Zn(NO_3)_2$ (10.8 to 86.3 grams of zinc per 100 liters of water) were applied with or without 0.5% urea ammonium nitrate (UAN; by weight) for three consecutive years (Table 1.2; Smith and Storey, 1979). In two out of three years, the addition of UAN to $ZnSO_4$ resulted in higher leaflet zinc concentrations than $ZnSO_4$ alone. With the exception of the lowest concentration of $Zn(NO_3)_2$, the addition of UAN resulted in greater leaflet zinc concentrations than $Zn(NO_3)_2$ alone.

Table 1.2. Resulting foliar leaf zinc concentrations of pecan trees treated with $ZnSO_4$, $Zn(NO_3)_2$, and UAN during 1973. 1974, and 1975 (Smith and Storey, 1979).

			Zn concentration ($mg \cdot kg^{-1}$)		
Treatment	**Zn**	**UAN**	**1973**	**1974**	**1975**
Control	0	-	23a	42a	23a
$ZnSO_4$	86.3	-	84d	266de	263d
$ZnSO_4$	86.3	+	195f	304e	350ef
$Zn(NO_3)_2$	86.3	-	170ef	409f	369g
$Zn(NO_3)_2$	86.3	+	271g	588g	612g
$Zn(NO_3)_2$	43.1	-	68cd	147b	125c
$Zn(NO_3)_2$	43.1	+	151c	233cd	387cd
$Zn(NO_3)_2$	21.6	-	37ab	77a	74ab
$Zn(NO_3)_2$	21.6	+	70d	181bc	144c
$Zn(NO_3)_2$	10.8	-	38b	38a	59ab
$Zn(NO_3)_2$	10.8	+	57bd	60a	81ab

*Yield expressed as $kg \cdot m^{-2}$ of cross-sectional trunk area.

Young developing leaf tissue absorbs foliar zinc more rapidly than older mature leaves with waxy surfaces (Wadsworth, 1970). A typical foliar fertilization program in Arizona entails spraying zinc sulfate at bud-break and continuing until vegetative growth has lessened (Kilby, 1985). Depending on the method of pruning and the overall vigor of the trees, in the southwest four to six foliar annual applications is typical (Wood, 2007).

Walworth et al. (2006) made fall applications of $ZnSO_4$ to pecan trees to determine whether zinc applied just prior to leaf senescence is translocated into the phloem or bud tissue and reused the next growing season, potentially reducing the need for early season foliar zinc applications in the subsequent year. In trees sprayed in the fall, buds collected the following February showed elevated levels of zinc. However, the zinc content of leaf samples from fall sprayed trees collected during the growing season was not higher than unsprayed controls.

Soil Applied Zinc

Soil application can be an alternative to foliar spray and may provide longer-term correction of zinc deficiency. Ideally, soil application of zinc fertilizer can provide optimal zinc nutrition, eliminating, or greatly reducing the need for foliar applications of zinc. In contrast to zinc sprayed onto foliage, that absorbed through roots is able to move throughout the tree, enhancing zinc nutrition of organs other than the foliage (Wadsworth, 1970; Wood, 2007: Walworth et al., 2017). However, soil applied zinc must remain phytoavailable to be effective, a challenge in high pH, calcareous soils. Important factors affecting soil zinc availability are the form of zinc applied, method of application, and the properties of the soil.

Zinc in the form of Zn-EDTA is suited to soil application in high pH, calcareous soils. Zinc is complexed by the organic EDTA chelate molecule forming a compound that protects the zinc from binding with soil hydroxyls and carbonates and maintains higher zinc phytoavailability relative to un-chelated zinc. The stability of Zn-EDTA is partially dependent upon the presence of metals such as iron that can displace zinc in the metal-chelate complex (Cheng et al., 1972). If concentrations of zinc and iron are equal in the soil the reaction of EDTA with iron is favored over that of zinc. In high soil pH conditions, iron precipitation is favored, so Zn-EDTA is more stable and effective (Thorne, 1957). The stability constants of other zinc chelates suggest that they may be better suited for low or moderate pH soils. For example, Norvell and Welsh (1993) found that HEDTA zinc chelate was stable in a lower pH (6.1) that is suitable for barley.

In zinc deficient, high pH soils, the amount of contact zinc has with the roots is a primary factor effecting zinc uptake (Zhao et al., 2015). Available zinc must reach feeder roots present in shallow soil (Worley, 2002). Wood and Payne (1997) found that disking soil following application of zinc oxide dispersed the zinc, allowed more root contact, and increased the speed of zinc response, elevating leaf concentrations in two years compared to four years in un-disked soil. This demonstrates that the rate of zinc diffusion through the soil is important to understanding plant response to zinc fertilization. Diffusion rates of Zn-EDTA were found to be unhindered by a high pH or a high $CaCO_3$ soil content, whereas $ZnSO_4$ diffusion was greatly restricted in these soils (Modaihsh, 1990). Additionally, high clay content, organic matter, and CEC slowed $ZnSO_4$ diffusion. Zhao et al. (2015) similarly observed that Zn-EDTA had a greater diffusion rate than $ZnSO_4$ in a high pH, calcareous soil. Zinc sulfate was detected 15 mm from the application site at the end of a 30-day incubation period versus Zn-EDTA, which was found 25 mm from the application point. Although Zn-EDTA was applied at a rate one fifth that of $ZnSO_4$, the DTPA-extractable zinc in the soil increased by 20% upon addition of either Zn-EDTA or $ZnSO_4$.

The availability of zinc in soil is low in the southeastern United States in areas with well-drained sandy low pH soils, as well as on soils that formed from phosphatic rock (Kubota and Allaway, 1972; Wood, 2007). The low nutrient and water holding capacity in sandy soils and high amounts of rainfall in the southeast results in increased loss of zinc through leaching. In addition, low soil pH increases leaching of zinc. He et al. (2006) found that the amount of zinc leached from sandy soils in column leaching and batch extraction experiments decreased linearly as pH of the solution was increased from 3 to 9.

Regardless, most soils of the southeast are well suited for effective soil application of zinc fertilizers because of their low native pH (Maelstrom et al., 1984). In southeastern soils, ZnO and $ZnSO_4$ are comparable in their ability to raise foliar zinc concentrations to sufficient levels, but ZnO may be more cost efficient (Wood and Payne, 1997). The low water solubility of ZnO requires that it be ground to a fine powder, making it difficult to handle or spread evenly. Therefore, $ZnSO_4$ is preferable when the fertilizers used are dry material (Sutradhar et al., 2016). $ZnSO_4$ has a higher water solubility than ZnO that may contribute to making $ZnSO_4$ more rapidly available to tree roots (Wood and Payne, 1997; Worley et al., 1972). According to Wood and Payne (1997) a disadvantage of $ZnSO_4$ is that it can acidify soil which is undesirable in the already acidic soils of the southeast.

Effective rates of $ZnSO_4$ application for calcareous high pH soils have been reported to be extremely high and impractical. Smith et al. (1934) found that 1.81 to 2.27 kg of $ZnSO_4$ per tree were needed to correct rosette when trenched 15 to 254 cm deep in a circle 61 to 76 cm from the trunks of trees growing in alluvial, high lime silt loam soil. Similar treatments applied to trees growing in heavier textured (silty clay loam or clay) high lime soils failed to alleviate pecan rosette caused by zinc deficiency. Smith et al. (1980) found that rates in excess of 20 kg per tree of $ZnSO_4$ were required to maintain foliar zinc concentrations above the target of 60 mg·kg^{-1} when broadcast and incorporated 15 cm into a deep calcareous sand.

A greenhouse experiment conducted with wheat demonstrated superior effectiveness of Zn-EDTA over $ZnSO_4$ in increasing wheat grain zinc concentrations when banded into high pH calcareous soils (Zhao et al., 2015). Although zinc sulfate was applied at 20 mg·pot^{-1} of zinc and Zn-EDTA at 4 mg·pot^{-1} of zinc, zinc concentrations in wheat grain were 66 mg·kg^{-1}, 41 mg·kg^{-1}, and 40 mg·kg^{-1} in Zn-EDTA, $ZnSO_4$, and the untreated control plants, respectively. As discussed previously, greater soil mobility was noted with Zn-EDTA than $ZnSO_4$.

Application of Zn-EDTA through micro sprinklers (fertigation) to pecans growing in southwestern soils has successfully alleviated zinc stress. In a five-year experiment conducted in San Simon, Arizona in a 'Wichita' orchard with a calcareous, alkaline sandy clay loam soil, Zn-EDTA was applied through micro sprinkler irrigation in split applications (Heerema et al., 2017; Walworth et al., 2017). The fertilizer was applied at two different rates (2.2 or 4.4 kg·ha^{-1} of actual zinc) plus an untreated control. During the course of this study, no foliar zinc was applied to the trees. Photosynthesis in trees receiving either rate of Zn-EDTA increased on multiple measurements taken during the third and fourth seasons relative to the untreated control. Leaf stomatal conductance and SPAD (leaf greenness) also increased compared with the control. Midseason photosynthesis rates did not increase at leaf zinc concentrations above the range of 14 to 22 mg·kg^{-1} (Heerema et al., 2017). Walworth et al. (2017) found that in the same study, leaf zinc concentrations were greater than that of the control at both rates of Zn application. Foliar Zn in trees treated with 4.4 kg·ha^{-1} ranged from 22 to 35 mg·kg^{-1}, trees treated with 2.2

kg·ha^{-1} ranged from 17-23 mg·kg^{-1}, and the control had leaf zinc concentrations ranging from 7 to 14 mg·kg^{-1}. Root, shoot, and nut kernel tissues also had higher concentrations than the control (Table 1.3). Growth of trunk diameter was increased by Zn-EDTA application, and most (but not all) signs of zinc deficiency symptoms were eliminated. Increases in nut yield were observed. Trees treated with 2.2 kg·ha^{-1} significantly out-yielded trees treated with 4.4 kg·ha^{-1} (Table 1.3).

Table 1.3. Dormant season 'Wichita' root and shoot zinc concentrations, harvested nut kernel zinc concentrations, and cumulative nut yield (Walworth et al., 2017).

Zn treatment level (kg·ha^{-1})	Shoot (mg·kg^{-1})	Root (mg·kg^{-1})	Kernel (mg·kg^{-1})	Cumulative Nut Yield (kg·ha^{-1})
		2014		
0	14.92	16.99	25.96	51.3
2.2	25.32	24.71	37.73	103
4.4	35.08	44.01	43.4	81.2
		2015		
0	12.95	24.53	10.09	123.4
2.2	19.7	25.27	15.41	475.3
4.4	29.57	45.26	31.91	330.9

Another method of applying zinc is broadcast application, with dry fertilizer evenly distributed over the soil surface. An experiment was performed with $ZnSO_4$ and Zn-EDTA on bean (*Phaseolus vulgaris L.*) and corn plants (*Zea mays L.*) in Othello, Washington in a non-calcareous silt loam soil with a pH of 7.2 (Boawn, 1973). These two formulations were broadcast at rates ranging from 0.22 to 10.69 kg·ha^{-1} of zinc and then leached into the soil with sprinkler irrigation. Equal rates of Zn-EDTA application consistently resulted in higher zinc uptake and plant tissue zinc concentrations, 57 mg·kg^{-1} in Zn-EDTA treated bean plants versus 27 mg·kg^{-1} in those treated with $ZnSO_4$.

In contrast to the relatively uniform soil distribution of Zn-EDTA afforded by fertigation and broadcast, banding concentrates application in a limited portion of the root zone. A four-year experiment was conducted during which both $ZnSO_4$ and Zn-EDTA were applied by soil banding to seven-year-old 'Wichita' pecan trees growing in a high pH, calcareous Pima clay loam soil (Nunez-Moreno et al., 2009). Zn-EDTA was applied at a rate of 19 $kg \cdot ha^{-1}$ of Zn, and $ZnSO_4$ at a rate of 74 $kg \cdot ha^{-1}$ of zinc. Zinc was applied only once at the beginning of the study in March of 2005; no foliar fertilizer was applied. Both formulations were injected in soil bands 18 cm deep at a distance of 1.2 m on both sides of the trunks. Neither treatment increased leaf zinc concentrations significantly compared to the control.

Sparks and Payne (1982) conducted a comparison of banding and broadcast application of $ZnSO_4$ in a non-irrigated orchard in Byron, Ga. over a six-year period. The trees in the orchard were 'Stuart' and 'Schley' of approximately 50 years in age. In the beginning of the study the trees had a mean leaf zinc concentration of 9 $mg \cdot kg^{-1}$. Zinc was applied using both methods at 0, 0.4, 0.8, 1.6, and 3.2 kg of zinc per tree. Broadcast applications were applied uniformly in a 14.2 m area around each tree, and banded applications in a 15 cm band, 14.2 m around each tree. After the first year of the experiment the trees that received broadcast application had greater leaf zinc concentrations at all rates. By the fifth year trees treated with 0.8, 1.6, and 3.2 kg of zinc per tree by broadcast reached leaf zinc concentrations greater than 50 $mg \cdot kg^{-1}$, whereas banded trees only achieved these concentrations after five years when treated with 3.2 kg of zinc per tree.

Soil *Salinity*

High levels of salinity/sodicity in soils have numerous deleterious effects on plant growth. High concentrations of salts in the soil result in areas of low water potential (Warrence et al., 2002). This causes osmotic stress resulting in growth inhibition, reduced amounts of new foliage, and a consequent reduction in photosynthesis. Accumulation of toxic levels of Na and Cl lead to cell damage and premature senescence of mature leaves (Munns and Tester, 2008). These stress factors may overlap, resulting in a variety of negative effects on plant functions (Tuteja, 2007). The severity of damage to pecan has been shown to be correlated with the electrical conductivity (EC) of both irrigation water and soil. Miyamoto and Cruz (1986) found that an irrigation water salinity of 1.1 and 4.3 $dS{\cdot}m^{-1}$ resulted in an increase in EC_e (electrical conductivity of a saturated paste extract) from 1.5 to 2.2 and 4.2 $dS{\cdot}m^{-1}$ at 0-60 cm depth. These conditions resulted in reduced trunk growth and overall nut yield, lower kernel percentage, and lower mass per nut in 11-year-old 'Western' pecan. Significant decrease in trunk size began at an EC_e of 2.0 $dS{\cdot}m^{-1}$ in the top 30 cm and 3.0 $dS{\cdot}m^{-1}$ in the top 60 cm. Tree growth almost completely stopped at an EC_e of 3.7 $dS{\cdot}m^{-1}$ in the top 30 cm and 4.2 $dS{\cdot}m^{-1}$ in the top 60 cm. In a separate experiment Miyamoto et al. (1985) found that pecan seedling growth was significantly reduced by an EC_e of 3.3 $dS{\cdot}m^{-1}$, minimal growth occurred at 5.2 $dS{\cdot}m^{-1}$, and branch die-back at 8.5 $dS{\cdot}m^{-1}$ when grown with a confined root zone depth of 50 cm.

Deb et al. (2013) conducted an experiment on one-year-old 'Western Schley' seedlings grafted on 'Riverside' rootstock potted in sandy loam soil and grown for two years with varying irrigation water salinity. Irrigation water salinity was 1.4 (control), 3.5, 5.5, or 7.5 $dS{\cdot}m^{-1}$. Seedling height and stem diameter growth was reduced, budbreak was delayed and in some instances inhibited, particularly in plants grown in irrigation water with EC levels of 5.5 and 7.5 $dS{\cdot}m^{-1}$. Salt injury occurred with irrigation water EC ranging from 0.89 to 2.71 $dS{\cdot}m^{-1}$. Scorched leaves were associated with elevated foliar chlorine and reduced foliar nitrogen levels. Seedlings irrigated with 5.5 and 7.5 $dS{\cdot}m^{-1}$ water did not survive until the end of the second growing season. The threshold $EC_{1:1}$ (1:1 soil:water extract), the lowest $EC_{1:1}$ at which plant damage occurred, was determined to be between 0.89 and 2.71 $dS{\cdot}m^{-1}$.

Faruque (1968) found that leaf damage to three and five-month-old 'Riverside' cultivar seedlings grown in quartz sand and treated with five levels each of NaCl, $CaCl_2$, and $NaSO_4$ corresponded to elevated foliar chlorine. Seedlings treated with the high chloride (NaCl and $CaCl_2$) irrigation water displayed symptoms of salt damage ranging from marginal burn to necrosis of the whole leaf. Necrosis occurred in plants grown in soil with chlorine concentrations of 1560 $mg{\cdot}kg^{-1}$ and a corresponding leaf tissue concentration of 5959 $mg{\cdot}kg^{-1}$. The most damage was caused by NaCl followed by $CaCl_2$. Minimal injury was caused by $NaSO_4$. Similarly, Harper (1946) found that pecan trees growing in river bottomland in Oklahoma were severely damaged when foliar chloride concentrations exceeded 6000 $mg{\cdot}kg^{-1}$. Injury to pecan was found to occur at dry soil chloride concentrations greater than 200 $mg{\cdot}kg^{-1}$.

Three distinctive types of adaptation mechanisms to salinity are resistance to osmotic stress, sodium exclusion, and tissue tolerance to accumulated salts (Munns and Tester, 2008). Munns (2002) mentions exclusion of chlorine as well as sodium. Munns and Tester (2008) note that whereas neither chlorine nor sodium is more toxic, some plant species are better at excluding one than the other. This may also be true of varieties within a species. A balance may need to be maintained between leaf sodium and chlorine to maintain turgor pressure and avoid toxicity (Munns and Tester, 2008).

