Nickel and Plant Disease

Bruce W. Wood and C. C. Reilly
Research Horticulturist and Research Plant Pathologist
USDA-Agricultural Research Service, Byron, Georgia

This article is a brief summary regarding nickel (Ni) and the present state of knowledge regarding Ni's roles in crop physiology and subsequent potential relevance to crop production enterprises. New Ni fertilizer products are appearing in the agrichemicals marketplace; as a result, the introduction of this new technology potentially triggers confusion among growers, consultants, and extension specialists as to the relevance, role and usage of Ni products. The present article reviews some of the USDA-ARS research documenting known primary roles of Ni directly affecting characteristics relevant to increasing the profitability of Ni requiring crops. Many of the examples come from pecan (*Carya illinoinensis*), as it is this crop that shows considerable Ni deficiency in field operations.

A careful reading of this article enables bridging of many knowledge gaps presently held by farmers, consultants, and extension specialist, and enables better understanding of the relevance of Ni in specific crop situations. It is important that farmers and extension specialists understand the key underlying principals driving management decisions; thus, enabling wise usage of resources and inputs while ensuring that environmental quality is not diminished.

Background. Nickel played a key and especially prominent role in the evolution and success of Earth life prior to about 0.6 billion years ago. This era was a time when the atmosphere was comprised predominately of reducing gases (e.g., ammonia, carbon monoxide, hydrogen, hydrogen sulfide, methane, etc). Primitive prokaryotic organs dominated this era, with Ni playing a critical role in the metabolism and success of these organisms under conditions of an electron-rich atmosphere. Beginning about 0.6 billion years ago, Earth's atmospheric composition tilted toward that of an oxidizing atmosphere, with the domination of dioxygen and carbon dioxide as atmospheric components. This change in the atmosphere was associated with an explosion in the evolution of complex life on Earth. While Ni remained essential for post-Cretacious Era life, the relative importance of its' role diminished. Metabolic functions previously satisfied by Ni, V, Ti, and Co were largely replaced by Zn, Fe, Mn, and Cu. However, not all metabolic roles for Ni in plant metabolism could be replaced by other trace elements—as it remains essential for one or more enzyme systems in all forms of complex life. Today, Ni is most aptly described as "the forgotten essential trace element"!

Nickel is an "*essential*" nutrient element for plants (Epstein and Bloom, 2005). Brown et al. (1987a, 1987b, 1990) discovered this fact, and it was validated by Wood et al., (2004c) with their discovered that pecan could not complete its life cycle without Ni. Ni initially received little attention by applied biologists because it was though that crops had such a low Ni requirement (1-10 ppb) that it would never show up in field or nursery settings. This was based on the fact that almost all soils contain enough Ni to more than

satisfy plant needs. Key points heretofore overlooked are that certain species have a relatively high Ni requirement (>1,000 ppb) and that soil and environmental conditions can limit the physiological availability of Ni to the crop.

Wood et al. (2004a) discovered that nickel (Ni) deficiency exists in certain real-world crop situations. Pecan appears to be the first crop in which field-level Ni deficiency is documented (Wood et al., 2004c). It has now been documented in potted river-birch trees (Ruter, 2005), and also occurs in several other plant species growing in specific field and nursery situations on a wide variety of soils or potting mixes. As an essential plant nutrient element, Ni is therefore expected to have a multitude of effects on plant metabolism and physiology that potentially impacts yields and profitability. Up until now, very little has been known regarding Ni nutrition in higher plants. Research on pecan has therefore expanded basic understanding of Ni for plants in general.

The relatively recent discovery that nickel (Ni) deficiency is a problem potentially having an adverse effect on yields from crops in fields, vineyards, yards and orchards (Wood et al. 2004c), naturally raises questions as to whether Ni nutrition is a profit-limiting factor in commercial operations, and whether long-term crop profitability can be increased by Ni usage. As is true for any new technology, its utility and value in specific situations is limited and often unknown.

Pathogens Interactions

The following bullet points are important to keep in mind when considering the interaction of Ni with pathogens.

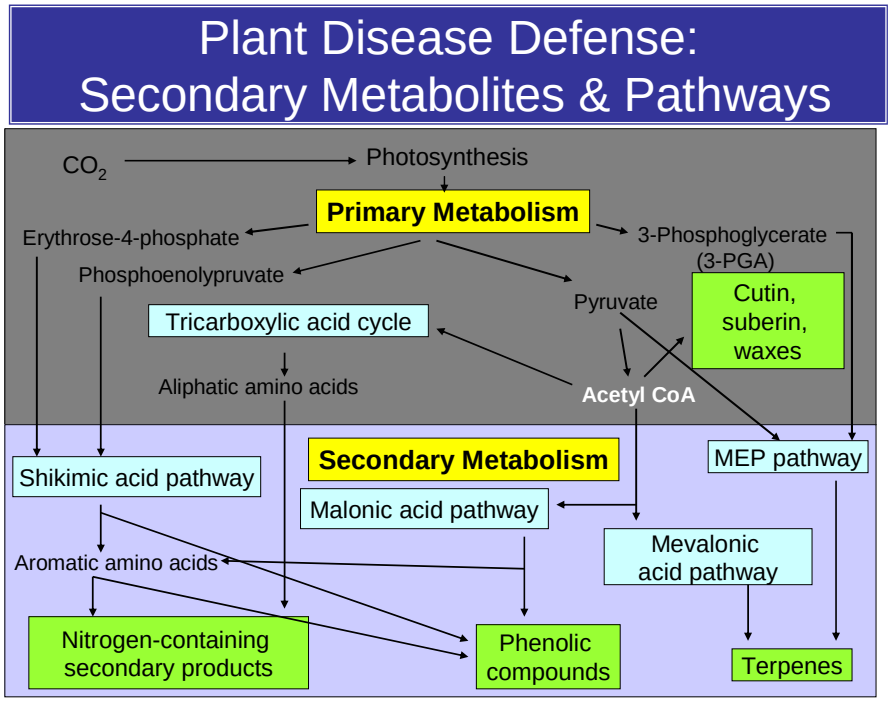
- Mineral element nutrition is one of the basic plant processes impaired by disease. (e.g., rootknot nematodes and Ni uptake!)
- Nutrient deficiency can predispose crops to infection that, once established, stress crops further by impairing nutrient acquisition and/or utilization.
- Resistance is the ability of host plants to limit penetration, development and/or reproduction of the invading pathogen, or limit the feeding of pests.
- Tolerance is the ability of the host plant to maintain its own growth despite infection or pest attack (e.g., ability of plant to “outgrow”, or compensate, effects of disease).
- Plants with optimal nutritional status possess the highest resistance to diseases.
- Susceptibility generally increases as nutritional status increasingly deviates from the optimum.
- Tolerance is lowered if plants suffer a mineral nutrient deficiency.
- Tolerance is increased by supplying the deficient nutrient.
- “Host plant resistance” is the main method of control for most diseases; thus, enhancement of disease resistance and/or tolerance is potentially a major component of control.
- An often overlooked aspect of disease control strategies is management to enhancement the natural resistance mechanisms already operating within the plant. One major factor affecting enhancement is <u>mineral nutrition status!!!</u>
- As “A Rule of Thumb”, the influence of mineral nutrition on plant resistance is relatively small in “highly susceptible” or “highly resistant” cultivars/varieties

