# FOLIAR APPLICATION OF NICKEL AND/OR COPPER ON PECAN PERFORMANCE IN CONTAINER AND THE FIELD

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FOLIAR APPLICATION OF NICKEL AND COPPER ON PECAN PERFORMANCE AND NITROGEN METABOLISM

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# CHAPTER I

## INTRODUCTION

Pecans [*Carya illinoinensis* (Wang.) K. Koch] are one of the most valuable commercial nuts in America and the only major nut crop native to the United States. Mouse-ear is a growth abnormality affecting pecan foliage, first reported in 1918 by Marz (1918). This disorder has been attributed to such causes as cold injury, a pathogen (Demaree, 1926), and later a manganese (Mn) deficiency (Gammon and Sharpe, 1956). In 2004, Wood et al. (2004) presented conclusive evidence that the cause of the disorder was a nickel (Ni) deficiency. Nickel has been added to the United States Department of Agriculture list of essential plant nutrients (Hull, 2003), and in 2005 was recognized as an essential plant nutrient by the Association of American Plant Food Control Officials (2005), thus enabling manufacture and sale of Ni fertilizer in the United States (US).

The plant's requirement for Ni is low and most soils have sufficient Ni to meet the requirement. Seed reserves of Ni can satisfy the Ni requirement of some annual plants' to complete their life cycle (Eskew et al., 1983). Pecan trees have higher Ni requirement than most crops (Wood et al., 2006).

Nickel is required for urease (EC 3.5.1.5, urea amidohydrolase) activation (Dixon

et al., 1975), and appears to function in additional enzymes affecting nitrogen (N) metabolism in pecan (Bai, et al., 2006, 2007). Urea was accumulated in the foliage of Ni deficient soybean [*Glycine max* (L.) Merr.] and cowpea (*vigna unguiculata* L.) due to impaired urease activity (Eskew et al., 1983, 1984; Walker et al., 1985). Pecan predominately transports ureide-N with less amide and amino-N in xylem sap (Bai et al., 2007). In pecan xylem sap, Ni deficiency increased citrulline and allantoic acid (Bai et al., 2007). Disruption of ureide catabolism caused by Ni deficiency affected other pathways such as amino acid metabolism and the citric acid cycle. This disruption of carbon metabolism resulted in toxic accumulations of oxalic acid and lactic acid and was attributed with causing mouse-ear symptoms in pecan (Bai et al., 2006). A survey of pecan leaf Ni concentrations suggested that orchards in southern Oklahoma and northern Texas, where native soil pH is alkaline, may be low in Ni (data not shown). One instance of mouse-ear symptoms has been observed in the Red River basin (C. Rohla, personal communication). Though the shortage is not typically sufficient to be symptomatic, trees may respond positively to foliar applied Ni.

Two orchards have been identified that may benefit from supplemental applications of Ni. The first is Johnny Dowd's orchard located near Nocona, TX growing on an alkaline soil. Leaf analysis results in 2007 indicated that Ni was apparently low (0.06 to 1.71 $\mu g\ g^{-1}$). Foliar application of Ni may enhance growth and production at this location.

The second orchard is Tim Montz's orchard located in Charlie, TX, where Smith et al. (2007) noticed kernel necrosis, a malady characterized by necrotic tissue at the basal end of the kernel that was prevalently on 'Pawnee' in this orchard, but rare or

nonexistent on ‘Pawnee’ at other locations. This orchard derives an unusually high concentration of N (34 mg $L^{-1}$ water applied) from nitrate contaminated irrigation water (Smith et al., 2007). It was hypothesized that excess N may be exacerbating kernel necrosis. In addition, concentrating N can reduce soil pH and occasionally phosphorus (P), potassium (K) and calcium (Ca) in soil (Worley, 1997). High N induced copper (Cu) deficiency by inhibiting the transport of Cu in plants under low Cu levels (Gilbert, 1951). Due to this reason, leaf Cu concentration was affected more by N than Cu supply. Another study (Chaudhry and Loneragan, 1970) suggested that N can accentuate deficiency symptoms in crops if grown under marginal zinc (Zn) or Cu supplies by inducing vigorous plant growth and diluting the concentrations of Cu and Zn in shoots and roots. Foliar application of Ni and Cu may be beneficial for this orchard to mitigate the negative effects of excess N by enhancing catabolism of urea and loss of excess N by ammonia ($NH_3$) volatilization, and increasing N metabolism.

A container study using seedling pecan trees tests Ni foliar applications to mitigate the negative effects of excessive N application. This allows partial duplication of conditions encountered in the field at the second orchard.

## Review of Literature

### Nitrogen Metabolism

An insight into N metabolism is important to crop management because the availability of N is one of the major factors limiting crop growth and yield. Nitrate ($NO_3^-$) and ammonium ($NH_4^+$) are the major available sources of nitrogen for most of the cultivated crops, although urea and amino acids are organic N available to plants. Crop

preference for the form of N uptake varies among species to maintain cation and anion balance (Wirén et al., 1997). However, some species have obligatory preference. Kronzuker et al. (1997) noted 20 times greater uptake of $NH_4^+$ than that of $NO_3^-$ in white spruce [*Picea glauca* (Moench) Voss] seedlings. Arctic sedge (*Eriophorum vaginatum*) preferred free amino acids (Chapin et al., 1993). A mixture of $NO_3$-N and $NH_4$-N was congenial for growth of maize (*Zea mays* L.) plants as compared to either one, though the proportion depended on total N in the growing medium (Xu et al., 1992). At a low concentration of N, a higher proportion of $NH_4$-N provided better growth, but at a high concentration $NO_3$-N was favored by maize.

Several studies have shown that all N, whether derived from nitrate, nitrogen fixation or ammonium ions, in a plant is converted to $NH_3$ before incorporating into organic compounds via several metabolic pathways beginning with glutamine synthetase (GS) enzyme activity (Imsande and Touraine, 1994; Tischner, 2000). Ureide transporter species like pecans transport either allantoin (ALN) and allantoic acid (ALC) or citrulline (CIT). Citrulline is normally synthesized from ornithine (ORN) and carbamoyl phosphate. Ureides (ALN and ALC) could be formed by the condensation of urea and glyoxylate or by the aerobic breakdown of purines (Reinbothe and Mothes, 1962). Purines may arise by the turnover of nucleic acids (DNA and RNA) or by *de novo* synthesis (Schubert, 1986).

## Effects of Ni and Cu on N Metabolism

### Nickel and N Metabolism

The mobilization and conversion of reserve N is critical during early spring as trees begin active growth and such conversion of N reserves to translocatable forms (amides, amino acids, ureides) is affected by Ni shortage. Nickel plays a great role in the metalloenzyme urease (Gerendás and Sattelmacher, 1997b; Gerendás et al., 1998b) which catalyzes the hydrolysis of urea to two moles of $NH_3$ and one mole of $CO_2$ (Reinbothe and Mothes, 1962; Witte et al., 2002). Thus, activation of urease to convert urea to ammonia requires $Ni^{2+}$ (Polacco, 1977). A lack of urease activation makes plants metabolically N deficient (Gerendás and Sattelmacher, 1997a). A reduction in overall N economy (arginine, precursor of urea) was detected in Ni deficient rice (*Oryza sativa* L.) plant (Gerendás et al., 1998b). Though growth of plants with ammonium nitrate ($NH_4NO_3$) was not affected by Ni supply, urease activity was significantly reduced irrespective of N source ($NH_4NO_3$ or urea) in plants grown without supplementary Ni (Gerendás and Sattelmacher, 1999). Leaf urease activity was suppressed in certain plant species [rye (*Secale cereale* M. Bieb.), wheat (*Triticum aestivum* L.), soybean, rape (*Brasscia napus* L.), zucchini (*Cucurbita pepo* L.), and sunflower (*Helianthus annuus* L.)] grown on urea- based nutrient media without supplementary Ni thereby causing accumulation of urea and reduction in dry matter and total N (Gerendás and Sattelmacher, 1997a). Brown et al. (1987b) noted 15-20 times higher levels of urea in leaf tips of Ni deficient wheat, barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) plants. Nickel deficiency symptoms were observed in apical tips of leaves, leaflets and

catkins due to the accumulation of urea in rapidly growing cells and tissues (Wood et al., 2006).

Though plant growth was not affected by Ni and cobalt (Co) supplies, urease activity was non-detectable in Co additions (Gerendás et al., 1998a). These results confirm that urease activation and recycling of endogenous urea requires Ni. Synergetic effect was observed between Ni and N (Palacios et al., 1998). Owing to the close relation between N metabolism and Ni, plants absorb N as different N forms and N form has a significant role in the behavior of Ni toxicity. With only $NO_3$-N, Ni inhibited growth but with simultaneous supply of $NO_3^-$ and $NH_4^+$, Ni stimulated growth (Zornoza et al., 1999).

Nickel affected N metabolism via disruption of ureide catabolism, amino acid metabolism and ornithine cycle intermediates, and also affected the respiration process via disruption of citric acid cycle (Bai et al., 2006). Nickel deficiency affected N metabolism, concentrations of malate and various inorganic anions in roots, shoots, and grains of barley (Brown et al., 1990). Nickel deficiency substantially increased citrulline and allantoic acid (ureido-N forms) and reduced asparagine (amide-N form), xanthine and ß-phenylethylamine (amino-N froms) concentrations in xylem sap (Bai et al., 2007). These results further support the hypothesis that Ni deficiency disrupts normal N-cycling via disruption of ureide metabolism.

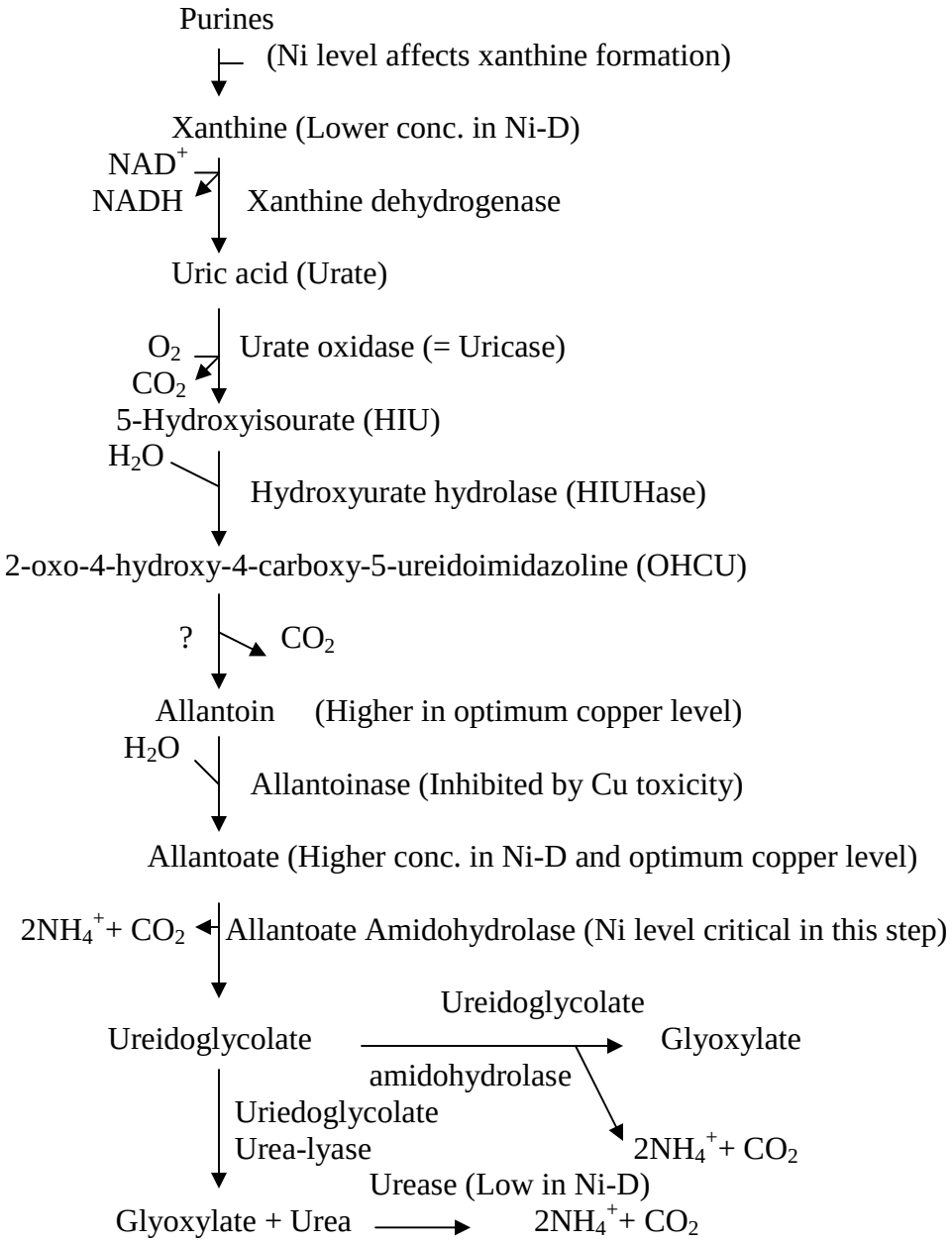


Fig. 1. The role of Ni in ureide catabolism (Bai et al., 2007; Buchanan et al., 2000; Marschner, 1995; Reddy et al., 1995).

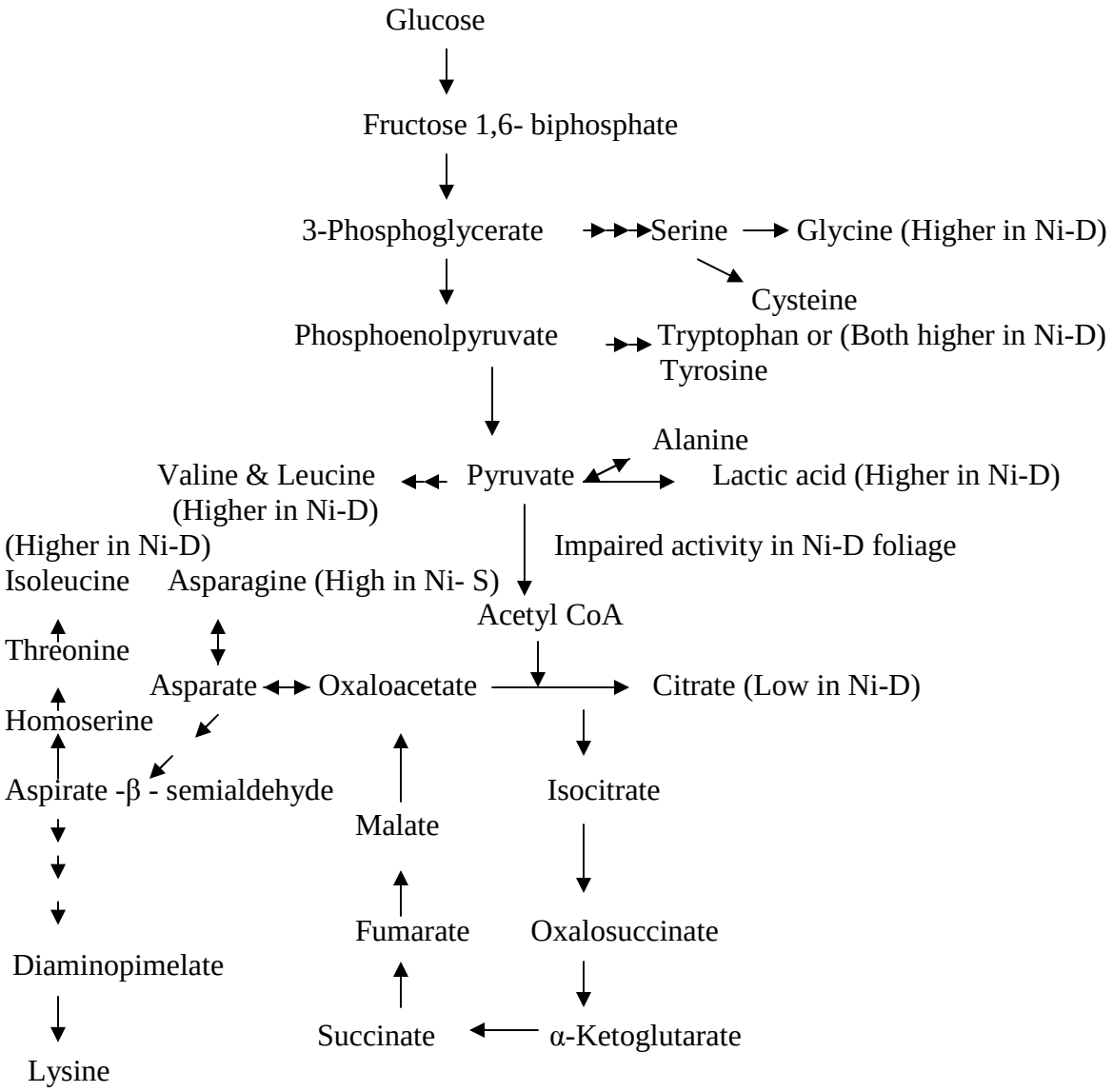


Fig. 2. The role of Ni on amino acid biosynthesis and organic acid metabolism (Bai et al., 2006; Mckee and Mckee, 1996).

