The impact of foliar nickel fertilization on urease activity in pecan trees

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Abstract

The aim of the present work is to analyze the effect of different foliar products of nickel (Ni) on the nutrient concentration, chlorophyll content, and enzymatic activity of urease as the possible bioindicator of the levels of Ni in the leaves of pecan [*Carya illinoensis* (Wangenh.) K. Koch]. The experiment consisted of the foliar application of Ni to pecan cv. Western Schley, using two commercial products: Nickel Plus®T₁ and Speedfol™ PecanoT₂, and a control T₀ received no Ni treatment. The following variables were evaluated: the total chlorophyll concentration, the concentration of macro- and micronutrients including Ni, and the activity of urease. The results demonstrated that the pecan trees treated with Ni increased in the concentration of this element and that the product Nickel Plus®T₁ increased by 41.24% with respect to control. The foliar application of Ni led to significant differences in the foliar concentrations of N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, and Ni. Chlorophyll showed a significant reaction to the foliar application of Ni. The enzymatic activity of urease proved to be positively related to the foliar level of Ni, and thus could be considered a good physiological bioindicator of the nutritional state of foliar Ni in the leaflets of the pecan tree.

Keywords: *Carya illinoensis*, metal, micro nutrients

1. Introduction

The pecan tree [*Carya illinoensis* (Wangenh.) K. Koch] has increased in crop profitability in recent years as well as socioeconomic importance in northern Mexico (Ojeda-Barrios *et al.*, 2014). The nutrition of this plant is a major topic in orchard management.

Nickel (Ni) has been recognized since 2004 by the Official American Association of Plant Nutrient Control as an essential element in plants (Wood *et al.*, 2004a) because of its relationship with urease as a catalyzer or inhibitor and constituent of this

enzyme (Bai *et al.*, 2007a). For plant metabolism, Ni is required in quantities of less than 0.001 mg kg⁻¹, dry weight. Several works have demonstrated that Ni is required for seed viability in barley (*Hordeum vulgare*) (Wagle *et al.*, 2011). This proposal has been strengthened by field observations of growth and responses to foliar applications of Ni in crops as diverse as wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), and broadbean (*Vicia faba*) (Rahmana *et al.*, 2005). Despite the studies made, little information is available on the metabolism or the metabolic function of Ni, compared with other microelements.

To date, the activation of urease appears to be the only enzymatic function of Ni in higher plants (Seregin and Kozhevnikova, 2006). Urease catalyses the hydrolysis of urea [CO(NH₂)₂], a reaction that splits the molecule into ammonium (NH₃) and carbon dioxide. Ni deficiency inhibits the action of urease, leading to the accumulation of urea, which can provoke the appearance of necrotic spots on the leaves (Reuter, 2005; Wood, 2006). In addition, this deficiency can interrupt the metabolism of ureides, amino acids, and organic acids, and can accumulate oxalatic and lactic acids. Urease contains two Ni ions at its active site (Palacco *et al.*, 2013). Evidence indicates that the transporter species of ureides, as in walnut trees, have a higher requirement of Ni than do transporter species of amides, thus increasing the possibility that the ureide transporters can have enzymes, except urease, which requires Ni for its activation or to intensify its activities (Wood, 2006). Under pecan cultivation, severe Ni deficiency can cause a growth abnormality (i.e. mouse ear) and interfere with transplants in orchards (Pradeep *et al.*, 2011). Such deficiency also interrupts N and C metabolism during leaf expansion (Bai *et al.*, 2007a). The necrotic spots associated with Ni deficiency correspond to the accumulation sites of urea or oxalatic and lactic acids, indicating that changes also occur in C metabolism,

in particular during the reduction of respiration (Wite Claus-Peter, 2011). Initially, this disorder (mouse ear) was attributed to various causes, such as cold damage, viral infections, or Mn or Cu. The foliar analysis of healthy leaves compared with affected ones revealed that the symptoms are provoked by Ni deficiency caused by low contents of this element or induced by excessive zinc in the soil (Wood *et al.*, 2004b).

Another study found that pecan trees transport mainly N such as ureides at the onset of spring and that the nutritional state of Ni in these trees affects the metabolism of ureides and amides as well as sap composition in the xylem. Meanwhile, Ni deficiency quantitatively affects the composition of xanthine in the sap, alantoic acid, asparagine, citruline, and β-phenyletylamine in the sap of the xylem in spring, and interrupts ureide catabolism and the urea-metabolism cycle (Wood *et al.*, 2004a). The effect observed of Ni on N metabolism constitutes evidence that Ni nutrition is more important for the management of N than for the transport of ureides in crops (Bai *et al.*, 2013).