and often substantial in "moderately susceptible" or "partially resistant" cultivars/varieties.

- The germination of conidia, or spores, on plant surfaces, and the development of the appressorium (i.e., a root-like structure) that penetrates host tissue, is *stimulated* by the presence of plant *exudates*. Thus, the *"flow of cellular exudates through walls and membranes"* greatly affects the success or failure of infection by most fungal disease pathogens. The flow of these exudates depends on cellular concentration (e.g., N, K, B, Cu…) and permeability of membranes (e.g., Ca, B,) and the corresponding diffusion gradient (e.g., evapotranspiration).
- The success of invasive fungi and bacteria also generally depends on the chemical *composition* of the apoplast (i.e., the solution existing between cells and in xylem vessels) and:
    1) Ions affecting ability of host enzymes to produce phytoalexins:
        - Chemicals that inhibit growth or kill pathogens
        - Boron and transition element metals (Fe, Mn, Cu, Ni, Zn) enables metabolism of phytoalexins

    2) Ions affecting formation of oxygen radicals (e.g., O2-, $OH^{\cdot}$) and hydrogen peroxide (H_2O_2)
        - Factors involved in hypersensitive reactions, and injury to pathogens
        - Transition metals (Fe, Mn, Cu, Ni, Mo, Zn) influence these radicals

    3) Ions affecting enzyme activity of pathogens:
        - Pectolytic enzymes excreted by the pest to dissolve the middle lamella is strongly inhibited by Ca^{2+}
            – Thus, there is often a high correlation between Ca^{2+} content of tissues and their resistance to pathogens.
- Nickel potentially affects disease resistance via any one of several ways, but is likely to be primarily via secondary metabolites affecting resistance. Impact is likely to be greatest in species that transport nitrogen as ureides (vs. amides or amino acids).
- The impact of Ni on disease resistance is usually greatest on moderately susceptible varieties/cultivars/genotypes. Thus, there is likely to be little impact on highly susceptible or highly resistant genotypes, unless Ni is especially limiting.
- The production of secondary matabolites plays a major role in the protection of plants from disease. While the production of "physical barrier" substances, such as cutin, suberin, and waxes, as a consequence of primary plant metabolism, plays an important role in protecting plants from pathogens, the production of metabolites via secondary metabolism also is critically important. Key secondary pathways are illustrated below. They produce general chemical products described as a) nitrogen-containing products, b) phenolics compounds, and c) terpenes. Nickel potentially affects both the quantitative and qualitative composition of each of these three categories of defense agents. The influence of Ni on the production of acetyl CoA is especially noteworthy, as acetyl CoA is important to the production of each of these categories of anti pathogenic agents.



Adapted from Taiz and Zeiger, Plant Physiology, third edition.

Figure. 1. Photoassimilated carbon can end up in one of four categories of products that plants use to protect against disease pests. The products of primary metabolism can end up as chemicals that function as physical barriers. Products from secondary metabolism can be shunted through any of three major pathways producing secondary chemical products that act to protect against pathogens. The availability of acetyl CoA is critical to the production of all four classes of protectants. Nickel deficiency potentially affects the synthesis of acetyl CoA via its synthase.

Non Pathogenic Disease

This section addresses specific cases where Ni deficiency occurs in orchard situations involving pecan trees. Similar responses might also occur in certain other crops and in certain situations.

1) ***Ni cures "mouse-ear" disease.*** Gammon and Sharpe, 1956, mistakenly concluded that mouse-ear (ME), an increasingly common and potentially devastating growth malady, is due to a manganese (Mn) deficiency, not realizing that it was likely the Ni impurities in low grade Mn-fertilizers that actually corrected ME. During the 1960-80's timely Mn application often cured ME trees; yet, the putative Mn cure became

increasingly ineffective over the last 2-3 decades as improved manufacturing produced low Ni contaminated Mn-fertilizers. A similar curative effect also occurred with copper (Cu), in that certain Cu products occasionally and partially cured ME; thus, there was emerging evidence that low Cu caused ME. Again, as with Mn, depending on the Ni composition of the scrap copper used to manufacture Cu products, Ni contaminated Cu sources, upon occasion, partially cured ME. The mistake in cause is perfectly understandable considering the relatively small amounts of Ni required to meet tree needs and the fact that Ni contaminated Mn or Cu fertilizers corrected the problem. A research effort to better understand the malady led to the discovery that Ni was the underlying cause. Scientific data demonstrating this is viewable in three HortScience papers (Wood et al., 2003, 2004a, 2004b, 2004c), a peer reviewed scientific journal readily available to state extension specialists.

Many different analytical grade, high purity, chemical forms of Ni were tested to rule out the possibility of there being an unknown active contaminating element within these Ni products.

