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Do foliar applications of nickel increase urease activity and nutrient levels in pecan leaflets?

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Abstract: Nickel (Ni) deficiency limits the production of pecan. Concentrations of mineral nutrients and urease activity in leaflets of young pecan plants cv. Pawnee were evaluated in response to foliar applications of Ni as Ni sulfate or Ni citrate, or as chelates with EDDHA, EDTA, or DTPA. Significant variations were found in N-total concentration with Ni-EDDHA (27.17 ± 0.98 g/kg) and Ni-sulphate (28 ± 0.89 g/kg), where the latter together with Ni-citrate were high in phosphorus concentrations. Levels of Ni^{2+} (3.70 mg/kg), Mn^{2+} (222.73 mg/kg) and Zn^{2+} (38.69 mg/kg) were all increased in leaflets sprayed with Ni-EDDHA but the Mn^{2+} concentrations were similar for leaflets sprayed with Ni-sulphate. The Ni-DTPA spray significantly reduced foliar Fe^{2+} concentration (73.66 ± 3.44 mg/kg). No significant effects of the sprays were observed on the concentrations of total chlorophyll or carotenoids. The plants sprayed with Ni-EDDHA showed significant increases in leaflet area and leaflet dry weight and also in root dry weight. The urease showed maximum activity in leaflets sprayed with Ni-EDDHA, Ni-citrate and Ni-sulphate. This study suggests growers of the pecan should consider foliar sprays of Ni-EDDHA or Ni-sulphate to increase the levels of Ni in their trees if Ni is a growth-limiting factor.

Keywords: *Carya illinoinensis*; foliar fertilisation; availability of Ni; micronutrients; mouse-ear disease; photosynthetic pigments

The pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a valuable commercial crop of high nutritional value. The United States of America and Mexico together produce about 90% of the world's supply of harvested nuts (Moran-Duran et al. 2020). Ensuring an adequate supply of nickel (Ni) is one of the most important management objectives for pecan trees (Ojeda-Barrios et al. 2016). Among the physicochemical properties that limit the availability of Ni to the trees is the alkaline nature ($pH \geq 7.5$) of the calcareous soils in the main planted areas (Rodríguez-Jiménez et al. 2016). Nickel is an essential micronutrient whose deficiency significantly reduces both the yield and the quality of the nuts. These reductions seriously erode the economic benefit for pecan growers (Hernández-Lopez et al. 2020). Nickel deficiency affects the photosynthetic rate, biomass accumulation and foliar nutrient status (Bai et al. 2006, 2007).

Some research has sought to evaluate the effects of foliar sprays of Zn^{2+} and Ni^{2+} on the concentrations of these nutrients in pecan leaflets (Wagle et al. 2011, Ojeda-Barrios et al. 2009, 2016, Hernández-Lopez et al. 2020). However, the results have not been consistent due to the differing characteristics of the tree varieties involved, their ages and the phenological stage of the trees at the time of spray application. Also due to the use of different spray concentrations and different numbers of sprays (Balandrán-Valladares et al. 2021). There has also been an underlying variation arising from differences in nutrient sufficiency associated with the trees' alternate bearing behaviour and with the different geographical locations (microclimates, soils, etc.) of the production areas (Pond et al. 2006).

Uptake rates and costs (spray concentrations × spray numbers) also affect the supply of optimal levels of mineral nutrients along with the application method. One of the most efficient modes of Ni application is *via* foliar sprays (Aburto-González et al. 2021, Niu et al. 2021). Nickel is usually applied to mature pecan trees as a foliar spray but a wide range of Ni sources has been employed, including the lignosulphonate, sulphate, oxide, chloride, citrate and various chelates (Rodríguez-Jiménez et al. 2016). Thus the use of chelating agents, e.g., EDTA, DTPA, EDDHA, has been found to be an efficient way to correct Ni deficiencies in pecan trees in calcareous soils, by promoting the uptake, transport and storage of Zn^{2+} due to the ability of these agents to complex with metal ions, including with Zn^{2+}, ions and so improve their performance in soils of different pH (Bai et al. 2007, Lucasynski-Carlim et al. 2019). However, the use of chelating agents in agriculture is limited by their high cost, so is economic only with fruits and vegetables of high market value (Niu et al. 2021). Thus, from a commercial perspective, the optimal solution in any particular circumstance is the one that obtains the maximum economic gain for the least additional cost.