The aim of the present work was to assess the impact of two commercial foliar Ni fertilizers on the leaf mineral composition, including Ni, chlorophyll content and urease activity in the leaflets of the pecan tree.

2. Materials and Methods

2.1. Description of the study area

The study was conducted in Delicias, Chihuahua (Mexico), during the 2010 vegetative cycle. The orchard is located at 28° 11' latitude norths, 105° 28' longitude west, and an altitude of 1170 m, with 336.5 mm of annual precipitation. It is an arid region with a mean temperature of 18.6°C. The orchard was on a calcareous soil (Domino silt loam, Xerollic Calciorthid), has an arable layer of 0-35 cm with a

pH of 7.25 in 1:1 soil: water, 1.2% organic matter, 10.0% total CaCO_3 , $8.8 \text{ mg}\cdot\text{kg}^{-1} \text{ NO}_3^-$, and $0.48 \text{ mg}\cdot\text{kg}^{-1}$ DTPA-extractable Zn (Rivera-Ortiz *et al.*, 2003). The orchard consisted of 15-year-old pecan ($70 \text{ trees}\cdot\text{ha}^{-1}$). The pecan trees, cv. Western Schley, grafted to a native rootstock, were 25 years old. The planting scheme was $12 \times 12 \text{ m}$ with a density of 70 trees per ha. Three treatments were evaluated. Two commercial Ni-based foliar products were applied on 25 May and 24 June 2010: Nickel Plus®T₁ (total N 5%, S 3%, and Ni 5.4%) (N 2.85, S 1.87 and 0.73 mM) and Speedfol™ PecanoT₂ (N 5%, P 12%, K 18%, Mg 4.2%, and Ni 5.4%) (N 2.39, P 2.51, K 0.95, Mg 1.15, and Ni 0.76 mM), and a Control T₀. The application rates were 2000 mL for Nickel Plus®T₁ and 670 g for Speedfol Pecano™ T₂. A randomized block design was used with four replications per treatment. The experimental unit was one tree. In all formulations, 0.1% urea was added as transporter ion and $100 \text{ mg}\cdot\text{L}^{-1}$ Tween 20 (Thermo Scientific, Illinois, USA) was used as a surfactant. The pH of the solutions was adjusted with sulfuric acid to 6.5, a pH that is thought to facilitate foliar uptake of metal formulations (Ojeda-Barrios *et al.*, 2014).

Trees were sprayed between 7:00 and 9:00 a.m. using a mist sprayer PTO SNT-500-30E, covering the entire tree canopy to the drip point.

2.2. Orchard management

The trees were fertilized once with 120-80-80 NPK using ammonium sulfate (20.5% of N), diammonium phosphate (46% of P_2O_5), and potassium sulfate (50% of K_2O), respectively. Fertilizers were broadcasted on 30 March 2010 and the area was immediately raked and then irrigated. Trees were flood irrigated, using 120 to 140 mm of water, every 20 days from March to the end of October. The most common pests were yellow aphid (*Monelia caryella*) and the hickory shuckworm

(*Laspeyresia caryana*), while the most common disease was downy spot (*Mycosphaerella carygena*). Due to the low incidence, no control was applied.

2.3. Foliar sampling

Approximately 30 pairs of leaflets were collected five times during the growing season (18 May, 9 June, 12 July, 23 August, 6 September, and 4 October 2010) from the mid-canopy of each tree. Leaflets were collected mixing samples from the four cardinal directions and from vegetative and fruiting shoots. Six foliar samplings were taken: 18 May, 9 June, 12 July, 23 August, 6 September, and 4 October 2010. The leaves were sampled from the middle of the branch selected while the leaf sample was taken from the middle part of the leaf, taken from 30 pairs of leaflets per sample. The different samplings were used to evaluate the variables of total chlorophyll concentration, greenness of the leaf by the Minolta SPAD 502 chlorophyll meter, and the foliar concentrations of N, K, Ca, Mg, Fe, Cu, Mn, and Ni, as well as urease activity.