Proper and timely application of Ni has now been shown to cure the common forms of ME (Figure 1), as well as the severe form of ME referred to as "orchard replant disease" (Figure 2). Timely Ni treatment of literally hundreds of symptomatic orchard trees has never failed to correct the Ni deficiency and associated morphological symptoms manifested in subsequent growth. It must be emphasized that correction of distorted shoot and foliar growth requires treatment during the early stages of growth, as Ni treatment will not restore already distorted organs to normal shape and size. As with any other micronutrient, it only corrects the shape of subsequent post-treatment growth.

Figure 1. Influence of foliar sprays of Ni on mouse-ear (ME) shoots from Ni deficient trees. The shoot on the right is Ni deficient, whereas the shoot on the left has been treated with a foliar spray of Ni.

Figure 2. Influence of foliar sprays of Ni on trees exhibiting "orchard replant malady". The left side of the tree was treated with a early season foliar spray of Ni, whereas the right side of the tree was left non-treated.

There is abundant evidence that the cause of ME is due to a short-term physiological deficiency of Ni in expanding organs and tissues during early spring. This deficiency usually links to excessive soil and tissue concentrations of metals that compete with Ni uptake by roots or by the movement of Ni across cellular membranes, or trigger inactivation of cellular Ni via cellular metabolic responses to excessively high levels of certain other metals. The metals found to be most associated with Ni deficiency are excessive Zn, Cu, Ca, Fe, or Mn. Soils abnormally high in any single one, or combination, of these can limit Ni uptake and/or physiological availability. Some of the published scientific data supporting this fact is viewable in HortScience (Wood et al., 2004b, 2004c), plus there is also unpublished data clearly showing this relationship with Cu and Zn. It is entire possible that certain forms of ME can also be caused by a deficiency of another metal other than Ni, but no evidence of this has been documented.

Mouse-ear type Ni deficiency often occurs in both soil and soil-less based nursery enterprises. Proper use of Ni on such seedlings quickly corrects ME and restores normal

growth. The authors have treated many hundreds of potted trees with ME symptoms, and Ni has never failed to restore normal growth if applied shortly after budbreak.

"Cold injury" can trigger mouse-ear (Ni deficiency) in tree tissues or organs. It appears to be the opinion by some that ME symptoms to foliage are due to cold injury of tree buds, leaves, shoots, etc... This is sometimes true on the surface of things, but is fundamentally mistaken when viewed at the physiological level. Observations, to date, clearly indicate that ME is upon occasion associated with both a) cold early spring temperatures, or b) premature and hard autumn freezes. In the case of the former, if early spring temperatures are cold, then there is occasionally "cold injury"; yet close visual inspection of literally hundreds of trees with ME has provided no evidence that typical ME problems are fundamentally due to cold injury to tree tissues or organs; although in certain cases the ME disorder is *triggered* by cold.

The not so obvious linkage between the occurrence of Ni deficiency and cold is such that Ni deficiency symptoms are often common in growing seasons where early spring temperatures are abnormally cool and soils are abnormally dry. The cool and/or dry soil conditions reduce root activity and uptake of mineral nutrient elements, thus, leading to a reduction in Ni availability to expanding foliage. Additionally, high soil availability of Ca, Mg, Mn, Cu, or Zn potentially reduces Ni availability in young expanding shoots and foliage. This means that at first glance, what might logically be interpreted as cold injury, is in reality abnormal tissue and organ (usually most obviously manifested in emerging leaves) growth due to a temporary Ni deficiency induced as canopies expand during early spring under an unbalanced nutrient element state caused by an interaction between cold temperatures and Ni uptake-adverse soil conditions. In the case of premature autumn freezes, feeder roots are often damaged or killed; thus, potentially impairing the tree's ability the following spring to absorb micronutrients such as Ni.

Ni uptake is reduced if spring soils are cool, dry, and/or are abnormally high in certain other metals. Under such circumstances, field studies have shown that a timely foliar spray of a 50-100 ppm Ni solution soon after the "Parachute-stage" (or "Sombrero-stage", as termed by some) of budbreak corrects the *cold triggered Ni deficiency*, whether it is due to late autumn or early spring cold. Note that much like Zn, Ni does not move far in treated organs, *thus good spray coverage is essential when using Ni to correct this malady.*

Again, it must be emphasized that foliar Ni application does not fully correct *existing* misshaped foliage, only *subsequent* foliage. In other words, Ni must be applied before there is a significant growth distortion, as it will not cure previously existing distortions. If all other factors are equal, then an early spring foliar Ni application corrects a cold triggered Ni problem and ensures renewal of normal growth in affected foliage and shoots. Thus, depending on severity, timely application of a Ni spray to affected trees is highly likely to prove beneficial for optimal canopy development. Improvement of canopy leaf area and functionality has been show by many researchers over the decades to be important to improving tree nut production and quality.

2) Ni cures the orchard replant disease in pecan and might influence similar maladies in other crops. The pecan replant malady has become increasingly common in 2nd generation orchards in the southeastern U.S. It shows up when young transplants are

established at locations in existing orchards or on a site where an orchard was removed and is replanted. Trees expressing this malady are associated with soils with very high levels of Zn or Cu, depending on the site. The accumulation of these metals over decades of use act to limit Ni availability and cause a severe form of ME, known as "orchard replant disease". The malady can be so severe that trees grow very slowly, display no laminar foliage, and eventually die. Timely early spring or late autumn application of Ni corrects this problem, enabling trees to recover and return to normal growth (Figure 2).

3) Ni potentially improves the health of crops attacked by rootknot nematode. Nematodes are ubiquitous in soils. Certain species damage roots of pecan trees. We have found that trees damaged by root knot nematodes can experience everything from mild to severe Ni deficiency expressed morphologically as mouse-ear (Nyczepir et al., 2005) (Table 1).

Table 1. Mouse-ear (ME) severity in open pollinated 'Desirable' pecan seedlings grown in field microplots and evaluated nine, 21, and 33 months after inoculation with *Meloidogyne partityla* (a rootknot nematode) or *Criconemoides xenoplax* (a ring nematode) alone and in combination (n=9)[z].