Fertilisation programs for pecan consider nitrogen (N) and Zn^{2+} as the two nutrient supplements that offer the greatest economic benefit. However, Ni^{2+} deficiency inhibits the activity of urease and so reducing the conversion of urea to NH_4^+ ions and potentially inducing chlorosis and even necrosis of the leaflets. In addition, Ni^{2+} deficiency is also associated with the visual symptom "mouse ear", which arises from a disruption of amino acid metabolism and the citric acid cycle (de Queiroz-Barcelos et al. 2017, Moran-Duran et al. 2020). Urease is a ubiquitous enzyme that contains two Ni^{2+} ions in its structure in its active site (Lucasynski-Carlim et al. 2019, Hernández-López et al. 2020). On the other hand, the synthesis and function of other enzymes protecting against oxidative stress (e.g., hydrogenase, carbon monoxide dehydrogenase, nickel superoxide dismutase) are also affected by Ni supply (Polacco et al. 2013). Nickel availability has also been shown to affect the concentration of urea, and the dry matter and total N in wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), rapeseed (*Brassica napus* L.), zucchini (*Cucurbita pepo* L.) and sunflower (*Helianthus annuus* L.) (Almanza et al. 2009, Ghasemi et al. 2013).

As previously noted, Ni can be applied as a chelate. However, there are few studies that report its physiological behaviours when applied as a foliar spray to seedlings and young plants, including to pecan. In past years, the body of information on the application of foliar sprays of chelated Fe^{2+}, Zn^{2+} and Ni^{2+} to adult trees has increased (Ojeda-Barrios et al. 2009, 2016). However, the responses to applications of Ni to seedlings and young plants can be masked by various anatomical and morphological conditions. In other cases, the results are inferred from applications to pecan of Ni-sulphate, one of the most accessible and inexpensive sources of Ni. The objective of this study was to evaluate the concentrations of mineral nutrients and urease activity in leaflets of young plants of cv. Pawnee pecan in response to foliar applications of Ni as Ni-sulphate, or Ni-citrate or as chelates with EDDHA, EDTA or DTPA.

MATERIAL AND METHODS

Study area, plant material and crop management. The study was carried out from December 2018 to June 2019 in a greenhouse at the Faculty of Agrotechnological Sciences from the Autonomous University of Chihuahua (UACH), Mexico. The experiment starts with the collection of seeds from 10-year-old pecan trees cv. Pawnee in Chihuahua, Mexico (27°06'16"'N and 104°56'09"W; 1 321 m a.s.l.). The site's mean annual temperature is 18.6 °C and the mean annual precipitation is 369.8 mm (García 2004). The seeds were scarified with tap water for three days and sown in the sand as the substrate. They were irrigated every three days for 54 days (the time to emergence). The young seedlings (about 2 months old) were then transplanted into the sand in black polyurethane bags (0.5 m^3), one seedling per bag.

Mineral nutrition was supplied to the bags every three days using 1.2 L per bag of a solution containing (mmol/L): 6 NH_4NO_3, 1.6 K_2HPO_4, 2.4 K_2SO_4, 4.0 $CaCl_2·2\,H_2O$, 1.4 $MgSO_4$ and (μmol/L) 5 Fe-EDDHA, 2 $MnSO_4·\,H_2O$, 1 $ZnSO_4$, 0.25 $CuSO_4·5\,H_2O$, 0.3 (NH_4), 6 $MO_7O_{24}·4\,H_2O$ and 0.5 H_3BO_3. The pH of the solution was 5.8 and the electrical conductivity (EC) was 2.7 dS/m.

Three foliar Ni applications were made – on April 12, April 26 and May 10, 2019 – when the young plants were about five or six months old and were of height

26.5 ± 2 cm and base stem diameter 2.85 ± 0.5 cm. Each canopy was sprayed with the appropriate Ni treatment solution using an atomiser spray (1.5 L). Spraying was continued to wetness but stopped before run-off so as not to contaminate the hydroponic solution or of the soil or the roots. To avoid any contact with the soil, each plant was sprayed separately, and a plastic film was used to cover the top of each pot before the spraying.

