Leaf Net Photosynthesis, Leaf Greenness, and Shoot Lignin Content of Nonbearing Pecan Trees at Two Nitrogen and Nickel Application Rates

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Abstract. In recent years, nickel (Ni) deficiency symptoms have been observed in commercial pecan [*Carya illinoensis* (Wangenh.) K. Koch.] orchards in New Mexico. Nickel deficiency can cause a reduction in lignin formation, which could affect the risk for breakage on pecan tree shoots. Ni deficiency might furthermore disrupt ureide catabolism in pecan and, therefore, could negatively affect nitrogen (N) nutrition in the plant. The objective of this study was to identify the effects of Ni and N fertilizer applications, at two rates, on net photosynthesis (P_n), leaf greenness (SPAD), and branch lignin concentration in New Mexico's nonbearing pecan trees. Sixty trees for year 2012 (Pawnee and Western cultivars) and 40 trees for year 2013 (Pawnee cultivar) were used at two New Mexico locations (Artesia and Las Cruces) to evaluate the effects of Ni and N on tree measures. Treatments were as follows: (1) High N plus Ni (+Ni); (2) Low N no Ni (–Ni); (3) High N –Ni; and (4) Low N +Ni. In 2012 and 2013, there was an increase in leaf greenness for each location and cultivar (tree group) through time (June to September). Photosynthesis measures in 2012 differed between tree group, time in the season, and N and Ni treatments. In 2013, P_n was influenced by tree group and time ($P < 0.0001$), but N and Ni interaction did not present a significant effect related to Ni benefits. Photosynthesis varied over time in 2012 and 2013, with an inconsistent pattern. In this study, Ni application at the high N rate had a negative effect on 'Pawnee' P_n early in the season at the Artesia site, but this application had a positive effect for 'Western' from Artesia at the low N level, also early in the season. Lignin content varied between tree groups only. The application of N and Ni did not affect lignin in pecan shoots. The results show an inconsistent pattern regarding the benefits of Ni on nonbearing pecan orchards for leaf greenness, P_n , and lignin content during the 2-year study. Future studies on Ni should focus on pecan trees exhibiting leaf Ni deficiency symptoms or on soils with less than 0.14 mg·kg^{–1} of DTPA extractable Ni, as well as the long-term effect of Ni on pecan growth and development to optimize the addition of Ni into an efficient fertilization program.

In 2018, New Mexico represented 34% of United States total production of improved cultivar pecan nuts (USDA-NASS, 2019).

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Pecan represents a major economic crop for the southwest growing region, and research focusing on plant mineral nutrition is crucial to improve pecan productivity. The nutritional status for pecan orchards may be assessed by comparing foliar tissue nutrient concentrations with published recommended levels (Flynn et al., 1999; Heerema, 2013). A shortage of N inhibits flower induction and can cause flower abortion in pecan trees (Acuña-Maldonado et al., 2003). Trees show leaf chlorosis (Epstein and Bloom, 2005) as a response to N deficiency, and in severe cases, leaves will prematurely senesce and abscise (Heerema, 2013; Taiz and Zeiger, 2010). In pecan trees, inadequate N levels in soils can compromise nut development and quality (Heerema, 2013; Heerema et al., 2014).

Pecan leaf greenness and P_n activity are affected by leaf tissue concentrations of N and micronutrients. Heerema et al. (2017) found that zinc (Zn) application via fertiga-

tion increased P_n on immature pecan trees. Sherman et al. (2017) observed an improvement in P_n under foliar applications of manganese (Mn) on nonbearing pecan trees. Heerema et al. (2014) suggested monitoring leaf greenness (i.e., SPAD) on pecan fruiting shoots to plan for an efficient N application in late-season to sustain optimum P_n levels. However, the interactions between N and Ni has not been studied.

Additionally, the application of N and micronutrients can positively influence yield and growth. For example, Caliskan et al. (2008) concluded that an application of N combined with an iron (Fe) chelate compound (FeEDTA) can positively improve early growth and final yield of soybean in Mediterranean-type soil. For pecan, Walworth et al. (2017) reported pecan nut yield increases and improved tree growth due to soil Zn fertilization. Furthermore, Badshah and Gohar (2013) reported that N (5% concentration) and Zn (0.25% concentration) significantly increase pecan seedling height, number of leaves, leaf area, root diameter, and number of roots.

Increased yield has long been reported in certain systems when Ni fertilizers are applied; for example, Roach and Barclay (1946) found that potato (*Solanum tuberosum* L.) had higher yields under foliar Ni applications. Years later, Brown et al. (1987) demonstrated that barley (*Hordeum vulgare* L. cv. Onda) failed to complete its life cycle, and plants did not produce viable grain, under solution culture growing conditions with low Ni concentration. The results of this study helped to support the role of Ni as an essential plant micronutrient.

Currently, the most beneficial response to Ni was observed when N was supplied as urea (Bai et al., 2006). Ojeda-Barrios et al. (2016) observed that urease activity was positively related to the foliar level of Ni in pecan leaves. Because pecan trees use a ureide-transporting mechanism that requires Ni for the proper functioning of urea metabolism, Ni deficiency might affect ureide catabolism and affect the availability of N for growth and development processes (Bai et al., 2006, 2008). In addition, low urease activity in leaves will result in large urea accumulation that causes leaf tip necrosis (Bai et al., 2006).