	Mouse-ear severity rating[y]		
Treatment	Apr. 2003	May 2004	May 2005
M. partityla (Mp)	8.3 a[x]	8.2 a	9.2 a
Mp + Cx	7.6 ab	5.2 c	7.4 a
Unpasteurized soil	5.9 bc	7.3 ab	6.9 a
C. xenoplax (Cx)	5.9 bc	6.7 abc	6.8 a
Untreated control	4.6 c	6.6 bc	6.4 a

[z]Initial population density of *Meloidogyne partityla* = 267 eggs/100 cm^3 soil, *Criconemoides xenoplax* = 267 juveniles and adults/100 cm^3 soil, and Mp + Cx = 267 Mp + 267 Cx/100 cm^3 soil. [y]ME severity was based on the following foliar symptom class scale: 1 = no Ni associated morphological distortions of leaflets or leaves (i.e., normal); 2 = 1% to 25% of leaflets on the seedling exhibiting Ni deficient morphological distortions (i.e., slightly blunted); 3 = 26% to 50% of leaflets exhibiting some degree of Ni associated morphological distort; 4 = >50% of leaflets exhibiting morphological distortion; 5 = #4, plus leaflet cupping; 6 = #5, plus necrosis of leaflet tips; 7 = #6, plus necrosis of leaflet margins, crinkled leaflets, and dwarfed leaflets; 8 = #7, plus dwarfed shoots; 9 = #8, plus rosetting; and 10 = #9, plus tree death.

The deficiency is due to nematode induced damage to feeder and next higher order roots (unpublished data). Data indicate that the greater the damage to the root system, the greater the degree of Ni deficiency (unpublished data). Thus, trees and orchards with a lot of rootknot nematodes, and possibly certain other nematodes as well, can experience hidden hunger type Ni deficiency. In such a case 1 or 2 early season Ni sprays to foliage improves tree health and thus potentially impacts long-term nut production and profits (unpublished data and observations).

It is very difficult to suppress rootknot nematode levels in affected orchards to levels that do not harm trees or vines, thus attention to early season Ni nutritional status of the crop potentially offers a means of reducing the negative impact of nematodes. It is also likely that a similar positive impact occurs as a result of early season foliar application of certain other micronutrients (e.g., Zn, Cu, and Fe) to nematode infested plants.

Field observations to date indicate that all rootknot infested pecan trees respond favorably to timely Ni sprays, but not all Ni deficient trees are infested with rootknot nematodes. Thus, nematodes can cause Ni deficiency, but not all Ni deficiency is due to nematodes.

Section summary. The above discussion presents background information pertaining to the occurrence of visible (i.e., morphological) forms of Ni deficiency in trees and orchards. It also identifies four distinct situations where timely foliar application of Ni during early spring improves tree health. These situations are therefore distinct circumstances where usage of Ni in orchards is likely to benefit orchard productivity. Thus, usage of Ni products for these specific problems on pecan most definitely possesses merit. The benefit of Ni in these situations is so clear cut and fundamental that there is no compelling scientific evidence that demonstrated benefits would not be realized in any symptomatic tree growing in any pecan orchard in the world.

Relevance to Crop Profitability

A fundamental goal in agricultural enterprises is to ensure that crop management strategies allow crops to grow as close to the *"physiological optimum"* as feasible. Feasibility involves ensuring that efforts to achieve this optimum result in a profitable long-term return on the investment. The *"most limiting"* factor limits crop productivity, regardless of the degree of management excellence. A large number of biological and non-biological factors potentially limit realization of optimal returns from invested resources and inputs. Crop mineral nutritional physiology is one such realm. For example, essential trace micronutrients, although relatively low in tissue concentration, often emerge as the most limiting factor. Timely correction of visible and invisible symptoms of deficiency increases crop health and productivity until limited by the next most limiting factor. This means that practitioners are most likely to enjoy maximum profitability from orchard enterprises managed with a mindset focused on identification

and profitable correction of the *most limiting* factor within the hierarchy of limiting factors. A problem with either *essential or beneficial* plant nutrient elements is that it is difficult to recognize deficiency symptoms severe enough to affect profits, but not severe enough to exhibit easily detectable symptoms.

This section briefly summarizes key points regarding the role of nickel (Ni), a potential *"limiting factor"* in certain aspects of internal crop processes affecting health, function, and potential economic profitability. These processes are associated with fundamental biochemistry and physiology. While these may appear to many readers to be a bit boring and overly technical, the following nevertheless provides practitioners with key background information needed to understand *"basic principals"* governing orchard management decisions regarding the usage of Ni in agricultural enterprises. These basic principals enable bridging of knowledge gaps affecting the practical relevance of Ni, and its associated technology, to "*individual"* crop situations; hence, enabling practitioners to understand *"why"* they might want to consider Ni in their orchard management strategy and better recognize *"when"* Ni is likely to be a profit limiting factor in orchard enterprises. The following is a brief summary of research documenting key roles, and modes of action, for Ni that potentially affect crop profitability.

1) Ni deficiency disrupts primary metabolism.

Research published in Plant Physiology and Molecular Biology documents the disruption of critical metabolic processes taking place within pecan trees during the early spring growth phase (Bai et al., 2006). This disruption occurs in at least four major biochemical pathways critical to shoots, foliage, and fruit. These processes are: the Ureide Pathway, the primary pathway for movement of nitrogen from roots to growing points; the Urea Cycle, a pathway involved in the incorporation of nitrogen into organic compounds required for growth; the Krebs Cycle (i.e., Citric Acid Cycle), a pathway involved in the conversion of sugars to organic energy needed for biosynthesis and the Shikimic Acid Pathway, a pathway for the biosynthesis of many critical organic molecules.

Organic Nitrogen pathways. The Ureide Pathway is critical for the conversion of storage forms of organic nitrogen (N) that perennial crops stockpile going into the dormant season. The plant must convert these stockpiles, during early spring, to ammonia or ammonium before stored organic N is integratable into critically important biochemicals that regulate functionality and structural characteristics of cell, tissues, and organs. Examples of affected key biochemical classes, depending on timely availability of organic N, include peptides, structural proteins, enzymatic proteins, and nucleic acids (i.e., both DNA and RNA).