There were five Ni treatments (T), with the Ni^{2+} supplied as reagent grade $NiSO_4 \cdot 7\ H_2O$, and water control (C). The treatments were T1 $NiSO_4$ chelated with DTPA (Ni(II)-DTPA), T2 $NiSO_4$ chelated with EDTA (Ni(II)-EDTA), T3 $NiSO_4$ chelated with EDDHA (Ni(II)-EDDHA), T4 Ni-sulphate with no chelating agent and T5 Ni-citrate with no chelating agent. The water control contained no Ni^{2+} and no chelating compound. The five Ni sprays each contained 25 mg Ni^{2+}/L and were pH adjusted to 6.0. The five sprays and the water control, all contained 1 mL/L of the surfactant INEX-A^{TM} (Cosmocel®, Guadalajara, Mexico) to render coverage and penetration more uniform. The experiment was established in a completely randomised design with six replications per treatment.

Sampling and foliar concentration of nutrients. The leaflets were harvested and promptly transported for analysis to the Plant Nutrition Laboratory of the Faculty of Agrotechnological Sciences, Chihuahua, Mexico. A triple wash of the leaves was first carried out with tap water, then with a 4 mol/L solution of HCl and then with deionised water. Surface moisture was completely removed from the leaflets by blotting at room temperature and the leaflets were then dried to constant weight in a ventilated oven (Heratherm VCA 230®, Thermo Scientific, Waltham, USA) at 70 °C for 24 h. Each sample was then homogenised in a mill (Willey R-TE-650/1, with a 1 mm mesh, Tecnal, São Paulo, Brazil). The concentrations of the cations K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Zn^{2+} and Ni^{2+} were determined following triacid digestion (HNO_3, $HClO_4$ and H_2SO_4) (25 mL of the mixture in a 10:10:25 ratio). Total N was assayed by the micro-Kjeldahl method. In all cases, an atomic absorption spectrophotometer was used (Thermo Scientific Analysis 3000®, Thermo Scientific, Waltham, USA). The total-P concentration was determined by the ammonium-molybdate vanadate colourimetric method (Wagle et al. 2011).

Total chlorophyll (TChl), total carotenoids (TC), leaflet area (LA), leaflet dry weight and root dry weight. Extraction and quantification of photosynthetic pigments (TChl and TC) were carried out by the method of Wellburn (1994). Briefly, samples were placed in 100 mL flasks with 80% acetone. Absorbance was recorded at 665, 653 and 470 nm using a UV/visible spectrophotometer (Lambda 25®, PerkinElmer, Waltham, USA). The results are expressed in µg/g. The leaf area was measured using a leaf area meter (CI-202®, Cid Bio-Science, Camas, USA). For dry weight, samples were dried for 72 h at 70 °C in a drying oven (Heratherm VCA 230®, Thermo Scientific, Waltham, USA). The dry weight (g) was determined using a (0.01 g) portable electronic balance (Scout® Pro SP202, Ohaus, Nänikon, Switzerland).

Urease activity (E.C. 3.5.1.5). Urease extraction and analysis was carried out using the method described by Ojeda-Barrios et al. (2016) with slight modifications. Briefly, 0.5 g of fresh tissue was placed in a test tube with 5 mL of KH_2PO_4 100 mmol at pH 7.5 and propanol (5%) with 0.2 mol/L urea. The tube was subjected to a continuous vacuum for 5 min at 30 mm Hg and incubated in a water bath at 100 °C for 3 min. Quantification of the released ammonia was carried out with 1 mL of the extract to which 6 mL of KH_2PO_4 100 mmol (pH 7.5), 4 mL of (15%) salicylate-nitroprusside-Na and 2 mL of 5.35% Na hypochlorite were added. The mixture was incubated for 20 min at 37 °C in the dark. The absorbance of samples with urea and without urea at 560 nm was recorded against an NH_4^+ standard. Enzyme activity was expressed as µmol NH_4^+/min/g.

Statistical analyses. Prior to statistical analysis, the raw data were subjected to tests for homogeneity of variance using the Levane test and when heterogeneity was detected, they were transformed with $\log_{10}$ (Sokal and Rohlf 1995). A multiple analysis of variance and a comparison of means were carried out with a Tukey's test ($P \leq 0.05$). The degree of association between the parameters evaluated was determined by a Pearson's correlation analysis. In all cases, statistical analysis software SAS version 9.3 was used (SAS Institute Inc., North Carolina, USA).