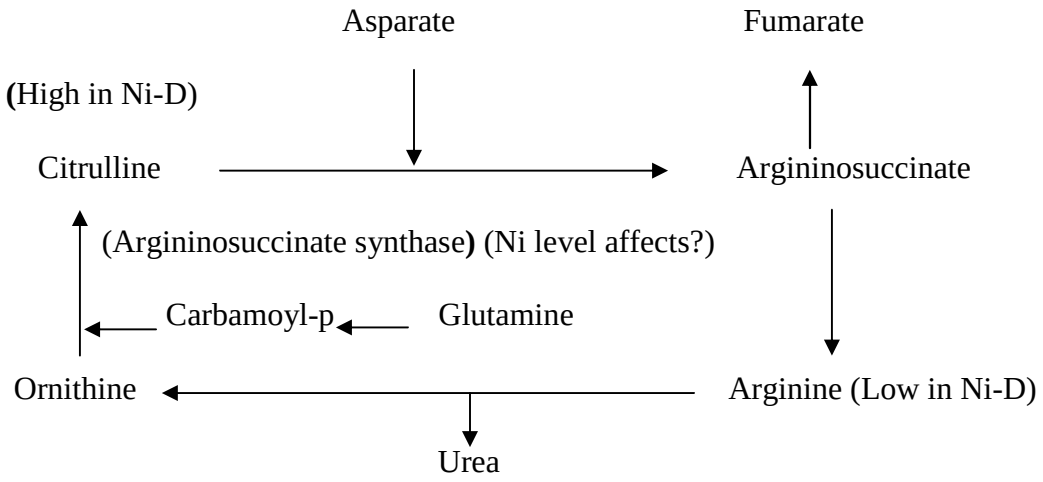


Fig. 3. The synthesis of ornithine, citrulline and arginine (Dennis et al., 1997).

## Copper and N Metabolism

Copper deficiency does not directly affect N metabolism or protein synthesis, but N supply to the plant influences Cu metabolism possibly through inactivation of Cu ions by the increased protein content (Gilbert, 1951). Xiong et al. (2006) reported an adverse effect of a high concentration of Cu on N metabolism and plant growth. They found that excess Cu increased free amino acid concentrations in the leaves while decreasing nitrate reductase (NR) activity in shoots and roots, and total chlorophyll content in the leaves. A reduction in NR activity, which is required for the assimilation of the main nitrogen source, nitrate, was observed with excess Cu (Llorens et al., 2000). Primary N metabolism is reduced in leaves to a lesser extent than in roots (Llorens et al., 2000).

There was a significant correlation between N and Cu concentrations in corn and bromegruss (*Bromus inermis* Leyess) (Soon et al., 1980). However, Kumar et al. (1990) noted a mutual antagonistic effect of N and Cu affecting plant concentrations. Available soil-N after harvest and N-level in plants significantly decreased with increasing Cu

concentrations and ammonium sources had greater antagonism than nitrate compounds (Kumar et al., 1990).

Copper deficient plants had elevated levels of amino acids as compared with control, and the root and shoot Cu concentrations decreased in the order $NO_3$>$NH_4NO_3$>$NH_4$ regardless of solution Cu concentration (Tills and Alloway, 1981). Growth and development of plants receiving higher levels of N without Cu was adversely affected (Cheshire et al., 1982). They noted more amino acids in Cu-deficient tissues and the reduction of amino acid with addition of Cu. Exposure of high Cu (20 mg $L^{-1}$) to rooting media induced synthesis of certain amino acids (glutamine, histidine, asparagine, nicotianamine) having high stability constants with Cu (Liao et al., 2000).

Urease activity in soil was completely inhibited by 120 mg Cu $Kg^{-1}$ (Nor, 1982). Allantoin and allantoic acid, dry weight, and total N content were increased up to certain level of Cu concentration due to increase in nirogenase activity and then decreased at higher concentration due to the inhibition of allantoinase (EC 3.5.2.5) (which converts allantoin to allantoic acid) activity and nitrogenase activity (Reddy et al., 1995). Copper ions, which catalyze glycine-glyoxylate non-enzymatic transamination, enhanced glycine, the immediate precursor of allantoin biosynthesis, production (Fleming and Crosbie, 1960; Metzler et al., 1954). Higher $Cu^{2+}$ reversed/inhibited the transamination in the presence of pyridoxal phosphate which could remove $Cu^{2+}$ from solution (Fleming and Crosbie, 1960).

## Crop Response to Ni and Cu

### Crop Response to Ni

Low Ni- plants had less dry matter production with a chlorotic appearance which is characteristic of a metabolic N deficiency due to the lack of urease activation (Gerendás and Sattlemacher, 1997b). Grains of barley containing less than 30 nanograms of Ni per gram dry weight were not viable due to the disruption of plants' normal grain filling and maturation processes (Brown et al., 1987a). It is also noted that barley plants could not complete their life cycle in the absence of Ni and the addition of Ni to the growing media fully alleviated the maternal plant's deficiency symptoms.

Necrosis developed at the leaflet tips of soybean grown in a Ni deficient nutrient solution, while no necrosis was observed in plants grown from seeds containing 160 nanograms Ni (Eskew et al., 1984). Cowpeas grown in nutrient solutions without Ni accumulated urea (up to 3.1% dry weight in necrotic leaf tips) in most of the tissues (Walker et al., 1985), and about 1% of total N was urea and tissue Ni levels ranged from less than 0.01 to 0.15 µg. Nickel deprivation in soybean plants resulted in accumulation of toxic concentrations of urea (2.5%) in necrotic lesions on leaflet tips, delayed nodulation and reduction of early growth. Addition of Ni at 1 µg $L^{-1}$ to the nutrient media prevented urea accumulation (Eskew et al., 1983).

Plant species, pedological factors and soil pH affect Ni uptake by plant roots. Absorption of $Ni^{2+}$, magnesium ($Mg^{2+}$), iron ($Fe^{2+}$), $Mn^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ appears to be competitive, thus an excess of one can result in a shortage of the other with the lowest availability (Palacios et al., 1998; Wood et al., 2004). Excess Ni induced Fe deficiency by

inhibiting Fe translocation (Aller et al., 1990). Palacios et al. (1998) recorded synergetic effect between N and Ni, positive interaction between Ni level and K uptake, and antagonistic interaction between sodium (Na) and K. Nickel deficient oat plants matured early (15 days ahead) and had lower root and shoot weights (Brown et al., 1987b). Increasing Ni in the nutrient solution reduced translocation of Cu and Fe from roots to tops but the translocation of Mn and Zn was unaffected (Rahman et al., 2005).

## Crop Response to Cu

The dry matter yields of wheat shoot and root increased with up to 5 µg $g^{-1}$ of Cu but decreased at higher Cu levels (Kumar et al., 1990). A higher concentration of Cu adversely affects plant growth characterized by fewer leaves, lesser chlorophyll content and shorter roots (Xiong et al., 2006). Copper uptake reduced the chlorophyll content and induced oxidative stress by increasing lipid peroxidation and leakage of ions (Rama Devi and Prasad, 1998). Copper mainly affected the synthesis or degradation of chlorophyll 'a' within 12 hours of application and $Cu^{2+}$ concentration was dependent on the decline in protein, DNA and RNA content (Gupta, 1986).

There were differences between roots and leaves in response to Cu exposure (Llorens et al., 2000). Root length and biomass of maize decreased with increasing Cu in nutrient medium and Cu concentration was more in roots than the shoots (Ouzounidou et al., 1995). Excess Cu substantially reduced root Ca and Fe, and caused extensive damage to root epidermal cells. Copper concentration of 5 µM in the nutrient solution inhibited root growth completely (Arduini et al., 1995). Higher concentrations of Cu reduced dry

weight (Reddy et al., 1995). Root and shoot growth, and protein and chlorophyll contents in barley were reduced by high Cu concentrations (Stiborova et al., 1986). A reduction of ribulose-1, 5-biphosphate carboxylase activity by heavy metal ions might be the main effect of heavy metal ions on photosynthesis (Stiborova et al., 1986).

Excess Cu inhibited a large number of enzymes, respiration and N fixation processes, vegetative growth, and also disturbed several aspects of plant biochemistry and plant physiology including photosynthesis, fatty acid and protein metabolism, and induced senescence as well (Fernandes and Henriques, 1991). Excessive $Cu^{2+}$ levels induced dramatic changes in plant cell plasmalemma permeability and caused ionic imbalance, loss of turgor and breakdown of cell metabolism (Demidchick et al., 1997).

Time of exposure, biological availability of the metals and interactions with other metals in the soil, nutritional status and age of plants were the factors influencing the degree of toxicity (Påhlsson, 1989). The concentrations of 100 to 200 $\mu g\ L^{-1}$ Cu disturbed metabolic process and growth of forest plant species (Påhlsson, 1989). Copper treatment (10 $\mu g\ L^{-1}$) induced proline accumulation and inhibited chlorophyll, and the effect of Cu was more than that of Zn on both proline accumulation and chlorophyll inhibition (Bassi and Sharma, 1993). Copper treatment induced Fe deficiency and consequently reduced the leaf chlorophyll concentration (Pätsikkä et al., 2002).

## Objectives

The objectives of these studies were to 1) evaluate the interaction between N rate and foliar Ni application on growth of container-grown pecan seedlings, 2) determine the

response of bearing pecan trees to foliar Ni application, and 3) assess the impact of foliar Ni and Cu treatments to mitigate the problems associated with excess N.

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CHAPTER II

# Is Supplemental Nickel Beneficial for Container-grown Pecans Receiving Varying Nitrogen Rates?

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ABSTRACT. Dormant 1-year-old pecan [*Carya illinoinensis* (Wang.) K. Koch] trees grown in containers from 'Peruque' seed were transferred from cold temperatures (cooler) to satisfy their chilling requirement to an outside container production bed. Treatments were initiated when the seedlings had three to four expanded leaves (a month after transfer to the production bed). Nitrogen (N) was applied to growth media each irrigation at 0, 50, 100 and 200 µg $ml^{-1}$ N from a 28% N solution. Nickel (Ni) was applied at 0 and 0.32 ml $L^{-1}$ $NiSO_4.6H_2O$ (0.023 g $L^{-1}$ Ni) to the foliage until runoff at two week intervals. Trees were harvested three months after transfer to the production bed when terminal buds were shed. Seedling height, leaf, trunk and root dry weights, total tree leaf area, and specific

leaf weight were not affected by treatment. Leaf phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), and manganese (Mn) concentrations were unaffected by Ni and N treatments. Nitrogen treatment linearly reduced leaf iron (Fe) concentration probably caused by proton ($H^+$) driven high nitrate ($NO_3^-$) uptake rates that blocked Fe uptake into cells. Zinc sulfate as a foliar spray was applied as a standard management practice to all seedlings at two week intervals. A reduction in leaf zinc (Zn) concentration by Ni treatment suggests the competitive absorption of these cations. Nitrogen and Ni treatments increased leaf N and Ni concentrations, respectively. Average leaf Ni concentration was 2.99 μg $g^{-1}$ dry weight in control trees and 12.99 μg $g^{-1}$ dry weight in treated trees. The lack of an N and Ni interaction on growth responses of seedling pecan trees indicates that a leaf Ni concentration of 2.99 μg $g^{-1}$ dry weight satisfies the tree's requirement and N metabolism was unaffected.

**Keywords:** *Carya illinoinensis,*urea, nitrogen metabolism, ureide catabolism, leaf elemental concentration.

Nitrogen is a major plant nutrient affecting crop growth and yield. Pecans predominately transport N as ureides (allantoin) (Bai et al., 2007). Ureide catabolism eventually results in breakdown into urea and glyoxylate. Urea N is not available for N metabolism unless converted to ammonia ($NH_3$) (Marschner, 1995). All N in a plant is converted to $NH_3$ before incorporation into organic compounds via several metabolic pathways beginning with glutamine synthetase (GS) enzyme activity (Imsande and Touraine, 1994; Tischner, 2000). Urease catalyzes the hydrolysis of urea to $NH_3$. Activation of urease to convert urea to $NH_3$ requires $Ni^{2+}$ (Polacco, 1977). A lack of urease activation makes plant metabolically N deficient (Gerendás and Sattelmacher,

1997a). Gerendás et al. (1998b) noted the reduced levels of N economy (arginine, precursor of urea) in Ni deficient rice (*Oryza sativa* L.) plants. Nickel deficiency in soybean [*Glycine max* (L.) Merr.] and cowpea (*Vigna unguiculata* L.) caused accumulation of urea in the foliage due to impaired urease activity (Eskew et al., 1983, 1984; Walker et al., 1985).

The plant's requirement for Ni is low and most soils have sufficient Ni to meet the plant's requirement. Seed reserves of Ni are sufficient for some annual plants to complete their life cycle (Eskew et al., 1983). Being a ureide-N transporting crops, however, pecans have higher Ni requirement than most crops (Wood et al., 2006) since enzymes involved in ureide catabolism require Ni for activation (Fig. 4). Nickel affected N metabolism via disruption of ureide catabolism, amino acid metabolism and ornithine cycle intermediates, and also affected the respiration process via disruption of citric acid cycle (Bai et al., 2006). Increasing Ni concentrations significantly increased N content, i.e. synergetic effect between N and Ni (Palacios et al., 1998). Thus, Ni is required for urease (EC 3.5.1.5, urea amidohydrolase) activation (Dixon et al., 1975), and appears to function in additional enzymes affecting N metabolism in pecan (Bai, et al., 2006, 2007).

This study evaluates the interaction between N rate and foliar Ni application on growth of container-grown pecan seedlings and tests Ni foliar applications to mitigate the negative effects of excess N by enhancing catabolism of urea and loss of excess N by $NH_3$ volatilization, and increasing N metabolism.