Organic nitrogen and carbon. Research published in the Journal of the American Society of Horticultural Science (Bai et al., 2007a, 2007b, Bai et al., 2005) found that Ni deficient trees can exhibit major disruptions in the composition of nitrogen-containing ureides that pecan trees used to transport stored N and C through the xylem system to the growing parts of the tree during early spring. Additionally, the cycling of N through the Urea Cycle is disrupted via effects on urease and at least one other enzyme requiring Ni for enzymatic activity.

The bottom line is that Ni deficiency disrupts the activity of at least 3-4+ critical enzymes, in key biochemical pathways, that regulate cycling and usage of N by trees

during early spring; thus, depriving the expanding canopy, fruit, and other growing points of the proper organic forms of N required for normal physiology and growth. This means that Ni deficiency directly affects the ability of perennial crops to use stored organic N, as well as indirectly affecting the inorganic N taken up by roots from soils during early spring. Within this context, Ni deficiency greatly disrupts the N economy of perennial crops, especially during early spring when an expanding leaf canopy requires a great deal of organic N if trees are to fully realize their potential for maximum leaf area and functionality. This includes key processes like gathering of sunlight and usage of this energy to drive metabolic [e.g., fixation of C, N, sulfur (S), and phosphorus (P)] and growth processes regulating the profitability of crop enterprises.

Organic carbon. Much of the newly assimilated C, as well as stored organic C, passes through the Krebs Cycle (i.e., a critically important energy producing metabolic pathway) in order to produce key feedstock biochemicals necessary for specialized growth functions of the various plant organs. If the Krebs Cycle does not function, or does not function at optimal speed, then plant physiological processes are limited; thus, eventually affecting profit associated processes that either directly or indirectly limits returns. Bai et al. (2006) also discovered that Ni deficiency blocks the availability of Coenzyme-A, a key chemical needed to convert pyruvate, a key organic C compound, to citric and other key organic acids. Ni is a critical component of Coenzyme-A Synthase, the enzyme that produces Coenzyme-A, a compound critical to *many* downstream processes affecting tree performance and profitability. The absence of sufficient Coenzyme-A means that pyruvate coming out of a C cycle, called Glycolysis, cannot form Acetyl Coenzyme-A to feed the Krebs Cycle. This causes reduced energy production and a buildup of lactic acid. Lactic acid is what builds up in our muscles during exercising when oxygen supply is reduced during strenuous exercise. Excessive lactic acid is toxic to young expanding shoots and leaves; thus, Ni ensures that toxic acids do not accumulate and that organic C forms are available for thousands of plant needs.

Recall from above that Ni deficiency greatly alters the concentrations of certain organic nitrogen (N) and organic acids in rising xylem sap and in young expanding foliage just after bud break (Bai et al., 2007). Pecan trees transport reduced (i.e., energized) N around in chemical forms termed ureides, amino acids and amides. Much of the stored and fixed carbon (C) must eventually pass into the Citric Acid (i.e., Krebs) Cycle before it is available to support downstream energy and C requiring processes within the tree.

The adverse influence of Ni deficiency on early season C and N usage potentially affects early season growing points such as leaves, fruit, cambial zones, roots, and new buds where there lies a great deal of metabolic activity and subsequently demand for these N and C resources.

It is reported that plant roots can directly uptake urea from soil solutions without waiting for the urea to convert to nitrate or nitrite (i.e., lower energy forms of N). Urea is a common form of N-fertilizer used in the pecan industry, often applied during early spring. If this root uptake of urea occurs for pecan, then Ni nutrition during the early stages of spring growth is very important from the standpoint of enabling the pecan trees to break down the urea to the ammonia and ammonium ions needed for producing amino acids and their incorporation into structural and enzymatic proteins, and also into critically important nucleic acids. It has been observed by the authors and others that

excessive foliar application of urea to trees can sometime result in urea toxicity at growing points. This means that urease, a Ni activated metalloenzyme, was insufficiently active for breaking down the surge of urea at growing points before it reached toxic levels in cells and tissues. Thus, usage of Ni can potentially improve urease activity, and therefore improve tree N nutrition.

2) Ni nutrition and disruption of secondary metabolism. Ni deficiency also disrupts "secondary" metabolic processes in plants. Secondary metabolites are used for plant defense against pests. These metabolites are comprised of three chemically distinct groups: terpenes, phenolics, and nitrogen-containing compounds. Nickel affects all three groups via any one of a multitude of primary or secondary intermediates that are required for production of these plant-protecting compounds. The terpenes are affected via the Melavonic Acid Pathway; phenolics via the Shikimic Acid Pathway; and nitrogenous compounds via several pathways. Thus, Ni associated disruptions to timely availability of these secondary biochemical products potentially affects resistance to certain disease pests. For example, certain rust diseases (Reilly et al., 2005; Mishra and Kar, 1974; Wood and Reilly, 2007) and pecan scab disease (unpublished data) are potentially influenced.

Nickel's indirect influence on the Shikimic Acid Pathway also has important implications in that the pathway influences production of certain plant hormones, integrity of structural components, and products influencing disease resistance. For example, impaired pathway activity adversely affects formation of lignin, phenolics, tannins, quinines, and similar compounds that indirectly affect tree health, performance, and orchard productivity. A classic example is wood strength. The abnormally brittle and easily broken limbs of trees with severe Ni deficiency (Wood et al., 2004a) is a manifestation of a Ni associated series of physiological processes that influence the amount of lignin laid down in shoots and branches to strengthen cell walls and limb structure. Thus, improving Ni nutrition can in at least certain cases, strengthen tree structure through its effect on lignification of wood. Possible benefits therefore include less limb breakage.

3) Ni is important to certain key enzymes. There are at least 9 enzymes proven to require Ni ions at the reactive site in the enzyme molecule if it they are to perform their function in plants (Wood and Reilly, 2007). There are also several additional enzymes requiring Ni as a structural component; thus, Ni deficiency adversely affects the production and/or functionality of many enzymes. A thorough search will undoubtedly identify many more.