RESULTS AND DISCUSSION

Foliar nutrient concentration. The concentrations of nutrients in the leaflets is shown in Table 1. Good mineral nutrition is critical for yield and quality in pecan (Smith et al. 2012). Ni-EDDHA and Ni-sulphate both significantly increased total N concentration, with values varying between 27.17 ± 0.98 (1.14 g/plant) (Ni-EDDHA) and 28 ± 0.89

Table 1. The concentration of foliar nutrients in young pecan plants of cv. Pawnee sprayed with nickel

Treatment	Macronutrient (g/kg)				
	N-total	P	K^{+}	Ca^{2+}	Mg^{2+}
Control	19.83 ± 2.14^{d}	1.88 ± 0.37^{bc}	5.67 ± 0.51^{b}	8.37 ± 0.31^{c}	2.40 ± 0.24^{d}
Ni-DTPA	24.00 ± 1.79^{c}	1.63 ± 0.23^{bc}	6.10 ± 0.54^{b}	15.36 ± 3.25^{a}	2.70 ± 0.14^{cd}
Ni-EDTA	25.00 ± 2.00^{bc}	1.55 ± 0.16^{c}	5.75 ± 0.22^{b}	13.91 ± 2.20^{a}	3.15 ± 0.23^{ab}
Ni-EDDHA	27.17 ± 0.98^{ab}	1.72 ± 0.18^{bc}	7.20 ± 0.15^{a}	13.18 ± 3.29^{ab}	3.42 ± 0.25^{a}
Ni-sulphate	28.00 ± 0.89^{a}	2.08 ± 0.41^{ab}	5.62 ± 0.23^{b}	9.66 ± 0.85^{bc}	3.00 ± 0.26^{bc}
Ni-citrate	23.67 ± 1.21^{c}	2.47 ± 0.05^{a}	4.70 ± 0.14^{c}	13.71 ± 2.39^{ab}	2.73 ± 0.34^{cd}
	Micronutrient (mg/kg)				
	Ni^{2+}	Fe^{2+}	Cu^{2+}	Mn^{2+}	Zn^{2+}
Control	1.79 ± 0.13^{c}	96.50 ± 19.48^{a}	6.92 ± 0.38^{a}	175.61 ± 3.50^{d}	20.35 ± 0.89^{e}
Ni-DTPA	2.87 ± 0.68^{bc}	73.66 ± 3.44^{b}	7.08 ± 0.49^{a}	206.47 ± 4.25^{bc}	25.64 ± 0.83^{d}
Ni-EDTA	3.03 ± 0.48^{b}	78.00 ± 17.96^{ab}	7.00 ± 1.22^{a}	197.11 ± 7.26^{b}	24.26 ± 0.51^{d}
Ni-EDDHA	3.70 ± 0.05^{a}	83.50 ± 5.88^{ab}	8.16 ± 1.69^{a}	222.73 ± 2.85^{a}	38.69 ± 0.29^{a}
Ni-sulphate	3.45 ± 0.05^{b}	79.16 ± 5.85^{ab}	6.66 ± 0.93^{a}	217.66 ± 1.84^{a}	34.22 ± 2.27^{b}
Ni-citrate	3.05 ± 0.46^{b}	93.00 ± 5.83^{ab}	6.50 ± 1.41^{a}	174.96 ± 15.75^{d}	28.53 ± 1.61^{c}

Mean values ± standard deviation (n = 6); values with the same letters within each column do not differ significantly ($P \leq 0.05$; Tukey's test); DTPA – diethylene-triamine penta-acetic acid; EDTA – ethylene diamine-tetra-acetic acid; EDDHA – ethylene-diamine-di-(o-hydroxyphenylacetic acid); Ni – nickel

(1.03 g/plant) g/kg (Ni-sulphate), respectively. This suggests increased stimulation of the activity of urease with the hydrolysis of urea and the consequently increased availability of N (Moran-Duran et al. 2020). Foliar deficiency for Ni^{2+} in pecan is associated with decreased N metabolism (Bai et al. 2006). Furthermore, an antagonistic relationship between divalent ions such as Zn^{2+} and Cu^{2+} can be indicated (Wagle et al. 2011). Freitas et al. (2019) when applying Ni-sulphate observed increased yield and growth in soybean, which indicates improved functioning of N metabolism induced by Ni fertilisation. The Ni-EDDHA indicates a higher affinity for Ni^{2+} and an increase in uptake and transport (Niu et al. 2021). Among the traditional sources of Ni^{2+} is Ni-sulphate, whose outstanding characteristics are high solubility and a near-neutral pH (6.0–6.5). These make it an excellent source of Ni for plants. The values found in this study for foliar total-N are in the range of "Ni sufficiency" as reported for pecan by Pond et al. (2006) and Smith et al. (2012). On the other hand, working with cv. Squirrel Delight pecan, Hernández-López et al. (2020) reported a reduction in the concentration of total-N with foliar applications of Ni^{2+} for doses of 100, 150, 200 and 250 mg/L of Speedfol Pecano SP® (Ni-oxide), a result attributed to a dilution effect induced by the increase in foliar area.