The response to high levels of salinity may vary among pecan cultivars. The research of Hanna (1972) suggests that genetics may be related to exclusion of chlorine. He found that even seedlings of the same parentage may exhibit varying patterns in absorption and accumulation of chlorine as well as toxicity symptoms. Miyamoto et al. (1985) compared the response of ‘Apache’, ‘Burkett’, and ‘Riverside’ pecan seedlings to irrigation water with levels of salinity ranging from 0.8 to 6.2 $dS{\cdot}m^{-1}$. He found that as levels of salinity were increased the percent of leaves exhibiting leaf damage increased more in ‘Apache’ and ‘Burkett’ than in the ‘Riverside’ cultivar. He also found that at high levels of soil sodium, ‘Riverside’ had the lowest sodium accumulation in its foliage. This was also true of chlorine content. This study suggests that ‘Riverside’ may be better suited to saline environments than the other two cultivars and that the salt exclusion adaptation mechanism may be genetically controlled.

Irrigation water is a primary source of salinity in irrigated soils (Deb et al., 2013). Alluvial soils with poor drainage, as well as evaporation rates that exceed rainfall, also contribute to soil salinity issues in semi-arid and arid environments. Clayey soils with low permeability and high specific surface area tend to accumulate salts more than sandy well-drained soils (Warrence et al., 2002). Miyamoto and Cruz (1986) evaluated the variability of soil salinity in five orchards in the El Paso Valley of Texas. In one 13.6 hectare orchard, salinity from saturated paste extracts ranged from 0.7 to 6.3 $dS{\cdot}m^{-1}$. In this orchard soil texture variability accounted for 73% of the variability in salinity levels.

It has been found that adequate zinc helps prevent damage from saline soils in various plants (Saxena and Rewari, 1990; Gupta and Gupta, 1984; Norvell and Welsh, 1993). Cakmak (2000) noted that in zinc deficient plants iron concentrations increase, promoting the production of Reactive Oxidative Species (ROS) which cause disturbances to plant growth.

In a study conducted in Chihuachua, Mexico 'Western' pecan seedlings grown in a sand culture were subjected to treatment with three doses of $ZnSO_4 \cdot 7H_2O$ containing 0, 50, 100, and 200 µmol of zinc applied by fertigation in an irrigation water with an EC of 2.7 $dS \cdot m^{-1}$ (Balandran-Valladares et al., 2021). The concentration of foliar nutrients, leaflet area, chlorophyll content, and dry weight of leaflets and roots were measured as well as changes in oxidative metabolism. It was found that at a dose of 200 µmol of zinc foliar nitrogen levels were elevated and chlorophyll content and dry weight of leaflets and roots increased significantly in the seedlings. Reduction in superoxide dismutase (SOD) occurred with doses of 100 and 200 µmol of zinc (Balandran-Valladares et al., 2021). This could be an indication that reactive oxidative species (ROS) levels were higher in the control and addition of foliar zinc inhibited their production, thereby reducing the need for SOD.

Orchard Variability and Sample Size

Tree-to-tree variability is often ignored in commercial orchard management. For example, fertilizer is commonly applied to all trees equally as it is difficult to tailor treatments for individual trees or areas with specific nutrient requirements (Brown, n.d.). Sources of tree-to-tree variability may include: irregular soil physical and chemical properties, uneven distribution of irrigation water and fertilizer, weed and pest distribution, differences in sunlight penetration and air flow, as well as plant genetics. A high level of genetic diversity exists in pecan due to the open pollinated nature of rootstocks, potentially exacerbating tree-to-tree variability within an orchard. Variability is not always readily apparent within an orchard. For example, some nutrient deficiencies can be seen with the naked eye, whereas others may not exhibit visible symptoms (Heerema, 2013).

It is not practical to sample each tree in an orchard block. Therefore, mean leaf nutrient concentrations are determined for composite samples collected from selected trees. This method provides an estimate of average nutrient concentrations, but includes a certain amount of variability (standard deviation), the magnitude of which is generally unknown.

Understanding the accuracy provided by composite samples in relation to the true population mean of the orchard requires some knowledge of tree-to-tree variability within an orchard or orchard block. There is some recognition of this. For example, McCraw et al. (n.d.) stated that “You must judge the uniformity of your own trees to determine the number of samples necessary for accurate recommendations”.

Standard leaf sampling protocol involves collecting the middle pair of leaflets from the middle of fruiting shoots (Heerema, 2013; O’barr and Mcbride, 1980; Smith et al., 2012). Orchard block sampling recommendations generally specify the number of trees that should be sampled, but rarely provide rationalization for sample size selection. Ten or more trees are often recommended regardless of the size of the orchard (O'barr and McBride, 1980; Pyzner n.d.; Robinson et al., 1997; Wells, 2009). Considering the margin of error, but without specifying methodology, Brown et al. (n.d.) determined that to estimate the true mean nitrogen concentration within 5% in almond (*Prunus dulcis L.*) orchards, samples from 18-28 trees were needed depending on the number of trees (acreage) and the level of confidence required (90 or 95%).

Evaluating variability within an orchard is a challenge that has been addressed in a variety of ways. Kriging is a technique used to interpolate the value of interest in unsampled areas based on known values at georeferenced sites (Bramley, 2005). Armindo et al. (2012) found that the creation of maps of isolines for leaf macro and micronutrient concentrations throughout a citrus orchard in Sao Paolo, Brazil generated by kriging using a semivariogram model gave an accurate depiction of the spatial variability of leaf nutrient concentrations within the orchard. They found that the variability of certain elements within the orchard could have been caused by many factors including: the variability of the soil microbiome, nutrient interactions, and spatial distribution of soil properties such as bulk density. Lopez-Granados et al. (2003) performed geostatistical analysis to determine spatial variability in an olive (*Olea europaea L.*) orchard in southern Spain by generating contour maps using kriging based on semivariograms with the goal of creating a site-specific fertilization plan. They found that this technique allowed the elimination of unneeded fertilizer applications in some parts of the orchard. This technique has also been used to describe spatial variability in levels of select metals, salts, and nitrogen in the soil, and to determine the affect they have on the growth of pecan trees (Assadian et al., 1999).

Surucu et al. (2020) conducted a study to determine variability in nutrient uptake, yield, and nut quality of various pistachio (*Pistacia vera L.*) cultivars grafted to the same seed propagated rootstock. The data from this experiment were analyzed to determine relationships between traits related to nutrient uptake, yield, and nut quality. Principle component analysis (PCA) showed that leaf nutrients were significantly different among the cultivars studied. Significant differences were also determined in yield and nut quality as well as variation in salinity tolerance and drought resistance. These authors concluded some of the variability could be attributed to the plants genetic makeup and environmental selection (Surucu et al., 2020).

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Does Foliar Zinc Application Boost Leaf Photosynthesis of 'Wichita' Pecan Fertigated with Zn-EDTA?

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Abstract

Many growers fertigating their orchards with Zn-EDTA are still using supplemental zinc foliar sprays due to 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, Arizona on 8-year-old ‘Wichita’ trees growing in an alkaline, calcareous Vekol loam soil to evaluate the effectiveness of supplemental foliar zinc (Zn) sprays. All trees were fertigated with 6.0 $kg \cdot ha^{-1}$ of Zn in the form of zinc-ethylenediaminetetraacetic acid (Zn-EDTA) in 2018 and 11.0 $kg \cdot ha^{-1}$ of Zn in 2019 and did not exhibit visible signs of Zn deficiency. Foliar treatments of 3.75 $ml \cdot L^{-1}$ urea-ammonium nitrate (UAN), 3.6 $g \cdot L^{-1}$ zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$), 3.6 $g \cdot L^{-1}$ $ZnSO_4 \cdot H_2O$ with 3.75 $ml \cdot L^{-1}$ UAN, 11 $ml \cdot L^{-1}$ 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. Mid-day stem water potential (MDSWP) was measured to verify that water stress was not limiting photosynthesis. Both measurements were taken approximately two to four weeks following applications of foliar treatments. MDSWP measurements indicated a lack of water stress and therefore no effect on photosynthesis. Leaf samples collected from non-treated branches indicated that the average foliar Zn concentration of untreated leaves was 21.3 $mg \cdot kg^{-1}$ in 2018 and 15.7 $mg \cdot kg^{-1}$ 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.

Introduction

Pecans [*Carya illinoinensis (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 semi-arid southwestern United States. In these alkaline soils Zn binds with hydroxyls and carbonates forming 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 $mg{\cdot}kg^{-1}$ of Zn. Zinc 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 and net photosynthesis (P_n) were concomitantly reduced by low Zn. Heerema et al. (2017) determined a threshold leaf Zn concentration between 14 to 22 $mg{\cdot}kg^{-1}$ 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, while measurements in August showed leaf Zn concentration thresholds on the lower end. Zinc concentrations close to or below 14 $mg{\cdot}kg^{-1}$ 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 $mg{\cdot}kg^{-1}$ (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 five-year period at rates of 2.2 $kg \cdot ha^{-1}$ and 4.4 $kg \cdot ha^{-1}$ of 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 these recommended minimum concentrations. The highest leaf Zn concentrations obtained during this study were 35 $mg \cdot kg^{-1}$, but P_n showed no significant increase when foliar Zn concentrations exceeded approximately 22 $mg \cdot kg^{-1}$ indicating that Pn 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 approximately 2 to 4% of 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 $ZnSO_4$ and $Zn(NO_3)_2$. 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 $ZnSO_4$ or $Zn(NO_3)_2$ sprays was enhanced by including UAN in the spray mixture. In other crops, a mixture of $MnSO_4$, $ZnSO_4$, and $FeSO_4$ salts were applied to soybean, fava bean, pea, and wheat with and without the addition of 1% urea. In all cases 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 $ZnSO_4$ or $ZnCl_2$ than in the organic Zn-EDTA form. Cuticular sorption of Zn-EDTA was approximately 5 $nM \cdot cm^{-2}$ after 72 hours versus approximately 41 $nM \cdot cm^{-2}$ for $ZnSO_4$. In pea plants, Zn uptake rates were approximately 1.45 times greater when Zn was applied as $ZnSO_4$ versus 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 $ZnSO_4$ (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 $ZnSO_4$. However, Zn concentrations of unsprayed leaves on branches adjacent to those sprayed with Zn-EDTA were increased significantly compared to the control, whereas leaves on branches adjacent to $ZnSO_4$ treatments were not, suggesting greater mobility of Zn-EDTA within the tree than $ZnSO_4$. In pecans, foliar applications of 50, 100, and 150 $mg \cdot L^{-1}$ of 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 $mg \cdot kg^{-1}$. They did, however, achieve the 14 to 22 $mg \cdot kg^{-1}$ 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 $mg \cdot kg^{-1}$ is adequate to maximize rates of P_n, recommendations for commercial orchards are generally much higher (at least 40 to 60 $mg \cdot kg^{-1}$; Heerema, 2013; Robinson et al., 1997; Smith et al., 2012; Sparks, 1993; Sparks and Payne, 1982). In part, these higher mean leaf Zn concentration recommendations are due to 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 foliarly, as continues to be the common practice, the Zn impacts 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, $ZnSO_4 \cdot H_2O$ 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 was a randomized complete block design (RCBD). Each tree is considered a block and each treated branch an experimental unit. The orchard's climate is semiarid and has an average annual precipitation of approximately 24 cm (Western Regional Climate Center). The orchard is irrigated through a micro-sprinkler system approximately 24 times per year (~152 cm annually). Nitrogen, P, and K were applied through the fertigation system on five occasions, March through June, at rates of 213 $kg \cdot ha^{-1}$, 50.5 $kg \cdot ha^{-1}$, and 50.5 $kg \cdot ha^{-1}$, 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 $kg \cdot ha^{-1}$ of Zn in 2018 and 11.0 $kg \cdot ha^{-1}$ of Zn in 2019. Trees did not exhibit observable signs of Zn deficiency. In both years, 2.24 $kg \cdot ha^{-1}$ of K and 1.12 $kg \cdot ha^{-1}$ of Ni were foliarly applied in late spring (April/May), and 4.48 $kg \cdot ha^{-1}$ of K, and 2.24 $kg \cdot ha^{-1}$ of Fe were applied foliarly 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 $kg\cdot ha^{-1}$ in 2018 and 2019, respectively; [United States Department of Agriculture (USDA), (n.d.)]. In 2018 yields were 2501 $kg\cdot ha^{-1}$ for 'Western' and 2829 $kg\cdot ha^{-1}$ for 'Wichita', and in 2019, 3262 $kg\cdot ha^{-1}$ for 'Western' and 3292 $kg\cdot ha^{-1}$ 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 $g\cdot nut^{-1}$ and 'Wichita' 7.8 $g\cdot nut^{-1}$ in 2018, and 6.6 $g\cdot nut^{-1}$ for 'Western' and 7.7 $g\cdot nut^{-1}$ 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 $ml\cdot L^{-1}$ urea ammonium nitrate

3) Zn Sulfate - 3.6 $g\cdot L^{-1}$ $ZnSO_4\cdot H_2O$ (36% Zn)

4) Zn Sulfate plus UAN - 3.6 $g\cdot L^{-1}$ $ZnSO_4\cdot H_2O$ + 3.75 $ml\cdot L^{-1}$ UAN

5) Zn-EDTA - 11 $ml\cdot L^{-1}$ of Zn-EDTA (applied only in 2019)

In order 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 further assure that all leaf surfaces were coated, and the bag removed. Applications were made on May 24 and June 25, 2018, and May 24 and July 1, 2019.

Leaf samples and P_n measurements. Gas exchange measurements were taken on middle leaflets from non-terminal, sun-lit leaves on each treated shoot in June and July of 2018 and 2019, approximately two to four weeks following 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 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Light saturation of P_n for pecan is reached between a PAR of 1500 to 1700 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Anderson, 1994; Lombardini et al., 2009). Reference CO_2 concentration was kept at 400 $\mu mol \cdot mol^{-1}$, near the global mean atmospheric concentration (U.S. Department of Commerce, 2020). Once the P_n and stomatal conductance (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), mid-day stem water potential (MDSWP) was measured for each tree on the same dates as the gas exchange measurements were taken. 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 water potential was measured immediately using a Scholander pressure chamber (PMS Instrument Co., Albany, OR).

Approximately thirty leaflets were collected from untreated fruiting shoots on the opposite side of each tree from the treated branches on June 25, 2018 and June 7, 2019 to determine background Zn concentrations. On June 18, 2018 twenty leaflets were also collected from the treated shoots. All leaflets were washed in a phosphorus-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 hours at 65^{o} C and ground using a cyclone mill (UDY Cyclone Sample Mill, Belt Drive, Model 3010 – 030, 120 Volt ac).

A 0.5 g aliquot of ground leaf tissue was ashed at 500^{o} C for 5.5 hours, dissolved in 10 ml of 2.0 N HCl 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 software (SAS Institute, Cary, NC) was used to perform ANOVA. Connecting letters reports to show differences in the means for all the data in figure and table form were obtained using Each Pair Student's t tests. JMP software was also used for linear regressions performed for the determination of 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 the sprayed branches) in the current study were 21.3 $mg{\cdot}kg^{-1}$ in 2018 and 15.7 $mg{\cdot}kg^{-1}$ in 2019. In 2018, leaf tissue zinc concentrations from shoots treated with water alone (21.1 mg·kg^{-1}) or water + UAN (22.5 mg·kg^{-1}) were not statistically different to that of untreated leaves sampled from the opposite side of the canopy (21.3 mg·kg^{-1}) (Table 2.1). The leaf Zn concentrations from shoots treated with Zn containing sprays were significantly higher than those of shoots treated with water alone or water + UAN. The addition of UAN to $ZnSO_4 \cdot H_2O$ treatments significantly increased leaf Zn concentrations relative to $ZnSO_4 \cdot H_2O$ 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 Figure 1.1. No significant differences were noted. There was no significant difference in stomatal conductance on any of the measurement dates (data not shown). The overall average stomatal conductance from all four measurement dates was 0.210 mol·m^{-2}·s^{-1}. Only one significant difference was found in levels of intercellular CO_2; the water + UAN treatment had significantly lower levels than the other treatments on July 18, 2018 (Table 2.2).

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 are applied exclusively to the soil. Maximum P_n rates in southwestern 'Wichita' pecans has been reported 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 below typical maximum rates. Both Heerema et al. (2017) and Hu and Sparks (1991) found that maximum P_n was observed in pecan 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 if 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 pecan, 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 lower than -0.9 MPa reduced pecan 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 versus MDSWP and linear regressions performed on data from each measurement date to evaluate effect of MDSWP on P_n (Figure 1.2). Statistical analysis showed no indication that P_n corresponded to MDSWP. Although combining the data indicates a non-significant weak trend (r^2= 0.0415) towards 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. Mid-day stem water potential 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 $mg \cdot kg^{-1}$ range, as 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 higher 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 higher average Zn concentration is likely needed to ensure that most, or all, trees exceed this range. Additionally, further testing is needed to determine if there are any other horticulturally important benefits from zinc sprays in these orchards.

Contributions

Our findings contribute to research in the fields of horticulture, soil fertility, and plant nutrition as well as present valuable information for pecan growers. The question addressed by this research about the necessity of higher mean leaf zinc concentration recommendations on an orchard wide scale is explored further in the third experiment presented in this thesis "Pecan Tree-to-Tree Variability: Implications for Leaf Sampling and Nutrient Recommendations".

My involvement in this research experiment included sample collection and measurements, sample processing, laboratory analysis, compiling and interpreting data, and research and writing (as the first author). Dr. Walworth and the other co-authors began the experiment and helped create this paper by editing and providing peer review and making suggestions regarding data analysis and presentation.

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Table 2.1. Leaf Zn concentrations averaged from all individually sampled pecan trees for each of four treatments taken July 18, 2018, 23 days following treatment application and leaf Zn concentrations averaged from untreated samples taken from the backside of each tree on June 25, 2018.