Nickel absorption by plant roots can be limited in alkaline soils that exhibit a significantly high pH (Wood and Reilly, 2007b) and causes Ni deficiency that reduces plant growth, Fe uptake, disrupts N metabolism, and accelerates plant senescence (Bai et al., 2006). Ni deficiency can predispose trees to diseases that contribute to inconsistency in quality and quantity of nut production, which causes a reduction in pecan orchard profit (Wood and Reilly, 2007c). Various authors had proposed a pecan leaf Ni concentration to be above 2.5 mg·kg^{–1} (Heerema, 2013; Smith et al., 2012) and a normal leaf Ni concentration to be within 8.5 to 14.2 mg·kg^{–1} (Pond et al., 2006).

Nickel deficiency symptoms have been reported in pecan orchards from New Mexico

and Arizona, further adding to the need to evaluate Ni in pecan nutrition (R. Heerema, personal communication). Nickel deficiency is expressed as a disorder known as mouse ear (ME; Wood and Reilly, 2007a; Wood et al., 2004a, 2004b). Mouse ear in pecan is characterized by small, roundish leaflets (Wood et al., 2004a, 2004b), and it was originally thought to be caused by Mn, copper (Cu), or Zn deficiency (Gallaher and Jones, 1976; Gammon and Sharpe, 1956; Wood et al., 2004b). Wood et al. (2004b) recommended the application of Cu or phosphorus (P; which contained trace amounts of Ni) to correct ME. Wood et al. (2004c) provided evidence of a possible role of Ni in higher plants. Further evidence from that study indicated that foliar Ni applications on pecan trees, soon after budbreak, prevent or minimize the incidence of ME.

Another disorder related to pecan Ni deficiency is water stage fruit split (WSFS) (Wood and Reilly, 2006a). This disorder expresses itself as splits over the length of the shell; the affected nuts eventually drop prematurely, typically occurring after periods of heavy rain and high humidity (Heerema, 2013; Heerema et al., 2010). Nickel deficiency might influence WSFS (Wells and Wood, 2008), because the shell is mostly composed of lignin, a molecule that provides rigidity to different plant structures. If Ni deficiency is present, the shell might not be hard enough to retain the liquid endosperm, creating a shell rupture (Wood and Reilly, 2006a). Nickel may act as a cofactor agent in precursor metabolic processes that produce lignin (Wood and Reilly, 2006a); consequently, insufficient Ni condition may cause a measurable reduction in lignin formation.

Lignin is a complex molecule formed in the cell walls of living plants (Taiz and Zeiger, 2010). Lignin provides strength to cell walls, facilitates water transport (Hatfield and Fukushima, 2001), and serves as protection to the plant when it binds to cellulose and proteins. This protection blocks the growth of pathogens by acting as a barrier to further infection or wounding (Taiz and Zeiger, 2010). In pecan trees, lignin is found in the nut shell and in large amounts in the trunk, branches, leaves, fruit, and in xylem cells (Kutscha and Gray, 1970).

Wood et al. (2006b) demonstrated that, under Ni deficiency conditions, pecan branches and shoots showed visible symptoms of brittle, and they were easily broken by hand or in the presence of high winds. A potential side effect of improving Ni nutrition is the impact that might have on lignin content; therefore stronger woody tissue will be expected (Wood and Reilly, 2007b) and other metabolic process (e.g., P_n) might be affected.

The objective of this study is to determine the effects of Ni and N application rates on P_n activity, leaf greenness (SPAD), and shoot lignin concentration over time in nonbearing pecan trees. This study hypothesizes that pecan trees receiving higher amounts of N

and Ni will have higher P_n levels, leaf greenness, and branch lignin concentration than trees not receiving Ni and N over a 2-year period.

Materials and Methods

Study sites. This study was conducted at two locations, during the years of 2012 and 2013. Location one was the NMSU Agricultural Science Center, Artesia, NM (lat. 32°45'4.84" N, long. 104°22'57.65" W, 1026 m elevation). The area features an annual precipitation of 329 mm, an average annual high temperature of 24.9 °C, and a 6.2 °C low. The soil series in this field is primarily Harkey very fine sandy loam (coarse-silty, mixed, superactive, calcareous, thermic typic Torrifluent). The soil has a pH of 7.6, 10.38 mg·kg⁻¹ P, 256 mg·kg⁻¹ K, 0.50 mg·kg⁻¹ Zn, 3.64 mg·kg⁻¹ Fe, 0.4 mg·kg⁻¹ Ni, and 1.42 dS·m⁻¹ ECe, and it has 14% native calcium carbonate. Twenty 'Western' (synonym 'Western Schley') and twenty 'Pawnee' trees were selected from within two rows of each cultivar, for a total of forty trees. Trees had been planted at a distance of 6.1 m between trees by 12.2 m between rows in Apr. 2010. The soil and trees were flood irrigated using a system supplied from an artesian groundwater source.

Location two was the NMSU Leyendecker Plant Sciences Research Center, 14.5 km south of Las Cruces, NM (lat. 32°11'56.66" N, long. 106°44'30.50" W, 1174 m elevation). The area features an annual precipitation of 249 mm, an average annual high temperature of 25.4 °C, and a 16.9 °C low. The soil is a mixture of Harkey clayey silt loam (coarse-silty, mixed, calcareous, thermic typic Torrifluent) and Glendale clay loam (fine-silty, mixed, calcareous, thermic typic Torrifluent). The soil has a pH of 7.6, 6.05 mg·kg⁻¹ P, 154.36 mg·kg⁻¹ K, 0.30 mg·kg⁻¹ Zn, 8.26 mg·kg⁻¹ Fe, 0.14 mg·kg⁻¹ Ni, and 0.82 dS·m⁻¹ ECe, and its lime content varies from 3 to 10%. A total of twenty 'Pawnee' trees were selected at this location. Trees were planted at a distance of 9.1 m between trees by 9.1 m between rows in Apr. 2010. Trees were flood irrigated from a groundwater source.