The leaflets treated with Ni-sulphate and Ni-citrate allow us to observe variation in the concentrations of P (2.08 ± 0.41 (0.08 g/plant) (Ni-sulphate) and 2.47 ± 0.05 (0.08 g/plant) g/kg (Ni-citrate), respectively. However, the values found for P in our experiment were in the range of P sufficiency (Pond et al. 2006). These results could be linked to the synergistic effect of Ni with N and P through the citric acid pathway that induces sprouting, biomass accumulation and fruit-set in pecan (Ojeda-Barrios et al. 2016). The foliar application of Ni as Ni-sulphate showed variable effects as it has with soybean (*Glycine max* L.), where no change was observed in P concentration. However, N did show an increase (de Queiroz-Barcelos et al. 2017). In cv. Apache pecan seedlings, P concentration increased after 100 mg/L of Ni (Ni-sulphate) was sprayed (de Oliveira et al. 2021). On the other hand, in umbu (*Spondias tuberosa* Arr.) after applying 0.03 mmol/L of Ni-sulphate in the nutrient solution, Neves et al. (2007) found an increase in the foliar concentration of P.

A significant response to the application of Ni-EDDHA was observed in foliar concentrations of K^{+} (7.20 ± 0.15 g/kg) (0.30 g/plant) and Mg^{+} (3.42 ± 0.25 g/kg) (0.14 g/plant), however, the Mg^{+} concentration was similar to that with Ni-EDTA. These K^{+} concentrations do not coincide with that indicated by Smith et al. (2012). The Ca^{2+} concentrations were similar in our plants treated with Ni-DTPA, Ni-EDTA or Ni-EDDHA. Previous studies in cv. Western Schley pecan were similar for foliar applications of 2 000 mL of Nickel plus® (Ni-lignosulfonate) and 670 g of Speedflo pecano SP® (Ni-oxide) in 1 000 L of water (Ojeda-Barrios et al. 2016). Likewise, in cv. Apache pecan seedlings the concentrations of K^{2+} and Mg^{2+} increased when 100 mg/L of Ni (Ni-sulphate) was sprayed. In contrast, foliar Ca^{2+} concentration did not show any treatment effects (de Oliveira et al. 2021). Variations in the concentrations of foliar nutrients can be linked to both Ni dose and Ni source (Bai et al. 2006).

Our young pecan plants of cv. Pawnee sprayed with Ni-EDDHA showed higher concentrations of Ni^{2+} (3.70 mg/kg) (0.16 mg/plant), Mn^{2+} (222.73 mg/kg) (9.37 mg/plant) and Zn^{2+} (38.69 mg/kg) (1.62 mg/plant) compared with the controls. However, Mn^{2+} levels were similar to the concentration in the Ni-sulphate plants. On the other hand, Ni-DTPA caused a significant reduction in foliar Fe^{2+} concentration (73.66 ± 3.44 mg/kg) (2.27 mg/plant). Concentrations of Cu^{2+} did not vary significantly among treatments. Similar results were previously reported with cv. Apache pecan seedlings sprinkled with 100 mg/L of $NiSO_4$ (de Oliveira et al. 2021) where Cu^{2+} was not significantly affected. In barley (*Hordeum vulgare* L.) cv. Minorimugi the supply of Ni^{2+} as 1 to 10 μmol Ni-sulphate in the nutrient solution decreased the concentrations of Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} in the shoots (Rahman et al. 2005). Similarly, Freitas et al. (2019) applied $NiSO_4 \cdot 6\ H_2O$ to the soil and found an antagonistic effect between Ni^{2+} and Fe^{2+} in the leaves of soy (*Glycine max* L.). There is good evidence that the micronutrient Ni^{2+} does not use specific cation transporters, and so competes with Cu^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}, so inhibiting their uptake and transport (Wagle et al. 2011, de Queiroz-Barcelos et al. 2017).