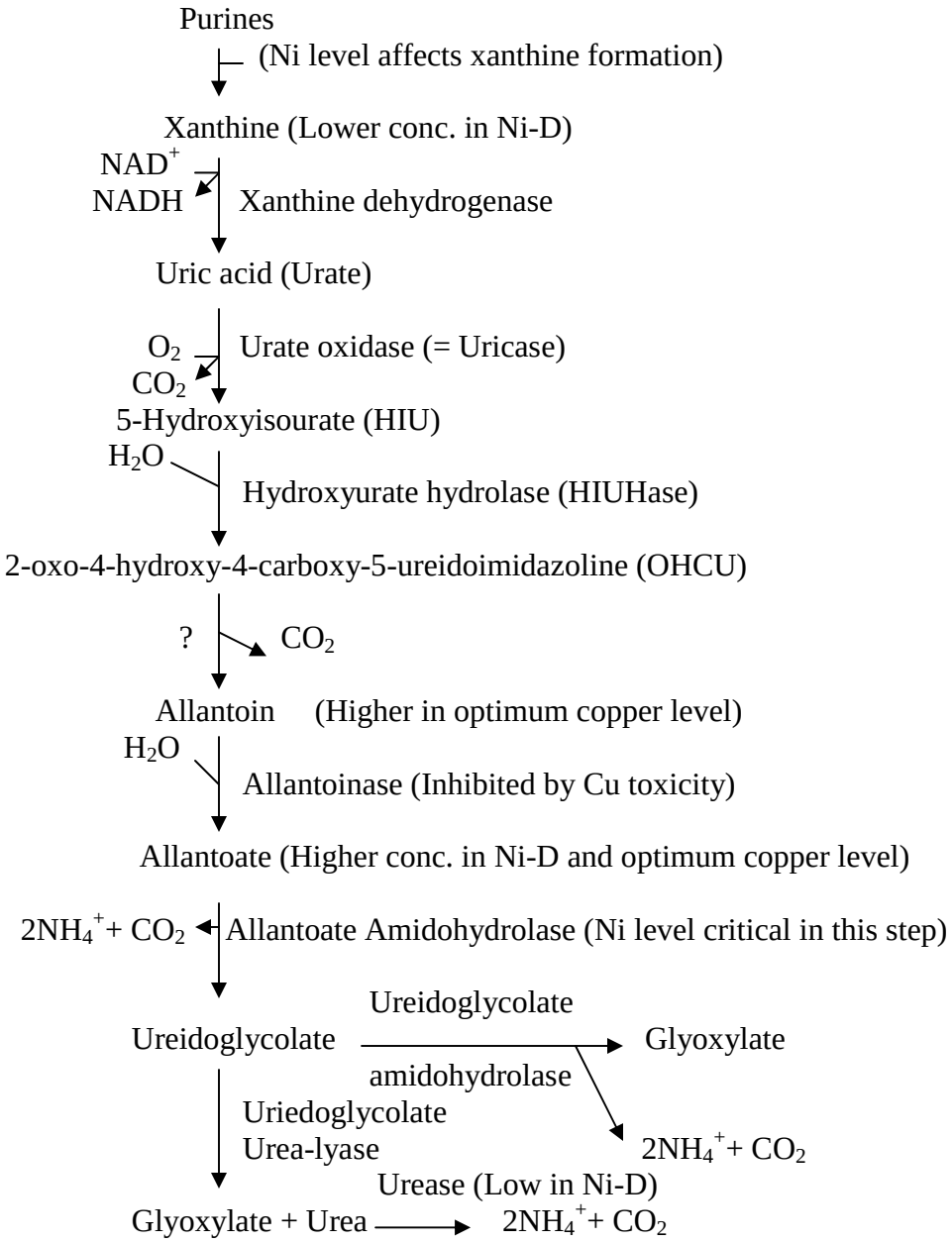


Fig. 4. The role of Ni in ureide catabolism (Bai et al., 2007; Buchanan et al., 2000; Marschner, 1995; Reddy et al., 1995).

## Materials and Methods

The experiment was conducted at Cimarron Valley Research Station, Perkins, OKlahoma. Dormant 1-year-old pecan trees grown in 10 x 10 x 25 cm containers from 'Peruque' seed were transferred from cold storage to satisfy their chilling requirement to an outside container production bed on 15 April 2008. Trees were pruned before overwintering in cooler and when transferred to the production bed in April the trunk was pruned to 7.5 cm above soil line. The growth media was Metro Mix 300 containing vermiculite, bark, peat moss, perlite, processed bark ash, starter nutrient charge, dolomitic limestone and a wetting agent. Trees were irrigated with rural water until treatment applications began.

Treatments were arranged in a split plot design with four N rates (0, 50, 100 and 200 µg $ml^{-1}$ N from a 28% N solution) as the main plot, and two levels of Ni [0 and 0.32 ml $L^{-1}$ $NiSO_4.6H_2O$ (0.023 g $L^{-1}$ Ni)] as the subplot with two sub samples per treatment combination and ten replications. The first treatment was applied when the seedlings had three to four expanded leaves (a month after transfer to the production bed). Nitrogen was applied to growth media each irrigation, except at approximately two week intervals plain water irrigation was utilized to leach excess salts from the media. Nickel was applied to the foliage until runoff at two week intervals. Surfking Plus (surfactant with buffering agent) was mixed with Ni at concentration of 0.62 ml $L^{-1}$ water during each treatment. Five Ni applications were made in total. Foliar Zn applications were made from 36% $ZnSO_4$ at 1.8 g $L^{-1}$ (0.65 g $L^{-1}$ Zn) at two week intervals (on same days of Ni treatment) as a standard management practice to all seedlings and single application of Peters soluble trace element mix (Scotts-Sierra Horticultural Products Company Marysville,

OH) at 0.15 g $L^{-1}$ water was applied to growing media during growing period. All treatments were applied until mid July when additional trunk and shoot expansion ceased.

Trees were harvested 3 months after transfer to the production bed (July 16) when terminal buds were shed. All leaves were collected for determination of leaf area, dry weight, and nutritional concentration. Leaf area was measured using Li-Cor model 3100 area meter. Leaves were washed in tap water followed by 0.1 N HCl, soapy (P-free detergent) distilled water and then two deionized water rinses. Trunk growth was measured after harvesting the leaves. Trunks were cut at soil line and roots were collected for measuring dry weight and Ni concentration. Leaves, trunks and roots were dried at $70^{o}$ C, and then their weights were recorded. Leaves, trunks and roots were ground to pass a 20 mesh (850 µm) screen and stored in air tight glass jars until analysis. Leaf elemental concentrations of N were determined using a modification of the Dumas method with a Leco N analyzer (St. Joseph, MI). Phosphorous was determined colorimetrically. Potassium, Ca, Mg, Cu, Fe, Zn and Mn were analyzed using atomic absorption spectroscopy (Perkin Elmer model 2380, Waltham, MA). Nickel was analyzed using an inductively coupled plasma emission spectrometry.

Data were analyzed using a mixed model for analysis of variance in SAS software. Treatment means were compared to the control using the protected $LSD_{0.05}$.

## Results and Discussion

Seedling height, leaf, trunk and root dry weights, total tree leaf area, and specific leaf weight (the ratio of leaf dry weight to leaf area) were not affected by Ni and N treatments (Tables 1, 2). Though Ni is an essential nutrient (Marschner, 1995), substantial growth responses of Ni may not be seen with plants getting mineral N due to the low Ni requirement of plants. There was no effect of Ni on growth of zucchini (*Cucurbita pepo* convar. *giromontiina*) plants (Gerendás et al., 1998a). In another study, Ni treatment did not affect dry matter production of zucchini plants receiving $NH_4NO_3$ (Gerendás and Sattelmacher, 1997a). Sparks and Baker (1975) observed toxic effects of N on growth of pecan seedlings receiving 180 μg $ml^{-1}$ N, but no toxic effects were found in seedlings receiving 200 μg $ml^{-1}$ N in this study. Tree Ni level may be a possible reason behind it.

Studies have shown different results regarding tissue mineral contents altered by Ni treatment. Gerendás and Sattelmatcher (1997b) reported that Ni enhanced plant growth and reduced the concentration of macronutrients by dilution, but the concentrations of Fe, Mn, Cu, and Zn were not affected. Leaf N, P, K, Ca, Mg, Fe, Cu, Mn, cobalt (Co), and molybdenum (Mo) were unaffected by Ni treatment in this study (Table 3). Nickel treatment increased leaf Ni (Table 3), trunk and root Ni concentrations (Table 4). Nickel treatment reduced leaf Zn concentration (Table 3). Foliar applications of Zn sulfate were applied as a standard management practice to all seedlings at two week intervals. A reduction in leaf Zn concentration by Ni treatment indicates the competitive absorption of these divalent cations. Absorption of $Ni^{2+}$, $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ appears to be competitive (Palacios et al., 1998; Wood et al., 2004).

Nitrogen treatment did not affect leaf P, K, Ca, Mg, Zn, Mn, Cu, and Ni (Table 5). Nitrogen treatment increased leaf N concentration (the linear, quadratic, and cubic trends were significant at 0.0001, 0.0001, and 0.05 respectively) (Fig. 5). There was no N and Ni interaction on leaf N and Ni concentrations (data not shown). Leaf Zn concentration was affected by N and Ni interaction (Table 6). Nitrogen treatment linearly suppressed leaf Fe concentrations (Fig. 6), probably caused by $H^+$ driven high $NO_3^-$ uptake rates that blocked Fe uptake into cells. Nitrogen treatment linearly reduced leaf Co concentration (Fig. 7) but root and trunk Co concentrations were not affected (Table 7). This suggests the inhibition of Co translocation to shoots. Nitrogen treatment reduced leaf Mo (Fig. 8) and root Mo (Table 7) concentrations probably by acidification of rhizosphere and reduced uptake of Mo but trunk Mo was unaffected.

Plant tissue concentration of less than 0.09-0.1 μg $g^{-1}$ dry weight is considered Ni-deficient in barley (*Hordeum vulgare* L.) (Brown et al, 1987a, b). Eskew et al. (1984) noted a much lower critical Ni level in soybean (*Glycine max* L.) tissue of 0.002–0.004 μg $g^{-1}$ dry weight. The critical level of Ni is below 0.025 μg $g^{-1}$ dry weight for rape (*Brassica napus* L.) receiving mineral N (Gerendás and Sattelmatcher, 1999). Growth of Zucchini plants was not impaired at Ni level of as low as 0.025 μg $g^{-1}$ dry weight (Gerendás and Sattelmatcher, 1997a). These findings show that not all plants have the same Ni requirement. Pecans requirement for Ni is high. Wood (2007) suggested that tissue levels of 3-5 μg $g^{-1}$ Ni can satisfy pecan trees Ni requirement. Average leaf Ni concentration was 2.99 μg $g^{-1}$ in control trees and 12.99 μg $g^{-1}$ dry weight in treated trees in this study.

The lack of an interaction between N and Ni treatments on growth responses of seedling pecan trees suggests that the sufficiency level of Ni for pecan seedlings is below 2.99 μg $g^{-1}$ dry weight. No synergetic effect of Ni on N concentration indicates that leaf Ni concentration of 2.99 μg $g^{-1}$ in the control was sufficient for N metabolism, and synergetic effect is not evident once tree Ni nutritional status is sufficient for N metabolism.

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Table 1. The influence of foliar Ni application [$NiSO_4 \cdot 6H_2O$ at 0 and 0.32 ml $L^{-1}$ (0.023 g $L^{-1}$ Ni)] on growth responses of seedling pecan trees.

| Treatment | Tree height (cm) | Trunk dry wt (g) | Root dry wt (g) | Leaf dry wt (g) | Leaf surface area ($cm^2$) | Specific leaf wt ($mg\ m^{-2}$) |
|---|---|---|---|---|---|---|
| None | 25.4[z] | 2.9 | 6 | 5.7 | 1007 | 572 |
| Ni | 26.2 | 2.9 | 5.3 | 6.3 | 1104 | 567 |

[z] Nickel treatments were not significantly different from the control for any parameter listed above.

Table 2. The influence of N application rates from a 28% N solution on growth responses of seedling pecan trees.

| N rate ($\mu g\ ml^{-1}$) | Tree height (cm) | Trunk dry wt (g) | Root dry wt (g) | Leaf dry wt (g) | Leaf surface area ($cm^2$) | Specific leaf wt ($mg\ m^{-2}$) |
|---|---|---|---|---|---|---|
| 0 | 25.5[z] | 3 | 6.7 | 5.8 | 1013 | 594 |
| 50 | 23.5 | 2.5 | 5 | 5.5 | 1017 | 515 |
| 100 | 26 | 2.8 | 5.2 | 5.4 | 933 | 594 |
| 200 | 28 | 3.2 | 5.6 | 7.3 | 1258 | 574 |

[z] Nitrogen treatments were not significantly different from the control for any parameter listed above.

Table 3. The influence of foliar Ni application [$NiSO_4 \cdot 6H_2O$ at 0 and 0.32 ml $L^{-1}$ (0.023 g $L^{-1}$ Ni)] on the leaf elemental concentrations of seedling pecan trees in July.

| | Dry wt (%) | | | | | Dry wt (μg $g^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | N | P | K | Ca | Mg | Fe | Mn | Cu | Zn | Co | Mo | Ni |
| None | 2.56 | 0.28 | 1.35 | 1.10 | 0.57 | 89 | 285 | 3.13 | 447 | 0.28 | 0.32 | 2.99 |
| Ni | 2.52 | 0.28 | 1.30 | 1.15 | 0.57 | 89 | 280 | 3.37 | 355 | 0.25 | 0.32 | 12.99 |
| Significance | NS | NS | NS | NS | NS | NS | NS | NS | * | NS | NS | * |

NS, * Means within columns are nonsignificant (NS) or significant at $P \leq 0.05$ (*).

Table 4. The influence of foliar Ni application [$NiSO_4 \cdot 6H_2O$ at 0 and 0.32 ml $L^{-1}$ (0.023 g $L^{-1}$ Ni)] on the trunk and root elemental concentrations of seedling pecan trees in July.

| | Trunk concn (μg $g^{-1}$ dw) | | | | Root concn ( μg $g^{-1}$ dw) | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Ni | Cu | Co | Mo | Ni | Cu | Co | Mo |
| None | 2.53 | 2.49 | 1.17 | 0.18 | 2.51 | 3.66 | 0.65 | 0.31 |
| Ni | 9.54 | 2.67 | 1.30 | 0.16 | 5.02 | 3.61 | 0.56 | 0.28 |
| Significance | * | NS | NS | NS | * | NS | NS | NS |

NS, * Means within columns are nonsignificant (NS) or significant at $P \leq 0.05$ (*).

Table 5. The influence of N application rates from a 28% N solution on the leaf elemental concentrations of seedling pecan trees in July.

| N rate | Dry wt (%) | | | | Dry wt (μg $g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| (μg $ml^{-1}$) | P | K | Ca | Mg | Zn | Mn | Cu | Ni |
| 0 | 0.28[z] | 1.37 | 1.20 | 0.58 | 414 | 256 | 3.25 | 8.92 |
| 50 | 0.29 | 1.36 | 1.12 | 0.58 | 387 | 255 | 3.39 | 8.15 |
| 100 | 0.28 | 1.30 | 1.09 | 0.56 | 410 | 250 | 3.13 | 8.27 |
| 200 | 0.28 | 1.28 | 1.08 | 0.56 | 392 | 370 | 3.21 | 6.63 |

[z] Nitrogen treatments were not significantly different from the control for leaf concentrations of any elements listed above.

Table 6. The influence of N and Ni interaction on the leaf Zn concentration of seedling pecan trees in July.

| Ni treatment | N rate (µg $ml^{-1}$) | Zn (µg $g^{-1}$ dw) |
|---|---|---|
| None | 0 | 454cd[z] |
| | 50 | 402bc |
| | 100 | 488d |
| | 200 | 444cd |
| Ni | 0 | 374ab |
| | 50 | 373a |
| | 100 | 331a |
| | 200 | 341a |

[z] Means followed by the same letter are not significantly different by protected LSD, 5% level.

Table 7. The influence of N application rates from a 28% N solution on the trunk and root elemental concentrations of seedling pecan trees in July.

| N rate | Trunk concn (µg $g^{-1}$ dw) | | | | Root concn (µg $g^{-1}$ dw) | | | |
|---|---|---|---|---|---|---|---|---|
| (µg $ml^{-1}$) | Ni | Cu | Co | Mo | Ni | Cu | Co | Mo |
| 0 | 5.46a[z] | 2.26a | 1.13a | 0.16a | 3.22a | 3.14a | 0.64a | 0.34a |
| 50 | 6.48a | 2.76a | 1.52a | 0.16a | 4.12a | 3.93a | 0.64a | 0.31ab |
| 100 | 5.65a | 2.64a | 1.12a | 0.20a | 3.70a | 3.74a | 0.59a | 0.28b |
| 200 | 6.55a | 2.66a | 1.18a | 0.16a | 4.01a | 3.74a | 0.54a | 0.26b |

[z] Means within columns followed by the same letter are not significantly different by protected LSD, 5% level.