4) Ni is tightly bound to cell walls. The unique physical characteristics of double positive charged Ni++ ions is similar to that of double positive charged copper (Cu++) ions in that both Ni and Cu ions taken up by roots during early spring binds to the strong negative charged binding surfaces of newly formed cell walls in the regenerating xylem cells of trunks, limbs, branches, and shoots. The literature indicates that Ni++ ions are more strongly held to charged surfaces than almost any other first period transition series element. This means that for a short period of time after growth begins in early spring, the availability of both Cu++ and Ni++ to growing points at tops of plants (i.e., new

canopy and fruit) is subject to limitation via adsorption to new cell walls. Availability is subject to further limitation if the relative concentration of certain competing metals are abnormally high in cells and in intercellular solutions; or soil conditions do not favor good Ni uptake by roots (i.e., soils are cool and dry during early spring; or are abnormally high in Ca, Mg, Fe, Mn, Zn, or Cu; or are substantially alkaline).

5) Temporary limitations in physiological availability of Ni. Two factors limiting early season availability of Ni to expanding perennial crop organs and tissues are a) timely transport across cell walls and membranes, and b) inactivation, or binding, of Ni to cellular peptides.

Movement across cell walls and membranes. In order for Ni to be available for critical cellular processes it must first get to the sites of action by moving through cell walls and across intracellular membranes within cells. This transport involves ion channels that move Mn, Co, Cu, Zn, and Ni (and to a certain degree, Fe). These specific channels are not element specific, thus the relative abundance of these ions in the solution at the channel entrance determines the relative amounts of each of these ions moving across the transport channels. This competition among ion of these different elements cause competitive inhibition among these elements. Thus, if zinc (Zn) ion concentration in the intracellular or extracellular sap solution is excessive, then Zn out competes the Ni ions for an opportunity to move across the cell wall or membranes. This results in relatively few Ni ions getting to metabolic sites where they are needed. This is one reason why Ni deficiency shows up most severely on soils with excessively high Zn or Cu. It is important to understand that it is not only relative concentrations of individual metal ions that is important, but also the total concentration of these ions to that of Ni. Therefore, a soil showing soil test results with 2 or more of the elements of Mn, Co, Cu, and Zn being "high", with none being "very high", the "total or combined" concentration of these elements relative to Ni can cause Ni deficiency in the tree, even if Ni is high in the soil.

Inactivation of Ni within cells. Excessively high levels of certain metals, such as Zn, and of phosphorus (P) within cells results in the cell being triggered to produce a special type of peptide that binds with Zn (and Fe, Mn, Co, Cu, and Ni). This binding results with these metals being removed from being available for meeting their required roles in cellular metabolism. This is why excessive leaf P levels can cause a Zn deficiency. A leaf analysis will show that there is plenty of leaf Zn, but in reality Zn is tied up in physiologically unavailable forms—with leaves subsequently showing Zn chlorosis, etc. The same is potentially true for Ni and Cu. It remains to be shown, but it may be that in certain cases excessive early spring application of foliar Zn may actually reduce the physiological availability of Ni and Cu. This is only one of several reasons why an early spring application of Ni to developing foliage is probably important to many orchard situations.

Section summary. The above notes a few of the many ways in which Ni affects physiological processes that ultimately influence tree health and orchard profitability. Space does not permit a detailed review of Ni's importance and roles. It should be abundantly clear from this brief review that Ni deficiency is especially relevant to internal tree processes involving nitrogen and carbon, which means that there is potential for direct and indirect effects on a multitude of physiological processes that in turn affect

growth, development, yields, and profits. This orientation provides growers and extension specialists with sufficient background information to understand basic principles underlying Ni usage. This understanding enables practitioners to sufficiently grasp how Ni affects plant processes influencing a wide variety of horticultural traits impacting orchard profitability.

We emphasize that just because Ni is essential for optimal tree health and performance, it does not necessarily mean that Ni is biologically limiting to all pecan trees or economically limiting to all orchard enterprises. The challenge facing practitioners and extension specialists is to understand site characteristics and basic physiological processes well enough to recognize when Ni is likely to be profit limiting. As with any other nutrient element or pest control or cultural practice, this involves good information, experience, and judgment. The latter characteristic, "judgment", involves making calculated risk assessments, a task that is essentially visceral for successful long-term farming.

The second section of this article documents four specific cases where Ni deficiency clearly affects crop health and production. The third section documents a) how Ni affects a multitude of key processes potentially influencing productivity and profits, b) provides a basis for understanding how Ni potentially affects a wide variety of horticultural traits, and c) identifies how Ni deficiency can potentially be a limiting factor in productivity and profitability. The next section documents cases in which Ni's usage improves horticultural traits affecting orchard profitability of pecan; thus, identifying actual cases where Ni is potentially a limiting factor to profitability.

Impact on Horticultural Characteristics

This section presents a few examples derived from orchard-based experimentation that provides evidence that nickel (Ni) potentially affects horticultural and disease management factors impacting profitability of pecan production enterprises. These data are evidence that Ni possesses potential for benefiting production enterprises in subtle ways distinct from the obvious benefits pertaining to mouse-ear, an orchard replant malady, cold triggered mouse-ear and Ni deficiency, and rootknot nematode induced Ni deficiency. Subtle influences of micronutrients on tree physiology are not unique for Ni, but occur for all essential micronutrients. Our previous article in this Ni series addressed metabolic and physiological roles, and how Ni potentially influences pecan tree health and functionality. This article presents examples of how Ni appears to have influenced biochemical and physiological processes to affect horticultural traits relevant to orchard profitability.

1) Ni sprays increased fruit-set via reducing June-drop. The importance of Ni for conversion of stored organic forms of nitrogen (N) during the period of leaflet and canopy expansion possibly accounts for the observation that early spring Ni sprays can, under certain circumstance, influence June-drop of 'Desirable' pecan. Data from a tightly controlled and replicated study shows that three pre-pollination Ni sprays reduced June-drop of 'Desirable' cv. by about 6% at a $\geq$ 95% level of confidence (Table 2). Retained fruit persisted until ripening. This means that there is a $\geq$ 95% probability that

this Ni effect on fruit retention is real for this particular experimental situation. A similar Ni associated increase in fruit retention in 'Desirable' orchards was also observed in a second Ni study (by Dr. Lenny Wells; University of Georgia Pecan Extension Specialist); thus supporting the tree Ni nutrition potentially affects fruit-set of 'Desirable', and possibly other cultivars. Although the magnitude of the Ni-associated increase in fruit-set is small, it potentially results in a "current-season" increase in yield. Whether such an increase in fruit-set is ultimately good or bad for profits depends on the orchard situation.