In adult pecan trees of cv. Western Schley increases in foliar Ni^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} have been observed with the foliar applications of 2 000 mL of Nickel plus® (nickel lignosulfonate) and 670 g of Speedflo pecano SP® (Ni-oxide) in 1 000 L of water (Ojeda-Barrios et al. 2016). In other fruit tree species, such as umbus (*Spondias tuberosa* Arr.) significant increases in foliar Ni^{2+} have been reported with the edaphic applications of 132 g/ha of Ni-sulphate (Neves et al. 2007).

In the present study, with the exception of Zn^{2+} (60–150 mg/kg), the values found are similar to the ranges of sufficiency for Ni^{2+} (> 2.5 mg/kg), Mn^{2+} (100–2 000 mg/kg), Fe^{2+} (50–300 mg/kg) and Cu^{2+} (6–30 mg/kg) in Smith et al. (2012). In this sense, the efficiencies of the chelates are linked to their rapid uptake and transport, with the additional advantage of minimising the relative cost of applications and the environmental impacts associated with conventional fertilisers (Niu et al. 2021).

Abiotic stress in pecan trees can be quantified in terms of the concentration of leaf chlorophyll, however, its accumulation and synthesis are affected by foliar applications of Zn^{2+}, Mn^{2+}, Fe^{2+} and Ni^{2+} (Ojeda-Barrios et al. 2016, Freitas et al. 2019, Ma et al. 2019, Balandrán-Valladares et al. 2021). In this regard, Ojeda-Barrios et al. (2016) when applying lignosulfonate and Ni-oxide in pecan cv. Western Schley reported an increase in the concentration of chlorophyll determined using SPAD 502. Under the conditions of our experiment, the young cv. Pawnee trees did not show significant variations in chlorophyll concentration, where values fluctuated between 35.56 ± 1.29 and 42.40 ± 5.19 μg/g (Table 2). The solvent used to extract the photosynthetic pig-

Table 2. Photosynthetic pigments, leaflet area and dry weight in young pecan plants of cv. Pawnee sprayed with nickel

Treatment	TChl	TC	LA	DW
	(mg/kg)		(cm^2)	(g/plant)
Control	35.56 ± 1.29^{a}	3.38 ± 0.49^{ab}	15.50 ± 1.42^{c}	23.67 ± 0.54^{e}
Ni-DTPA	41.09 ± 4.16^{a}	2.59 ± 1.08^{b}	19.48 ± 8.68^{bc}	30.85 ± 0.57^{d}
Ni-EDTA	40.00 ± 2.73^{a}	2.42 ± 0.17^{b}	16.92 ± 2.38^{bc}	36.20 ± 0.93^{b}
Ni-EDDHA	41.45 ± 6.10^{a}	3.67 ± 0.78^{a}	27.28 ± 2.20^{a}	42.08 ± 0.59^{a}
Ni-sulphate	42.40 ± 5.19^{a}	3.45 ± 0.49^{a}	22.85 ± 1.51^{ab}	36.72 ± 1.08^{b}
Ni-citrate	40.81 ± 4.65^{a}	3.75 ± 0.49^{a}	19.03 ± 2.55^{bc}	33.33 ± 1.0^{4c}

Mean values ± standard deviation ($n = 6$); values with the same letters within each column do not differ significantly ($P \leq 0.05$; Tukey's test); TChl – total chlorophyll; TC – total carotenoids; LA – leaflet area; DW – dry weight ments, the phenological state and tree age, as well as the dose, solubility, spray drop size and drop volume and Ni source are all factors that affect the response of pecan leaflets to Ni applications. Freitas et al. (2019) demonstrated the active participation of Ni in photosynthesis as a photoprotector against the oxidation of chlorophylls *a* and *b* and also of the accessory pigments, including carotenoids. Another important factor in the functional performance of Ni is associated with the interaction with Cu^{2+}, as indicated by Wagle et al. (2011). The values obtained for TChl concentration are similar to those reported by Balandrán-Valladares et al. (2021).