Leaf samples	Untreated	Water	Water + UAN	$ZnSO_4 \cdot H_2O$	$ZnSO_4 \cdot H_2O$ + UAN
Average Zn concentration $mg \cdot kg^{-1}$	21.3 c	21.1 c	22.5 c	126.8 b	151.5 a

Table 2.2. Intercellular CO_2 concentrations ($\mu mol \cdot mol^{-1}$) on all four measurement dates. Values in each column that do not share the same letter are statistically different.

Treatment	6-22-2018	7-18-2018	6-7-2019	7-18-2019
Water	236.71 a	264.48 a	237.18 a	264.72 a
Water + UAN	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 + UAN	233.14 a	263.30 ab	239.93 a	261.09 a
Zn-EDTA	-	-	246.45 a	261.27 a

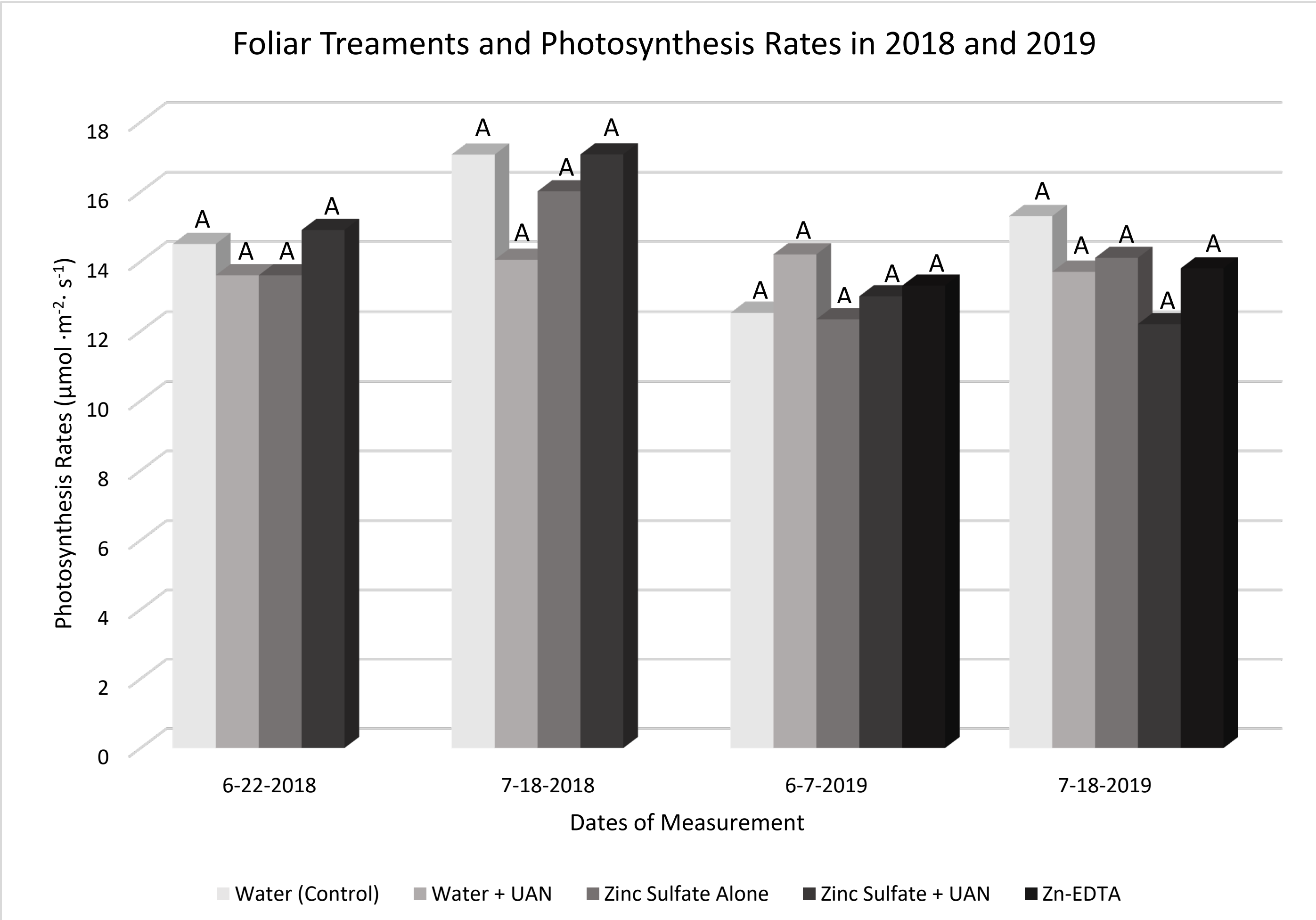

Figure 1.1. Photosynthesis rates (µmol·m^{-2}·s^{-1}) of sprayed shoots in 2018 and 2019. The mean photosynthesis rate for treatments from all seven pecan trees (replicates) on each of four measurement dates are compared. Treatments that do not share the same letter within the same sampling date are significantly different.

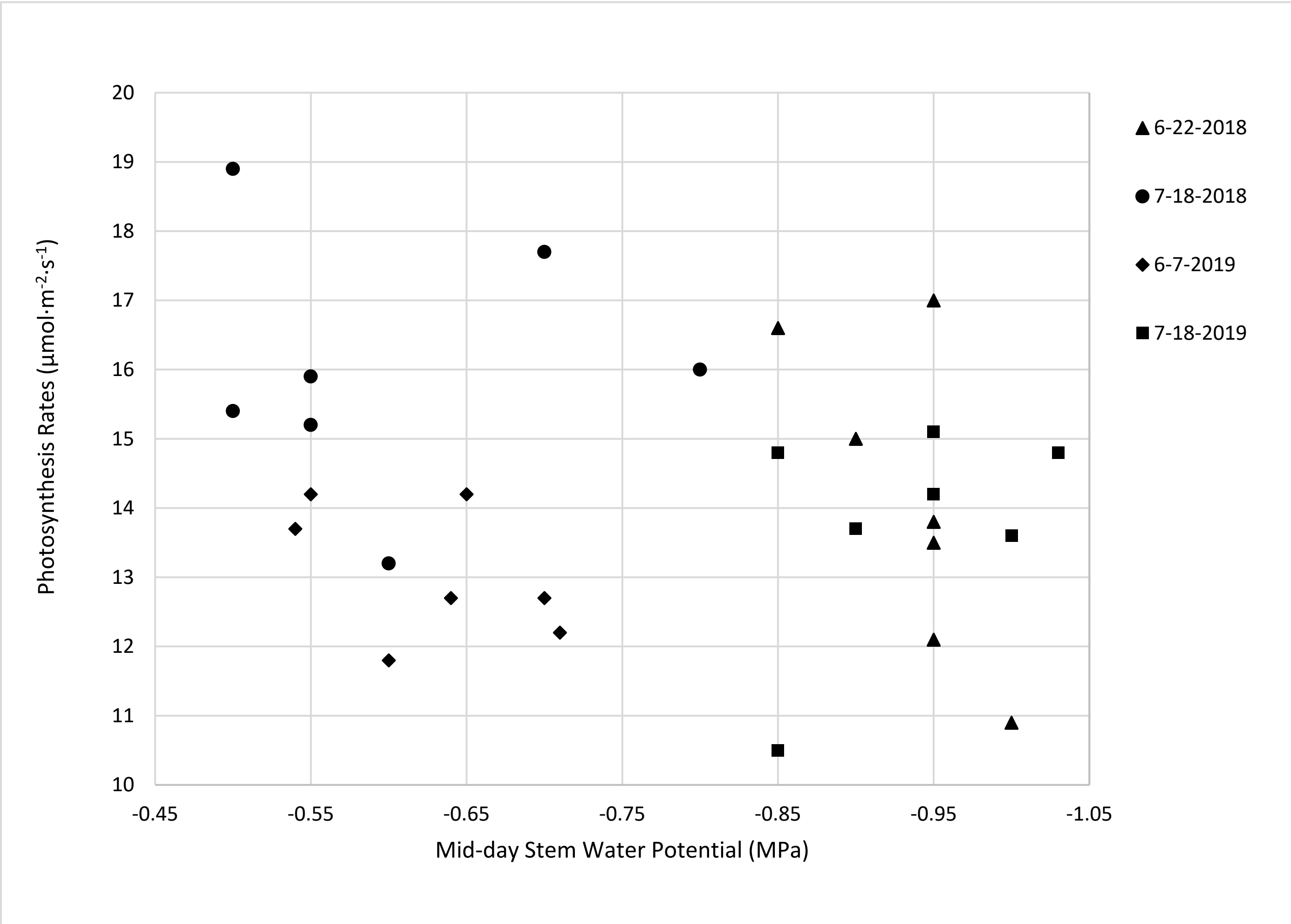

Figure 1.2. Relationship between MDSWP (MPa) and photosynthesis rates (µmol·m^{-2}·s^{-1}) for all seven pecan trees (replicates) in the experiment. P-values of 0.076 on 6/22/2018, 0.979 on 7/18/2018, 0.280 on 6/7/2019, and 0.292 on 7/18/2019 were obtained. With an alpha value of 0.05 none of these relationships were significant.

Impacts of Maternal Genotype on Pecan Seedling Performance in an Alkaline, Saline-Sodic Soil

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Abstract

A field study was conducted to evaluate tolerance of pecan rootstocks to soil salinity and sodicity. Seven cultivars (‘Elliott’, ‘Giles’, ‘Ideal’, ‘Peruque’, ‘Riverside’, ‘Shoshoni’, and ‘VC1-68’) were selected from a range of geographical regions of origin. The soil of the experimental plot is a poorly drained, saline-sodic Pima silty clay variant. The irrigation water is a moderately saline mix of Gila River and local groundwater with an EC of 2.8 $dS{\cdot}m^{-1}$ containing primarily ions of sodium (Na) and chlorine (Cl). Eighty seeds of each cultivar were planted in a greenhouse in late Feb. 2016 and 48 seedlings of each cultivar were transplanted into field plots in Feb. 2017. Half the trees received a soil-based application of Zn-EDTA at planting. The trees were observed and rated for both vigor and resistance to salt injury on seven separate occasions. Trunk diameter was measured each dormant season. Leaf samples were collected on 9 Oct. 2019 and 6 Oct. 2020 and analyzed for nutrient content. Zn-EDTA was not found to have a significant effect on vigor or resistance to salt injury. ‘Elliott’ exhibited greater tolerance for the alkaline, saline-sodic soil conditions than the other cultivars. ‘Giles’ and ‘Peruque’ were most severely affected. Resistance to salt injury (ranging from marginal leaf burn to necrosis of entire leaf), vigor, and growth were more strongly correlated with foliar Na concentrations than Cl or potassium (K) during 2019. Vigor and growth were not significantly correlated with foliar Na, Cl, or K concentrations in 2020. Foliar K:Na ratio had a nearly equal correlation with resistance to salt injury and a greater correlation with growth than that of Na alone in 2019. However, while the correlation of K:Na ratio with vigor was stronger than that of Cl or K, Na had the strongest correlation with vigor in 2019. In 2020 the only significant correlation of growth and vigor was

with K:Na ratio. The strongest correlation with resistance to salt injury in 2020 was with foliar Na concentration.

Introduction

Soil conditions, including sodicity, salinity, and poor drainage, limit distribution of pecan [*Carya illinoinensis (Wangenh.) K. Koch*] acreage in the southwestern United States. Multiple aspects of plant physiology and metabolism are affected by salinity stress (Tuteja, 2007). Low water potentials associated with high salt content in the soil solution make it more difficult for plant roots to acquire water. Sodic soils [soils with exchangeable sodium percentage (ESP) greater than 15%] are associated with unstable soil structure, poor drainage, and salt accumulation (Sumner, 1993). High levels of Na negatively impact K uptake in plants causing disturbance in the function of certain enzymes and stomata as well as osmotic balance (Tuteja, 2007).

Cell injury caused by ion excess in the leaves reduces growth and can lead to leaf senescence and plant mortality. Plants have been reported to have two phases of response to salinity stress; young leaf growth is inhibited by osmotic stress, and senescence of mature leaves is accelerated by an ionic phase in which toxic levels of salts accumulate (Munns and Tester, 2008). Rajendran et al. (2009) suggested that the three main components of salinity stress tolerance in cereals are Na exclusion, Na tolerance in plant tissues, and osmotic stress tolerance. Munns and Tester (2008) more broadly described these mechanisms as Na or Cl exclusion, tolerance to accumulated Na or Cl, and tolerance to osmotic stress, and speculated that because plants use Na and Cl to maintain turgor pressure in leaves, the plant may need to develop a balance between these ions to avoid ion toxicity.

It is unclear which of the ions in saline water or soil causes salt injury in pecans. Faruque (1968) treated ‘Riverside’ pecan seedlings growing in sand culture with various salt solutions, which they expressed in terms of osmotic pressure. They determined that salt injury to leaves was caused by Cl rather than Na. Seedlings treated with NaCl solutions began to exhibit significant injuries (measured by percent necrosis of leaf) when growing in a 0.15 MPa osmotic potential solution, and a 0.30 MPa solution resulted in death. Seedlings treated with $CaCl_2$ began to show significant injuries at 0.20 MPa, but no injury was caused by Na_2SO_4 solutions with osmotic potentials as great as 0.30 MPa, implicating Cl and not Na as the causal agent. Necrosis occurred in leaves of seedlings with whole plant tissue content of 5959 $mg \cdot kg^{-1}$ Cl when trees were treated with either NaCl or $CaCl_2$. Similarly, Harper (1946) found that leaf content of 6000 $mg \cdot kg^{-1}$ Cl caused severe damage to pecans. In contrast, Miyamoto et al. (1985) reported that Na content, but not Cl content, in pecan leaves demonstrated a strong negative correlation with leaf, stem, and root weights and soil solution. Na, but not Cl, was related to leaf weight.

Although few studies have investigated pecan cultivar sensitivity to salts, Hanna (1972) found that seedlings produced by hand pollination of identical parentage exhibited a wide range of patterns of Cl absorption. Miyamoto et al. (1985) reported that ‘Riverside’ seedlings absorbed less Na and showed less salt damage than ‘Apache’ and ‘Burkett’, the two other cultivars tested.

Foliar K:Na ratio has been found to be a factor in salinity tolerance. Gorham (1990) reported that salinity tolerance in *Aegilops* (goatgrass) species was related to the ability to maintain high leaf K:Na ratios. Munns and Tester (2008) indicated that foliar K:Na ratios were a function of ion transporter genes and that salinity tolerance in plants may be related to increased leaf tissue K:Na ratios. Almeida et al. (2017) noted that Na inhibits K uptake by cells and likely inhibits K transporters. Wakeel (2013) pointed out that an optimal K:Na ratio is essential for the activation of enzymatic reactions in the cytoplasm required for maintaining plant growth.

Soil physical properties can affect salt accumulation in the soil profile. Clayey soils with low permeability and greater specific surface area tend to accumulate more salts than porous, well-drained soils (Warrence et al., 2002). Irrigated pecan orchards with low permeability, (e.g., poorly drained alluvial soils) may exhibit salt accumulation (Miyamoto and Storey, 1995). Miyamoto et al. (1986) noted that 'Western' scions grafted to 'Riverside' rootstock grown in soils with high clay content (silty clays and silty clay loams) were stunted and had smaller trunk diameter than trees planted in coarser textured (loam) soil. Greater salt accumulation occurred in the clayey soils. In soils with an EC_e (saturated paste extract electrical conductivity) in the upper 30 cm greater than 2.0 $dS \cdot m^{-1}$, trunk diameter was reduced. Branch dieback occurred when EC_e exceeded 6.0 $dS \cdot m^{-1}$.

A major source of soil salts in irrigated systems is irrigation water. Deb et al. (2013) found that use of irrigation water with EC_{irr} (irrigation water electrical conductivity) between 1.4 and 3.4 $dS \cdot m^{-1}$ resulted in soil $EC_{1:1}$ (soil salinity of a 1:1 soil/water extract) of between 0.89 and 2.71 $dS \cdot m^{-1}$. This level of salinity resulted in bud break delay and inhibition, reduced seedling growth rate, and visible symptoms of salt injury on 1-year-old ‘Western’ grafted to ‘Riverside’ rootstock. Seedlings did not survive the two-year test period where EC_{irr} levels were 5.5 $dS \cdot m^{-1}$ or more.

Plant zinc (Zn) content has been reported to be related to salinity tolerance (Cakmak, 2000). Zinc promotes the synthesis and activity of antioxidative enzymes that can help prevent damage from oxidative stress factors, including salinity (Cakmak, 2000). Therefore, improving the Zn nutritional status of plants grown in arid and semi-arid regions with saline soils may be important for not only preventing Zn deficiency but also for protecting plants against the damage caused by excess salinity. Norvell and Welch (1993) suggested that increased Zn may reduce plant accumulation of Na. Zinc has been shown to increase salinity tolerance in chickpea (*Cicer arietinum* L.), evidenced by reduced levels of Na uptake and elevated levels of K in shoots (Saxena and Rewari, 1990). In soybeans (*Glycine max* L.) grown in two saline soils, one with primarily chloride salts, the other with sulfate salts, the uptake of Zn was suppressed in proportion to the salinity of the soil (Gupta and Gupta, 1984).

Long-term drought and expansion of pecan acreage in the semi-arid southwestern United States have increased use of low-quality irrigation water in pecan production. This has accentuated the need for rootstocks that are tolerant of sodic and saline soil conditions. To that end, we conducted a field study to evaluate the effect of maternal genotype on pecan seedling tolerance to such conditions, and to evaluate the effect of the application of Zn-EDTA on rootstock performance. We selected open pollinated seeds from several cultivars originating from environmentally diverse parts of the pecan native range. We expected that seedlings whose genetic origins lie in lower precipitation regions where saline and sodic soils are more common would be more tolerant of these soil conditions than those whose genetic origins lie in higher precipitation areas.