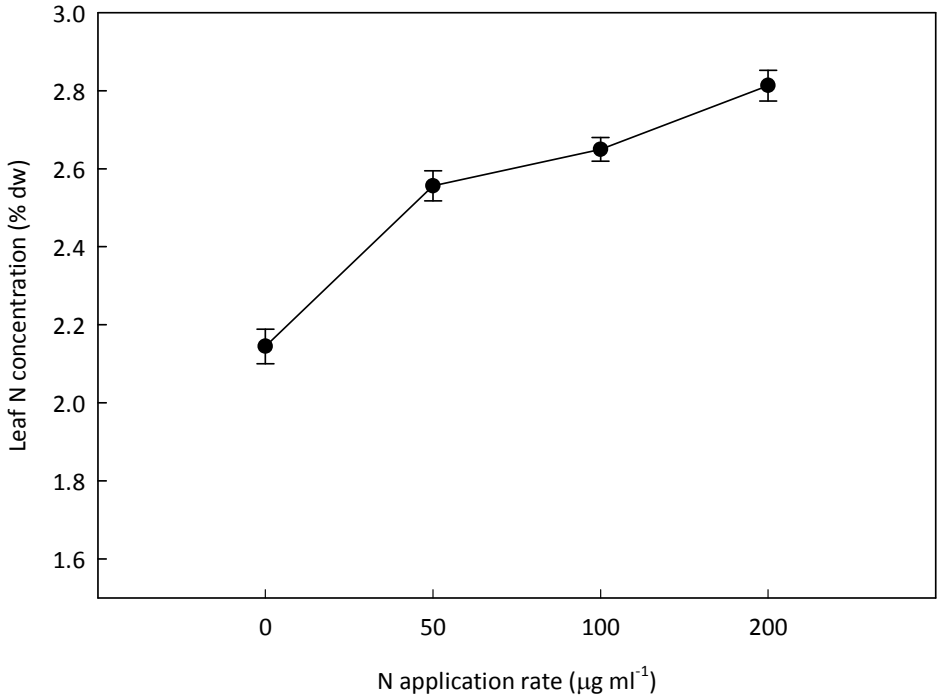


Fig. 5. The influence of N application rates from a 28% N solution on the leaf N concentration of seedling pecan trees in July. Treatments were initiated when the seedlings had three to four expanded leaves and applied to growth media each irrigation for two months until terminal buds were shed and additional trunk and shoot expansion ceased. The linear, quadratic, and cubic trends were significant at 0.0001, 0.0001, and 0.05 respectively. Standard errors of the means are shown.

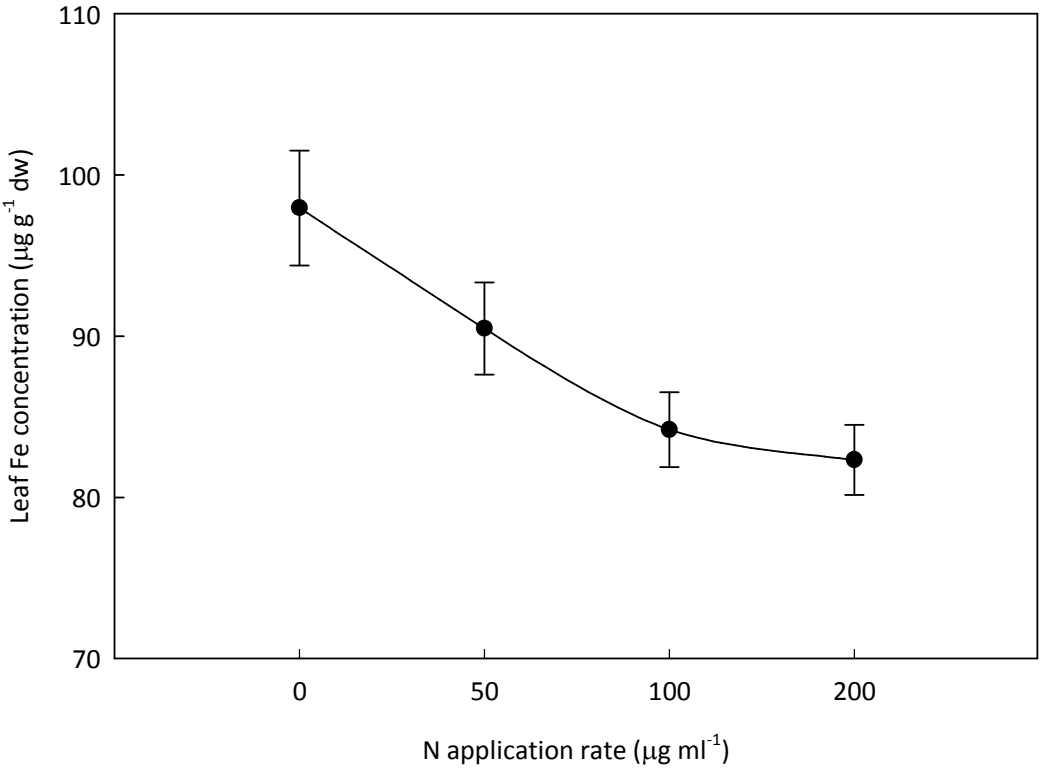


Fig. 6. The influence of N application rates from a 28% N solution on the leaf Fe concentration of seedling pecan trees in July. Treatments were initiated when the seedlings had three to four expanded leaves and applied to growth media each irrigation for two months until terminal buds were shed and additional trunk and shoot expansion ceased. The linear trend was significant at 0.0001. Standard errors of the means are shown.

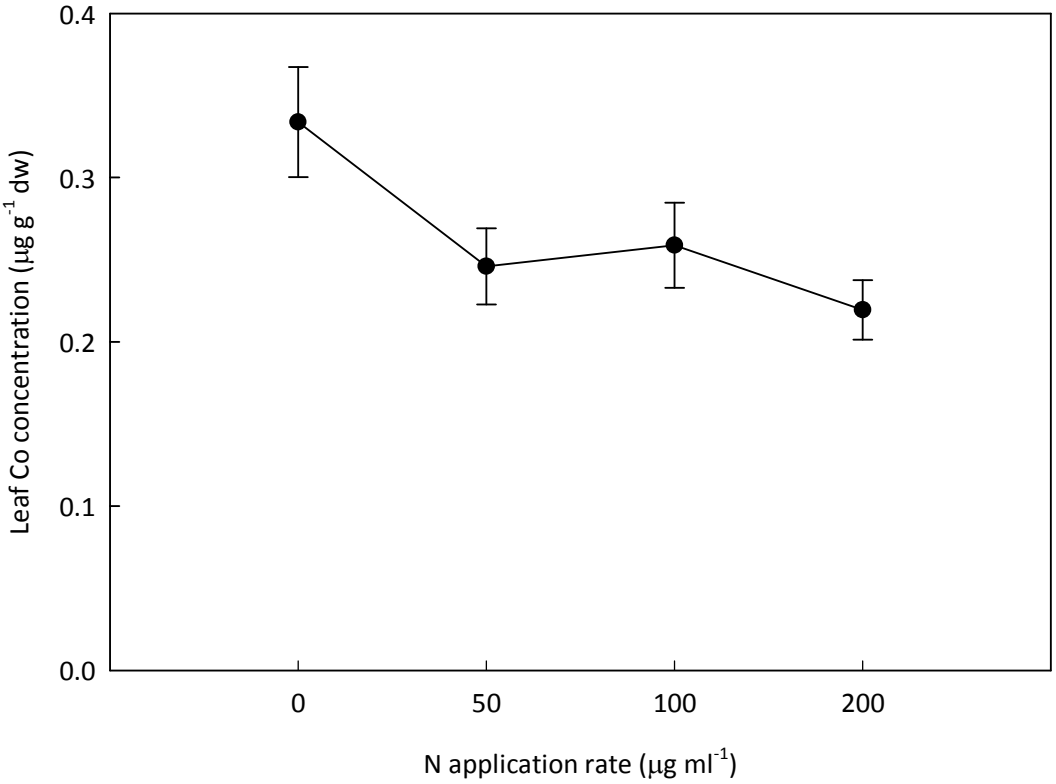


Fig. 7. The influence of N application rates from a 28% N solution on the leaf Co concentration of seedling pecan trees in July. Treatments were initiated when the seedlings had three to four expanded leaves and applied to growth media each irrigation for two months until terminal buds were shed and additional trunk and shoot expansion ceased. The linear trend was significant at 0.01. Standard errors of the means are shown.

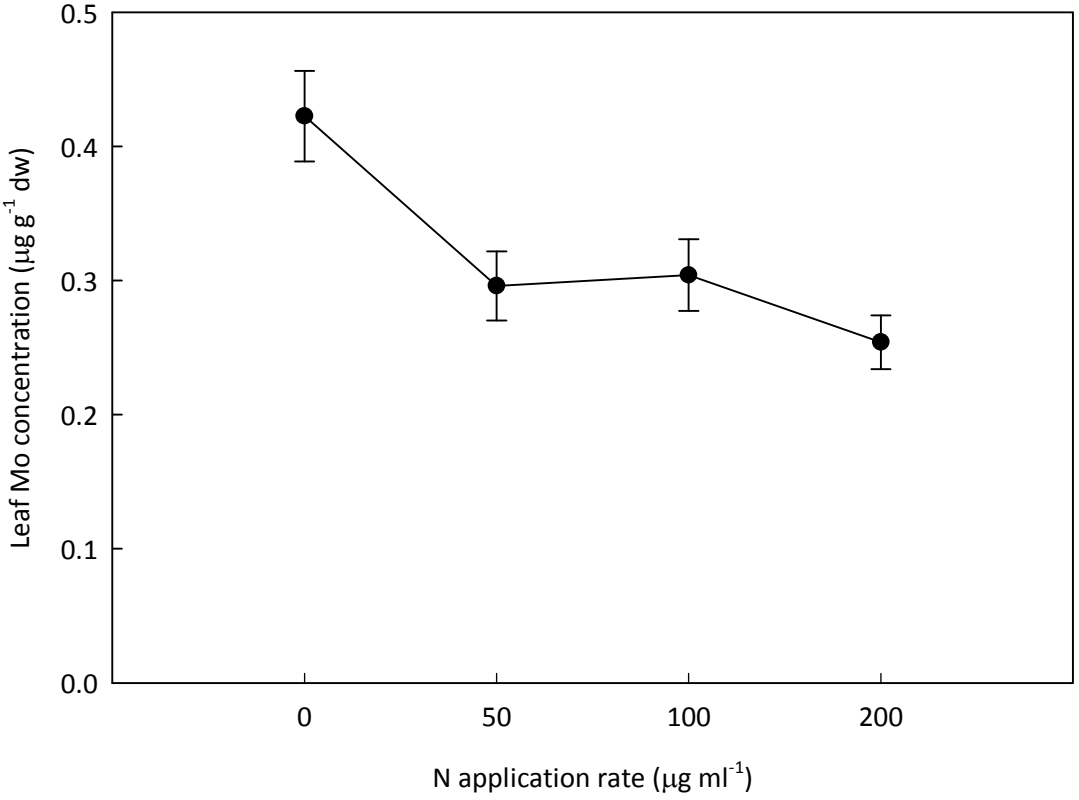


Fig. 8. The influence of N application rates from a 28% N solution on the leaf Mo concentration of seedling pecan trees in July. Treatments were initiated when the seedlings had three to four expanded leaves and applied to growth media each irrigation for two months until terminal buds were shed and additional trunk and shoot expansion ceased. The linear trend was significant at 0.0001. Standard errors of the means are shown.

# CHAPTER III

## Response of Bearing Pecan Trees to Foliar Nickel Application

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ABSTRACT. Pecans [*Carya illinoinensis* (Wang.) K. Koch] transport considerable nitrogen (N) as ureides. Nickel (Ni) shortage is more likely for ureide-N transporting crops since activation of enzymes involved in ureide catabolism requires Ni. Johnny Dowd's orchard located near Nocona, TX was selected for having low leaf Ni concentration compared to other orchards in Oklahoma and north Texas. Foliar Ni application from $NiSO_4.6H_2O$ at 0.78 ml $L^{-1}$ [0.055 g $L^{-1}$ Ni (82.3 g $ha^{-1}$)] was applied at 1496 L $ha^{-1}$ water to the trees at the parachute stage of leaf development followed by two additional applications at two week intervals. Yield, yield efficiency (the ratio of yield to cross sectional trunk area), trunk growth, leaf area, leaf dry weight, and specific leaf weight were

not influenced by treatment. Total yield and average yield efficiency over two years were unaffected. Treatment did not affect nut weight, nut quality, kernel percentage, kernel necrosis (a malady characterized by necrotic tissue at the basal end of the kernel), and opalescence (leakage of oil bodies into intercellular spaces in kernel). Leaf N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) were not altered by Ni treatment. Nickel increased leaf Ni concentration and reduced leaf zinc (Zn) and manganese (Mn) concentrations. Zinc sulfate and manganese sulfate were foliarly applied as a standard management practice to the control and tank mixed with Ni. A reduction in leaf Zn and Mn concentrations by Ni treatment suggests the competitive absorption of these chemically similar divalent cations. The lack of response to Ni treatment on tree growth, nut yield and quality in this study suggests that a leaf Ni concentration in the control (2.87 µg $g^{-1}$) met pecan tree's Ni requirement. Results indicate that additional Ni beyond the critical level was not beneficial.

**Keywords:** *Carya illinoinensis,* parachute stage, yield efficiency, kernel necrosis, opalescence, ureides, Ni critical level, leaf elemental concentration.

The mobilization and conversion of reserve N is critical during early spring when pecan trees begin growing actively. Conversion of reserve N to translocatable forms (amides, amino acids, ureides) is affected by Ni shortage. Pecans are ureide-N transporting crops (Bai et al., 2007). Ureides stored in dormant pecan roots are transported to emerging foliage through ascending xylem sap to be catabolised and assimilated into organic compounds (amino acids, proteins). Pecans transport either allantoin (ALN) and allantoic acid (ALC) or citrulline (CIT).

Ureide catabolism is influenced by plant Ni level since enzymes involved in ureide catabolism require Ni for their activation. Disruption of ureide catabolism caused by Ni deficiency affected several other pathways such as amino acid metabolism and the citric acid cycle (Bai et al., 2006). This disruption of carbon metabolism resulted in toxic accumulations of oxalic and lactic acids and was attributed with causing mouse-ear symptoms (leaves exhibit typical rounded, blunt leaflet tips) in pecan. Ureide catabolism eventually results in breakdown into urea and glyoxylate with $CO_2$ and ammonia as byproducts.

Urease (EC 3.5.1.5, urea amidohydrolase) catalyzes the hydrolysis of urea to ammonia and Ni is required for urease activation (Dixon et al., 1975). Several studies have found an accumulation of urea in the foliage of Ni deficient plants indicating that urease activity was impaired (Eskew et al., 1983, 1984; Walker et al., 1985), resulting in induced metabolic N deficiency (Gerendás and Sattelmacher, 1997).

Wood (2009) reported that high levels of Zn and Cu relative to Ni triggered Ni deficiency in Georgia pecan orchards. The induced Ni deficiency was apparently caused by a competitive interaction among these cations for transport into the cell. Early season chlorotic/necrotic leaf, loss of apical dominance, dwarfed of foliage and internodes, rosetting, curled leaf/leaflet margin, and an enhanced reddish pigmentation of young leaflets are key morphological symptoms of Ni deficiency in pecans (Wood et al., 2006). Pecan trees have higher Ni requirement than most crops but root uptake appears inefficient.

A survey of pecan leaf Ni concentrations in Oklahoma and northern Texas suggested that some orchards in the Red River basin may be low in Ni (data not shown).

Symptoms of Ni deficiency have not been observed in this area, except in one instance (C. Rohla, personal communication). Soil pH in this region is slightly acid to alkaline with most orchards experiencing Zn shortages if left untreated. Three to five foliar applications of Zn sulfate during a growing season is a common standard management practice in this area (pH was 8 in this orchard).

Johnny Dowd's pecan orchard located near Nocona, TX was identified as having a low leaf Ni concentration relative to other orchards in the area. It was selected for a trial using foliar application of Ni. Our objective was to determine if tree growth, production or nut quality could be enhanced by foliar applied Ni.

## Materials and Methods

Eight year old 'Pawnee' trees grafted onto Apache rootstock and spaced 10.7 x 10.7 m in a micro sprinkler irrigated Teller sandy loam soil (fine-loamy, mixed, thermic, Udic Argiustoll) were used for this study. Trees were in their second year of production at the beginning of this study. Uniform trees were selected in April before starting application.