At both locations, irrigation, pest management, and fertilization (other than N and Ni treatments) were regularly made according to standard agricultural practices established by the farm manager.

Experimental design. Four treatments were defined in the experiment that combined N and Ni at low and high concentrations in a 2 × 2 factorial treatment structure with five single-tree replications within each combination of location and cultivar. Treatments were combined as follows: 1) High N +Ni; 2) Low N -Ni (control); 3) High N -Ni; and 4) Low N +Ni. The variables (location and cultivar) were renamed as a "tree group" variable, with levels 'Pawnee' at Artesia (Art_Paw); 'Western' at Artesia (Art_WS); and 'Pawnee' at Leyendecker (Ley_Paw). Measurements that were sensitive to ambient

conditions (such as P_n) were obtained in blocks (i.e., grouped by measurement time), with each block containing the four treatments.

It is important to note that in 2013, only results for 'Pawnee' at the Artesia site were considered for the statistical analysis. This was due to a severe late freeze before the first Ni application in 2013 causing critical vegetative tissue damage to the 'Western' trees, which prevented the treatment application and data collection.

Treatment application. Nitrogen and Ni treatments were combined as follows: 1) High N +Ni; 2) Low N -Ni (control); 3) High N -Ni; and 4) Low N +Ni. Nitrogen was applied in a dry urea form (46-0-0), ≈30–60 cm away from each tree trunk, in a trench ≈10 cm deep—and then covered with soil. Nitrogen application was timed to correspond to within 1 to 3 d before irrigation. High N treatments received 120 g N per tree and low N treatments, 57 g N per tree. Nitrogen applications were made three times per season (May, July, and August), during the summers of 2012 and 2013.

Nickel treatments were foliar applied, with nickel lignosulfonate from Nickel Plus (5-0-0-5.4% Ni) (Nipan LLC, Valdosta, GA). The application was made on cool mornings when the wind was calm (less than 8 km·h⁻¹). Pecan foliage was sprayed until leaves were dripping. High Ni (+Ni) treatments received a solution of 1.9 mL of Nickel Plus fertilizer per liter of water. This application was made twice per season as recommended by the product's label. The first application was made at the parachute stage (onset of leaflet expansion) and the second application was made in mid-July. A 2.5 mL·L⁻¹ of non-ionic surfactant (NIS) was added to the Ni mixture. The no-Ni (-Ni) trees received 2.5 mL·L⁻¹ of NIS plus water. Nickel applications were made using a hand pump sprayer.

Data collection. Soils were sampled at both locations to a depth of 50 cm, in two, 25-cm increments. A total of 12 samples were pooled and mixed from different areas within the tree rows at Artesia. For the Leyendecker site, 11 samples were collected. Soil samples were analyzed for standard nutrients on high pH soils, pH, salinity, and DTPA extractable Ni.

Leaf samples were collected following NMSU guidelines (Flynn et al., 1999). An average of 24 middle leaflets were collected per tree in late August, and ≈15 d after treatment. Leaflets were collected in pairs of the middle shoot leaf and kept in a small paper envelope under cool conditions to avoid tissue damage. The leaves were scanned with a LI-COR leaf area meter model LI-3100C (LI-COR Inc., Lincoln, NE). Leaf area represents the total number of leaflets collected per tree.

Leaflets were dried at 65 °C for at least 48 h after the leaf area was determined. Dry weight was taken after leaflets were completely dry. Before leaf analysis, leaflets were ground through a 2-mm mesh using a Micro

Hammer/Cutter Mill (Glen Mills, Inc., Clifton, NJ). Two different procedures were implemented: microwave digestion for nickel (EPA 3051A, 2007) and TKN for N analysis (Technicon™ Auto Analyzer™ II, 1974).

The Standard Operating Procedure (SOP) for microwave digestion of plant, soils, and solids (EPA 3051A, 2007) was used for leaf Ni analysis. A 0.5-g subsample of ground leaf material was weighed and placed in a Teflon PFA vessel. Five milliliters of HNO₃, 2 mL of 30% H₂O₂, and 5 mL of deionized (DI) water were added to the vessel containing the sample. Vessels were sealed and placed in the microwave unit for 15 min. Samples were left overnight to cool. The following day, the liquid material was diluted to 100 mL using DI water and analyzed by an Inductively Coupled Plasma (ICP) (Perkin Elmer, Shelton, CT).

In total, 14 leaf samples were placed in a carousel containing the vessels at each time of the nutrient extraction. One vessel had the quality control sample; in this case, the standard QC 2009-210 (sugar beet leaf), from North American Proficiency Testing program (NAPT), was used. The other vessel contained a blank solution, in which only the chemicals were added; and another vessel was the duplicate of one of the samples being analyzed in the same batch.

The SOP for Total Kjeldahl Nitrogen (TKN) (Technicon™ Auto Analyzer™ II, 1974) was used for determining leaf N concentration. The TKN procedure used 0.2 g of ground leaf material placed in microdigestion tubes. Each sample, plus a blank and a quality control sample (QC 2009-211, oats), were analyzed, along with N standards at 2.5, 5.0, and 7.5 mL. For each batch (14 samples), there was a duplicate of one of the pecan leaflets' samples.