Materials and Methods

A field experiment was conducted at the University of Arizona Safford Agricultural Center in Safford, AZ (32^0 48’ 57˝ N, 109^0 40’ 48˝ W). This location receives average annual precipitation of approximately 24.6 cm (US Climate Data). Soil samples were collected on 25 July 2018. The soil is a saline-sodic alluvial Pima silty clay variant [Coarse-loamy, mixed (calcareous), thermic Typic Torrifluvents] (Post et al., 1977) with an $EC_{1:1}$ (soil salinity of a 1:1 soil/water extract) of 3.4 $dS{\cdot}m^{-1}$ at 0-15 cm depth, 2.8 $dS{\cdot}m^{-1}$ at 15-30 cm depth, and 2.1 $dS{\cdot}m^{-1}$ at 30-60 cm depth (Table 3.1). The plots were flood irrigated with a blend of Gila River water and groundwater with an EC of 2.8 $dS{\cdot}m^{-1}$ at a rate of 11 – 15 cm per irrigation event with approximately 10 events per year. The dominant irrigation water ions are Na and Cl.

In an attempt to maximize genetic variability with respect to salinity tolerance, seven pecan rootstock cultivars were selected based on probable location of origin (Grauke and Thompson, 2019; Table 3.2). ‘Elliott’ is a commonly used rootstock in the southeastern United States, ‘Peruque’ and ‘Giles’ in Midwestern states, ‘Riverside’ in the southwest, and ‘VC1-68’ in the West (Grauke, 2010). Eighty seeds of each of seven cultivars were planted in a greenhouse in late Feb. 2016. ‘Riverside’ and ‘Ideal’ seeds were collected from Farmers Investment Co. (FICO; Sahuarita, AZ) orchards in San Simon, AZ. The other five cultivars came from Linwood Nursery (La Grange, CA).

Forty-eight seedlings of each cultivar were transplanted into the field plots on 27 Feb. 2017. Seedlings of the seven pecan cultivars were arranged in a split-plot design with 12 replications. Each plot contained four trees, spaced 213 cm between trees and 6 m between rows. Main plots were cultivar, and sub-plots were Zn-EDTA treatment. Half the trees in each main plot were treated with 75 ml of 9% Zn-EDTA (Sequestar, Brandt Consolidated, Inc., Springfield, IL) placed in the tree hole at the time of planting.

Trees were visually ranked for vigor and for resistance to salt injury on 28 Sept. 2017, 18 June 2018, 12 Sept. 2018, 20 June 2019, 9 Oct. 2019, 26 June 2020, and 6 Oct. 2020. Observations on 28 Sept. 2017 consisted of resistance to salt injury ratings only. Resistance to salt injury ratings were based on amount of leaf tissue exhibiting typical salt injury symptoms (marginal leaf burn to necrosis of entire leaf). A rating of 5 was assigned to trees that exhibited no injury and a rating of 1 for severe injury, where all leaves exhibited extensive injury. Plant vigor ratings were based on overall appearance of foliage (other than salt injury), size of leaves, and amount of recent growth. A rating of 5 reflects healthy foliage and growth, and a rating of 1 indicates that the tree was alive but had very little or no green foliage or recent growth. Trunk diameters were measured 7 cm above the soil surface by electronic caliper in each dormant season. Annual tree growth was determined by comparing annual dormant season trunk caliper measurements.

Leaf samples were collected from each living tree that had enough leaf tissue to collect on 9 Oct. 2019. Due to the severity of salt injury and size of some seedlings, standard pecan leaf sampling protocols (Heerema, 2013) could not be followed. Whereas pecan leaf samples are usually collected in late July to early August, we waited until closer to the end of the growing season (October) to avoid defoliation injury to smaller plants. Some collected leaves had necrotic lesions, and leaf position on the tree was ignored (i.e., leaves were collected from wherever on the seedling they were present). Composite samples were created for each cultivar by combining samples from individual trees to accumulate enough tissue for analysis. In 2019 and 2020, the living trees were separated into four groups, dependent on number and location of living trees, by combining trees from separate replications. A total of four composite samples were derived for each cultivar except for 'Peruque' for which only two composite samples could be created. Some composite samples from 2019 contained trees that were either no longer alive or had too few leaves to be sampled in 2020. This resulted in 'VC1-68' and 'Giles' having three composite samples in 2020, whereas in 2019, each of these cultivars had four composite samples. The leaflets were placed in a 65^{o} C oven for 48 h, then ground using a cyclone mill (UDY Corporation, Fort Collins, CO). The samples were analyzed for complete nutrient contents (Brookside Laboratories, Inc. New Bremen, OH).

JMP software (SAS Institute, Cary, NC) was used to perform ANOVA. Connecting letters reports to show differences in means of data in figures and tables were obtained using the Each Pair Student's t tests. Microsoft Excel was used for linear and non-linear regressions.

Results

Application of Zn-EDTA at planting did not have a significant effect for any of the measurements. Therefore, all presented statistical analyses result from grouping those trees that did and did not receive Zn (i.e., main plots only).

On 28 Sept. 2017 'Elliott' and 'Riverside' exhibited the greatest resistance to salt injury (Table 3.3). 'VC1-68', 'Ideal', and 'Shoshoni' were intermediate, and the least resistance to salt injury was observed in 'Peruque' and 'Giles'. Differences were less distinct in June 2018. 'Elliott' displayed the most resistance to salt injury late in the 2018 season. Although not statistically different from some of the other seed source cultivars, 'Peruque' displayed the least resistance to salt injury throughout 2018. Similar to 2018, differences in June 2019 were smaller than later season resistance to salt injury ratings. In late 2019 'Elliott' showed significantly greater resistance to salt injury than all other seed source cultivars, repeating the pattern of 2018. 'Ideal', 'Riverside', 'Shoshoni', 'VC1-68', and 'Giles' were intermediate, and 'Peruque' had the least resistance to salt injury. Early in 2020, the most resistance to salt injury was seen in 'Ideal', 'Shoshoni', and 'Giles'. Late in 2020, 'Elliott' and 'Riverside' showed the most resistance to salt injury, while 'Peruque' and 'Shoshoni' were most affected.

Vigor ratings reflect general observable plant health and growth but do not consider leaf salt injury symptoms. Very little statistical difference in vigor was seen early in 2018 (Table 3.4). 'Elliott' and 'VC1-68' had the highest vigor ratings late in the 2018 season. Although not statistically different from some cultivars, 'Peruque' and 'Giles' were the least vigorous. 'Elliott' and 'VC1-68' continued to exhibit the highest ratings early in June 2019, and 'Peruque' was least vigorous. 'Elliott' was the most vigorous cultivar late in the 2019 season, and 'Peruque' continued to exhibit the least vigor. Early in 2020 'Elliott', 'Shoshoni', and 'VC1-68' were the most vigorous, and late in 2020 'Elliott' showed the greatest vigor, while 'Peruque' and 'Giles' had the lowest vigor ratings. Observed resistance to salt injury was related to seedling vigor more closely in late than in early season observations (Table 3.5).

'Elliott' stood out with the greatest growth each year and cumulatively over the course of the study (Figure 2.1). All other cultivars grew at similar rates. Over 70% of 'Giles' seedlings died early in the study (Figure 2.2). By June 2020, over 70% of 'Shoshoni', 'Peruque', and 'VC1-68' had also died. 'Elliott', 'Ideal', and 'Riverside' had the lowest mortality rates. Between June and Oct. 2020, the greatest mortality occurred in 'Giles', which lost 3 of its 10 remaining trees. No other cultivars lost more than 10% of their remaining trees during this time. Averaged over the life of the study, tree growth was more strongly related to ratings of tree vigor than with resistance to salt injury ratings, whereas tree survival was more closely related to resistance to salt injury than plant vigor (Table 3.6). There was a very weak relationship (r^2 = 0.136) between tree growth rate and survival (data not shown).

Cultivar differences in leaf P, Mg, Ca, Cl, K, and Na concentrations were significant on 9 Oct. 2019 (Table 3.7). Other nutrients did not differ significantly between cultivars. Notably, 'Elliott' had high K and shared the lowest Na concentrations with 'Ideal' and 'Riverside'. 'Shoshoni' leaf Cl concentrations were higher than 'Ideal', 'Riverside', 'Giles', and 'VC1-68' but not 'Peruque' and 'Elliott'. Leaf K:Na ratio was much higher in 'Elliott' than in other cultivars.

Differences in P, Mg, Ca, Cl, K, and Na concentrations were again significant among cultivars on 6 Oct. 2020 (Table 3.7). Notably, 'Elliott' shared the lowest Na concentration with 'Giles', 'VC1-68', and 'Riverside'. Although 'Peruque' and 'Giles' had the highest K concentrations, 'Elliott' had the highest K:Na ratio (although not significantly greater than 'VC1-68').

There was a linear relationship between leaf Na concentration and resistance to salt injury in Oct. 2019 (r^2 = 0.550) (Figure 3A; Table 3.8) and between leaf K concentration and resistance to salt injury (r^2 = 0.168), but that between leaf Cl concentration and resistance to salt injury was not significant (Table 3.8). There was also a linear relationship between leaf Na and resistance to salt injury in Oct. 2020 (r^2 = 0.412) (Figure 3B), the relationship of resistance to salt injury with Cl had an r^2 of 0.210, whereas K and resistance to salt injury were not significantly related (Table 3.8). The relationship of resistance to salt injury with leaf K:Na ratio (r^2 = 0.545) (Figure 4A) was nearly as strong as that of Na concentrations alone in 2019. In 2020, the logarithmic relationship of the K:Na ratio with resistance to salt injury was slightly weaker than the linear relationship of Na alone (r^2 = 0.365) (Figure 4B).

The leaf K:Na ratio was also related to cumulative growth in trunk diameter in 2019 (r^2 = 0.545) (Figure 5A; Table 3.8). In 2020, the correlation between increases in trunk circumference and K:Na ratio was weaker (r^2 = 0.370) than in 2019 (Figure 5B; Table 3.8). Notably, 'Elliott' had the largest K:Na ratio in 2019 and exhibited the greatest growth. 'Elliott' again had the largest K:Na ratio in 2020 but was not statistically different from 'VC1-68'.

Discussion

Overall, 'Elliott' ratings for both vigor and resistance to salt injury were either better than, or among the best of all cultivars. 'Elliott' growth rate, measured by trunk diameter increase, exceeded that of other cultivars throughout the experiment, and it was among the cultivars with the lowest mortality. 'Elliott' was also among the cultivars with the lowest leaf Na and highest leaf K concentrations and the highest leaf K:Na ratios (nearly three-fold greater than any other cultivar in 2019).

The correlation between the K:Na ratio and resistance to salt injury supports previous reports of the importance of K:Na ratio in salt stress tolerance for a range of other plant species (Gorham, 1990; Munns and Tester, 2008; Wakeel, 2013). Our results show an increased resistance to salt injury at a K:Na ratio between approximately four to six. Further research is needed to confirm this threshold. This knowledge may be beneficial in rootstock evaluation and selection and in diagnosing salinity issues in mature, grafted pecan orchards.

Because 'Elliott' maintained high leaf K and was among the cultivars lowest in leaf Na concentration, it is not possible to specifically attribute the observed tolerance to one or both of these elements. Our results conform to the findings of Miyamoto et al. (1985) in which the 'Riverside' cultivar accumulated the least amount of Na and displayed the least effect of salt on measured growth parameters relative to 'Apache' and 'Burkett', the other cultivars studied. Miyamoto et al. (1985) did not report leaf K concentrations.

In our study, leaf Cl concentrations in Oct. 2019 did not reach levels previously reported to cause leaf necrosis in pecans, perhaps explaining lack of correlation with resistance to salt injury, vigor, or growth. The highest leaf Cl concentrations were 3258 to 4454 $mg \cdot kg^{-1}$, found in 'Shoshoni', 'Elliott', and 'Peruque', whereas previously reported leaf Cl concentrations required for necrosis in pecan leaves are 5000 to 6000 $mg \cdot kg^{-1}$ (Faruque, 1968; Harper, 1946). Chloride concentrations in Oct. 2020 were greater in all cultivars than in 2019. Four cultivars ('Giles', 'Peruque', 'Shoshoni', and 'VC1-68') exceeded 5000 $mg \cdot kg^{-1}$. Even with this increase in Cl concentrations, the correlation between leaf Cl and resistance to salt injury was weaker than that of Na. This supports the finding of Miyamoto et al. (1985) that Na, rather than Cl, was more closely related to the degree of salinity injury in pecans.

Although the physiological mechanisms for differences in salt tolerance among cultivars are not well-understood, it is clear that the variation of performance is genetically controlled, which highlights the potential for rootstock improvement. Because all of the seeds were from open pollinated seed sources, we do not know the paternal contribution. The results of this experiment give evidence of the heavy influence of the maternal parent in seedstock tolerance to saline-sodic conditions. The likely Mexican ancestry of 'Elliott' may be an explanation of its genetic salt tolerance adaptations and superior performance to cultivars chosen from other geographic regions. The significant relationships between leaf Na concentration and K:Na ratio and both observed resistance to salt injury and tree growth rate suggest that these measures could be used to predict genotype salt tolerance in short-term evaluations.

Contributions

This experiment contributes to the limited research previously conducted regarding pecan tolerance to saline-sodic soil conditions. Our discovery that the K:Na ratio in pecan leaves growing in alkaline, calcareous, saline-sodic soil was strongly correlated with resistance to salt injury and also correlated with growth and vigor, agrees with the findings of research on other plants. The superior performance of 'Elliott' in this experiment and its high K:Na ratio make this cultivar of interest for future studies in salinity tolerance. This is a valuable contribution to the fields of horticulture, soil fertility, and plant nutrition as well as genetics and plant breeding.

My contribution to this research included sample collection, measurements and observations, sample processing, compiling and interpreting data, and research and writing (as the first author). Dr. Walworth and the other co-authors began the experiment and helped create this paper by editing and peer review and making suggestions regarding data analysis and presentation.

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Table 3.1. Complete soil analysis from samples taken on 25 July 2018. One sample was taken from each of twelve rows. Values at each depth are averaged from all samples.

			Soil Depth		
Method	Test	Units	0-15 cm	15-30 cm	30-60 cm
1:1	pH	SU	8.5	8.5	8.8
1:1	$EC_{1:1}$	$dS \cdot m^{-1}$	3.4	2.8	2.1
Calculated	EC_e	$dS \cdot m^{-1}$	7.5	6.5	5.2
NH4OAc (pH 8.5)	Ca	$mg \cdot kg^{-1}$	4050	4125	3592
NH4OAc (pH 8.5)	Mg	$mg \cdot kg^{-1}$	510	563	514
NH4OAc (pH 8.5)	Na	$mg \cdot kg^{-1}$	4408	3433	2700
NH4OAc (pH 8.5)	K	$mg \cdot kg^{-1}$	653	618	489
DTPA	Zn	$mg \cdot kg^{-1}$	1.9	2.1	2.0
DTPA	Fe	$mg \cdot kg^{-1}$	4.0	3.8	4.2
DTPA	Mn	$mg \cdot kg^{-1}$	4.7	4.2	4.1
DTPA	Cu	$mg \cdot kg^{-1}$	20.7	22.7	18.1
DTPA	Ni	$mg \cdot kg^{-1}$	0.1	0.1	0.1
Cd-Reduction	NO_3	$mg \cdot kg^{-1}$	24.1	22.9	17.5
Olsen	PO_4	$mg \cdot kg^{-1}$	8.1	7.4	5.6
Hot Water	SO_4	$mg \cdot kg^{-1}$	190	155	107
Hot Water	B	$mg \cdot kg^{-1}$	3.2	2.6	2.3
Acid Test	Free Lime		High	High	High
Calculated	ESP	%	42.0	35.5	32.2
Calculated	CEC	meq/100g	45.3	41.8	35.2

Table 3.2. Origin descriptions of selected pecan cultivars.

Cultivar	Description (based on Grauke and Thompson, 2019)
Elliott	A seedling selected from Milton, FL in 1912; probably from trees of Mexican origin
Giles	A native seedling from near Chetopa, KS, 1927
Ideal	A seedling selection from San Saba, TX selected about 1925
Peruque	A native seedling from St. Charles, MO in 1918
Riverside	A seedling selected in Big Valley, TX
Shoshoni	USDA-ARS cross of 'Odom' (seedling from MS) X 'Evers' (seedling from Arlington, TX) made in Brownwood, TX in 1944; released in 1972
VC1-68	A seedling of unknown origin selected in Phoenix, AZ in 1968

Table 3.3. Mean resistance to salt injury ratings on pecan rootstocks from seven seed source cultivars on all observation dates. A rating of five represents the most resistance to salt injury, a rating of one represents the least. Values in each column that do not share the same letter are statistically different.

Seed Source Cultivar	Observation Date						
	9-28-2017	6-18-2018	9-12-2018	6-20-2019	10-9-2019	6-26-2020	10-6-2020
Elliott	2.8 AB	3.9 A	3.6 A	4.4 AB	3.8 A	3.9 BC	3.8 A
Giles	1.8 D	3.7 ABC	2.8 BC	4.2 ABC	2.6 B	4.0 ABC	3.1 BC
Ideal	2.5 BC	4.1 A	3.0 B	4.5 A	2.9 B	4.1 AB	3.1 C
Peruque	1.8 D	3.2 C	2.2 D	3.9 C	1.7 C	3.5 C	2.3 D
Riverside	2.9 A	4.0 A	2.9 B	4.4 AB	2.7 B	3.9 BC	3.6 AB
Shoshoni	2.4 C	3.7 AB	2.5 CD	4.4 AB	2.8 B	4.4 A	2.7 CD
VC1-68	2.5 ABC	3.2 BC	2.9 BC	4.1 BC	2.8 B	3.8 BC	3.0 C

Table 3.4. Mean vigor ratings on pecan rootstocks from seven seed source cultivars on all observation dates. A rating of five reflects healthy foliage and growth, a rating of one indicates that the tree was alive but had very little or no green foliage or recent growth. Values in each column that do not share the same letter are statistically different.