TREATMENTS. Treatments were control (no Ni applied) and Ni treated [$NiSO_4.6H_2O$ at 0.78 ml $L^{-1}$ (0.055 g $L^{-1}$, 82.3 g $ha^{-1}$ Ni)]. Treatments were applied in 1496 L $ha^{-1}$ of water with an orchard sprayer as a foliar application at the parachute stage of leaf development in April followed by two additional applications at two week intervals. Foliar Zn from 36% $ZnSO_4$ at 4.49 g $L^{-1}$ [1.62 g $L^{-1}$ Zn (2.42 kg $ha^{-1}$)], and Mn from 32% $MnSO_4$ at 4.49 g $L^{-1}$ [1.44 g $L^{-1}$ Mn (2.15 kg $ha^{-1}$)] were applied as a standard management practice to the control and was combined with Ni. Surfking Plus (surfactant

with buffering agent) was tank mixed with Ni, Mn and Zn at concentration of 0.62 ml $L^{-1}$ water during each treatment. The experimental design was completely randomized with each treatment replicated eighteen times (three rows of trees and six trees in a single row) so that total trees under study were thirty six.

SOIL SAMPLE. Two soil samples were taken from 0-15 cm and 15-30 cm depths using a standard soil probe to determine baseline nutrient levels at the beginning of study. Soil was composited with at least 10 soil cores and two samples were collected for each depth and submitted to the Soil, Forage and Water Testing Laboratory at Oklahoma State University for analysis of pH, $NO_3$-N, P, K, Fe, Zn, boron (B) and Cu.

TRUNK DIAMETER MEASUREMENT. Trunk diameter was measured at 1.4 m above ground level at the beginning of study in April and each fall.

LEAF SAMPLE

1) Thirty middle leaves from the current season growth per tree were collected a week after the first treatment application for Ni analysis. Leaves were washed in tap water followed by 0.1 N HCl water, soapy (P-free detergent) distilled water and then two deionized water rinses. Leaves were dried at 70 $^{o}$C, ground to pass a 20 mesh (850 µm) screen and stored in air tight glass jars until analysis.
2) Twenty compound leaves from the middle of current season shoot per tree were harvested in July when leaf expansion ceased, and leaf surface area and leaf dry weight were measured. Leaf surface area was measured using Li-Cor model 3100 area meter. Leaf samples were dried at 70 $^{0}$C before weighing.

Specific leaf weight was calculated by dividing the dry weight of leaves by the area of a same portion of leaves.

3) In July, thirty middle leaflet pairs from the middle leaf on current season's growth were collected for nutrient analysis from each tree in the study. Collected leaf samples were washed, dried and ground as described earlier. Leaf elemental concentrations of N were determined using a modification of the Dumas method with a Leco N analyzer (St. Joseph, MI). Phosphorus was determined colorimetrically. Potassium, Ca, Mg, Cu, Fe, Zn, and Mn were analyzed using atomic absorption spectroscopy (Perkin Elmer model 2380, Waltham, MA). Nickel was analyzed using an inductively coupled plasma emission spectrometry.

NUT SAMPLE. Nut samples (2 - 20 nut samples per tree) were taken in October at harvest. Kernel percentage, nut weight, kernel quality, opalescence, and kernel necrosis were determined. Kernel halves were graded on 1 to 4 scales for quality with 1 being a perfectly colored and filled, 2 was not completely filled and/or colored, 3 poorly filled and/or discolored, and 4 for completely discolored and/or not filled. Kernel halves were graded on a scale of 1 to 4 for necrosis; 1 being no necrosis and 4 was severe necrosis (Fig. 9). Opalescence grading was done on 1 to 5 scale in which 1 was no opalescence or less than 20% intercellular spaces covered with leaked oil and 5 was severe opalescence (more than 80% intercellular spaces covered with leaked oil) (Fig. 10). Total yield per tree was determined annually.

DATA ANALYSIS. Data were analyzed using a mixed model for analysis of variance in SAS software. Treatment means were compared to the control using the protected $LSD_{0.05}$.

## Results and Discussion

Nickel treatment increased leaf Ni concentration and decreased leaf Zn and Mn concentrations (Table 8). Zinc and Mn sulfates were foliarly applied as a standard management practice to the control and tank mixed with Ni. The reduction in leaf Zn and Mn concentrations by Ni treatment suggests the competitive absorption of these chemically similar divalent cations. The absorption of Ni as $Ni^{2+}$ suppressed the uptake of divalent cations $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ (Palacios et al., 1998). Root and shoot concentrations of Zn and Mn in barley (*Hordeum vulgare* L. cv. Minorimugi) were suppressed with increasing Ni concentration in nutrient solution (Rahman et al., 2005). Nickel reduced roots, shoots and fruit Mn concentrations in cabbage (*Brassica oleracea var. capitata* L.) (Yang et al., 1996). A similar trend was reported by Palacios et al. (1998) in tomato (*Lycopersicon esculentum* M. cv. Marmande).

Nickel treatment did not alter leaf N, P, K, Ca, Mg, and Fe concentrations (Table 8). Leaf Cu level was unaffected by Ni treatment in the first year but increased in the second year. Nickel and Cu have competitive absorption and translocation. Nickel reduced root and stem Cu suppressing absorption but leaf and fruit Cu were unaffected in tomato (Palacios et al., 1998). Nickel reduced shoot Cu concentration in barley by inhibiting Cu translocation (Rahman et al., 2005). Copper contamination in Ni supply may be responsible for increased leaf Cu concentration in this study.

Treatment did not influence yield, yield efficiency, trunk growth, leaf area, leaf dry weight, and specific leaf weight (Table 9). Total yield and average yield efficiency over two years were unaffected. Nut weight, quality, kernel percentage, kernel necrosis, and opalescence were not affected by treatment (Table 10).

Wood (2007) suggested that tissue levels of 3-5 μg $g^{-1}$ Ni can satisfy pecan trees Ni requirement. Average July leaf Ni concentration was 5.71 μg $g^{-1}$ dry weight in control trees and 6.96 μg $g^{-1}$ in treated trees in the first year, and 2.87 μg $g^{-1}$ in control trees and 6.71 μg $g^{-1}$ in Ni treated trees in the second year. Results indicate that July leaf Ni concentration of as low as 2.87 μg $g^{-1}$ did not impair tree growth, yield, and N metabolism in this orchard. The expression of Ni deficiency was strongly related to the ratio of foliar Zn and/or Cu to Ni but not linked to the foliar Ni concentration (Wood, 2009). Data indicate that Zn and Cu concentrations were within or near the established sufficiency ranges for these elements (average leaf Zn concentrations were ranged from 50 to 104 μg $g^{-1}$ and Cu concentrations were ranged from 7 to 9 μg $g^{-1}$) in this orchard. Results demonstrate that no benefits from additional Ni beyond the critical level were detected on tree yield, nut quality, and tree growth in this study. Positive responses to essential elements are common when an element is in short supply but not sufficient to be symptomatic, i.e. showing visible symptoms. This is termed “hidden hunger” in many texts. Our results indicate that the range between hidden hunger and becoming symptomatic is narrow for Ni. Paying greater attention is, therefore, suggested to maintain optimum Ni concentration against long term applications of Zn and Cu fertilizers for the sustainable orchard profitability.

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Table 8. The influence of foliar application of $NiSO_4 \cdot 6H_2O$ on the pecan leaf elemental concentrations in July. Trees received three applications beginning at budbreak and followed at two week intervals.

| | Year 2008 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dry wt (%) | | | | | Dry wt ($\mu g\ g^{-1}$) | | | | |
| Treatments | N | P | K | Ca | Mg | Zn | Fe | Mn | Cu | Ni |
| Control | 2.56 | 0.11 | 1.09 | 1.57 | 0.37 | 104 | 80 | 123 | 7.11 | 5.71 |
| Ni | 2.61 | 0.11 | 1.07 | 1.51 | 0.38 | 76 | 86 | 100 | 7.33 | 6.96 |
| Significance | NS | NS | NS | NS | NS | * | NS | * | NS | * |
| | Year 2009 | | | | | | | | | |
| Control | 2.66 | 0.12 | 1.10 | 1.64 | 0.35 | 71 | 68 | 125 | 8.39 | 2.87 |
| Ni | 2.66 | 0.12 | 1.11 | 1.57 | 0.34 | 50 | 64 | 99 | 9 | 6.71 |
| Significance | NS | NS | NS | NS | NS | * | NS | * | * | * |

NS, * Means within year and columns are nonsignificant (NS) or significant at $P \leq 0.05$

Table 9. The influence of foliar application of $NiSO_4 \cdot 6H_2O$ on yield, yield efficiency and growth responses of pecan trees. Trees received three applications beginning at budbreak and followed at two week intervals.

| Treatments | Yield (Kg tree$^{-1}$) | Yield efficiency (Kg m$^{-2}$ trunk area) | Trunk growth (cm$^2$) | Leaf dry wt (g leaf$^{-1}$) | Leaf surface area (cm$^2$ leaf$^{-1}$) | Specific leaf weight (g m$^{-2}$) |
|---|---|---|---|---|---|---|
| | | | Year 2008 | | | |
| Control | 6[z] | 328 | 47 | 2.05 | 206 | 100 |
| Ni | 6.5 | 386 | 44 | 2.00 | 200 | 100 |
| | | | Year 2009 | | | |
| Control | 3.0 | 133 | 68 | 2.57 | 277 | 93 |
| Ni | 2.6 | 124 | 66 | 2.52 | 269 | 94 |

[z] Nickel treatments were not significantly different from the control for any parameter listed above.

Table 10. The influence of foliar application of $NiSO_4 \cdot 6H_2O$ on pecan nut weight, quality, kernel percentage, kernel necrosis and opalescence. Trees received three applications beginning at budbreak and followed at two week intervals.

| | Year 2008 | | Year 2009 | |
|---|---|---|---|---|
| Parameters | Control | Ni | Control | Ni |
| Nut weight (g) | 10.43[z] | 10.45 | 10.42 | 10.53 |
| Kernel % | 57.67 | 57.56 | 57.72 | 58.65 |
| Any necrosis (%)[y] | 7.2 | 8.3 | 7.1 | 7.7 |
| Severe necrosis (%)[x] | 6.6 | 7.4 | 3.5 | 4.4 |
| Any opalescence (%)[w] | 12.9 | 14.2 | 10.4 | 9.7 |
| Severe opalescence (%)[v] | 3.4 | 2.4 | 2.5 | 3.4 |
| Quality 1 (No. of nut halves/40 halves) | 33 | 33 | 34 | 34 |
| Quality 2 (No. of nut halves/40 halves) | 4 | 3 | 3 | 3 |
| Quality 3 (No. of nut halves/40 halves) | 3 | 4 | 3 | 3 |
| Quality 4 (No. of nut halves/40 halves) | 0 | 0 | 0 | 0 |

[z] Nickel treatments were not significantly different from the control for any parameter listed above.

[y] Kernel necrosis grade 2 or more.

[x] Kernel necrosis grade 3 or more.

[w] Kernel opalescence grade 2 or more.

[v] Kernel opalescence grade 3 or more.

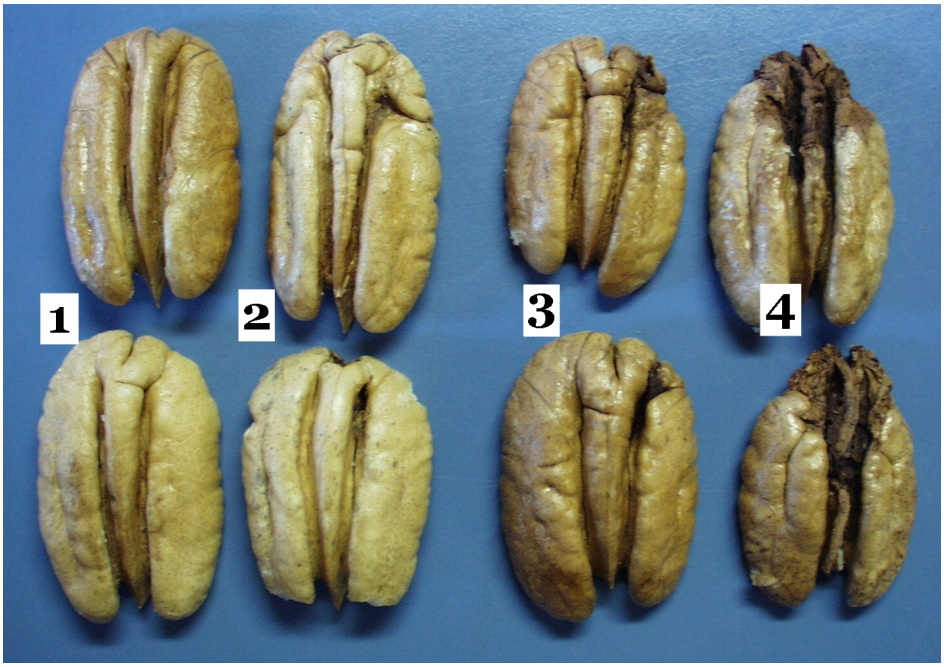


Fig. 9. Kernel necrosis grades of ‘Pawnee’ pecan. From left to right: grade 1 normal kernels; grade 2 darkening of testa in the dorsal grove at the basal (stem) end of the kernel; grade 3 necrotic tissue progressing outside of the dorsal grove at the basal end; and grade 4 encompasses the entire basal section of the kernel.


Fig. 10. Kerenl opalescence grades of ‘Pawnee’ pecan. Grade 1 no opalescence or less than 20% intercellular spaces covered with leaked oil; and grade 5 was severe opalescence (more than 80% intercellular spaces covered with leaked oil).

# CHAPTER IV

## Do Pecan Trees Receiving Excess Nitrogen Benefit from Supplemental Foliar Nickel and Copper Applications?

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ABSTRACT. The mobilization and conversion of reserve nitrogen (N) is critical for pecans [Carya illinoinensis (Wang.) K. Koch] during early spring as trees begin active growth. Conversion of N reserves to translocatable forms (amides, amino acids, ureides) is adversely affected by a nickel (Ni) shortage. Nickel is required for urease activation and appears to function in additional enzymes affecting N metabolism. The orchard used for study received an unusually high amount of N annually from nitrate contaminated irrigation water. High N induces copper (Cu) deficiency by inhibiting transport in plants. Copper, Ni and Ni + Cu were applied as a foliar spray application at the parachute stage of leaf development followed by two additional applications at two week intervals to

observe the effects of Ni and Cu treatments to mitigate excess N. One study was conducted two years using the same trees. A second study was initiated at an adjacent orchard using the same treatments and was conducted for one year. Results demonstrate that the influences of foliar treatments on yield and yield efficiency (the ratio of yield to cross sectional trunk area), tree growth, nut weight, kernel percentage and leaf elemental concentrations were inconsistent. Although Ni and Cu treatments reduced leaf N, kernel necrosis, a malady characterized by necrotic tissue at the basal end of the kernel, was unaffected by treatment. The cause of kernel necrosis remains unknown. Data indicate that all treatments, including the control, had leaf Ni concentration (> 8 μg. $g^{-1}$) above the level reported to be deficient. Leaf Cu concentration in the control was at the level reported as low (≈ 6 μg $g^{-1}$). Copper and Ni + Cu increased leaf Cu concentrations beyond the upper limit (30 μg $g^{-1}$) of desired range for Cu in pecans in the second year and second study. Some beneficial effects on growth were observed in the first year but not during the second year that were associated with Ni and/or Cu treatments. Conservation of Ni and/or Cu from the first year's treatments may be associated with the ineffectiveness of treatments in the second year or random variation may be associated with the positive responses noted during the first year. In the second study positive growth responses were not observed, suggesting that random variation was responsible for the growth responses in the first study.

**Keywords:** *Carya illinoinensis,* parachute stage, yield efficiency, kernel necrosis, nut quality, critical level, leaf elemental concentration.

Tim Montz's orchard at Charlie, Texas was selected for this study because a unique problem was identified that might affect the response to applied Ni or Cu. The orchard derives an unusually high amount of N from nitrate contaminated irrigation water (34 mg $NO_3$- N $L^{-1}$ water) (Smith et al., 2007). Smith et al. (2007) noticed kernel necrosis, a malady characterized by necrotic tissue at the basal end of the kernel that was prevalently on 'Pawnee' in this orchard, but rare or nonexistent on 'Pawnee' at other locations. It was hypothesized that excess N may be exacerbating kernel necrosis.