Leaf P_n, leaf SPAD, and midday stem water potential (MSWP) measurements were taken in June, Aug., and Sept. of 2012 and 2013. Two leaflets attached to a different branch away from each other were randomly selected from the same tree for these measures. The leaflet had to be exposed to full sun (facing east) at the time that P_n data and SPAD readings were recorded, and the leaflet had to be located in the middle canopy of the tree. The same leaflet was used for both P_n and SPAD.

Leaf P_n was measured using the LI-COR 6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE). The photosynthetic active radiation (PAR) was set to 'Track PAR' on bright sunny days, or set to 1700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ if scattered clouds could potentially shade the sunlight. The carbon dioxide (CO₂) was set constant to 390 $\mu\text{L}\cdot\text{L}^{-1}$ to mimic ambient CO₂ levels. The first seasonal P_n measurements were taken $\approx$ 30 to 35 d after the first seasonal Ni application (mid-May). The second set of measurements was made 20 to 25 d after the second seasonal application (mid-July), and the third P_n observations were collected 30 d after the second seasonal P_n measurement (mid-August).

Leaf greenness was measured using the SPAD 502 portable chlorophyll meter (Spectrum Technologies, Inc., Aurora, IL) immediately after P_n measurements. The SPAD meter was clamped to the middle section of the leaflet, avoiding clamping over the main vein of the leaflet. SPAD readings were averaged over two measurements per tree.

Midday stem water potential (MSWP) data were taken on leaves in the most shaded canopy location (usually the west side) with a Scholander pressure chamber (PMS Instrument Co., Albany, OR) after 12:00 PM and after P_n and SPAD data were collected, to show that trees were not under stress at the time of measurement. The selected leaf was placed into a sealed reflective bag while still attached to the tree. The bag remained sealed for at least 30 min. After this minimum amount of time, the leaf was cut at the base of the petiole. The sealed reflective bag containing the leaf was placed into the pressure chamber, and the data were reported in units of MPa.

In Jan. 2014, branch samples were collected from the previous season's new growth, $\approx$ 1.5 m from the ground. Branch samples were cut with a lopper, cleaned of any buds, cut into small pieces, and weighed. Branches were shredded and ground through a 2-mm sieve in a Wiley mill and then thoroughly mixed. They were analyzed for lignin using acid detergent fiber (ADF) procedures (ANKOM Technology, 1998) at Ward Laboratories, Inc. in Kearney, NE. This procedure creates an acidic detergent solution that dissolves soluble materials and leaves a residue of cellulose and lignin that is measured gravimetrically. A quality control sample was used to compare with the pecan branch samples. The results are presented as percentage (%) of dry weight.

Statistical analysis. The criterion for significance was established at $P < 0.05$. A mixed model analysis was used to assess the effects of N and Ni treatments, time (day of sampling), and tree group for each year (2012 and 2013) using SAS® software, version 9.3 (SAS Institute Inc., 2011). The three-way factorial analysis included fixed effects for N, Ni, tree group, time, and all interactions. The model included random effects for block and time $\times$ block, and it accounted for the repeated measurements through time by using either the compound symmetric or compound heterogeneous symmetric variance structure (SAS Institute Inc., 2011). Degrees of freedom were computed using the Kenward–Roger option (SAS Institute Inc., 2011). Least square (LS) means were created to compare each of the means when an effect was found to be significant, and SAS macro "pdmix800" was performed for further analysis (Saxton, 1998). If multiple higher-order effects were significant, then all the LS means are reported. In addition, all LS means were compared within time, and simple effects of time were assessed. Lignin, tree trunk diameter, leaf area, leaf dry weight, and leaf N and Ni concentration comparisons were made using a factorial analysis of var-

iance with factors N, Ni, and tree groups because only one sample per year was taken during the entire study.

Results and Discussion

Leaf area. The results for leaf area samples indicated no significant differences at the Artesia location (data not shown). The Art_Paw cultivar presented a leaf area of $246.12 \pm 13.03 \text{ cm}^2$ for 2012, and $368.47 \pm 13.82 \text{ cm}^2$ for 2013. The Art_WS cultivar showed a leaf area of $269.84 \pm 12.64 \text{ cm}^2$. However, Pawnee cultivar at Leyendecker showed a significantly higher leaf area, $481.48 \pm 12.64 \text{ cm}^2$ and $519.21 \pm 13.41 \text{ cm}^2$ for 2012 and 2013, respectively. The leaf area differences were most likely due to the differences in the growing conditions, soil chemistry, and cultural practices between the two locations.

Midday stem water potential. The results of MSWP measurements have been proven to be a good estimator of tree water status (Choné et al., 2001; Shackel et al., 1997). Midday stem water potential was measured to ensure that trees were not under water stress that could influence P_n measurements. Othman et al. (2014) showed a decrease in pecan leaf P_n when MSWP values were less than -0.9 MPa . The MSWP values for our study were higher than -0.9 MPa (data not shown).

SPAD. Tracking SPAD over time for all treatments assessed the effect of the fertilizer treatments on leaf greenness during the summer months. Leaf SPAD had previously been shown to be closely related to plant N status in orchard trees, including pecan (Heerema et al., 2014; Neilsen et al., 1995). For 2012, statistical significance for SPAD was found for time ($P < 0.0001$), tree group ($P = 0.0205$), tree group $\times$ time interaction ($P = 0.0061$), and tree group $\times$ time $\times$ N interaction ($P = 0.0176$). The Ni treatment had no significant effect on SPAD (data not shown). Through time, SPAD values were lowest in June and gradually increased through the season for both low and high N levels, and on all tree groups (Table 1). All tree groups had a significant increase in SPAD from minimum values in June, to final maximum values in September. Higher SPAD readings later in the season are a result of normal leaf development, as leaf increased thickness during the season (i.e., June to September) (Campbell et al., 1990). In 2013, SPAD data showed main effects for tree group ($P < 0.0001$), time ($P < 0.0001$), and tree group $\times$ time interaction ($P = 0.0061$). No significance was found for all N or Ni treatments. For Art_Paw, SPAD values increased progressively through time, whereas the Ley_Paw tree group increased from June to September (Table 2). When comparing across tree groups in June/July and August, Ley_Paw SPAD values were higher than those of Art_Paw. By September, both tree group's SPAD readings were essentially the same (Table 2).