Seed Source Cultivar	Observation Date					
	6-18-2018	9-12-2018	6-20-2019	10-9-2019	6-26-2020	10-6-2020
Elliott	3.5 A	3.5 A	3.6 A	3.5 A	4.0 A	4.0 A
Giles	3.1 AB	2.5 CD	3.1 B	2.3 BC	3.0 DE	2.8 CD
Ideal	3.4 A	3.1 B	3.2 B	2.5 BC	3.6 BC	3.4 B
Peruque	2.7 B	2.3 D	2.6 C	1.5 D	2.6 E	2.3 D
Riverside	3.1 AB	2.8 BC	3.0 B	2.7 B	3.2 CD	3.4 BC
Shoshoni	3.4 A	2.8 BC	3.0 B	2.2 C	3.7 AB	3.4 BC
VC1-68	3.1 AB	3.2 AB	3.3 AB	2.5 BC	3.5 ABCD	3.3 BC

Table 3.5. Linear regressions between visual resistance to salt injury and plant vigor ratings by date. Larger r^2 values indicate a stronger relationship.

Date	r^2
6-18-2018	0.016
9-12-2018	0.358
6-20-2019	0.122
10-9-2019	0.546
6-26-2020	0.090
10-6-2020	0.217

Table 3.6. Linear regressions between growth rates and resistance to salt injury and vigor as well as death rates and resistance to salt injury and vigor. Larger r^2 values indicate a stronger relationship.

	Resistance to salt injury rating	Vigor rating
Tree growth	$r^2 = 0.410$	$r^2 = 0.565$
Tree survival	$r^2 = 0.530$	$r^2 = 0.390$

Table 3.7. Mean leaf nutrient concentrations in $mg{\cdot}kg^{-1}$ and K:Na ratios on 9 Oct. 2019 and 6 Oct. 2020. Values in each column that do not share the same letter are statistically different.

Seed Source Cultivar	P	Ca	Mg	Cl	Na	K	K:Na
				2019			
Elliott	935 D	11725 AB	2725 BC	3258 AB	1182 C	9975 A	10.1 A
Giles	1080 ABC	10750 B	1743 D	2913 B	4638 AB	7050 B	3.5 B
Ideal	1078 ABC	10625 B	3353 B	2610 B	3393 BC	7350 B	2.3 B
Peruque	970 BCD	9450 B	2185 CD	3632 AB	5570 AB	8200 AB	1.5 B
Riverside	1143 A	15525 A	3820 A	2416 B	3485 BC	6375 B	1.9 B
Shoshoni	1110 AB	12250 AB	2465 BCD	4454 A	6783 A	6575 B	1.1 B
VC1-68	965 CD	11425 AB	2525 BCD	2727 B	4755 AB	6475 B	2.2 B
				2020			
Elliott	911 C	7974 B	2880 BC	4637 BC	2208 C	10100 B	14.5 A
Giles	958 ABC	8333 B	2362 C	5687 ABC	3266 BC	13050 A	7.0 BC
Ideal	1090 A	8717 B	3228 B	3174 C	4869 B	6958 C	2.8 BC
Peruque	977 ABC	8543 B	2434 C	7640 A	4834 B	12814 A	3.3 BC
Riverside	942 BC	11659 A	3874 A	3942 C	3840 BC	7294 C	3.3 BC
Shoshoni	995 ABC	8317 B	2555 C	6770 AB	8624 A	6342 C	0.9 C
VC1-68	1059 AB	9233 B	2783 BC	8128 A	3629 BC	10533 AB	8.2 AB

Table 3.8. Correlations (r^2 values) between leaf nutrient concentrations and resistance to salt injury, vigor, and growth on leaves sampled in 2019 and 2020. NS, *,** = nonsignificant, significant (Pr > F = 0.05), and highly significant (Pr > F = 0.01).

	Leaf Nutrient	2019	2020
Resistance to Salt Injury	Na	0.550*	0.412*
	K	0.168**	NS
	Cl	NS	0.210**
	K:Na	0.545*	0.365*
Vigor	Na	0.586*	NS
	K	0.198**	NS
	Cl	NS	NS
	K:Na	0.500*	0.231**
Growth	Na	0.321*	NS
	K	0.198**	NS
	Cl	NS	NS
	K:Na	0.545*	0.370*

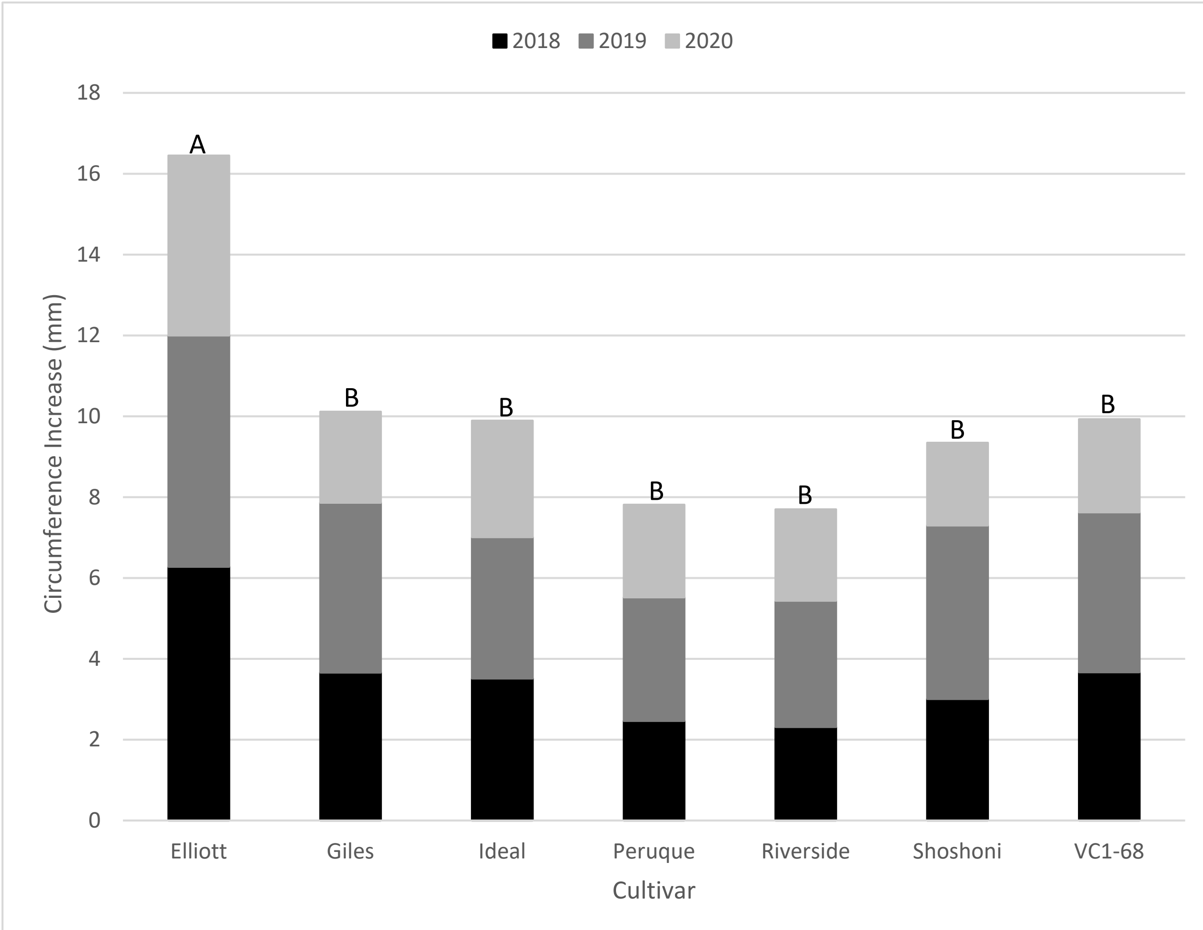


Figure 2.1. Annual pecan tree growth rates represented by dormant season trunk caliper measurements. Annual growth is represented by various colors in each bar, and total bar height reflects cumulative growth. Columns that do not share the same letter have statistically different overall circumference increases.

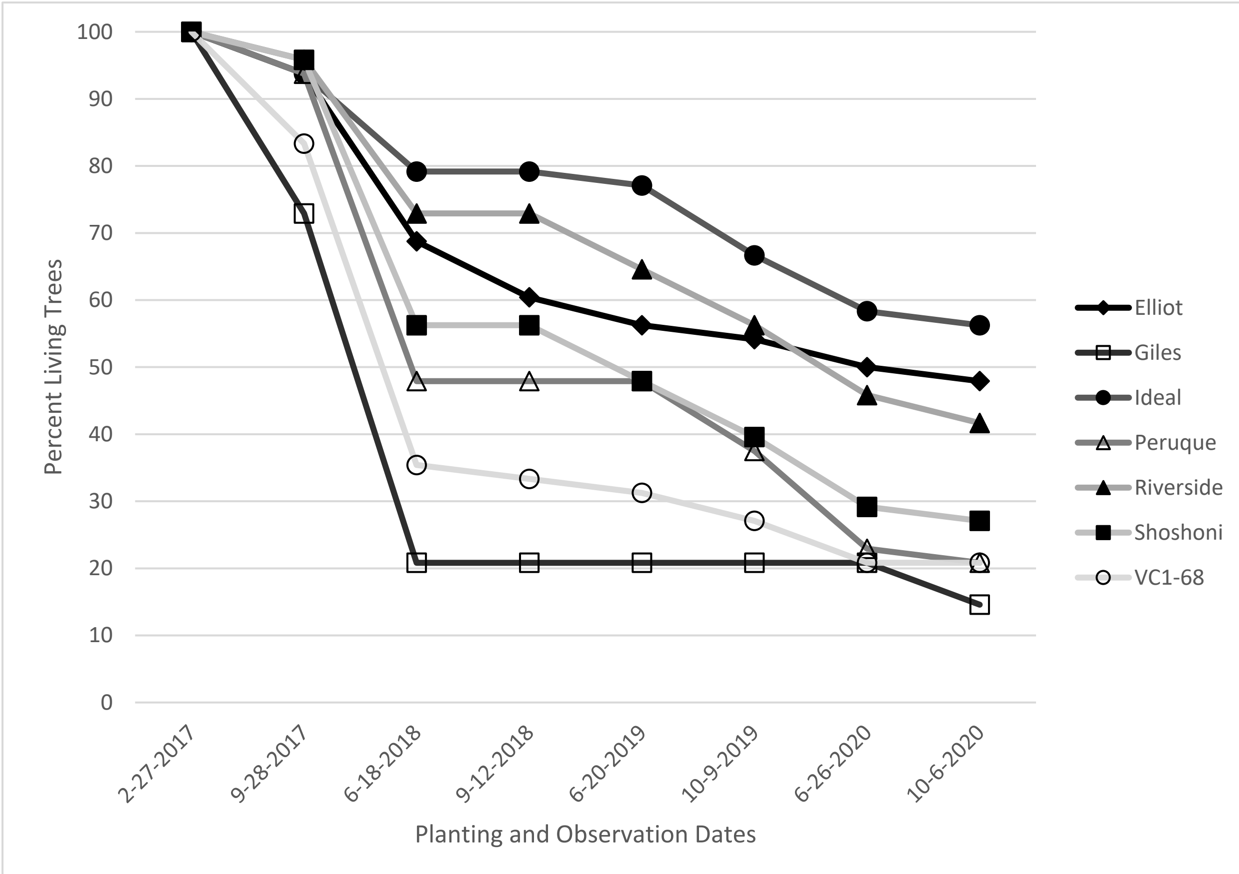
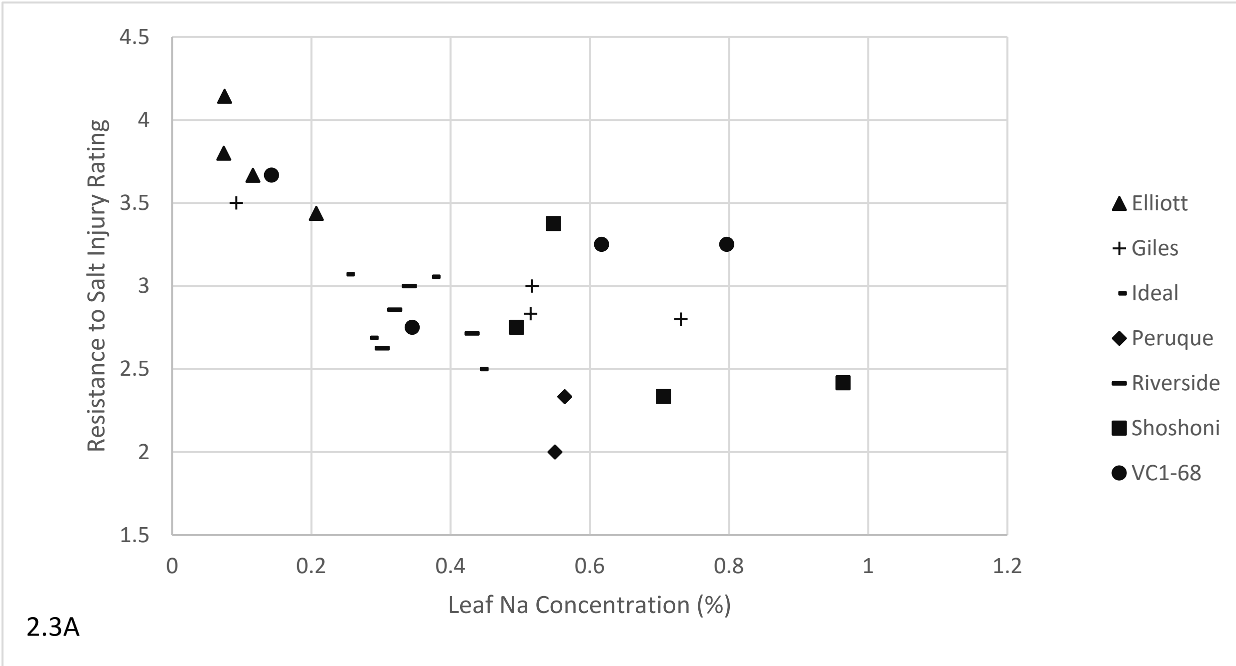
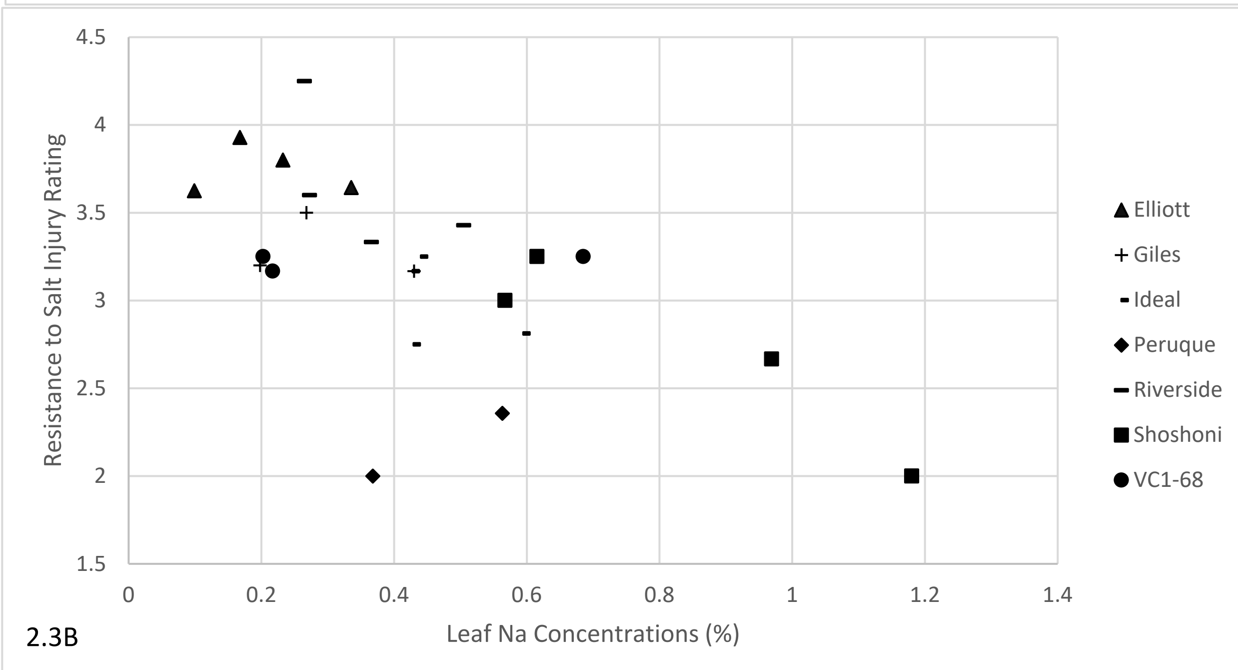
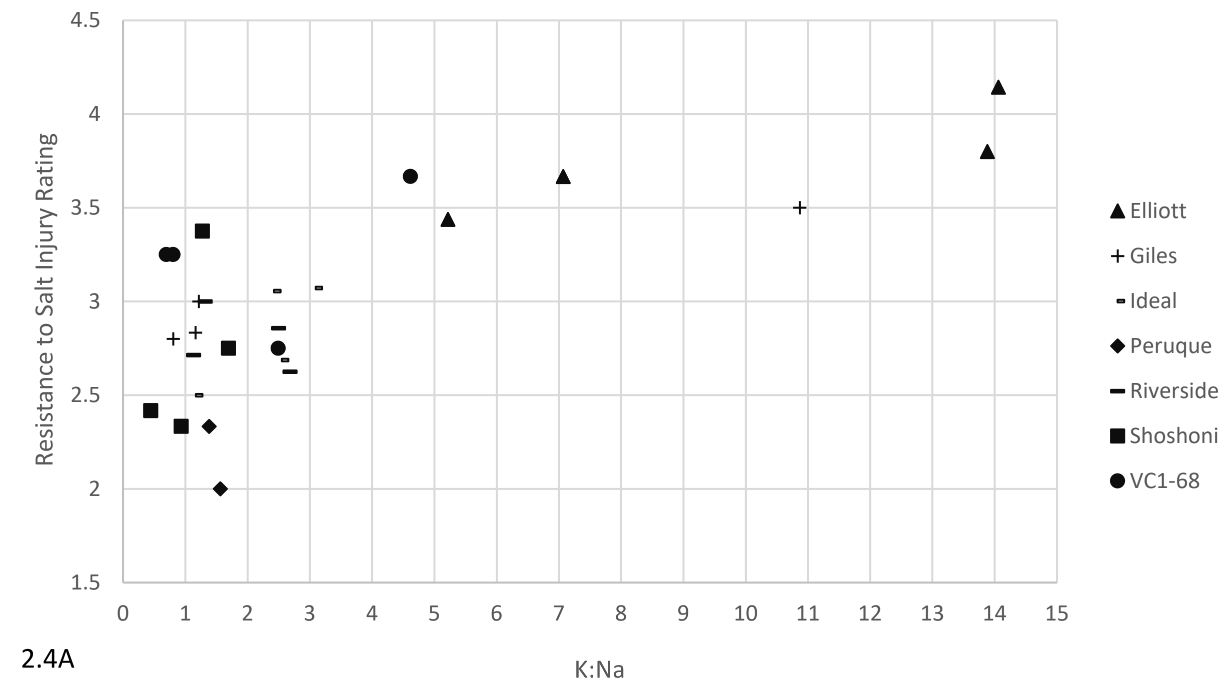


Figure 2.2. Rates of survival for all seven pecan cultivars throughout the study. Tree mortality determined on each observation date. The planting date of 27 Feb. 2017 is included.