The samples collected were analyzed in the laboratory of Plant Nutrition del Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD-Delicias). The samples were washed with running water, immediately afterwards with running water plus HCl 4N and then were rinsed with tap water and with deionized water. The leaves were left to drain and were dried at room temperature and finally were dried in a stove at 60°C for 24 h and milled using a 20-mesh screen (Ojeda-Barrios *et al.*, 2012). Nickel, Zn, Fe, Mn, Cu, K, Ca, and Mg were determined by triacid digestion (HNO_3 , HClO_4 , and H_2SO_4 in the ratio 10:1:0.25), and with an atomic absorption spectrophotometer (Thermo Scientific Analysis 3000) (Smith *et al.*, 2004). Nitrogen level was determined by the micro-Kjeldahl method (Wagle *et al.*, 2011).

2.4. Determination of total chlorophyll

The total chlorophyll concentration was quantified by extraction with methanol and reading the absorbance. For this, 60 mg of petiolules of 5 mm in diameter were weighed, placed in test tubes with 5 ml of methanol, and left 24 h or more until total discoloration of the plant material. The process was made at ambient temperature in darkness. Afterwards, the absorbance was read in 652 nm, the total chlorophyll content being expressed in $\text{mg}\cdot\text{kg}^{-1}$ (Ojeda-Barrios *et al.*, 2012).

2.5. Leaf greenness (SPAD units)

With the Minolta SPAD-502 chlorophyll-meter, 20 readings were taken for the chlorophyll from pecan leaflets, and in the middle part of the tree, in the current-year growth, the third leaflet of the leaf was taken, making sure that it was healthy, avoiding veins (especially the central one).

2.6. Urease activity (E.C. 3.5.1.5)

For the extraction of the urease activity, 0.5 g of petiolules of 3 mm in diameter were weighed and placed in test tubes with 5 ml of H_2PO_4 mM at pH 7.5 in the presence of at 5% with urea at 0.2 M and without urea. This was placed in a vacuum for 5 min at 30 mmHg and afterwards incubated in a double boiler at 100°C for 3 min. An extract was used to quantify the ammonium released, using one mL of the extract 6 mL of KH_2PO_4 mM at pH 7.5, 4 mL salicylate-nitroprussiate-Na at 15 %, and 2 mL of Na hypochlorite at 5.35%. The extract was incubated for 20 min at 37°C in darkness and finally the process was carried out at room temperature in

darkness. Afterwards, the absorbance was read at 560 nm, from the samples without urea, and with urea against a pattern of NH_4^+ . The enzymatic activity was expressed as μmol of NH_4^+ formed $\text{min}^{-1}\text{g}^{-1}$ p. f. (Palacco *et al.*, 2013).

2.7. Statistical analysis

All the data were submitted to an analysis of variance using the completely random model with a total of 3 treatments and 4 replicates. The differences between the means of the treatments were compared using the LSD test at 95% using SAS (2007). Finally, the significance levels were represented by * at $P < 0.05$ and N.S. for not significant).

3. Results and Discussion

3.1. Foliar nutritional concentration

Nutritional analysis assesses the state of nutrition of a crop and evaluates the availability of reserves in the plant (Wood *et al.*, 2004c). In the present study, significant differences were found in the foliar concentration after the application of the Ni-based products Nickel Plus® T_1 and Speedfol™ Pecano T_2 , for macronutrients (N, P, K, Ca, and Mg; Table 1) as well as micronutrients (Fe, Mn, Cu, Zn, and Ni; Table 2) (Pradeep *et al.*, 2011). The values found for these nutrients were within the sufficiency range (Medina, 2004). It has been reported that Ni has a synergetic relationship with Fe, Zn, Cu, and Mn (Wood *et al.*, 2004a) as well as with N and P through the interaction of Ni in the biochemical pathways that are critical for budding, canopy formation, and fruit formation in pecan trees (Wood *et al.*, 2004b).

Table 1. Effect of the application of Nickel Plus® and Speedfol Pecano™ on the macronutrient concentrations in pecan trees. The data are means $\pm$ standard error (n = 4).

Treatments of Nickel	Macronutrients (g.kg ⁻¹)				
	N	P	K	Ca	Mg
Control T ₀	[‡] 2.14b $\pm$ 0.12	0.157a $\pm$ 0.04	1.344a $\pm$ 0.05	0.775a $\pm$ 0.01	0.411a $\pm$ 0.03
Nickel Plus®T ₁	2.39b $\pm$ 0.14	0.154a $\pm$ 0.03	1.425a $\pm$ 0.07	0.726a $\pm$ 0.02	0.365b $\pm$ 0.03
Speedfol™ PecanoT ₂	2.26ab $\pm$ 0.09	0.147a $\pm$ 0.02	1.438a $\pm$ 0.04	0.633a $\pm$ 0.02	0.432a $\pm$ 0.04
Significance	*	ns	*	*	*

[‡]Data are means (n=4) and the significance level is represented by * at $P \leq 0.05$, ** at $P \leq 0.01$, *** at $P \leq 0.001$, and * at $P < 0.05$ (ns being not significant).