On the other hand, there is a relationship between chlorophyll concentration and the expression of accessory pigments, including carotenoids. Our young pecan plants sprayed with Ni-DTPA and Ni-EDTA showed decreases in TC concentration (Table 2). In this sense, the use of foliar sprays of these chelates could offer a valid option for minimising the abiotic stress-induced by Ni deficits. We are unaware of studies in pecan that indicate that changes in the concentration of TC could be associated with foliar applications of Ni^{2+}. However, correlations have been reported between the optimal performance of photosynthesis and tissue concentrations of Zn^{2+}, whose values in this study were maintained in the deficiency range (24.26 ± 0.51–38.69 ± 0.29 mg/g) (Smith et al. 2012). It would be worthwhile to carry out new evaluations with other doses, application times and at different phenological stages to expand our understanding of the effects of Ni^{2+} on the behaviour of carotenoids in pecan leaflets.

Among the factors determining the optimal level of solar radiation and the synthesis of photoassimilates is leaf area (Balandrán-Valladares et al. 2021). In our study, a LA value of 27.28 ± 2.20 cm^2 was obtained with Ni-EDDHA and 22.85 ± 1.51 cm^2 with Ni-sulphate (Table 2). These results are in contrast with previous studies by Wagle et al. (2011) and Moran-Duran et al. (2020) with cv. Pawnee trees (8 and 3 years old, respectively) with foliar applications of Ni-sulphate (0.78 mL/L, 0.055 g/L and 82.3 g/ha) and 1.9 mL/L of Nickel plus® (nickel lignosulfonate). However, our results are similar to those reported in adult pecan trees of cv. Squirrel Delight by Hernández-López et al. (2020) when applying 100 mg Ni-oxide/L. The same authors did not observe an effect of increasing doses from 150, 200 to 250 mg/L. Variation in leaflet area is determined by multiple factors, including Ni dose, number of applications, pecan cultivar, phenological stage, and tree age. It is important to emphasise that Ni uptake is associated with the formation of stable chelates that increase the efficiency of use for Ni (Niu et al. 2020).

The optimal growth and development of the canopy of a fruit tree, including of pecan, is realised when roots are healthy and uptake of water and nutrients is adequate (Aburto-González et al. 2021). Among the Ni chelates, Ni-EDDHA significantly increased the dry weights of the young plant with a value of 42.08 ± 0.59 g/plant (Table 2). An earlier study in pecans cv. Pawnee receiving foliar sprays of Ni-sulphate (0.78 mL/L) showed a similar behaviour (Wagle et al. 2011). Values of LA are similar to those obtained in cv. Western Schley pecan seedlings by Balandrán-

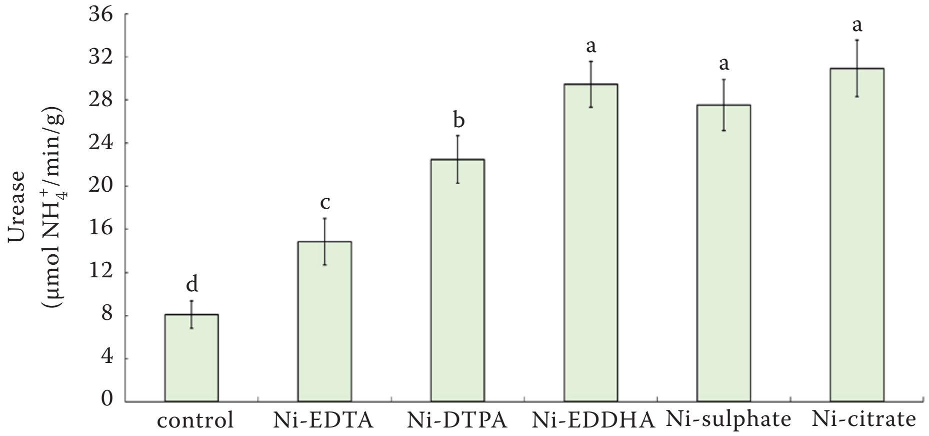

Figure 1. Urease activity in young pecan plants of cv. Pawnee sprayed with nickel. Bars with the same letter are equal ($P \leq 0.05$; Tukey's test). Error bars represent standard deviations (n = 6). DTPA – diethylene-triamine penta-acetic acid; EDTA – ethylene diamine-tetra-acetic acid; EDDHA – ethylene-diamine-di-(o-hydroxyphenylacetic acid); Ni – nickel

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Table 3. Pearson's correlation coefficients for foliar nutrients and some parameters evaluated in young pecan plants of cv. Pawnee sprayed with nickel