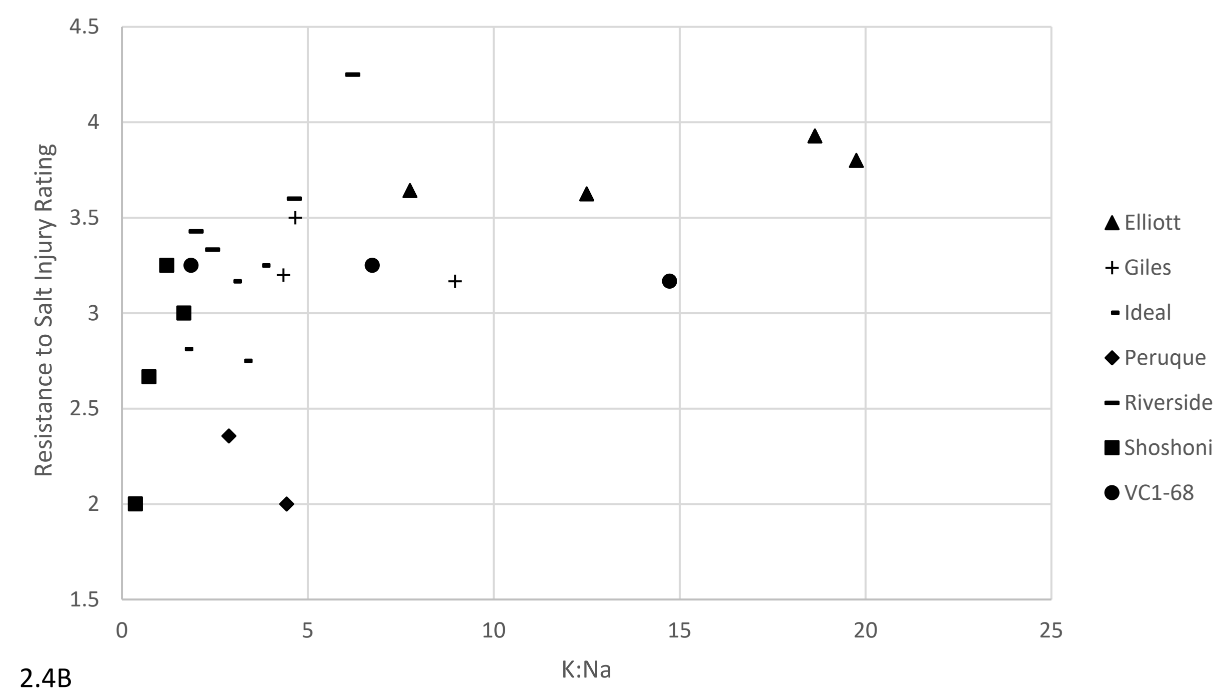




Figures 2.3A and 2.3B. Average resistance to salt injury ratings from composite samples and the corresponding Na percentages. Samples in figure 2.3A collected 9 Oct. 2019 ($r^2 = 0.550$) [$y = -0.523 * \ln(x) + 2.426$]. Samples in figure 2.3B collected 6 Oct. 2020 ($r^2 = 0.412$) ($y = -1.39x + 3.79$).

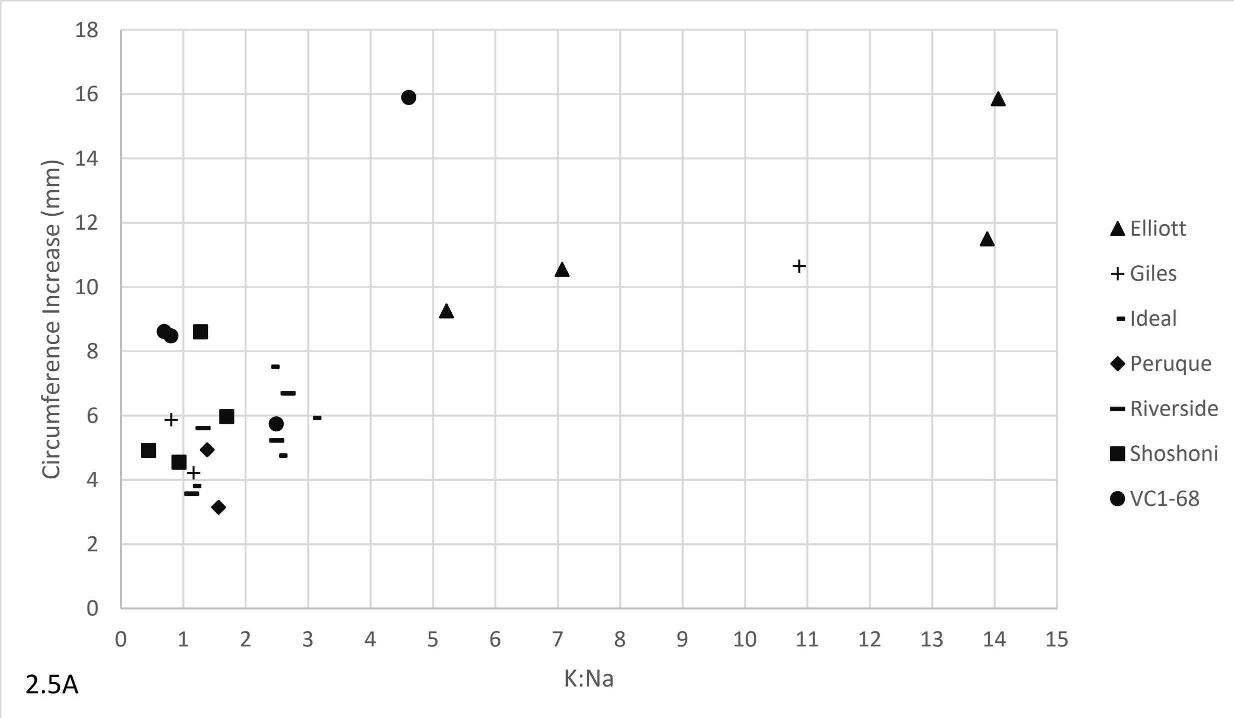


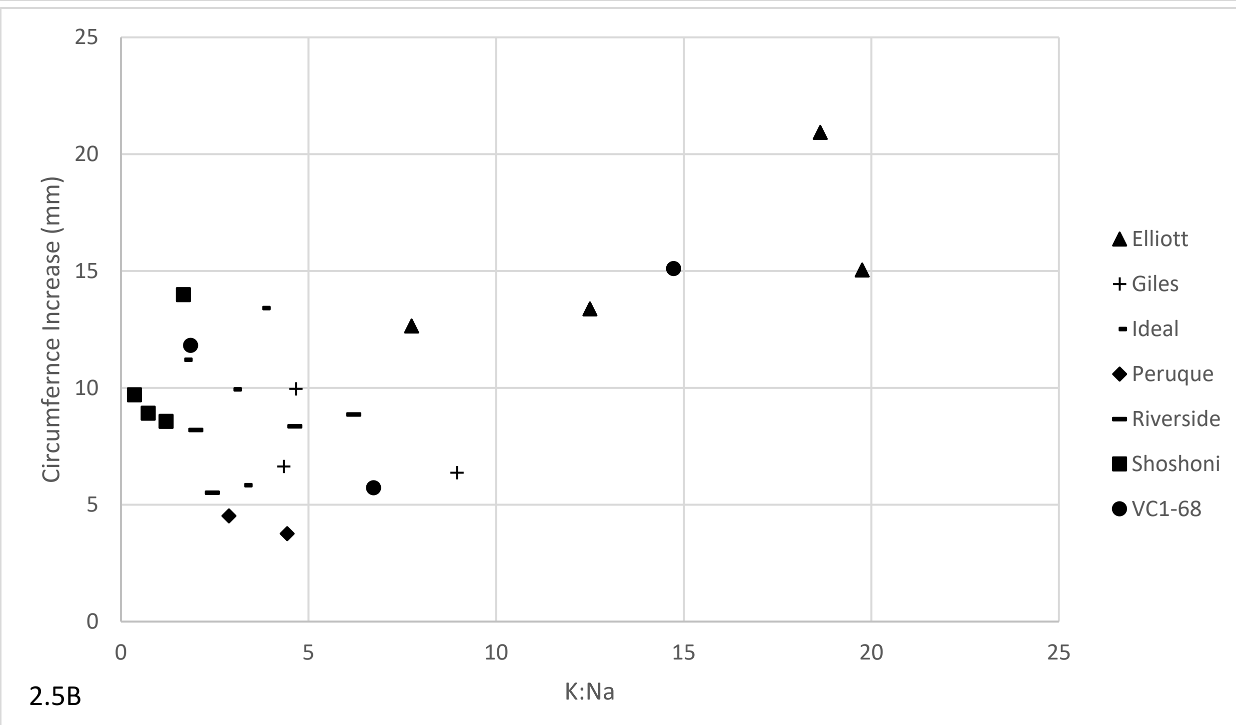
2.4A



2.4B

Figures 2.4A and 2.4B. Average resistance to salt injury ratings from composite samples and the corresponding K:Na ratios. Samples in figure 2.4A collected 9 Oct. 2019 ($r^2 = 0.545$) ($y = 0.0975x + 2.67$). Samples in figure 2.4B collected 6 Oct. 2020 ($r^2 = 0.365$) [$y = 0.336 * \ln(x) + 2.7361$].





Figures 2.5A and 2.5B. Average trunk circumference increases of trees in composite samples and the corresponding K:Na ratios. Samples in figure 2.5A collected 9 Oct. 2019 (r^2 = 0.545) (y = 0.651x + 5.04). Samples in figure 2.5B collected 6 Oct. 2020 (r^2 = 0.370) (y = 0.454x + 7.31).

Pecan Tree-to-Tree Variability: Implications for Leaf Sampling and Nutrient Recommendations

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Abstract

A field study was conducted in an orchard in San Simon, AZ to: (1) analyze associations between soil and leaf nutrients and between individual leaf nutrients. (2) determine the magnitude and sources of variability in leaf zinc (Zn) concentration within the experimental plot, (3) determine a reasonable sample size for practical use by calculating sample sizes for a range of relative margins of error based on measured tree-to-tree variability, and (4) establish a minimum acceptable orchard block-scale mean foliar Zn concentration to avoid or minimize Zn deficiency. The experimental plot consisted of seven rows in 2018 and nine rows in 2019 of seventeen trees each. Planted in 2011, the primary cultivar is 'Wichita', pollinated with 'Western'. The orchard soil is an alkaline calcareous Vekol loam. The plot was fertigated with 6.0 $kg \cdot ha^{-1}$ of zinc-ethylenediaminetetraacetic acid (Zn-EDTA) in 2018 and 11.0 $kg \cdot ha^{-1}$ in 2019. No foliar Zn applications were made. The trees did not exhibit visible signs of Zn deficiency during either year. Soil and leaf samples were collected from each tree on July 18, 2018 and August 1, 2019, and photosynthesis measurements taken on September 20, 2019 from 'Wichita' trees only. Trunk circumferences of each tree were measured annually. Analysis of associations between soil nutrients and leaf Zn concentrations, variability in mean leaf Zn concentrations between rows, and variability in mean leaf Zn concentrations by tree position within rows revealed cultivar differences and open pollinated rootstock to be the likely sources of tree-to-tree leaf Zn concentration variability. Calculations based on 2019 population data indicated that a sample size of 35 trees was needed to achieve a 10% relative margin of error at a 95% confidence level. An orchard block-scale mean leaf Zn concentration needed to avoid Zn deficiency in 95% of the trees in 2018 was calculated to be 21 $mg \cdot kg^{-1}$, and 25 $mg \cdot kg^{-1}$ in 2019.

Introduction

Many factors, including variation of soil characteristics, topography, irrigation, microclimate, pests, pathogens, and weeds may contribute to tree-to-tree variability within an orchard. In pecan *[Carya illinoinensis (Wangenh.) K. Koch]* orchards, where rootstocks are derived from open-pollinated seed, rootstock genetic variability may also contribute. Nutrient uptake variability has been noted in nut trees both within rootstocks from open-pollinated seed, and with different cultivars grafted on the same rootstock. For example, Surucu et al. (2020) conducted a study of 14 pistachio (*Pistacia Vera L.*) cultivars grafted to the same open-pollinated rootstock grown in an alkaline soil in Turkey and attributed variability in nutrient accumulation, nut quality, and yield to genetic differences in both seed-propagated rootstock and scion cultivar.

Nutrient management is most efficient if there is uniformity among trees. Usually, nutrients are applied evenly across each orchard block. In particular, where nutrients are applied via irrigation water (fertigated), a practice becoming routine in nut tree orchards (Brown, n.d.), there is seldom ability to vary application rates within a block, so a single rate of nutrients is applied to each tree, regardless of individual tree needs. Ideally, nutrients should be applied such that each tree receives at least the minimum acceptable levels of nutrients, and all trees maintain adequate leaf nutrient concentrations. To accomplish this, acceptable orchard block, as opposed to individual tree, nutrient levels must be defined.

The magnitude of variability that exists within an orchard block is not always readily apparent. Nutrient deficiencies can exist without visible symptoms, i.e. “hidden hunger”

(Heerema, 2013). Even where visible symptoms occur, identification of visible nutrient deficiencies requires close inspection, as symptoms can appear on as little as one branch of a tree (Sparks and Payne, 1982).

Both leaf and soil sampling are helpful for evaluating the magnitude and the sources of variability in an orchard block. Lindsey (1972) reported that plant tissue sampling and analyses are more sensitive for evaluating tree nutrient status than soil analyses, although Wear and Cope (1976) found significant correlations between soil and leaf Zn, Ca, Mg, and P in 'Stuart' and 'Schley' pecan cultivars growing in coarse to medium textured soil in central Alabama, and concluded that analyzing the top 0-6 inches of soil is satisfactory for determining pecans fertility status. Contrary to this, Alben and Hammar (1944) determined that because of the immobility of Zn in the soil, leaf analyses provide a more accurate determination of Zn deficiency. Brown and Uriu (1996) noted that the primary tool for making fertilization decisions is leaf analysis.

It is impractical and not economically feasible to sample and analyze leaves from each tree in an orchard block, so usually we do not know the true leaf nutrient concentration mean, how much variability exists, or minimum or maximum foliar nutrient levels within a block. Instead, orchard block sampling generally consists of collecting 40 to 100 leaflets from at least 10 trees randomly located within the block (Wells, 2014; McCraw et al., n.d.; Pyzner n.d), resulting in a value that represents the average nutrient level in the sampled area. Ideally, an orchard manager would sample the minimum number of trees necessary to obtain results that approximate the true population mean within an acceptable margin of error for practical decision making. Perhaps more important than the orchard block mean is determination of the margin of error associated with the mean. It is important to understand the possible deviation from the true mean, or margin of error, that may be present in smaller, practically-sized leaf samples so that accurate fertilization decisions can be made. Management decisions made in the absence of knowledge about spatial variation can lead to under or over-application of nutrients in various areas of the orchard (Lopez-Granados et. al., 2004).