Overall, SPAD readings increased over time during both years of the study (Tables 1

Table 1. Least square (LS) means for SPAD for 2012 pooled across Ni treatment for tree group by time, by N level.

Time	SE	Group ²							
		Art_Paw		Art_WS				Ley_Paw	
		Low N	High N	Low N	High N	Low N	High N	Low N	High N
June	1.07	39.53	a ^y B ^x	36.15	b C	36.56	b B	38.20	ab C
August	1.01	43.27	abc A	42.25	bc B	43.94	abc A	41.71	c B
September	1.01	43.88	c A	44.70	c A	44.54	c A	45.04	bc A
								47.74	ab A
									49.06

²Group Art_Paw (Artesia_Pawnee); Art_WS (Artesia_Western); Ley_Paw (Leyendecker_Pawnee).

^yRow comparisons. LS means within time and across tree group and N level sharing the same lowercase letter are not statistically different ($P > 0.05$).

^xColumn comparisons. LS means for each tree group across time sharing the same uppercase letter are not statistically different ($P > 0.05$).

Table 2. Least square (LS) means for SPAD for 2013 pooled across N and Ni levels for tree group by time.

Time	Group mean $\pm$ SE ²			
	Art_Paw		Ley_Paw	
June/July	38.39 $\pm$ 0.73	b ^y C ^x	43.27 $\pm$ 0.68	a B
August	40.64 $\pm$ 0.90	b B	44.70 $\pm$ 0.85	a AB
September	45.16 $\pm$ 0.63	a A	46.38 $\pm$ 0.60	a A

²Group Art_Paw (Artesia_Pawnee); Ley_Paw (Leyendecker_Pawnee).

^yRow comparisons. LS means within time and across tree group sharing the same lowercase letter are not statistically different ($P > 0.05$).

^xColumn comparisons. LS means for each tree group across time sharing the same uppercase letter are not statistically different ($P > 0.05$).

and 2). Monitoring leaf greenness using the SPAD meter can help to evaluate N status to avoid N deficiency that can limit crop productivity (Neilsen et al., 1995). In the present study, pecan leaf SPAD readings progressively increased in time over each of the two seasons (Tables 1 and 2). This indicated that time of year can influence SPAD measurements.

Photosynthesis. All treatments affected photosynthesis in 2012. Significant main effects were observed for tree group ($P = 0.0028$); time ($P = 0.0221$); for the interaction of tree group $\times$ N ($P = 0.0284$); tree group $\times$ time ($P < 0.0001$); tree group $\times$ time $\times$ Ni ($P = 0.0017$); and time $\times$ N $\times$ Ni ($P = 0.0403$). Because of the two, three-way interactions involving all factors, significant means for tree group, time, N, and Ni combinations are reported in Table 3.

Photosynthesis increased through time in low N level and -Ni treated trees for Art_Paw and Ley_Paw groups (Table 3). Both tree groups (Art_Paw and Ley_Paw) show lower P_n in June compared with September. In contrast with Art_Paw and Ley_Paw, Art_WS presented a decrease in P_n in August and September at the low level of N and when Ni was applied. However, under the high N rate, no depression in P_n was observed.

For this study, in June, no P_n rate comparison was significant between high and low N made for a given combination of tree group and Ni rate (Table 3). In August, P_n was higher for Art_WS compared with Art_Paw at the high N and -Ni level (Table 3). In August and when Ni was applied, no significant differences were found between both N levels for any of the tree groups.

Lower P_n at the high N rate (-Ni) for the Art_Paw tree group was observed compared with Art_WS, where simple effects revealed higher P_n for the high N and -Ni level across time (Table 3). A significant P_n difference of $3.88 \pm 1.04 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was observed between Art_Paw and Art_WS in September

and at the high N level and -Ni. In September, P_n for Art_Paw (high N and -Ni) also differed when compared with both N levels in Ley_Paw. In September, the Ley_Paw tree group had higher P_n rates over Art_WS at low N and when Ni was applied. That is, Ley_Paw at low N had a P_n rate that was $2.72 \pm 1.04 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ higher than for Art_WS.

Photosynthesis measures in 2013 were affected by the interaction of N $\times$ Ni ($P = 0.0152$) and by the tree group $\times$ time ($P < 0.001$). The estimated P_n rates for the low N and -Ni levels were lower ($15.21 \pm 0.34 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) than for low N and +Ni ($16.45 \pm 0.37 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) or high N -Ni level ($16.59 \pm 0.37 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). High N and +Ni had an intermediate level of P_n ($15.99 \pm 0.34 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and did not statistically differ from any other mean.