A high concentration of N reduced soil pH and occasionally P, K and Ca in soil (Worley, 1997). Nitrogen and Cu are antagonistic affecting plant concentrations (Kumar et al., 1990). High N induced Cu deficiency by inhibiting Cu transport in plants at low Cu levels (Gilbert, 1951). Another study reported vigorous growth induced by high N diluted the concentrations of Cu and Zn in shoots and roots of wheat plants (Chaudhry and Loneragan, 1970). Growth and development of plants receiving higher levels of N without Cu were adversely affected. Addition of Cu mitigated the toxic effects of excess N and reduced the tissue N level (Chesire et al., 1982).

A survey of pecan leaf Ni concentrations suggested that orchards in southern Oklahoma and northern Texas may be low in Ni (data not shown) though the shortage is typically not sufficient to be symptomatic. One instance of mouse-ear symptoms has been observed in the Red River basin (C. Rohla, personal communication). Native soil pH is near neutral to alkaline (pH 7.2 at this orchard). Due to unavailability of soil Zn at this soil pH, three to five foliar applications of Zn sulfates during a growing season is a common standard management practice. Accumulating more soil Zn from years of Zn applications may be inducing Ni deficiency through antagonistic impact of high

concentrations of Zn on absorption and sequestration of Ni. Nickel deficient plants accumulate urea in foliage due to impairment of urease (EC 3.5.1.5, urea amidohydrolase) activity (Eskew et al., 1983, 1984; Walker et al., 1985) since Ni is required for urease activation (Dixon et al., 1975) to convert urea to ammonia ($NH_3$). Supplemental applications of Ni and Cu may enhance growth and production in this orchard via improved Ni and Cu nutrition and by mitigating any negative effects associated with excess N by enhancing catabolism of urea and loss of excess N by $NH_3$ volatilization, and increasing N metabolism. This study evaluates the effects of foliar applied Ni and Cu on kernel necrosis, growth and yield of pecans, and tests the impact of foliar Ni and Cu treatments to mitigate the problems associated with excess N.

## Materials and Methods

### FIRST STUDY

Twenty-one year old 'Pawnee' trees grafted onto Apache rootstock and spaced 12.2 x 12.2 m in a micro sprinkler irrigated Teller sandy loam soil (fine-loamy, mixed, thermic, Udic Argiustoll) were used for this study. Twelve uniform trees were selected randomly for each treatment in April before starting application.

TREATMENTS. Four treatments, control (no Ni or Cu applied), Cu [$CuSO_4.5H_2O$ at 1.8 g $L^{-1}$ (0.45 g $L^{-1}$; 0.56 kg $ha^{-1}$ Cu], Ni [$NiSO_4.6H_2O$ at 0.94 ml $L^{-1}$ (0.066 g $L^{-1}$; 82 g $ha^{-1}$ Ni)] and Ni + Cu (same rates), were applied in 1244 L $ha^{-1}$ of water with an orchard sprayer as a foliar application at the parachute stage of leaf development (mid April) followed by two additional applications at two week intervals. All treatments

were tank mixed with 36% $ZnSO_4$ at 5.4 g $L^{-1}$ [1.95 g $L^{-1}$ Zn (2.42 kg $ha^{-1}$)] and Surfking Plus (surfactant with buffering agent) at 0.62 ml $L^{-1}$.

SOIL SAMPLE. Two soil samples were taken from 0-15 cm and 15-30 cm depths using a standard soil probe to determine baseline nutrient levels at the beginning of study. Soil was composited with at least 10 soil cores and two samples were collected for each depth and submitted to the Soil, Forage and Water Testing Laboratory at Oklahoma State University for analysis of pH, $NO_3$-N, P, K, Fe, Zn, boron (B) and Cu.

TRUNK DIAMETER MEASUREMENT. Trunk diameter was measured at 1.4 m above ground level at the beginning of study in April and each fall.

LEAF SAMPLE

1) Thirty middle leaves from the current season growth per tree were collected a week after the first treatment application for Ni analysis. Leaves were washed in tap water followed by 0.1 N HCl water, soapy (P-free detergent) distilled water, and then two deionized water rinses. Leaves were dried at 70 $^{o}$C, ground to pass a 20 mesh (850 µm) screen and stored in air tight glass jars until analysis.

2) Twenty compound leaves from the middle of current season shoots per tree were harvested in July when leaf expansion ceased, and leaf surface area and leaf dry weight were measured. Leaf surface area was measured using Li-Cor model 3100 area meter. Leaf samples were dried at 70 $^{0}$C before weighing. Specific leaf weight was calculated by dividing the dry weight of leaves by the area of the same portion of leaves.

3) In July, thirty middle leaflet pairs from the middle leaf on current season's growth were collected for nutrient analysis from each tree in the study. Collected leaf samples were washed, dried and ground as described earlier. Leaf elemental concentrations of N were determined using a modification of the Dumas method with a Leco N analyzer (St. Joseph, MI). Phosphorous was determined colorimetrically. Potassium, Ca, Mg, Cu, Fe, Zn and Mn were analyzed using atomic absorption spectroscopy (Perkin Elmer model 2380, Waltham, MA). Nickel was analyzed using an inductively coupled plasma emission spectrometry.

ENDOSPERM ANALYSIS. Analysis of reduced N compounds in the liquid endosperm was done by high performance liquid chromatography (HPLC). Samples were collected at the full ovary expansion in the water stage from control, Ni, and Cu treated trees. Duplicate endosperms were collected from each tree using at least 5 fruit per sample. One milliliter (ml) of endosperm was added to a vial containing 1 ml phenyl mercuric acetate (PMA) (enzyme inhibitor). Vials were packed on dry ice and transported to the laboratory. All samples from Tim Montz's orchard were exposed to high $NO_3$-N from contaminated irrigation water. Endosperm samples were collected from Jonny Dowd's orchard, Nocona, TX, in the first year, and from Hoffman's orchard, Stillwater, OK, in the second year from control trees at the same stage of nut development as collected at Tim Montz's to get normal N application rate for comparison.

NUT SAMPLE. Nut samples (2 - 20 nut samples per tree) were taken in October at harvest. Kernel percentage, weight/nut, kernel quality, opalescence, and kernel necrosis were determined. Kernel halves were graded on 1 to 4 scales for quality with 1

being a perfectly colored and filled, 2 was not completely filled and/or colored, 3 poorly filled and/or discolored, and 4 for completely discolored and/or not filled. Kernel halves were graded on a scale of 1 to 4 for necrosis; 1 being no necrosis and 4 was severe necrosis (Fig. 11). Opalescence grading was done on 1 to 5 scale in which 1 was no opalescence or less than 20% intercellular spaces covered with leaked oil and 5 was severe opalescence (more than 80% intercellular spaces covered with leaked oil) (Fig. 12). Total yield per tree was determined annually.

DATA ANALYSIS. Data were analyzed using a mixed model for analysis of variance in SAS software. Treatment means were compared to the control using the protected $LSD_{0.05}$.

SECOND STUDY

A second study was initiated at an adjacent orchard using the same treatments and was conducted for one year. Twenty-four uniform ‘Pawnee’ trees were selected randomly for each treatment in April before starting application.

SOIL SAMPLE. Collection and analysis were performed as in the first study.

TRUNK DIAMETER MEASUREMENT. Trunk diameter was measured at 1.4 m above ground level at the beginning of study in April and in fall.

LEAF SAMPLE

In July, thirty middle leaflet pairs from the middle leaf on current season’s growth were harvested for nutrient analysis from each tree in the study. Sample preparation and analysis techniques were the same as in the first study.

NUT SAMPLE. Same as described in the first study.

DATA ANALYSIS. Same as described in the first study.

## Results and Discussion

### FIRST STUDY

Nickel and Ni + Cu increased leaf Ni (Table 11). Nickel, Cu, and Ni + Cu increased leaf Cu concentrations (Table 12). Treatment did not alter leaf P, K, Ca, Mg, and Mn concentrations. Leaf N was reduced by Cu and Ni treatments in the first year and by Ni + Cu in the second year. Enhanced urea catabolism via Ni enhanced urease activity resulting in loss of N by $NH_3$ volatilization probably caused to reduce tissue N concentration. Elevating leaf Cu was expected to suppress leaf N concentration by antagonism. Xiong et al. (2006) reported an adverse effect of a high concentration of Cu on N metabolism. Copper reduced toxic effects of N on growth and decreased tissue N level (Chesire et al., 1982).

Foliar treatments increased leaf Zn concentration compared to the control (Table 12), perhaps the result of Zn contamination in the Cu and Ni compounds. However, Ni + Cu reduced leaf Zn concentration in 2008, a result that might be attributable to competitive absorption among similar cations. Foliar Zn sulfate was applied as a standard management practice to the control and was tank mixed with the Ni and/or Cu treatments. Some previous studies corroborate this result. Absorption of $Ni^{2+,}$ $Mg^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ appears to be competitive (Palacios et al., 1998; Wood et al., 2004). Zinc

reduced Cu concentration by inhibiting Cu absorption (Chaudhry and Loneragan, 1970). Foliar Cu (50 μM) treatment reduced Zn concentration in soybean (*Glycine max* L.) plants (Bernel et al., 2007).

Leaf Fe was unaffected in the first year, and was higher in Ni + Cu treated trees in the second year (Table 12). Gerendás et al. (1998) also reported variable Fe concentrations; however, higher concentration in Ni treated soybean plants. The high Ni concentration increased Fe content in *Matricaria chamomilla* leaf rosettes (Kováčik et al., 2009).

Foliar treatments increased leaf area and leaf dry weight, but specific leaf weight was unaffected in 2008 (Table 13). In 2009, leaf area and leaf dry weight were unaffected by treatment but specific leaf weight was reduced by Ni.

The Ni + Cu treatment reduced yield and yield efficiency (Fig 13, 14), and Ni, and Ni + Cu increased the individual nut weight in the first year (Table 14). A lower yield in the combination treatment may result from elevated metal accumulation or it may be the effect of nut yield in the previous year. A small crop load on the Ni + Cu treated trees may contribute to larger nuts. Tree yield and individual nut size are reported to be negatively correlated in pecans (Nu´n˜ez-Moreno et al., 2009). Cluster size on lateral shoots was inversely related to pecan nut weight and kernel percentage (Rohla et al., 2005). Kernel percentage, quality and individual nut weight were improved by fruit thinning in 'Mohawk' pecan trees (Smith et al., 1993). Yield, yield efficiency and nut weight were not affected by treatment in the second year. However, the lowest total yield and average yield efficiency over two years were observed in Ni + Cu treated trees that

were attributed to the low tree yield in the first year. Trunk growth was increased by Ni in 2008 and less trunk growth was observed in Ni + Cu treated trees as compared to the control in 2009 (Fig 15).

Kernel necrosis and nut quality were unaffected by treatment (Table 14). Nickel, Cu and Ni + Cu treatments increased opalescence, perhaps the result of increased oil content by the treatments, in the first year but not in the second year. Foliar application of nickel sulfate and copper sulfate increased oil content in geranium (*Pelargonium sp.*) (Prasad et al., 2008). Nickel and Cu were supplied from Ni and Cu sulfates in this study. Increase in oil content may contribute to higher kernel percentage by Ni in the first year and by Ni and Ni + Cu in the second year (Table 14).

Leaf tissue analysis results show that Ni concentrations in July leaf were 11.27, 10.42, 13.76, and 13.57 μg $g^{-1}$ in 2008, and 8.98, 8.17, 14.79, and 14.79 μg $g^{-1}$ in 2009 in control, Cu, Ni, and Ni + Cu treated trees, respectively. July leaf Cu concentrations were 5.94, 18.62, 7.66, and 23.08 μg $g^{-1}$ in 2008, and 6.42, 51.25, 9.58, and 40.83 μg $g^{-1}$ in 2009 in control, Cu, Ni, and Ni + Cu treated trees, respectively. Data indicate that Cu level in control trees was at or below the minimum sufficiency level. The desired range for Cu in pecan is 6-30 μg $g^{-1}$ (Wells, 2009). Increment in Cu concentrations by Cu and Ni + Cu treatments was within the desired range in the first year, but beyond the range in the second year. Results indicate that some of the beneficial effects of foliar treatments on trunk and leaf growth in the first year might be related to improved Ni and/or Cu nutrition via increasing chlorophyll concentration and photosynthesis. Copper plays important roles in activities of photosystem I (PSI) and PSII, plastocyanin (a component of electron transport chain of PSI), and other chloroplast enzymes (Marschner, 1995).

Nickel treatment increased chlorophyll concentration and availability of photosynthates (Moraes et al., 2009). Ineffectiveness of treatment during the second year may be attributed to conservation of Cu and/or Ni from the first year's treatments or random variation may be associated with the positive responses noted during the first year.

SECOND STUDY

Nickel treatment increased leaf Ni concentration (Table 15). Copper and Ni + Cu increased leaf Cu concentration. Nickel treatment increased leaf N level. Palacios et al. (1998) recorded synergetic effect between N and Ni, increasing Ni concentrations significantly increased N content. Leaf P, Mg, and Mn were not altered by treatment. Copper and Ni + Cu reduced leaf K concentrations. Copper treatment reduced leaf Ca concentration, and Ni + Cu decreased leaf Fe concentration. Increased metal accumulation might influence uptake and translocation of Fe, Ca and K. Some previous studies substantiate this result. Higher concentrations of Ni and Cu reduced plant Fe contents suppressing root Fe uptake due to inhibition of root $Fe^{3+}$ reductase activity (Alcantara et al., 1994). Antagonistic effect between Fe and Cu was observed in spinach (*Spinacia oleracea*) (Ouzounidou et al., 1998). The high Ni concentration reduced K content in *Matricaria chamomilla* leaf rosettes (Kováčik et al., 2009). The higher Zn concentration in Ni, and Ni + Cu treated trees might be associated with Zn contamination in the Cu and Ni compounds (most likely in Ni).

Nickel treatment reduced yield and yield efficiency (Fig 16, 17). The Ni + Cu increased individual nut weight but kernel necrosis, opalescence and nut quality were unaffected by treatment (Table 16). Greater nut weight may be related to lower yields in

the treatment receiving Ni and Cu or increased tissue Ni and/or Cu concentration by Ni + Cu treatment. The reduction in yield and yield efficiency by Ni treatment is not clear. It may be related to previous year's crop load or random error. Nickel and Ni + Cu treated trees had less trunk growth as compared to control (Fig 18). It is unlikely that Ni and Cu concentrations were sufficiently high to suppress growth and yield since they were within or near the established sufficiency ranges for these elements.

## Conclusion

Leaf tissue analysis results show that a leaf Ni concentration (>8 μg $g^{-1}$) in the control was sufficient to satisfy tree's Ni requirement. Leaf Cu concentration was below or near the critical level (≈ 6 μg $g^{-1}$) in control trees. Although Ni and Cu treatments reduced leaf N, kernel necrosis was unaffected by treatment. The cause of kernel necrosis remains unknown. Results demonstrate that the influences of foliar treatments on tree yield, trunk and leaf growth, and leaf elemental concentrations were inconsistent. Some beneficial effects of Ni and/or Cu treatments on growth were observed in the first year but not during the second year. In the second study positive growth responses were not observed, suggesting that random variation was responsible for the growth responses in the first study.

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Table 11. The influence of foliar applied nutrients on the pecan leaf Ni concentration in May and July (First study). Trees received three applications beginning at budbreak and followed at two week intervals.

| | Year 2008 | | Year 2009 | |
|---|---|---|---|---|
| Treatment | Leaf Ni concn in May ($\mu g\ g^{-1}$ dw) | Leaf Ni concn in July ($\mu g\ g^{-1}$ dw) | Leaf Ni concn in May ($\mu g\ g^{-1}$ dw) | Leaf Ni concn in July ($\mu g\ g^{-1}$ dw) |
| Control | 8.90 | 11.27 | 14.42 | 8.98 |
| Cu | 8.65 | 10.42 | 12.49 | 8.17 |
| Ni | 12.64* | 13.76* | 14.4 | 14.79* |
| Ni + Cu | 13.30* | 13.57* | 14.49 | 14.79* |

* Means within year and columns are significantly different from the control by LSD, 5% level (*).