Table 2. Effect of the application of Nickel Plus® and Speedfol Pecano™ on the micronutrient concentration in pecan trees. The data are means $\pm$ standard error (n = 4).

Treatments of Nickel	Micronutrients (mg kg ⁻¹)				
	Cu	Fe	Mn	Zn	Ni
Control T ₀	[‡] 9.89a $\pm$ 0.63	56.79a $\pm$ 3.06	55.61a $\pm$ 3.91	56.94a $\pm$ 2.54	3.38b $\pm$ 0.04
Nickel Plus®T ₁	10.75a $\pm$ 0.57	56.96a $\pm$ 4.60	67.01a $\pm$ 7.63	60.62a $\pm$ 1.71	5.94a $\pm$ 0.06
Speedfol™ PecanoT ₂	11.16a $\pm$ 0.40	57.96a $\pm$ 4.47	60.65a $\pm$ 8.40	58.54a $\pm$ 3.51	4.79ab $\pm$ 0.10
Significance	*	ns	**	ns	*

[‡]Data are means (n=104) and the significance level is represented by * at $P \leq 0.05$, ** at $P \leq 0.01$, *** at $P \leq 0.001$, and * at $P < 0.05$ (ns being not significant).

3.2. Foliar nitrogen

Nitrogen is the element most required by nut trees, as no other nutrient is as determinant of vigor, yield, and nut quality (Smith *et al.*, 2004). In the form of urea, N is not available for metabolism by the plant, unless hydrolyzed to NH₃ and CO₂ by urease. In this study, the product Nickel Plus® T₁ 0.73.mM boosted

N status in comparison to Speedfol™ Pecano T₂ 0.76 mM and control T₀ (Table 1). The values found in the two treatments in which Ni was applied were higher and control lower than the sufficiency range (Medina, 2004). The metabolism of N is affected by ureide catabolism, amino acid metabolism, and the

intermediary cycle of ornithine, while it affects the citric acid cycle of carbonate metabolism. Perhaps determination of N fractions could be better to understand Ni effect. This indicates that Ni deficiency can potentially influence the metabolism and the physiology of N in the plant (Bai *et al.*, 2007a). The reaction catalyzed by ureases is essential so that N from urea is accessible to plants (Wood, 2006; Ruter, 2005). In this study, Ni and urease presented a value significative of $r^2=0.6468$

3.3. Foliar zinc

Zinc is one of the most nutrient required by pecan trees. Zinc deficiency causes yellowing between foliar veins, which has been related to the stabilizing role of Zn on the chlorophyll molecule (Nuñez-Moreno *et al.*, 2009; Ojeda-Barrios *et al.*, 2012; Ojeda-Barrios *et al.*, 2014). The results found in foliar sprays with Ni were highly significant for the case of foliar-Zn content (Table 2). Nickel Plus® T₁ had the higher Zn leaf level with 60.62 mg kg⁻¹, which proved to be deficient (Medina, 2004). The Zn values found revealed a possible interaction with Ni, as for other nutrients such as Fe, Cu, and Mn (Wood, 2006).

3.4. Foliar-Ni dynamics

Nickel is essential for growth, productivity, and yield in the pecan tree (Wood *et al.*, 2004a). In the present study, it was found that the Ni-based products had a direct effect on the foliar Ni content in comparison with control (Figure 1ab show Urease level), underscoring that the product Nickel Plus®T₁

improved the foliar N in comparison with the product Speedfol™ Pecano T₂ and control T₀. This makes the product more effective to prevent mouse ear in the pecan tree (Wood *et al.*, 2004b). It is important to mention that the values found even for the product that achieved the best results were lower than those reported in Arizona (11.4 mg.kg⁻¹) (Pond *et al.*, 2006). In addition, it was found that the treated leaflets of the plants presented a concentration of 7 mg kg⁻¹, while the affected leaflets with mouse ear symptoms had a concentration of 0.5 mg kg⁻¹, this coinciding with reports elsewhere (Wood *et al.*, 2004a). The response of these effects was due possibly to the fact that Ni was transported rapidly to the metabolically active sites, bringing about multiple effects in growth, as previously established (Wood *et al.*, 2004c). In nutritional dynamics, it was found that the product Nickel Plus® T₁ applied on 25 May and 24 June increased the Ni concentration, as reflected in Figure 1cd. The highest foliar concentration corresponded to Nickel Plus® T₁ on the sampling dates of 9 June and 12 July, after the foliar applications of Ni, in which this nutrient was demanded because the tree had entered the rapid growth phase of the fruit. Finally, there was an increase in the Ni concentration on 6 September, possibly related to the retranslocation of nutrients in the ecodormancy in deciduous trees. Ni is critical for the tree during the first growth stages in spring in order to break down the urea into ammonium ions in order to produce amino acids for incorporation into protein structures, enzymes, and nucleic acids (Wood *et al.*, 2004c). Yield, nut weight and nut quality were unaffected by treatments (not shown)