For tree group $\times$ time interaction, the June/July 2013 readings for Art_Paw increased from June ($14.29 \pm 0.46 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to September ($17.75 \pm 0.32 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) (Table 4). Ley_Paw had P_n values that increased in August ($18.86 \pm 0.34 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) over June/July measurements ($15.10 \pm 0.44 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and decreased to lower levels by September to $14.22 \pm 0.31 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

For this 2-year study, P_n rates varied in 2012 and 2013. Nickel treatment did not appear to have a consistent effect for 2012. It is possible that Ni did not show a significant impact because Ni levels already present in the soil could have supplied the trees with an adequate amount of Ni, even under the alkaline soil conditions. Therefore, Ni application might not have been a major factor that could have significantly affected P_n . However, other studies, in different species, demonstrated a positive effect on P_n levels, chlorophyll content, and yield when Ni was applied. Moraes et al. (2009) stated that the growth of rice plants depended on the N source provided, and they found that P_n levels decrease when Ni or molybdenum (Mo) was absent.

Gheibi et al. (2009) found that wheat showed higher chlorophyll content when Ni was applied. In addition, Dahiya et al. (1994) showed increased dry matter yield of wheat (*Triticum aestivum* L.) when N and Ni were applied to the soil. Research on rice (*Oryza sativa* L.) demonstrated an increase in dry weight and amino acids on plants grown with urea-N, plus a supply of Ni, at a rate of $0.5 \text{ mmol}\cdot\text{m}^{-3}$ (Gerendás et al., 1998). Therefore, an indirect relationship could exist between Ni supply to plants and P_n levels (Moraes et al., 2009).

Lignin. No significant effects of N and Ni on lignin concentration were observed (Table 5). Leyendecker had lower soil DTPA extractable Ni ($0.14 \text{ mg}\cdot\text{kg}^{-1}$) compared with Artesia, which had an average of $0.40 \text{ mg}\cdot\text{kg}^{-1}$. Both locations had a soil pH of 7.6. According to Liu et al. (2010), higher pH can result in Ni deficiency in trees. However, no trees at either location exhibited symptoms of Ni deficiency.

The pecan trees at the Leyendecker location show means with significantly higher lignin concentration (Table 5). The Ley_Paw tree group had a higher lignin concentration (of 17.31 ± 0.19) than did the trees at the Artesia location, despite the soil pH having a lower amount of native Ni in the soil at Leyendecker. More favorable growing conditions could be the underlying cause of having higher lignin levels at the Leyendecker location.

The results in this study show a non-consistent pattern of the benefits of Ni on nonbearing pecan orchards. In addition, few effects of applied N on leaf greenness (SPAD) were detected for 2012 and 2013. However, at both N levels (low and high), SPAD readings increased through time in 2012. When comparing N levels within the same tree group for the same sampling time, significance in SPAD was present only one time: during June for Art_Paw (Table 4), where SPAD under low N conditions was actually higher than under high N conditions. The lack of differences among N levels could possibly be due to a difficulty in determining N response by trees in the short term.

In the present study, P_n for 2012 was not consistently affected by either N or Ni treatments. In 2013, P_n data indicated no significant effect for either N or Ni treatment. In addition, lignin content was not statistically different among any of the Ni treatments.

A 2-year study, as in the present research, may be too short a time frame to evaluate any

Table 3. Least square (LS) means for net photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) over time for tree groups as affected by Ni and N levels in 2012.

Time	Ni	Group ^z							
		Art_Paw				Art_WS			
		Low N		High N		Low N		High N	
June	–	13.18	ef ^y B ^x	14.28	de A	17.20	bc A	18.81	ab A
	+	12.59	ef E ^w	11.28	f E	19.37	a D	18.17	ab D
August	–	15.78	abc AB	13.68	c A	17.25	abc A	19.12	a A
	+	17.16	abc D	15.34	bc D	15.49	bc E	16.24	abc D
September	–	16.03	abc A	13.65	d A	15.37	bcd A	17.53	a A
	+	16.38	abc D	15.65	abcd D	14.18	cd E	16.69	ab D

^zGroup Art_Paw (Artesia_Pawnee); Art_WS (Artesia_Western); Ley_Paw (Leyendecker_Pawnee).^yRow comparisons. LS means within time across Ni, N, and tree group sharing the same lowercase letter are not statistically different ($P > 0.05$).^xColumn comparisons. LS means within tree group and N level, Ni rate, and across time sharing the same uppercase letter are not statistically different ($P > 0.05$).^wColumn comparisons. LS means within tree group and N level, +Ni rate, and across time sharing the same uppercase letter are not statistically different ($P > 0.05$).Table 4. Least square (LS) means for net photosynthesis ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) pooled across N and Ni treatments for 2013 tree groups.

Time	Group mean $\pm$ SE ^z			
	Art_Paw		Ley_Paw	
June/July	14.29 $\pm$ 0.46	a ^y C ^x	15.10 $\pm$ 0.44	a B
August	16.15 $\pm$ 0.36	b B	18.86 $\pm$ 0.34	a A
September	17.75 $\pm$ 0.32	a A	14.22 $\pm$ 0.31	b C

^zGroup Art_Paw (Artesia_Pawnee); Ley_Paw (Leyendecker_Pawnee).^yRow comparisons. LS means within time and across tree group sharing the same lowercase letter are not statistically different ($P > 0.05$).^xColumn comparisons. LS means for each tree group across time sharing the same uppercase letter are not statistically different ($P > 0.05$).

Table 5. Least square (LS) means pooled across N and Ni treatments for lignin concentration (% of dry weight) by tree group.