Table 2. Influence of pre-pollination foliar sprays of nickel on fruit retention of 'Desirable'. Nutrient elements were applied via foliar sprays with a first application in April prior to flowering, a second application in early May just prior to female flower maturity, and a third application in mid May after flower maturity. Fruit-set is based on fruit remaining in clusters by late June. Trees were routinely treated with pesticides to prevent fruit-drop due to nut casebearer and other insects. Statistical differences are based at $a = 0.05$ (i.e., 95% probability that the treatments are indeed different). Means within a treatment are different if followed by different letters. Separation is based on ANOVA. Experimental design consisted of 5 Blocks (i.e., trees), with drop data from 25 fruiting clusters per tree. The treated and non-treated treatments are on opposite major limbs within the same tree to overcome tree-to-tree variability in crop-load and fruit-drop.

Spring nutrient sprays	% retention in treated fruit	% retention in non-treated fruit
Ni	69a	63b
Zn	40b	48a
Ni + Zn	53a	52a

It is unlikely that this apparent Ni induced "nut-retention effect" occurs in all orchards and all varieties, but data to date indicates that it likely occurs in certain 'Desirable' orchards. It is noteworthy that these orchards were devoid of visible Ni deficiency symptoms, such as mouse-ear. These results indicate that in certain cases, timely improvement in tree and fruit Ni nutrition can increase fruit-set. While three applications of Ni are used in the above-described study, it is not know whether a single application might have been just as effective as three applications.

The apparent positive influence of Ni on fruit-set is consistent with the fact that rapidly growing fruit require access to considerable amounts of organic nitrogen and Coenzyme-A for normal growth and metabolism—and Ni deficiency obviously affects metabolic processes affecting timely availability of these chemicals. Additionally, young fruit are poorly connected to the parent tree via xylem connections; meaning that young fruit may potentially be Ni deficient, as Ni transport in the phloem component of the trees vascular system appears to be little or none during early spring. A multitude of unique site conditions also potentially limits the physiological availability of Ni during the spring growth period.

Practitioners should note that it is highly unlikely that foliar spraying of trees in early spring with Ni will ensure increase fruit-set in all orchards. Such an effect is likely to occur only in orchards where tree growth processes are temporarily Ni limited. Common Ni limiting causes are excessive soil or tissue levels of Zn, Cu, Co, Mn, Fe, Ca, Mg or P; unusually cold and/or dry soil during early spring; roots infested with rootknot (and possibly other species) nematodes; or soils that are excessively alkaline in pH. We are further testing this apparent effect of Ni on fruit-set.

2) Ni can potentially influence water-stage-fruit-split (WSFS). WSFS is a major problem for growers of certain varieties in certain years. We have shown that tree water status several days prior to, and during, late water-stage of fruit development greatly influences the incidence of WSFS (Wood and Reilly, 1999). Fruits split when the turgor pressure generated by the liquid endosperm within the developing fruit exceeds the capacity of the seed coat, or shell, to contain that pressure without tissues rupturing. The capacity of the immature shell to contain this pressure is partly regulated by the degree of shell lignification. Lignin is analogous to a biological glue that plants use to strengthen and provide rigidity to their structures. The timely activity of key enzymes regulates lignin biosynthesis. The timely physiological availability of certain essential micronutrients influences the activity of these enzymes. Copper, as well as other metals, is especially key to lignin formation; yet, Ni is also indirectly involved via its influence on the activity of CoA-synthase, and probably other down-stream lignin associated enzymes.

A 2006 field study on 'Oconee', a variety often exhibiting considerable WSFS in trees with heavy crop loads, found that trees receiving early spring Ni sprays exhibited a slight reduction in WSFS during late August. The percent reduction was not great, with WSFS being only 79% that of the untreated check (i.e., a 21% reduction in split) (Table 3). Treatment means differed at a 99.5% level of confidence; meaning that there is less than a 1% probability that this difference occurred by chance rather than due to a Ni. The study utilized 16 blocks of trees, carefully matched for equivalent trunk diameters and crop loads. The study is being repeated this year to determine if the result was real or a rare statistical anomaly (a 1 in 200 chance of there being a difference when in fact there was none). It is noteworthy that the amount of WSFS exhibited by trees is tightly linked to crop load, water stress, and boron and potassium nutrition; hence, an assessment of treatment effects on WSFS must be made using trees nearly identical in these characteristics.

Table 3. Influence of foliar sprays of nickel on water-stage-fruit-split (WSFS) of 'Oconee' pecan. Trees received three treatments at 1 pint/acre (i.e., 100 gallons) at two weeks intervals beginning in mid April. Trees were blocked based on size of crop load. The different letters following the treatment means reflects statistical significance at a = 0.005 (i.e., 99.5% probability that the treatments are indeed different) via ANOVA testing.

Nickel	34b
Treatment	**Percentage of split fruit in mid August**
Untreated check	43a

The apparent beneficial effect of early season foliar Ni sprays on reducing losses caused by WSFS is consistent with the fact that Ni nutrition potentially influences lignin metabolism within the immature shell of the developing fruit. Additional circumstantial evidence supporting this is that severely Ni deficient trees (i.e., severe mouse-ear trees) typically possess brittle shoots, branches, and limbs (Wood et al., 2004a). It is highly probable that a Ni-linked interference in wood lignification is the root cause; although, lignin levels in Ni deficient tissues has not been quantified. This is why it is likely that a potential side effect of improving tree Ni nutrition is improved lignin biosynthesis and deposition; thus, potentially providing stronger shoots, branches, limbs, and shells.

Nickel usage will not prevent WSFS, no more than protecting against water stress during the fruit-split sensitive phase will prevent WSFS; however, Ni appears to be one of many interacting factors that potentially influence WSFS and good tree Ni nutrition will certainly contribute to the likelihood of less crop loss due to WSFS. This means that a slight reduction in the frequency of WSFS, in at least certain cases, is likely a beneficial side effect arising from a Ni application during early spring budbreak.