Figure 1ab. Effect of the application of three treatments of Nickel on the nutritional dynamics of foliar; A) Urease Activity; B) Total Nitrogen; C) Foliar Nickel and D) SPAD Units in pecan trees.

Figure 1cd. Effect of the application of three treatments of Nickel on the nutritional dynamics of foliar; A) Urease Activity; B) Total Nitrogen; C) Foliar Nickel and D) SPAD Units in pecan trees.

3.5. Urease activity

The importance of urease is linked to the hydrolysis of urea for the metabolism of N and the activation of this enzyme is the only enzymatic function involving Ni in higher plants (Witte Claus-Peter, 2011). Previous results have indicated that the Ni applications stimulate the activity of urease in pecan leaves (Bai *et al.*, 2013). In our study, we found statistical differences in urease activity after the application of the Ni-based products in pecan trees (Figure 1ab), findings reaffirmed by Bai *et al.*, 2007b(). The increases in urease by the products Nickel Plus® T1 and Speedfol™ Pecano T₂ with respect to control were 32% and 21%, respectively. Thus, the foliar application of Ni increased the activity of urease in the leaf, activating and preventing the accumulation of foliar urea (Wood *et al.*, 2004c). The relation

between Ni and urease has an $r^2=0.6468$ (Figure 2). The use of Ni can improve the activity of urease and thereby improve N nutrition in pecan (Wood *et al.*, 2004c). The evidence indicates that the transporter species of ureides, such as those contained in the pecan tree possess high Ni requirements, more than the transporter species of amides, and therefore urease can be used as a bioindicator of the Ni concentration in the pecan tree (Wood, 2006). In the present study, a higher urease activity was found on the sampling dates of 9 June and 6 September, proportional to the Ni concentration (Figure 1cd). The results indicate that the Ni applications augmented urease activity, preventing urea accumulation in the leaflets of the pecan tree (Wagle *et al.*, 2011). For the June sampling date, the tree was in rapid fruit growth while on the September date the tree was at the fruit-maturation stage, requiring greater activity of N metabolism related to ureide production (Redjela *et al.*, 2010)

Figure 2. Correlation between Urease and Nickel concentration of treatments during vegetative cycle. $Y=0.6468x+0.3764$. $R^2=0.6468$.

3.6. Total Chlorophyll

Total chlorophyll is an indicator of the nutritional state of crops and, for the pecan tree, has been related to the contents of N and Zn (Ojeda-Barrios *et al.*, 2009; De La O-Quezada *et al.*, 2011). The values found for chlorophyll by SPAD 502 significantly differed due to the effect of foliar application of Ni, the mean being 44.38. In this sense, the best treatment was the product Nickel Plus® T₁, with a Ni concentration of 5.94 mg kg⁻¹. For chlorophyll determined by methanol, the values reflected that there was significance only for Speedfol™ Pecano T₂ mM with a Ni concentration of 4.79 mg kg⁻¹, this being the best treatment, while the mean for the total chlorophyll concentration was 22.76 mg mL⁻¹. The above findings are shown in Figure 2. It bears highlighting that the product Nickel Plus® T₁ was considered in concentrations of sufficiency, this coinciding with previous reports (Ojeda-Barrios *et al.*, 2012).

4. Conclusions

In short, considering our overall results, we conclude that the foliar applications of Ni increased the foliar Ni concentration in the leaves of Western Schley pecan trees, the product Nickel Plus® T₁ being the better choice to supply Ni to the trees. On the other hand, Ni deficiency can have potentially harmful consequences for the metabolism and physiology of pecan trees. The application of the Ni products exerted a positive effect on the nutritional state of foliar N, indicating a synergism between Ni and N. Urease activity proved to be positively related to the foliar level of Ni, and therefore can be considered a good physiological bioindicator of the nutritional state of foliar Ni in the leaflets of pecan.

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