Table 12. The influence of foliar applied nutrients on the pecan leaf elemental concentrations in July (First study). Trees received three applications beginning at budbreak and followed at two week intervals.

| | Dry wt (%) | | | | | Dry wt (μg $g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment | N | P | K | Ca | Mg | Zn | Fe | Mn | Cu |
| | | | | Year 2008 | | | | | |
| Control | 2.81 | 0.12 | 0.92 | 1.52 | 0.63 | 321 | 55 | 680 | 5.94 |
| Cu | 2.70* | 0.12 | 0.94 | 1.51 | 0.63 | 364* | 54 | 562 | 18.62* |
| Ni | 2.70* | 0.12 | 0.87 | 1.42 | 0.63 | 405* | 58 | 578 | 7.66* |
| Ni + Cu | 2.73 | 0.12 | 0.97 | 1.52 | 0.58 | 264* | 57 | 586 | 23.08* |
| | | | | Year 2009 | | | | | |
| Control | 2.79 | 0.11 | 0.85 | 1.41 | 0.56 | 218 | 43 | 667 | 6.42 |
| Cu | 2.75 | 0.12 | 0.76 | 1.34 | 0.53 | 285* | 41 | 532 | 51.25* |
| Ni | 2.72 | 0.11 | 0.83 | 1.40 | 0.58 | 279* | 41 | 625 | 9.58* |
| Ni + Cu | 2.64* | 0.11 | 0.93 | 1.47 | 0.53 | 294* | 47* | 695 | 40.83* |

* Means within year and columns are significantly different from the control by LSD, 5% level (*).

Table 13. The influence of foliar applied nutrients on the pecan leaf growth (First study). Trees received three applications beginning at budbreak and followed at two week intervals.

| Treatment | leaf dry wt (g $leaf^{-1}$) | leaf surface area ($cm^2$ $leaf^{-1}$) | Specific leaf wt (g $m^{-2}$) |
|---|---|---|---|
| | | Year 2008 | |
| Control | 2.47 | 268 | 92.2 |
| Cu | 2.73 | 302* | 90.3 |
| Ni | 2.95* | 313* | 94.3 |
| Ni + Cu | 2.92* | 317* | 92.3 |
| | | Year 2009 | |
| Control | 2.57 | 315 | 81.5 |
| Cu | 2.44 | 304 | 80.4 |
| Ni | 2.36 | 312 | 75.5* |
| Ni + Cu | 2.46 | 311 | 79.2 |

* Means within year and columns are significantly different from the control by LSD, 5% level (*).

Table 14. The influence of foliar applied nutrients on pecan nut weight, quality, kernel percentage, kernel necrosis and opalescence (First study). Trees received three applications beginning at budbreak and followed at two week intervals.

| | | Year 2008 | | |
|---|---|---|---|---|
| Parameters | Control | Cu | Ni | Ni + Cu |
| Nut weight (g) | 7.78 | 7.93 | 8.42* | 8.74* |
| Kernel % | 57.96 | 58.07 | 58.87* | 58.63 |
| Any necrosis (%)[z] | 4.1 | 4.5 | 4.4 | 6.0 |
| Severe necrosis (%)[y] | 2.4 | 3.3 | 3.4 | 3.5 |
| Any opalescence (%)[x] | 8.9 | 15.9* | 15.8* | 17.1* |
| Severe opalescence (%)[w] | 1.6 | 3.2 | 4.2* | 4.2* |
| Quality 1 (No. of nut halves/40 halves) | 33 | 33 | 36* | 34 |
| Quality 2 (No. of nut halves/40 halves) | 6 | 5 | 3* | 4 |
| Quality 3 (No. of nut halves/40 halves) | 1 | 2 | 1 | 2 |
| Quality 4 (No. of nut halves/40 halves) | 0 | 0 | 0 | 0 |
| | | Year 2009 | | |
| Nut weight (g) | 8.88 | 8.83 | 8.66 | 8.61 |
| Kernel % | 58 | 58.64 | 59.55* | 60.34* |
| Any necrosis (%)[y] | 17.0 | 15.3 | 14.9 | 13.2 |
| Severe necrosis (%)[x] | 11.0 | 11.2 | 9.5 | 9.4 |
| Any opalescence (%)[w] | 13.9 | 13.5 | 15.2 | 16.5 |
| Severe opalescence (%)[v] | 3.0 | 2.9 | 2.9 | 4.8 |
| Quality 1 (No. of nut halves/40 halves) | 30 | 31 | 32 | 33 |
| Quality 2 (No. of nut halves/40 halves) | 3 | 3 | 3 | 2* |
| Quality 3 (No. of nut halves/40 halves) | 6 | 6 | 5 | 5 |
| Quality 4 (No. of nut halves/40 halves) | 1 | 0 | 0 | 0 |

* Means within year and rows are significantly different from the control by LSD, 5% level (*).

[z] Kernel necrosis grade 2 or more. [y] Kernel necrosis grade 3 or more. [x] Kernel opalescence grade 2 or more. [w] Kernel opalescence grade 3 or more.

Table 15. The influence of foliar applied nutrients on the pecan leaf elemental concentrations in July (Second study). Trees received three applications beginning at budbreak and followed at two week intervals.

| | Dry wt (%) | | | | | Dry wt (μg $g^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | N | P | K | Ca | Mg | Zn | Fe | Mn | Ni | Cu |
| Control | 2.67 | 0.12 | 0.91 | 1.95 | 0.46 | 255 | 52 | 1244 | 8.92 | 8 |
| Cu | 2.72 | 0.12 | 0.77* | 1.82* | 0.44 | 278 | 49 | 1055 | 8.08 | 38.46* |
| Ni | 2.73* | 0.12 | 0.84 | 1.94 | 0.43 | 372* | 53 | 1213 | 11.69* | 7.42 |
| Ni + Cu | 2.72 | 0.12 | 0.78* | 1.86 | 0.43 | 293* | 46* | 1091 | 9.89 | 36.71* |

* Means within columns are significantly different from the control by LSD, 5% level (*).

Table 16. The influence of foliar applied nutrients on pecan nut weight, quality, kernel percentage, kernel necrosis and opalescence (Second study). Trees received three applications beginning at budbreak and followed at two week intervals.

| Parameters | Control | Cu | Ni | Ni + Cu |
|---|---|---|---|---|
| Nut weight (g) | 7.35 | 7.62 | 7.3 | 7.7* |
| Kernel % | 56.13 | 56.91 | 55.79 | 56.96 |
| Any necrosis (%)[z] | 0.6 | 1.1 | 1.1 | 1.4 |
| Severe necrosis (%)[y] | 0.5 | 0.7 | 0.8 | 1.1 |
| Any opalescence (%)[x] | 7.5 | 6.3 | 7.5 | 8.6 |
| Severe opalescence (%)[w] | 0.2 | 0.4 | 0.7 | 0.4 |
| Quality 1 (No. of nut halves/40 halves) | 36 | 36 | 35 | 36 |
| Quality 2 (No. of nut halves/40 halves) | 4 | 3 | 4 | 3 |
| Quality 3 (No. of nut halves/40 halves) | 0 | 1 | 1 | 1 |
| Quality 4 (No. of nut halves/40 halves) | 0 | 0 | 0 | 0 |

* Means within rows are significantly different from the control by LSD, 5% level (*).

[z] Kernel necrosis grade 2 or more.

[y] Kernel necrosis grade 3 or more.

[x] Kernel opalescence grade 2 or more.

[w] Kernel opalescence grade 3 or more.

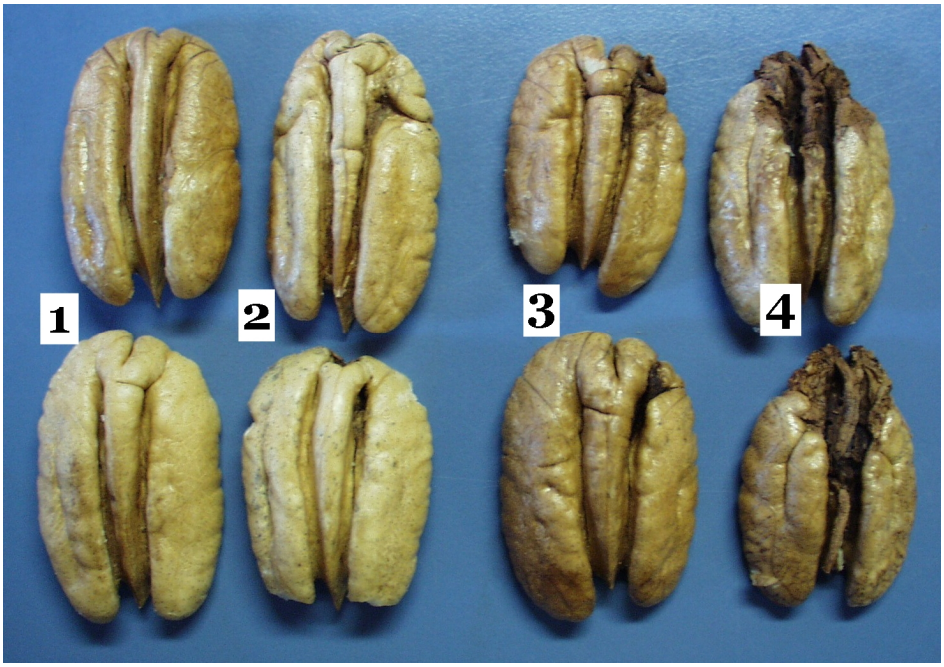


Fig. 11. Kernel necrosis grades of ‘Pawnee’ pecan. From left to right: grade 1 normal kernels; grade 2 darkening of testa in the dorsal grove at the basal (stem) end of the kernel; grade 3 necrotic tissue progressing outside of the dorsal grove at the basal end; and grade 4 encompasses the entire basal section of the kernel.


Fig. 12. Kerenl opalescence grades of ‘Pawnee’ pecan. Grade 1 no opalescence or less than 20% intercellular spaces covered with leaked oil; and grade 5 was severe opalescence (more than 80% intercellular spaces covered with leaked oil).

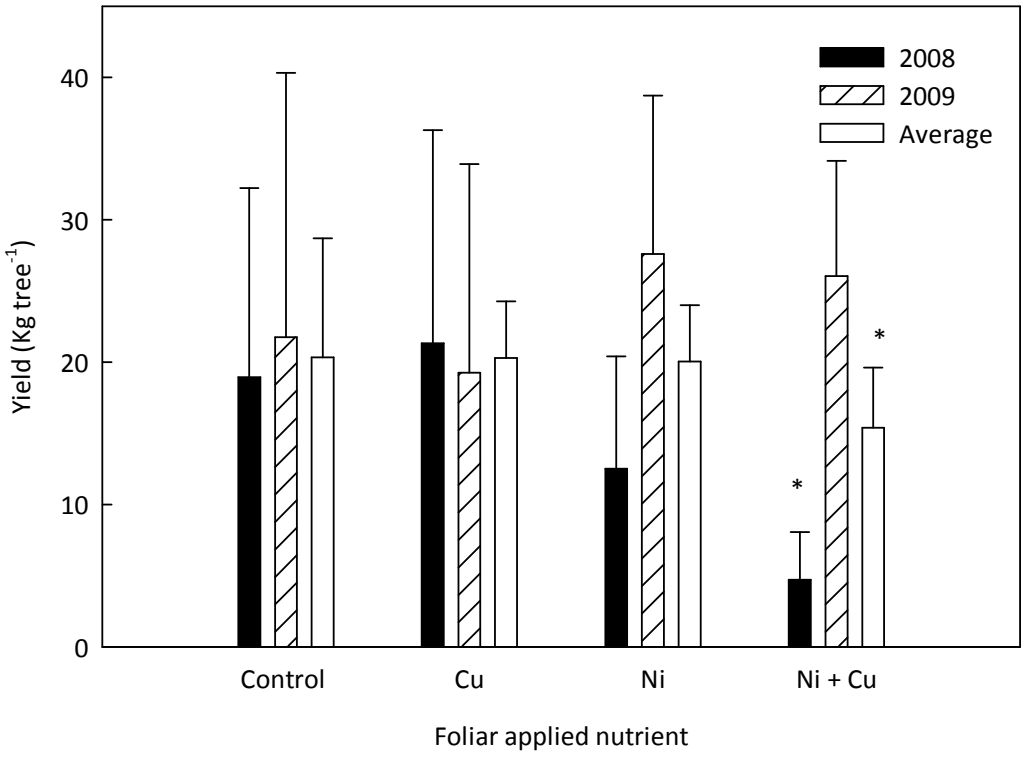


Fig. 13. The influence of foliar applied nutrients on the pecan yield (First study). Trees received three applications beginning at budbreak and followed at two week intervals. Significantly different within year from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

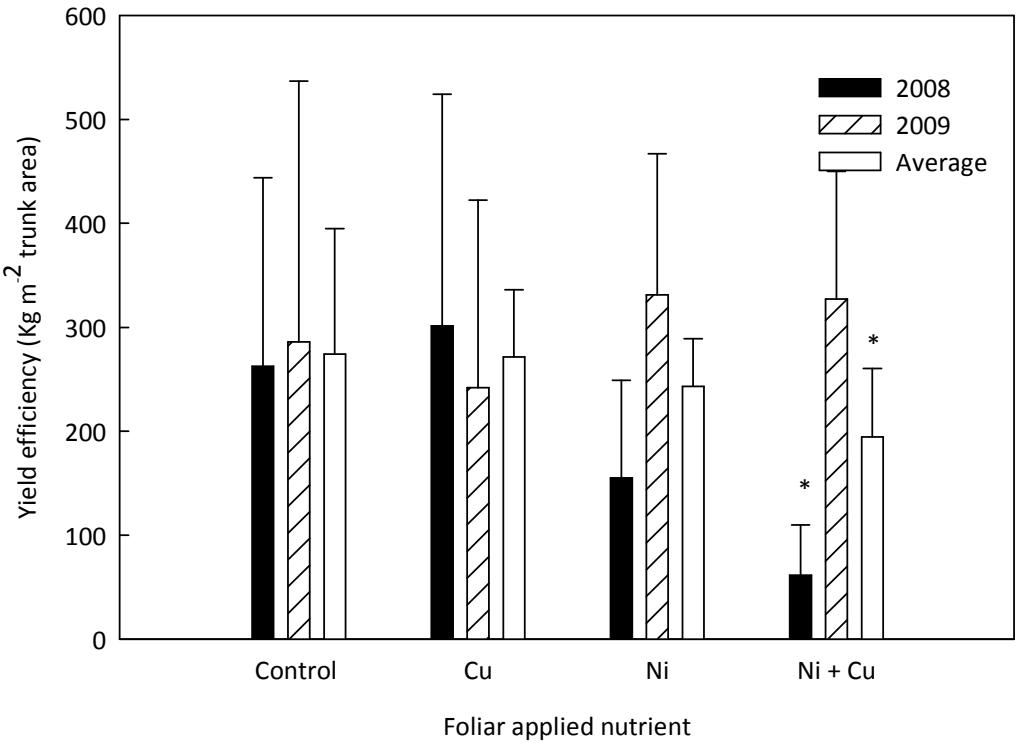


Fig. 14. The influence of foliar applied nutrients on the pecan yield efficiency (First study). Trees received three applications beginning at budbreak and followed at two week intervals. Significantly different within year from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