	Urease (μmol NH_4^+/min/g)	LA (cm^2)	TChl	TC
			(mg/kg)	
N-total	0.615**	0.514**	0.291	0.102
P	0.177	–0.040	–0.086	0.402*
K^+	–0.030	0.350*	0.102	0.018
Ca^{2+}	0.325	–0.122	–0.134	–0.011
Mg^{2+}	0.620**	0.444**	0.261	0.142
Fe^{2+}	–0.235	–0.140	–0.221	0.213
Cu^{2+}	–0.041	0.306	0.007	0.058
Mn^{2+}	0.297	0.541**	0.310	0.047
Zn^{2+}	0.582**	0.710**	0.354*	0.291
Ni^{2+}	0.742**	0.602**	0.479**	0.052

LA – leaflet area; TChl – total chlorophyll; TC – total carotenoids; *$P \leq 0.05$, **$P \leq 0.05$

Valladares et al. (2021). Likewise, similar behaviour has been observed in shoots and roots of soybean (*Glycine max* L.) genotype TMG2158 with edaphic applications of $NiSO_4$ (3.0 mg/kg) (Freitas et al. 2019). As previously mentioned, Ni is a micronutrient that affects the stability of chlorophyll and so can support the synthesis of essential organic compounds for the growth of new leaflets (Queiroz-Barcelos et al. 2017).

Urease activity. The enzyme urease plays an important role in urea hydrolysis and so contributes to N metabolism in plants (Ojeda-Barrios et al. 2016, de Queiroz-Barcelos et al. 2017). The urease activity in our leaflets treated with different Ni sources varied between 14.84 ± 1.27 and 30.93 ± 2.62 μmol NH_4^+/min/g, where the maximum values were obtained with Ni-citrate, Ni-EDDHA and Ni-sulphate ($P \leq 0.05$) (Figure 1). In this sense, the availability of Ni^{2+} is affected by the plant tissue pH but associated with citrate tends to stabilise (Rahman et al. 2005). Urease is activated by the two Ni^{2+} atoms, so a Ni deficiency can block or reduce the activity of this enzyme (Rodríguez-Jiménez et al. 2016). Pecan trees are often found to be Ni-deficient. These can develop leaflets with "mouse ear" symptoms which are associated with the roles of Ni in the configurations of a number of enzymes, including urease (Bai et al. 2006). Urease participates in the catabolic pathway of ureides, the urea cycle, the citric acid cycle, amino acid synthesis and the carbon-nitrogen relationship (Polacco et al. 2013).

Other reports offer similar results with the pecan cvs. Western Schley and Apache when sprayed with lignosulfonate (2 000 mL/1 000 L, Nickel Plus®) (Ojeda-Barrios et al. 2016) and Ni-sulphate (100 mg/L) (de Oliveira et al. 2021). Thus, the monitoring and correction with Ni of the "mouse ear" symptom can be observed 12 days after the Ni application with the normal growth of the shoots, i.e., with no signs of marginal necrosis (Hernández-Lopez et al. 2020, de Oliveira et al. 2021). Likewise, there is evidence in soybean (*Glycine max* L.) and chilacayote (*Cucurbita ficifolia* Bouché) of the role played by interaction between Ni and urease activity in the assimilation of N (Almanza et al. 2009, Lucasynski-Carlim et al. 2019).

On the other hand, after N and Zn^{2+}, Ni^{2+} plays a key role in determining the commercial productivity of a pecan orchard. In our study, the highest Pearson's correlation values between the various parameters evaluated was for urease and total-N (0.615), urease and Mg^{2+} (0.620), urease and Zn^{2+} (0.297), urease and Ni^{2+} (0.742), LA and total-N (0.514), LA and K^+ (0.350), LA and Mg^{2+} (0.444), LA and Mn^{2+} (0.541), LA and Zn^{2+} (0.710), LA and Ni^{2+} (0.602), TChl and Zn^{2+} (0.354), TChl and Ni^{2+} (0.479), and TC and P (0.402) (Table 3). Pecan is perhaps the only Ni-deficient woody species, where the Ni and urease interaction and its effects on the reduction in leaflet growth have been investigated in any depth (Bai et al. 2007, Polacco et al. 2013, Hernández-López et al. 2020). Likewise, Ni is a micronutrient that serves as a photoprotector against the oxidation of chlorophyll (Freitas et al. 2019). On the other hand, the severity of Ni-deficiency is related to the optimal concentration of Zn^{2+} or Cu^{2+} in the plant tissue (Wagle et al. 2011, Ghasemi et al. 2013, Ma et al. 2019).

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