Location_Cultivar (group ^z)	Mean $\pm$ SE ^y
Art_Paw (Group 1)	16.41 $\pm$ 0.199 b
Art_WS (Group 2)	16.52 $\pm$ 0.194 b
Ley_Paw (Group 3)	17.31 $\pm$ 0.194 a

^zGroup Art_Paw (Artesia_Pawnee); Ley_Paw (Leyendecker_Pawnee).^ya, b = LS means sharing the same letter indicate no significant difference at $P \leq 0.05$.

carryover effect that Ni could potentially have over time. Additionally, the study was conducted in an open field, where trees were exposed to other natural or human factors, such as weather, fertilization, weed competition, biotic and abiotic stress, and other soil factors including native soil Ni and residual N content.

Future considerations might focus on including different cultivars of pecan, variable application rates of Ni, or evaluating Ni under a more controlled environment that would help researchers to more fully understand the impacts of Ni on the physiology of pecan. In addition, a better understanding of residual soil Ni and N, along with a longer-term study, should be considered.

Further research on the effect of Ni application at different pecan growth stages could result in creating an optimum Ni fertilization plan for pecan orchards. This is especially true if background Ni levels were lower than the present study. A fertilization plan promotes an efficient use of resources for pecan growers and, including Ni, could help growers to prevent Ni deficiencies that could limit yield and nut quality.

Literature Cited

- Acuña-Maldonado, L.E., M.W. Smith, N.O. Maness, B.S. Cheary, and B.L. Carroll. 2003. Influence of nitrogen application time on nitrogen absorption, partitioning, and yield of pecan. *J. Amer. Soc. Hort. Sci.* 128:155–162.
- ANKOM Technology. 1998. Method for determining acid detergent fiber. Ankom 200/220 Fiber Analyzer. Ankom Technology, Fairport, NY.
- Badshah, N.L., and A. Gohar. 2013. Effect of different concentrations of nitrogen and zinc on the growth of pecan nut seedlings. *J. Agr. Biological Sci.* 8(4):337–343.
- Bai, C.C., C.C. Reilly, and B.W. Wood. 2006. Nickel deficiency disrupts metabolism of ureides, amino acids, and organic acids of young pecan foliage. *J. Plant Physiol.* 140:433–443.
- Bai, C.C., C.C. Reilly, and B.W. Wood. 2008. Insights into the nutritional physiology of nickel. *Acta Hort.* 772:365–368.
- Brown, H.P., R.M. Welch, and E.E. Cary. 1987. Nickel: A micronutrient essential for higher plants. *J. Plant Physiol.* 85:801–803.
- Caliskan, S., I. Ozkaya, M.E. Caliskan, and M. Arslan. 2008. The effects of nitrogen and iron fertilization on growth, yield and fertilizer use efficiency of soybean in a Mediterranean-type soil. *Field Crops Res.* 108(2):126–132.
- Campbell, R.J., K.N. Mobley, R.P. Marini, and D.G. Pfeiffer. 1990. Growing conditions alter the relationship between SPAD-501 values and apple leaf chlorophyll. *HortScience* 25:330–331.
- Choné, X., C. VanLeeuwen, D. Dubourdieu, and J.P. Gaudillère. 2001. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* 87(4):477–483.
- Dahiya, D., V. Kumar, and J. Singh. 1994. Nitrogen uptake in wheat as influenced by the presence of nickel. *Arid Soil Res. Rehabil.* 8(1):51–58.
- Environmental Protection Agency. 2007. Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils, and oils.
- Epstein, E. and A.J. Bloom. 2005. Mineral nutrition of plants: Principles and perspectives. 2nd ed. Sinauer Associates, Inc., Sunderland, MA.
- Flynn, R., S.T. Ball, and R.D. Baker. 1999. Sampling for plant tissue analysis. Cooperative Extension Service, College of Agriculture and Home Economics, New Mexico State University. Guide A-123.
- Gallaher, R.N. and J.B. Jones, Jr. 1976. Total extractable, and oxalate calcium and other elements in normal and mouse-ear pecan tree tissue. *J. Amer. Soc. Hort. Sci.* 101:692–696.
- Gammon, N. and R.H. Sharpe. 1956. Mouse-ear: A manganese deficiency of pecans. *Amer. Soc. Hort. Sci.* 68:195–200.
- Gerendás, J., Z. Zhu, and B. Sattelmacher. 1998. Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (*Oryza sativa* L.). *J. Expt. Bot.* 49(326):1545–1554.
- Gheibi, M.N., M.J. Malakouti, B. Kholdebarin, F. Ghanati, S. Teimouri, and R. Sayadi. 2009. Significance of nickel supply for growth and chlorophyll content of wheat supplied with urea or ammonium nitrate. *J. Plant Nutr.* 32(9):1440–1450.
- Hatfield, R. and R.S. Fukushima. 2001. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using acetyl bromide spectrophotometric method. *J. Agr. Food Chem.* 49(7):3133–3139.
- Heerema, R. 2013. Diagnosing nutrient disorders of New Mexico pecan trees. Cooperative Extension Service, College of Agricultural, Consumer and Environmental Sciences. Guide H-658.
- Heerema, R.J., D. VanLeeuwen, M.Y. Thompson, J.D. Sherman, M.J. Comeau, and J.L. Walworth. 2017. Soil-application of Zinc_EDTA increases leaf photosynthesis of immature ‘Wichita’ pecan trees. *J. Amer. Soc. Hort. Sci.* 142:27–35.
- Heerema, R.J., D. VanLeeuwen, R.S. Hilaire, V.P. Gutschick, and B. Cook. 2014. Leaf photosynthesis in nitrogen-starved ‘Western’ pecan is lower on fruiting shoots than non-fruiting shoots during kernel fill. *J. Amer. Soc. Hort. Sci.* 139:267–274.
- Heerema, R., N. Goldberg, and S. Thomas. 2010. Diseases and other disorders of pecan in New Mexico. Cooperative Extension Service, College of Agricultural, Consumer and Environmental Sciences. Guide H-657.
- Kutscha, N.P., and J.R. Gray. 1970. The potential of lignin research. *Maine Agricultural Experiment Station. Bul.* 41.
- Liu, W., H. Wang, Y. Shi, Y. Ago, Z. Zhang, H. Duane, J. Fang, and F. He. 2010. The effect of different N, P, K rates on photosynthesis rate and chlorophyll content of leaves of walnut saplings. *Acta Hort.* 861:283–288.