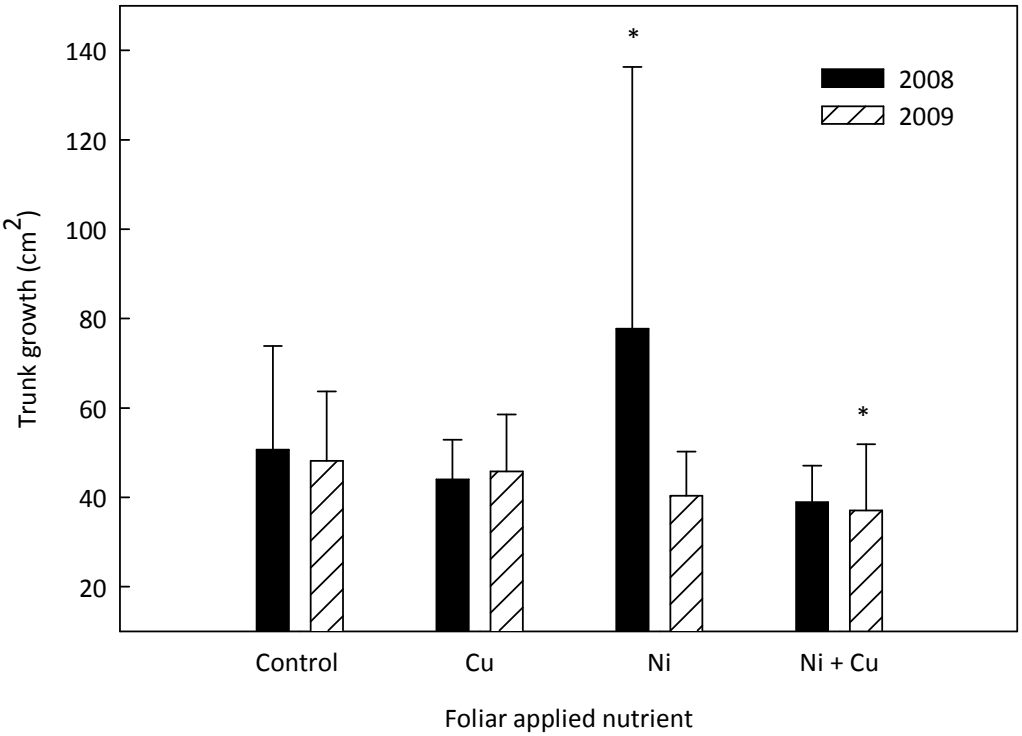


Fig. 15. The influence of foliar applied nutrients on the pecan trunk growth. (First study) Trees received three applications beginning at budbreak and followed at two week intervals. Trunk diameter was measured at 1.4 m above ground level at the beginning of study in April and each fall. Trunk growth was calculated by subtracting trunk area in previous year from trunk area in the current year. Significantly different within year from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

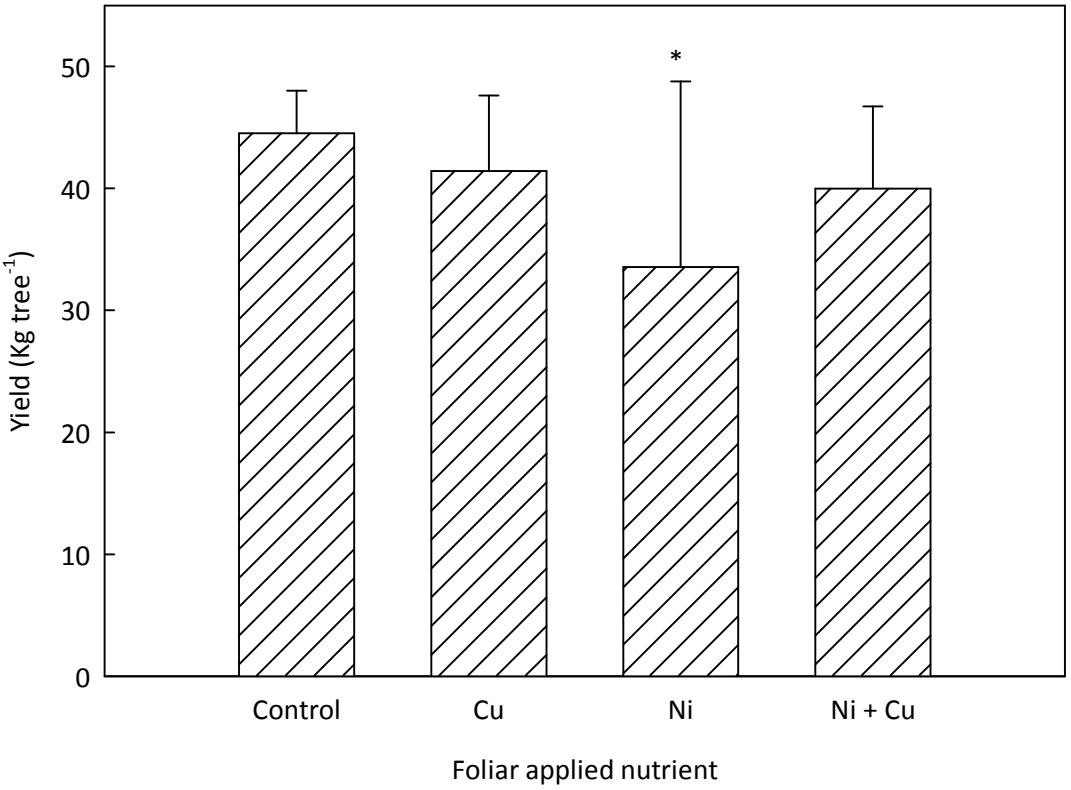


Fig. 16. The influence of foliar applied nutrients on the pecan yield (Second study). Trees received three applications beginning at budbreak and followed at two week intervals. Significantly different from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

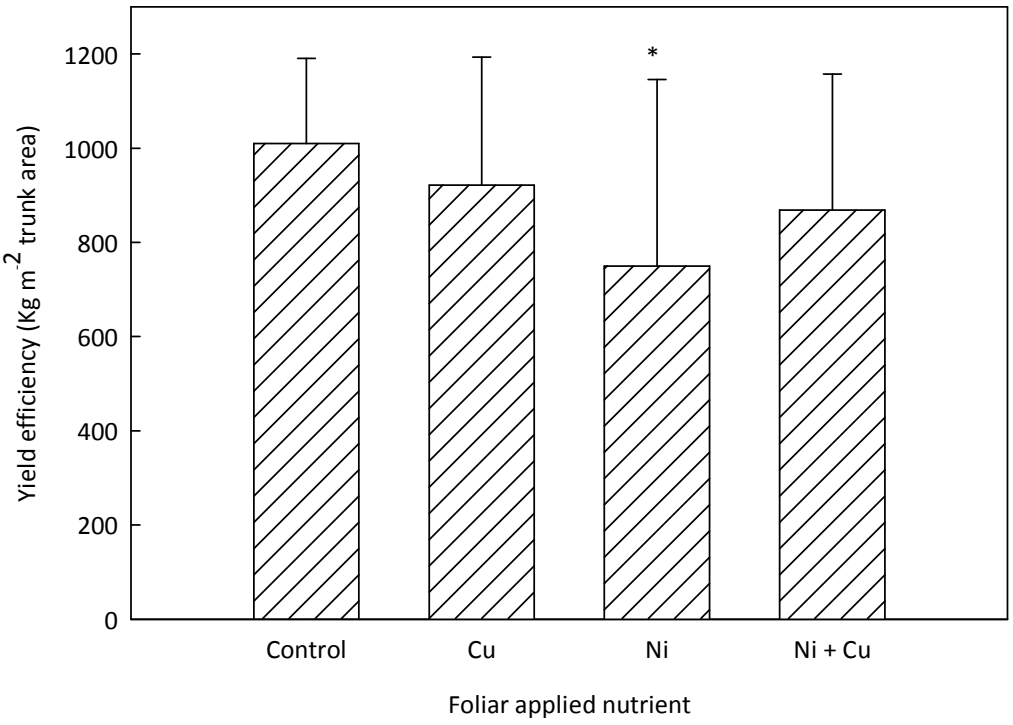


Fig. 17. The influence of foliar applied nutrients on the pecan yield efficiency (Second study). Trees received three applications beginning at budbreak and followed at two week intervals. Significantly different from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

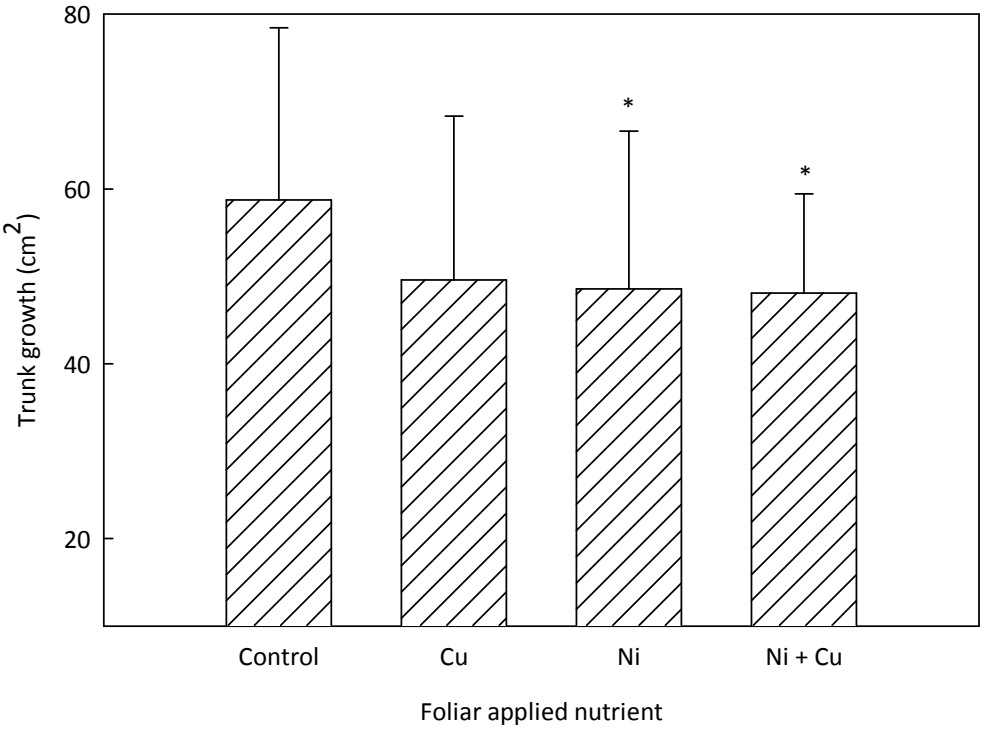


Fig. 18. The influence of foliar applied nutrients on the pecan trunk growth (Second study). Trees received three applications beginning at budbreak and followed at two week intervals. Trunk diameter was measured at 1.4 m above ground level at the beginning of study in April and in fall. Trunk growth was calculated by subtracting trunk area in April from trunk area in fall. Significantly different from the control by LSD, 5% level (*). Vertical bars indicate the standard error of the mean.

# CHAPTER V

## CONCLUSION

This research project covers three experiments. The first study was conducted to determine the interaction effect of nickel (Ni) and nitrogen (N) on growth of container-grown pecan seedlings and to test the effect of foliar Ni applications on N metabolism. This study can be considered as a partial duplication of conditions encountered in the field at Charlie, the third experiment. Dormant 1-year-old 'Peruque' seedlings overwintered in cooler to satisfy their chilling requirements were transferred to an outside container production bed. Nitrogen at 0, 50, 100 and 200 $\mu g\ ml^{-1}$ from a 28% N solution were applied to the growth media each irrigation and Ni was applied at 0 ml $L^{-1}$ and 0.32 ml $L^{-1}$ $NiSO_4.6H_2O$ (0.023 g $L^{-1}$ Ni) to the foliage until runoff at two week intervals. Trees were harvested 3 months after transfer to the production bed when terminal buds were shed and additional trunk and shoot expansion ceased. Seedling's growth responses in terms of tree height, leaf area, leaf dry weight, specific leaf weight, and trunk and root dry weights were determined. Leaf elemental concentrations, root and trunk Ni concentrations were also determined. The effect of an N and Ni interaction on growth responses of seedling pecan trees was not visible. Leaf elemental concentrations were unaltered for most of the elements. The synergetic effect of Ni on N was not evident. Results indicate that a leaf Ni concentration of 2.99 $\mu g\ g^{-1}$ dry weight in control trees met the tree's requirement and was adequate for N metabolism.

The purpose of the second study was to evaluate the response of bearing pecan trees grown at soils apparently low in Ni. Nickel shortage is more likely for ureide-N transporting crops like pecans. Foliar Ni application [$NiSO_4.6H_2O$ at 0.78 ml $L^{-1}$ (0.055 g $L^{-1}$, 82.3 g $ha^{-1}$ Ni)] was applied to the trees at the parachute stage of leaf development (mid April) followed by two additional applications at two week intervals. Nickel treatment did not alter the leaf elemental concentrations of most of the elements except suppressing Zn and Mn concentrations due to competitive absorption of similar divalent cations. Foliar Zn from 36% $ZnSO_4$ at 4.49 g $L^{-1}$ [1.62 g $L^{-1}$ Zn (2.42 kg $ha^{-1}$)], and Mn from 32% $MnSO_4$ at 4.49 g $L^{-1}$ [1.44 g $L^{-1}$ Mn (2.15 kg $ha^{-1}$)] were applied as a standard management practice to the control and tank mixed with Ni. Soil pH in this region is slightly acid to alkaline with most orchards experiencing Zn shortages if left untreated. Three to five foliar applications of Zn sulfate during a growing season is a common standard management practice in this area (pH was 8 in this orchard). Soil in the orchard is accumulating more Zn from years of Zn applications. Increasing supply of Zn may be inducing Ni deficiency through antagonistic impact of high concentrations of Zn on absorption and sequestration of Ni. Results indicate that a leaf Ni concentration in the control (2.87 μg $g^{-1}$) did not impair tree growth, yield, and N metabolism in this orchard if leaf Zn and Cu concentrations were within or near the established sufficiency ranges (average foliar Zn concentrations were ranged from 50 to 104 μg $g^{-1}$ and Cu concentrations were ranged from 7 to 9 μg $g^{-1}$). Additional Ni beyond the critical level was not beneficial. However, paying greater attention is suggested to maintain optimum Ni concentration against long term applications of Zn and Cu fertilizers for the sustainable orchard profitability.

The third study was used to determine whether supplemental applications of Ni and Cu may enhance growth and production in this orchard via improved Ni and Cu nutrition and by mitigating any negative effects associated with excess N. Copper, Ni and Ni + Cu were applied as a foliar spray application at the parachute stage of leaf development followed by two additional applications at two week intervals. Yield, yield efficiency, nut weight, nut quality, kernel percentage, kernel necrosis, opalescence, trunk growth, leaf area, leaf dry weight, specific leaf weight, and leaf elemental concentrations were determined. Leaf tissue analysis results show that a leaf Ni concentration in the control (> 8 μg $g^{-1}$) was sufficient to satisfy tree's Ni requirement. Leaf Cu concentration was at or below the sufficiency level in control trees (≈ 6 μg $g^{-1}$). The effects of treatment on tree yield, nut quality, and tree growth were inconsistent. Although Ni and Cu treatments reduced leaf N, kernel necrosis was unaffected by treatment. The cause of kernel necrosis remains unknown. Some beneficial effects of Ni and/or Cu treatments on growth were observed in the first year but not during the second year. Conservation of Ni and/or Cu from the first year's treatments may be associated with the ineffectiveness of treatments in the second year or random variation may be associated with the positive responses noted during the first year. In the second study positive growth responses were not observed, suggesting that random variation was responsible for the growth responses in the first study.

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Scope and Method of Study:

Nickel (Ni) shortage is more likely for ureide-nitrogen transporting crops like pecans [*Carya illinoinensis* (Wang.) K. Koch]. Soil pH in the Red River basin is near neutral or alkaline restricting the availability of many nutrient cations such as zinc (Zn), copper (Cu) and Ni. Nickel and/or Cu treatments were applied as a foliar application at the parachute stage of leaf development followed by two additional applications at two week intervals. The objectives of the study were to 1) evaluate the interaction between (nitrogen) N rate and foliar Ni application on growth of container-grown pecan seedlings, 2) determine the response of bearing pecan trees to foliar Ni application, and 3) assess the impact of foliar Ni and Cu treatments to mitigate the problems associated with excess N.

Findings and Conclusions:

Leaf Ni concentration of 3 μg $g^{-1}$ dry weight met pecan tree's Ni requirement if leaf Zn and Cu concentrations were within or near the established sufficiency ranges. No benefits from additional Ni beyond the critical level were detected on tree yield, nut quality, tree growth, and N metabolism. Positive responses to essential elements are common when an element is in short supply but visible symptoms are not present. This is termed "hidden hunger" in many texts. Our results indicate that the range between hidden hunger and becoming symptomatic is narrow for Ni.

It was hypothesized that excess N may be exacerbating kernel necrosis, necrotic tissues at the basal end of kernel, at Tim Montz's orchard where trees derive an unusually high amount of N from nitrate contaminated irrigation water. The supplemental applications of Ni and Cu may enhance growth and production in this orchard via improved Ni and Cu nutrition and by mitigating any negative effects associated with excess N. Although Ni and Cu treatments reduced leaf N, kernel necrosis was unaffected by treatment. The cause of kernel necrosis remains unknown. The effects of Ni and/or Cu treatments on tree yield, nut quality, and tree growth were inconsistent.

ADVISER'S APPROVAL: Dr. Michael W. Smith