Noordzij et al. (2011) pointed out that small sample sizes may result in unacceptable data, whereas unnecessarily large sample sizes result in excess time and money expenditures. The University of Oklahoma Cooperative Extension emphasized that it is necessary to judge the level of uniformity of the trees within an orchard to determine the requisite number of samples, but did not provide guidance for making this determination (McCraw et al., n.d). Brown et al. (n.d.) determined the number of almond (*Prunus dulcis* L.) trees to sample to obtain a mean nitrogen concentration within 5% of the true population mean to be between 18 and 28. More broadly, they indicated that the number of trees required corresponds to the desired level of confidence and the number of trees in the sampled orchard block, but they did not specify a method for establishing the sample size. Carvalho et al. (2020) established a minimum sample size for estimation of citrus flush patterns by defining an acceptable relative sampling error from the true population mean and determining a sample size based on the relationship between relative sampling error and the existing standard deviation and mean using the equation

$$\mathrm{n} = [(\sigma/(\mathrm{Er} \cdot \mu))]^2 \qquad \text{Eq(1)}$$

where n=sample size, σ=standard deviation, Er=relative sampling error [(standard error of the mean)÷mean, the degree of accuracy required or tolerated], and μ=population mean.

Miyamoto and Cruz (1986) used this equation to determine the sample size needed to obtain a soil salinity mean within 15% of the true orchard block mean when soil sampling pecan orchards in the El Paso Valley of Texas.

Research based on individual tree responses can be used to determine acceptable nutrient levels for maximizing tree performance (Heerema et al., 2014 and 2017; Sherman et al., 2017). Applying individual tree optima to orchard blocks is problematic, however, because of the tree-to-tree variability inherent in orchards. The goal of orchard block nutrient management must be to ensure that most or all trees in a block contain optimum levels of nutrients. Knowledge of variability within a block is required so that the needs of all trees can be addressed. In other words, the level of variability might be used to convert individual tree data to orchard-scale recommendations. Hu and Sparks (1990) noted that variability within an orchard has a “masking effect”, and that average values must be higher to ensure adequate nutrition in all trees.

For example, reduction in carbon assimilation rates was reported when leaf zinc concentrations fell below 14 to 22 $mg \cdot kg^{-1}$ (Heerema et al., 2017) or 14 $mg \cdot kg^{-1}$ (Hu and Sparks, 1991). Ideally, all or most of the individual trees in a management area should have foliar Zn concentrations above this threshold. To utilize the Zn threshold observed by Heerema et al. (2017) and Hu and Sparks (1991) on an orchard block scale, it is necessary to know what block-wide mean leaf Zn concentration is required to ensure only a minimal number of trees within an orchard block will have foliar concentrations below this threshold. This is reflected in recommended orchard leaf Zn concentrations which are generally much higher than the reported individual tree threshold. Sparks and Payne (1982) indicated that, although most diagnostic laboratories recommend maintaining leaf Zn concentrations of greater than 50 or 60 $mg \cdot kg^{-1}$ due to variability within the orchard, actual Zn deficiency does not occur until leaf Zn concentrations fall below 20 $mg \cdot kg^{-1}$.

The current experiment was conducted using exhaustive sampling from a single orchard block with the following objectives: (1) analyze associations between soil and leaf and between individual leaf nutrients, (2) determine the magnitude and sources of variability in leaf Zn concentration within the experimental plot, (3) determine a reasonable sample size for practical use by calculating sample sizes for a range of relative margins of error based on measured tree-to-tree variability, and (4) establish a minimum acceptable orchard block-scale mean foliar Zn concentration to avoid or minimize Zn deficiency.

Materials and Methods

A field experiment was conducted in an orchard near San Simon, AZ (lat. 32°15′20.2” N, long. 109°10’29.8” W, elevation 1118 m) established in 2011. In 2018, the studied area consisted of 7 rows with 17 trees each, beginning with the 11th tree from the end of each row, in the interior of an orchard block. Six rows were ‘Wichita’ and one row was ‘Western’ (every fourth row in the orchard block). In 2019, two more rows of ‘Western’ with 17 trees each were included such that the study included six rows of ‘Wichita’, and three of ‘Western’. Both scion cultivars were on open-pollinated ‘Ideal’ rootstock. The soil was Vekol loam (Fine, mixed, superactive, thermic Typic Haplargids). The orchard’s climate is semi-arid and it receives approximately 24 cm of precipitation annually (Western Regional Climate Center). The orchard was irrigated through a micro-sprinkler system, with one sprinkler between adjacent trees, 24 times per year at a rate of approximately 6.4 cm per irrigation (approximately 152 cm annually). Nitrogen, P, and K were applied uniformly through the fertigation system on five occasions, March through June each year, at annual rates of 213 $kg \cdot ha^{-1}$, 50.5 $kg \cdot ha^{-1}$, and 50.5 $kg \cdot ha^{-1}$, respectively. Nine percent Zn-EDTA was applied on eight occasions by fertigation at a total rate of 6.0 $kg \cdot ha^{-1}$ of Zn in 2018 and 11.0 $kg \cdot ha^{-1}$ of Zn in 2019. No foliar Zn applications were made to the trees during this study. In both years, 2.24 $kg \cdot ha^{-1}$ K and 1.12 $kg \cdot ha^{-1}$ Ni were foliarly applied in April/May, and 4.48 $kg \cdot ha^{-1}$ K, and 2.24 $kg \cdot ha^{-1}$ Fe were applied foliarly in June. Standard commercial weed and insect control measures were conducted by the grower-cooperator.

In 2018 and 2019, foliar samples were collected from fruiting branches of each tree in the experimental plot following a standard leaf sampling protocol (Heerema, 2013). All leaflets were washed in a phosphorus-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 hours at 65^{o} C and ground using a cyclone mill (UDY Cyclone Sample Mill. UDY Corporation, Fort Collins, CO.). Samples were analyzed for total nutrient content (Brookside Laboratories, Inc. New Bremen, OH).

Soil samples, each consisting of four combined sub-samples, were collected from near the base of each tree in the experimental plot in 2018 and 2019 to a depth of 0-12 inches. The soil samples were oven-dried at a temperature of 65^{o}C, ground and sorted using a 2mm sieve. Samples were analyzed for extractable nutrient contents and pH (Brookside Laboratories, Inc. New Bremen, OH). Trunk diameters were measured at 75 cm above the ground surface on February 28, 2019 and January 14, 2020.

Leaf gas exchange was measured on middle leaflets from non-terminal, sun-lit leaves from each tree in the 'Wichita' rows on September 20, 2019 using a portable Pn (photosynthesis) system (LI-6800; LI-COR, Lincoln, NE) equipped with a red/blue light source (LICOR 6800-02). Photosynthetically active radiation (PAR) in the chamber was maintained at 1700 mmol$\cdot$ m^{-2} $\cdot s^{-1}$. Light saturation of Pn for pecan is reached between a PAR of 1500 to 1700 mmol$\cdot$ m^{-2} $\cdot s^{-1}$ (Anderson, 1994; Lombardini et al., 2009). Reference CO_2 concentration was kept at 400 mmol$\cdot mol^{-1}$, near the global mean atmospheric concentration (U.S. Department of Commerce, 2021). Once the Pn and gS (stomatal conductance) 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.

Relationships between soil and leaf and within leaf nutrients, mean leaf nutrient concentrations and tree growth and pH, as well as Pn relationships with tree growth and mean leaf nutrient concentrations were assessed using Spearman's p correlations. Relationships were assessed individually for each cultivar during each year of the experiment.

Determination of the required sample size to obtain a mean with a predetermined relative margin of error from the true population mean using combined 2019 leaf Zn concentration data [the nutrient with the highest coefficient of variance (CV)] from both cultivars was accomplished using Eq(2).

The relative margin of error at a specified confidence level ($Mr_{\alpha/2}$) was expressed as a percentage and is defined by

$$Mr_{\alpha/2} = (Z_{\alpha/2} \cdot (\sigma/\sqrt{n}))/\mu$$

where $Z_{\alpha/2}$ = desired level of confidence n = sample size. All other terms are defined as in Eq(1).

To determine n with a specified confidence level the Z-value was added to the equation.

$$n = [Z_{\alpha/2} \cdot (\sigma/(Mr_{\alpha/2} \cdot \mu))]^2 \qquad \text{Eq(2)}$$

One thousand random samples without replacement were simulated using the Survey Select Procedure (SAS version 9.4) to confirm the adequacy of the standard approach previously described (which is based on independence, constant variance, and normality assumptions, without the inclusion of a finite population correction factor) for the combined 2019 leaf Zn concentration data. The approximate percent margin of error was obtained from the difference between the 2.5 percentile and 97.5 percentile mean leaf Zn concentrations divided by the true population mean.

To determine a mean leaf Zn concentration required to ensure that less than 5% of the trees in the experimental plot fell below a prescribed minimum of 15 $mg \cdot kg^{-1}$ at which Pn would not be reduced (based on the data of Heerema et al., 2017 and Hu and Sparks, 1991) the population data (complete data for the experimental plot) was used in the following equation

$$m \geq \ln(x) + Z(\sigma) \qquad \text{Eq(3)}$$

where m = natural log of required mean leaf Zn concentration, x = the target mean leaf Zn threshold, Z = percent of trees below a desired threshold, and σ = the natural log standard deviation of the complete population data. The data were normalized by natural log transformation. ‘m’ is then exponentiated to determine the required mean leaf Zn concentration in the original units ($mg \cdot kg^{-1}$).

JMP Pro 15 software (SAS Institute, Cary, NC) was used to perform nonparametric Spearman's p correlations. The Survey Select Procedure (SAS version 9.4) was used for simple random sampling without replacement. An alpha value of 0.05 was used in all statistical tests.

Results

Foliar concentrations of most essential nutrients were measured and evaluated (chlorine, molybdenum, and nickel were not included) (Table 4.1). Because leaf Zn had the greatest variability (based on combined 2019 data), and because a goal of this study was to determine an orchard level target mean leaf nutrient Zn concentration, the presented results are focused on Zn nutrient variability. Recommended sample size determination was similarly based on leaf Zn concentration data.

Averaged throughout the course of the study 'Wichita' had approximately 6% greater variability than 'Western' in mean leaf Zn concentration. 'Western' mean leaf Zn concentrations were higher (22.75 $mg{\cdot}kg^{-1}$ in 2018, 25.12 $mg{\cdot}kg^{-1}$ in 2019) than 'Wichita' (19.44 $mg{\cdot}kg^{-1}$ in 2018, 17.76 $mg{\cdot}kg^{-1}$ in 2019) both years (Table 4.1). 'Wichita' mean leaf Zn concentrations declined from 2018 to 2019, whereas 'Western' Zn concentrations rose during that period.

Although there was generally a decrease in mean 'Wichita' leaf Zn concentrations from 2018 to 2019, the majority of 'Wichita' leaf Zn concentrations fell between 15 and 25 $mg{\cdot}kg^{-1}$ both years (Figure 3.1). In 2018, 7.8% percent of 'Wichita' trees fell below the minimum threshold of 15 $mg{\cdot}kg^{-1}$ versus 27.5% that fell below this value in 2019. 'Western' had a higher mean leaf Zn concentration in 2019 than 2018. In 2018, 5.9% of the trees had less than 15 mg·kg^{-1} of Zn, whereas none fell into this category in 2019. In 2018, just 23.5% of 'Western' trees contained more than 25 mg·kg^{-1}. This fraction more than doubled to 49.1% in 2019.

Individual tree Zn status is shown in Figure 3.2 (for 2018) and Figure 3.3 (2019). Higher leaf Zn concentrations were generally found in 'Western' rows versus those of 'Wichita'. Analysis by tree position (tree number) within rows revealed little difference in mean leaf Zn concentrations; 18.27 to 21.30 mg·kg^{-1} in 2018, and 17.88 to 21.44 mg·kg^{-1} in 2019 (data not shown).

In addition to Zn, concentrations of leaf N, P, Ca, S, B, Fe, Mn, and Cu in 'Wichita' trees were lower in 2019 than in 2018. Potassium and Mg concentrations increased. With the exceptions of Zn and Ca, a similar pattern was observed for these leaf nutrients in 'Western'.

The highest leaf nutrient variabilities in both years and both cultivars were found in B, Mn, and Zn. The least variability was observed in leaf N (Table 4.1). Both Zn and N were applied entirely by fertigation through the permanent microsprinkler system, so uniformity of distribution for these nutrients was likely similar. Zinc concentration variability was highest in 'Wichita' in 2019 (CV=25.51%) and lowest in 'Western' during 2018 (CV=18.60%) (Table 4.1).

Nonparametric Spearman's p correlations showed that 'Wichita' had nearly five times as many consistent, significant within leaf nutrient correlations in 2018 and 2019 than 'Western' (Table 4.2). However, only three leaf-to-leaf nutrient concentration correlations were consistently significant during both years and in both cultivars. These were positive correlations between Ca and Mg, Ca and Mn, and between Zn and Cu.

We assessed association between soil and leaf nutrient levels to determine the extent to which variability of soil nutrient levels could explain the observed variability of leaf composition. A consistent positive soil to leaf correlation was found only between soil Cu and leaf S in ‘Wichita’. In ‘Western’ the sole consistent (positive) soil to leaf correlation was between soil Zn and leaf S (data not shown). No consistent correlations were found between pH and any leaf nutrients. Increase in trunk diameter from February 2019 to January 2020, and 2019 leaf Zn concentrations were positively correlated (R^2= .2361) (data not shown). Other leaf nutrient concentrations correlated with tree growth included a positive correlation with N and negative correlations with K and Cu (data not shown). Measured rates of photosynthesis were not significantly correlated with any foliar nutrient concentrations, nor with growth of trunk diameter.

The relative margins of error associated with the sample sizes obtained from Eq(2) at 95% confidence (Table 4.3) track with the approximate percent margins of error obtained from simple random sampling (Table 4.4). However, the results from simple random sampling show a slightly smaller percentage of error than the relative margins of error calculated using the standard equation due to the assumption of an infinite population and lack of a finite population correction factor in Eq(2). With an increase in the coefficient of variance, the relative margin of error becomes larger for a given sample size. This can be seen in the difference in relative margins of error for each leaf nutrient with a sample size of 35 trees (Table 4.5).

Using the log transformed population data with Eq(3) in 2018 we found that the mean leaf Zn concentration required to keep equal to or less than 5% of the trees below 15 $mg{\cdot}kg^{-1}$ in our plot would be 21 mg·kg^{-1}. Using 2019 leaf Zn data, the corresponding sample size was calculated to be 25 mg·kg^{-1}. The corresponding mean leaf Zn concentrations required to ensure that no more than 1% of the trees fall below this threshold were 24 mg·kg^{-1} based on 2018 data, and 30 mg·kg^{-1} using 2019 data.

Discussion

The mean leaf Zn concentration of 'Wichita' was lower and the variability relative to the mean higher than those of 'Western' during both years. Walworth et al. (2017) also found that leaf Zn concentrations were slightly higher in 'Western' than 'Wichita' in a study conducted in a nearby orchard. Herrera (2005) and Heerema (2013) both noted that when grown in high pH, calcareous soils 'Wichita' pecans are more susceptible to Zn deficiency than 'Western' trees.

No consistent relationships between soil nutrients and leaf Zn concentrations were found, suggesting that soil nutrient availability was not a major source of leaf Zn concentration variability. No consistent relationship was found between leaf Zn concentration and pH. The orchard block is uniformly irrigated and weed and pest management is well maintained. Because end rows and end trees were not included in this study, and sampled trees were in the interior of the orchard block, exposure to sunlight is expected to be uniform and air flow throughout the trees consistent. The orchard block is level and visually, soil characteristics are uniform. This lack of variability sources as well as little difference in row to row average mean leaf Zn concentrations or in mean leaf Zn concentrations of tree position within rows in either year, suggests that the tree-to-tree variability in leaf Zn concentration is not due primarily to position in the field, but likely related to cultivar differences and individual genetics of the open-pollinated rootstocks. This is compatible with the findings of Surucu et al. (2020) who found variable Zn uptake among pistachios grafted to open-pollinated rootstock. In contrast, two studies conducted in different locations with 18 of the same pecan cultivars found no significant difference in foliar Zn levels among any of the cultivars, suggesting limited genetic influence on variability in Zn uptake (Sparks and Madden, 1977; Worley and Mullinix, 1993).

Variability in relation to the mean, reflected in the CV, is the primary factor affecting calculation of the sample size needed to obtain results that approach the true population mean of the orchard within an acceptable margin of error. This relationship can be seen in the study by Miyamoto and Cruz (1986) who found that as the CV increased for mapping units of an orchard in the El Paso Valley, the sample size required to obtain a soil salinity mean within 15% of the true mean increased. This author used a modified version of Eq(2) to determine sample sizes.

A sample size of 35 trees was determined from the nutrient with the highest CV (Zn) for the combined 2019 data to be necessary to achieve a relative margin of error of 10% and 95% confidence using Eq(2). All other nutrients have a smaller CV than Zn, and therefore lesser margins of error at a sample size of 35. This sample size is considerably larger than the 10 trees recommended by Wells (2014), McCraw et al. (n.d.), or Pyzner (n.d) for pecan, and slightly larger than the 18 to 28 trees suggested by Brown (n.d.) for almonds.

The relative margins of error of sample sizes obtained using Eq(2) are larger than the approximate percent margins of error obtained through simple random sampling due to the assumption of an infinite population and lack of finite population correction factor in the equation. This calculation errs on the side of caution, as the margin of error obtained through real random sampling in an orchard should be similar to the results of simple random sampling without replacement. Ultimately, the choice of sample size will depend on the time dedicated to sample collection contrasted with the desired level of accuracy.