- Moraes, M.F., A.R. Reis, L.A.C. Moraes, J. Lavres-Junior, R. Vivian, C.P. Cabral, and E. Malavolta. 2009. Effects of molybdenum, nickel, and nitrogen sources on the mineral nutrition and growth of rice plants. *Commun. Soil Sci. Plant Anal.* 40(21-22):3238–3251.
- Neilsen, D., E.J. Hogue, G.H. Neilsen, and P. Parchomchuk. 1995. Using SPAD-502 values to assess the nitrogen status of apple trees. *HortScience* 30:508–512.
- Ojeda-Barrios, D.L., E. Sánchez-Chávez, J.P. Sida-Arreola, R. Valdez-Cepeda, and M. Balandran-Valladares. 2016. The impact of foliar nickel fertilization on urease activity in pecan trees. *J. Soil Sci. Plant Nutr.* 16(1):237–247.
- Othman, Y., D. VanLeeuwen, R. Heerema, and R.S. Hilaire. 2014. Midday stem water potential values needed to maintain photosynthesis and leaf gas exchange established for pecan. *J. Amer. Soc. Hort. Sci.* 139:537–546.
- Pond, A.P., J.L. Walworth, M.W. Kilby, R.D. Gibson, R.E. Call, and H. Núñez. 2006. Leaf nutrient levels for pecans. *HortScience* 41:1339–1341.
- Roach, W.A. and C. Barclay. 1946. Nickel and multiple trace element deficiencies in agricultural crops. *Nature* 157(3995):696.
- SAS Institute Inc. 2011. SAS/STAT® 9.3 User's guide. SAS Institute Inc., Cary, NC.
- Saxton, A.M. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. *Proceedings of the 23rd SAS Users Group International*, 1243–1246.
- Shackel, K.A., H. Ahmadi, W. Biasi, R. Buchner, D. Goldhamer, S. Gurusinge, and J. Yeager. 1997. Plant water status as an index of irrigation need in deciduous fruit trees. *HortTechnology* 7:23–29.
- Sherman, J., R.J. Heerema, D. VanLeeuwen, and R.S. Hilaire. 2017. Optimal manganese nutrition increases photosynthesis of immature pecan trees. *HortScience* 52:634–640.
- Smith, M.W., C.T. Rohla, and W.D. Goff. 2012. Pecan leaf elemental sufficiency ranges and fertilizer recommendations. *HortTechnology* 22:594–599.
- Taiz, L. and E. Zeiger. 2010. *Plant physiology*. Sinauer Associates Inc., Sunderland, MA.
- Technicon™ Auto Analyzer™ II. 1974. Total Kjeldahl Nitrogen. Ammoniacal Nitrogen/BD Acid Digest. BD-20/BD-40. Industrial Method No. 321-74A.
- United States Department of Agriculture, National Agricultural Statistic Service (USDA-NASS). 2019. Noncitrus fruits and nuts 2018 summary. <<https://downloads.usda.library.cornell.edu/usda-esmis/files/zs25x846c/0z7096330/7s75dp373/ncit0619.pdf>>.
- Walworth, J.L., S.A. White, and M.J. Comeau. 2017. Soil-applied ZnEDTA: Vegetative growth, nut production, and nutrient acquisition of immature pecan trees grown in an alkaline, calcareous soil. *HortScience* 52:301–305.
- Wells, M.L. and B.W. Wood. 2008. Foliar boron and nickel applications reduce water-stage fruit-split of pecan. *HortScience* 43:1437–1440.
- Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004a. Mouse-ear of pecan: I. Symptomatology and occurrence. *HortScience* 39:87–94.
- Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004b. Mouse-ear of pecan: II. Influence of nutrient applications. *HortScience* 39:95–100.
- Wood, B.W., C.C. Reilly, and A.P. Nyczepir. 2004c. Mouse-ear of pecan: A nickel deficiency. *HortScience* 39:1238–1242.
- Wood, W.B. and C.C. Reilly. 2007a. Nickel: Impact on leaf morphology and growth. *Pecan South* 40(5):13–16.
- Wood, W.B. and C.C. Reilly. 2007b. Nickel: Impact on horticultural characteristics. *Pecan South* 40(7):24–33.
- Wood, W.B. and C.C. Reilly. 2007c. Nickel: Relevance to orchard profitability. *Pecan South* 40(6):16–19.
- Wood, W.B. and C.C. Reilly. 2006a. Nickel and plant disease. *International Plant Nutrition Institute*. 12 Oct. 2013. <http://www.nutricao-de-plantas.agr.br/site/downloads/unesp_jaboticabal/Niquel_doencas_BruceWood.pdf>.
- Wood, W.B., C.C. Reilly, and A.P. Nyczepir. 2006b. Field deficiency of nickel in trees: Symptoms and causes. *Acta Hort.* 721:83–97.