Using accepted recommended nutrient concentration ranges and the calculated margin of error for each nutrient (Table 4.5) one can compare the impact of the error associated with a sample size of 35 trees on interpretation of nutrient analyses. For a leaf sample containing 2.75% N [the median of the "adequate" nutrient concentration range cited by Heerema (2013)] the margin of error is estimated to be 0.047% N, so the sample should contain between 2.7 and 2.8% N. This is likely an acceptable level of precision to allow accurate diagnosis. Similarly, the expected concentration range of a leaf sample falling in the middle of the 0.14 to 0.19% P "adequate" range is 0.159 to 0.171% P. For K, the "adequate" range is 1.2 to 2.5%, compared to an expected margin of 1.79 to 1.91%. Boron and Mn exhibited the greatest variability. For B, a sample at the midpoint of the "adequate" range (50 to 150 $mg{\cdot}kg^{-1}$), the sample margin would be 91 to 109 $mg{\cdot}kg^{-1}$. Similar values for Mn are 100 to 300 $mg{\cdot}kg^{-1}$ and 182 to 218 $mg{\cdot}kg^{-1}$. Sample collection for Zn analysis is likely to be different for trees sprayed with Zn solutions where leaf Zn concentrations are much higher than unsprayed trees such as those in this study. Based on the average Zn concentrations measured in this study (20.1 $mg{\cdot}kg^{-1}$) the expected sample margin would be 18.1 to 22.1 $mg{\cdot}kg^{-1}$.

Commercially, sample size selection will result from a balance of cost and practicality of sampling versus an acceptable relative margin of error. Of course, the results obtained in this study are strictly valid for the conditions of our data only, but should provide a reasonable starting point for determining the requisite number of trees to sample in an orchard block.

In research, it is often desirable to intensively sample all the treated trees in a study in order to produce accurate data and to maximize the ability to detect tree responses. Such data are also used to develop detailed relationships between leaf nutrient concentrations and plant performance measures such as rates of growth or photosynthesis, nut yield, etc. (Heerema et al., 2014 & 2017; Sherman et al., 2017), but it is challenging to apply these at an orchard block scale. To make field-scale mean leaf Zn concentration recommendations, determination of a target mean leaf nutrient concentration needs to consider variability of trees in a sampled block and the relative margin of error associated with the collected leaf sample. Individual tree mean leaf Zn concentration in unsprayed trees fertigated with Zn-EDTA needs to be maintained at a minimum of approximately 15 $mg \cdot kg^{-1}$ (slightly above the minimum concentration noted by Heerema et al., 2017 and Hu and Sparks, 1991) to ensure photosynthesis is not Zn limited. The mean leaf Zn concentration of an orchard block sample required to ensure a sufficient Zn concentration in 95% of the trees is, in part, dependent on the level of tree-to-tree variability. As variability increases so does the mean leaf Zn concentration required to avoid trees limited by unacceptably low Zn concentrations. Based on our 2019 data, an appropriate field-scale minimum Zn concentration to maintain at least 15 $mg \cdot kg^{-1}$ of foliar Zn in 95% of trees is approximately 25 $mg \cdot kg^{-1}$. A more conservative threshold of 30 $mg \cdot kg^{-1}$ is needed to ensure that 99% of trees have at least 15 $mg \cdot kg^{-1}$.

This guideline is based on the studied orchard block, but provides an initial target value for pecan orchards in general. More precise orchard management guidelines require an assessment of individual orchard or orchard block variability (McCraw et al., n.d.), or else determination of tree-to-tree variability in additional pecan orchards.

Contributions

This experiment contributes to the field of horticulture with our analysis of complete population data and presentation of a mean leaf zinc concentration required to ensure photosynthesis in 95% or 99% of the trees will not be zinc limited, giving growers a guideline for crop health management decisions. Our determination of the relative margin of error from the true population mean associated with small sample sizes also contributes to horticulture by assisting the pecan grower in making fertilization decisions. Individual tree data can be converted to orchard-block scale nutrient recommendations based on variability with better understanding of the level of accuracy associated with the samples obtained. Our analysis of sources of variability contribute to research in the fields of horticulture and soil fertility and plant nutrition and presents information for genetic research in the future.

My contribution to this research included sample collection, measurements, sample processing, compiling and interpreting data, and research and writing (as the first author). Dr. Walworth and the other co-authors began the experiment and helped create this paper by editing, providing peer review and making suggestions regarding data analysis and presentation.

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Table 4.1. Leaf nutrient concentration means (in percentage or mg·kg^{-1}), SD, and CV (expressed as a percentage) for 'Wichita' and 'Western' pecan trees sampled individually in late July and early August of 2018 and 2019.

		N	P	K	Ca	S	Mg	B	Fe	Mn	Cu	Zn
		(%)						(mg·kg^{-1})				
2018	Wichita	2.20	0.10	1.37	1.64	0.20	0.36	145.97	126.4	188.44	6.63	19.44
	SD	0.14	0.01	0.18	0.23	0.02	0.06	43.78	18.92	55.75	0.60	4.18
	CV	6.29	8.02	13.51	14.15	9.79	16.2	29.99	14.97	29.58	8.98	21.5
	Western	2.27	0.11	1.18	1.44	0.19	0.37	149.65	133	184.76	6.90	22.35
	SD	0.12	0.01	0.19	0.25	0.02	0.06	51.88	20.53	41.22	0.40	4.16
	CV	5.30	8.75	16.36	17.68	11.3	16.79	34.67	15.44	22.31	5.86	18.6
2019	Wichita	2.13	0.09	1.66	1.54	0.19	0.37	98.06	95.78	108.27	6.19	17.76
	SD	0.11	0.01	0.16	0.25	0.02	0.07	23.60	14.35	30.68	0.47	4.53
	CV	5.07	9.33	9.68	16.48	12.76	18.11	24.07	14.98	28.34	7.64	25.51
	Western	2.18	0.09	1.59	1.48	0.19	0.40	97.4	97.93	125.07	5.89	25.12
	SD	0.11	0.01	0.17	0.23	0.03	0.05	28.58	14.58	35.80	0.57	5.86
	CV	4.82	11.48	10.51	15.58	12.97	12.55	29.34	14.89	28.63	9.65	23.34

Table 4.2. Consistent significant (Pr > F = 0.05) leaf to leaf nutrient correlations in 2018 and 2019 for 'Wichita' and 'Western' cultivars. Spearman's p correlation coefficients for each year shown separately. + = a positive correlation, - = a negative correlation.

Wichita		2018	2019
Ca	Mn +	0.683	0.674
Cu	Zn +	0.356	0.388
	N +	.519	.212
Fe	S +	0.326	0.298
	B +	0.398	0.225
K	Mn -	.299	.260
Mg	Ca +	0.451	0.686
	B +	0.347	0.366
	Fe +	0.416	0.458
	Mn +	0.356	0.542
P	Ca +	.271	.255
	Cu +	.450	.280
	N +	0.282	0.354

Western		2018	2019
Ca	Mn +	.547	.780
	Mg +	.670	.648
Cu	Zn +	.716	.418

Table 4.3. Number of trees required at 95% and 90% confidence for the corresponding relative margin of error from the true population mean of the combined 2019 leaf Zn concentration data. Data calculated using Eq(2).

Relative margin of error (expressed as a percentage)	Number trees at 95% confidence	Number of trees at 90% confidence
15	15	11
14	18	13
13	21	15
12	24	17
11	29	20
10	35	25
9	43	30
8	54	38
7	71	50

Table 4.4. Results of simple random sampling without replacement from combined 2019 leaf Zn concentration data (1000 simulations for each sample size). Sample sizes were chosen based on results from Table 4.2. Approximate percent margin of error from the true population mean is ½ the difference between the 2.5 percentile and 97.5 percentile mean leaf Zn concentrations obtained in $mg \cdot kg^{-1}$ divided by the true population mean (20.22 $mg \cdot kg^{-1}$) (comparable to 95% confidence).

Number of trees sampled	15	18	21	24	29	35	43	54	71
Standard error	1.57	1.43	1.33	1.24	1.13	1.03	0.93	0.83	0.72
2.5 percentile	17.4	17.7	17.9	18.2	18.4	18.5	18.7	18.9	19.2
97.5 percentile	23.3	23.0	22.6	22.5	22.1	22.0	21.8	21.5	21.3
Approximate % margin of error ±	14.6	13.1	11.6	10.6	9.15	8.65	7.67	6.43	5.19

Table 4.5. Coefficients of variance (expressed as a percentage) and the relative margin of error (expressed as a percentage) from the true population mean at a sample size of 35 trees and 95% confidence for combined 2019 leaf nutrient concentration data. Relative margins of error calculated using Eq(2). Nutrients arranged in ascending order of relative margin of error.

Leaf nutrient	N	Cu	P	K	S	Fe	Ca	Mg	B	Mn	Zn
	%	$mg \cdot kg^{-1}$	%	%	%	$mg \cdot kg^{-1}$	%	%	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$	$mg \cdot kg^{-1}$
SD	0.11	0.525	0.009	0.166	0.025	14.4	0.248	0.063	25.3	31.3	6.09
Mean	2.15	6.09	0.086	1.64	0.194	96.5	1.52	0.381	97.8	114	20.2
CV (%)	5.1	8.6	10.5	10.1	12.9	14.9	16.3	16.5	25.9	27.5	30.1
Relative margin of error (%)	1.7	2.9	3.4	3.4	4.3	5	5.4	5.5	8.6	9.1	10

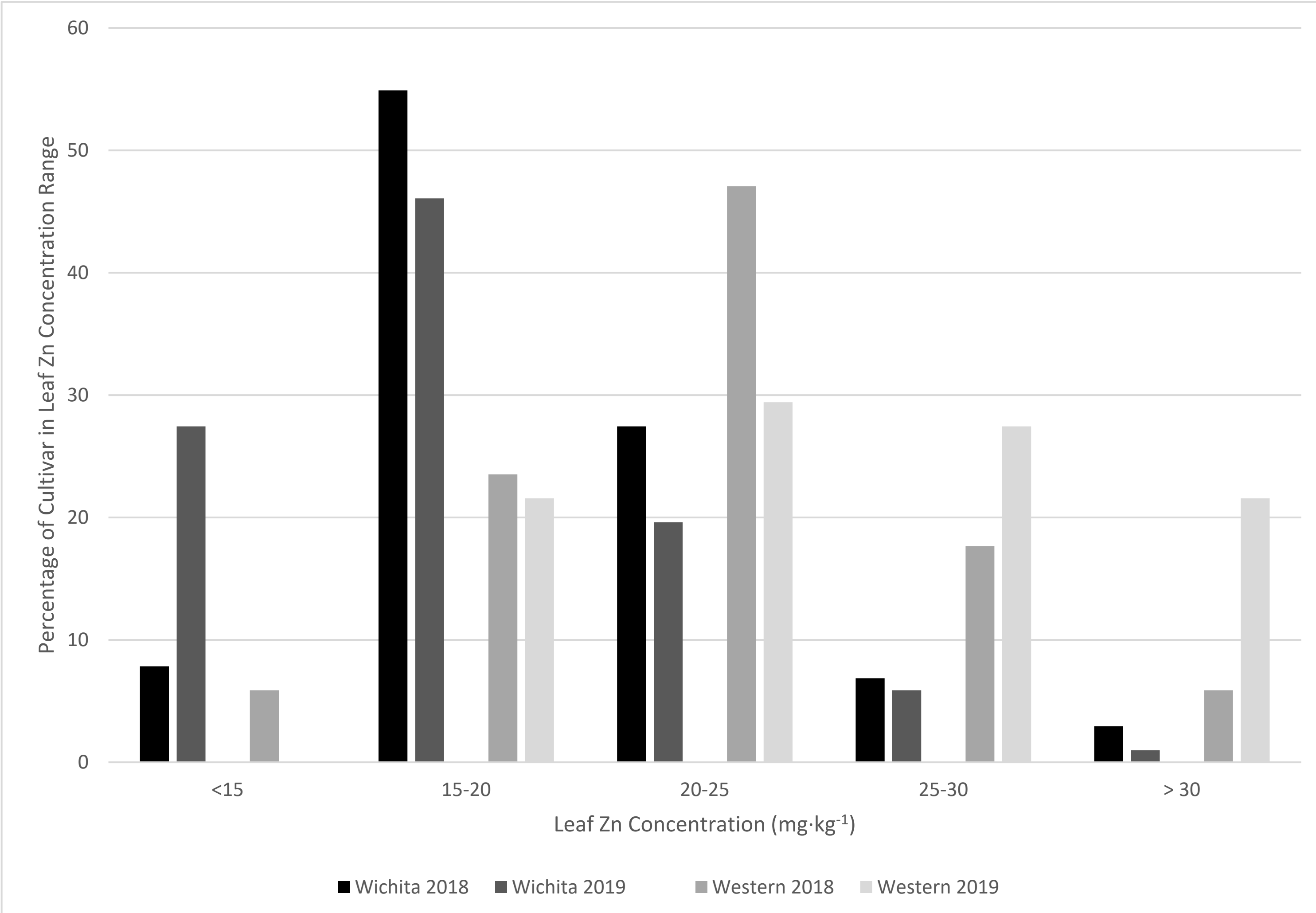

Figure 3.1. Distribution of pecan trees by leaf Zn concentration in 2018 and 2019. 'Western' comprises 14% of trees sampled in 2018 and 33% of trees sampled in 2019.

Row Number → Tree Number ↓	Wichita R11		Wichita R10		Wichita R9		Western R8		Wichita R7		Wichita R6		Wichita R5	
T11	17	•	18.8	•	16.8	•	23.5	●	24.3	●	19.3	•	29.2	●
T12	15.3	•	16.4	•	16.2	•	14	•	28	●	15.3	•	22.7	●
T13	14.9	•	17.8	•	15.1	•	26.6	●	20.3	•	19.7	•	19.3	•
T14	20.1	•	22.2	●	15	•	24.7	●	25.8	●	20.6	•	17.1	•
T15	17.1	•	21.7	●	12.4	•	20.7	•	17.3	•	23.9	●	27.1	●
T16	18.9	•	23.9	●	11.8	•	19.6	•	18.7	•	18.1	•	24.5	●
T17	22.6	●	17.2	•	17.4	•	22.5	●	16.9	•	18.5	•	21.2	•
T18	16.9	•	15.9	•	16	•	21.9	•	16.8	•	20.7	•	23.5	●
T19	27.8	●	13.5	•	21	•	25	●	19.6	•	14.2	•	20.8	•
T20	23.5	●	17.8	•	18.6	•	19.4	•	19.5	•	17.1	•	19.3	•
T21	17.8	•	18.2	•	15.4	•	24.9	●	17.4	•	29.3	●	21.2	•
T22	18.4	•	17.5	•	18.7	•	21.6	•	12.7	•	21.3	•	31.9	●
T23	21.4	•	16.6	•	16.6	•	31.6	●	15.9	•	15.6	•	31.4	●
T24	22.2	●	15.5	•	16.7	•	17	•	16.2	•	20.6	•	30	●
T25	17.8	•	19.1	•	16.4	•	17.6	•	17.9	•	23.3	●	23.2	•
T26	21.8	•	21.5	•	12.5	•	24	●	17.5	•	16.8	•	25.6	●
T27	17.5	•	20.8	•	23	•	25.4	●	16.3	•	14.8	•	19.5	•
Row Average	19.5	•	18.5	•	16.4	•	22.4	•	18.9	•	19.4	•	24.0	•

Figure 3.2. Representation of experimental plot in 2018. Size of circles correspond to leaf Zn concentration of individual trees (larger circles = higher leaf Zn concentrations). Actual leaf Zn concentrations in $mg \cdot kg^{-1}$ are given next to circles.

Tree Number ↓ Row Number →	Western R12	Wichita R11	Wichita R10	Wichita R9	Western R8	Wichita R7	Wichita R6	Wichita R5	Western R4
T11	45.1	17.4	17.4	12.7	19.9	17.4	21.6	14.2	17.4
T12	36.4	10.9	14.1	11.5	16.0	23.0	12.8	15.5	20.7
T13	33.8	10.8	17.9	11.9	21.6	28.0	14.1	16.6	24.3
T14	32.3	9.9	19.9	12.7	33.9	15.7	20.0	17.4	30.2
T15	32.5	11.1	14.2	12.8	19.1	15.5	17.6	20.3	24.8
T16	17.4	14.7	21.0	12.9	16.1	15.3	21.9	30.7	32.1
T17	21.9	10.4	13.4	16.9	24.6	14.9	20.7	26.4	26.5
T18	19.1	18.4	11.2	15.3	21.1	17.0	18.7	26.2	25.5
T19	30.8	18.3	9.9	28.5	21.6	15.9	12.2	26.7	27.2
T20	18.7	18.1	19.5	13.6	20.4	16.6	22.1	23.6	27.2
T21	30.0	19.5	12.5	16.6	29.2	15.9	19.7	24.6	25.0
T22	23.4	18.6	11.8	17.2	28.4	22.0	18.7	21.9	23.6
T23	17.9	16.4	19.9	15.2	25.6	16.1	24.9	24.4	28.5
T24	17.5	17.3	17.5	22.0	23.2	15.5	18.7	24.6	28.5
T25	19.4	21.0	19.9	19.6	27.5	17.9	26.1	22.1	26.0
T26	24.4	12.4	20.3	15.1	21.4	14.2	16.1	19.4	26.3
T27	21.0	16.3	17.7	19.8	25.2	14.1	24.9	17.7	30.7
Row Average	26.0	15.4	16.4	16.1	23.2	17.4	19.5	21.9	26.1
Average Circumference Increase (mm)	6.14	4.99	5.38	5.35	6.29	4.89	5.66	5.39	6.47

Figure 3.3. Representation of experimental plot in 2019. Size of circles correspond to leaf Zn concentration of individual trees (larger circles = higher leaf Zn concentrations). Actual leaf Zn concentrations in $mg \cdot kg^{-1}$ are given next to circles.