The ultimate value of Ni sprays to farmers as a tool to prevent WSFS is likely to be minimal; nevertheless, evidence to date indicates that it likely impacts the amount of loss to WSFS in certain situations. Orchards most likely to benefit from improved Ni nutrition with regard to WSFS or limb strength are those where Ni is limiting during early spring. These soil-tree circumstances are above-described under the effect of Ni on fruit-drop and in the second section of the article, and will be expanded upon in the following section. It is highly unlikely that all orchards, regardless of situation, will benefit from Ni sprays to increase lignification and associated strength of organs; however, evidence to date indicates that there are situations where there would be benefit.

3) Ni impacting pecan scab disease. Previously reported data indicates that Ni potentially affects leaf susceptibility to pecan scab disease. Plant Ni nutritional status and Ni exposure can affect disease by certain fungal disease organism (Wood and Reilly, 2007). This is why The International Nickel Company developed a research project and database on the use of Ni compounds as fungicides (Anonymous, 1964), and why a major agrichemicals company patented the use of Ni for disease control during the 1950's with the intent of using Ni as a fungicide, or as a fungicidal ingredient.

The authors conducted a preliminary greenhouse study of the effect of Ni on pecan scab. Results clearly demonstrated a degree of activity against the pecan scab disease on pecan. A large follow up field study confirms that Ni can, to some degree, suppress nut scab in orchard situations. Dry spring weather has, to date, not enabled field research to progress to the point to allow for a thorough evaluation of whether, or how, Ni can play a significant role in scab disease control. However, research data clearly indicates that scab disease on young nuts can potentially be impacted by Ni (Table 4).

Table 4. Influence of foliar sprays of nickel on pecan scab severity of developing fruit and on nuts per pound. "Farm Treatment Fungicide" and "Farm Treatment Fungicide + Ni" was applied twice, early June and mid July. Spray treatments were applied at 100 gallons per acre. Nickel was 2 pints (Nickel Plus) per acre and applied concurrently with the fungicide sprays. The different letters following the treatment means reflects statistical significance at $a = 0.02$ (i.e., 98% probability that the treatments are indeed different) via ANOVA testing.

Scab control treatment	Nut scab severity rating (% coverage)	Nuts per pound
Farm Treatment Fungicide Check	29.8a	60a
Farm Treatment Fungicide + Nickel	26.3b	57b

One large field study involving 'Desirable' trees in 32 blocks of trees within several commercial orchards across Georgia, and involved thousands of individual measurements. Trees were either treated with "Farm Treatment Fungicides + Nickel" (FTF+Ni) or "Farm Treatment Fungicides" (FTF) alone. Developing fruit on trees treated with "FTF+Ni" had fruit covered with pecan scab at 31% whereas those treated with "FTF" had 38% disease coverage of the fruit. Therefore, the inclusion of Ni resulted in only 82% as much scab on developing fruit. This reduction is not great; nevertheless, it is in all probability real as statistical analysis indicates a 99.5% level of confidence that the Ni associated difference is real. Additionally, analysis also indicated a 97.7% probability that Ni usage resulted in fewer nuts per pound (i.e., larger and/or better-filled nuts; 57 vs. 60 nuts per pound), and a 98.8% probability of improved kernel filling. These results support conclusions drawn in the 1950-'60's by agrichemical companies that Ni can indeed suppress certain plant diseases (Anonymous, 1964).

In the case of pecan, data clearly indicate that Ni possesses a degree of efficacy against pecan scab disease on developing fruit and can subsequently influence crop yields. Thus, Ni potentially affects pecan scab disease on nuts when used with standard fungicides. Whether the impact is enough to warrant Ni usage as an additive to fungicides for scab disease management is not clear; however, there is considerable evidence that early season Ni usage confers a side-effect that potentially reduces losses to pecan scab disease.

At present, it is unknown if the mode of action is directly on the scab organism, or is indirect via effects on tree physiology and associated scab resistance. Circumstantial evidence indicates that impact is via both a direct role on the organism and an indirect role via the ability of the foliage or fruit of trees to resist the disease, presumably via enhanced production of secondary metabolites that influence either infection or fungal growth. This is not surprising when one recalls that Ni plays a major role in the production of many of the biochemicals linked to secondary plant metabolites, and the qualitative and quantitative aspects of these metabolites influence pest resistance.

The value of integrating Ni as an active component of pecan pest management remains to be determined; however, there is substantial data and evidence that Ni could indeed play a major role. Evidence to date indicates that many pecan trees likely experience a transitory hidden-hunger type Ni deficiency during the first few weeks after budbreak, when new tissues are most susceptible to scab infection. This likely accounts

for part of the reason why Ni has demonstrated a degree of efficacy against scab. This provides evidence that an early season foliar Ni supplement is likely to confer positive benefits regarding certain diseases, especially that of pecan scab. We have previously observed that Ni sprays control rust disease on daylily (Reilly et al., 2005). The practice is rapidly becoming the primary means for control in yard and commercial daylily plantings. Similar benefits also occur on rice.

The bottom line is that Ni alone will not control pecan scab when applied at reasonable concentrations, but there is field data and considerable evidence that timely Ni application can potentially reduce the severity of fruit scab. Nickel effects on leaf and fruit scab need further investigation. The apparent relatively high Ni requirement of pecan trees, compared to that of many other crop species, is suggestive that Ni likely performs a relatively unique role in pecan and other ureide-N transporting crop species (Wood et al., 2006; Bai et al., 2006, 2007a, 2007b). Such a role is not apparent in amide-N or amino acid-N transporting crop species possessing very low Ni requirements.

Section summary. We have described above four specific cases where Ni deficiency affects tree health and orchard production (mouse-ear, a type of orchard replant malady, cold induced Ni deficiency, and rootknot nematode induced Ni deficiency). We have also addressed known situations where Ni affects critical metabolic and physiological processes that potentially influence crop health and profitability. The above section presents data and information linking Ni usage to four horticultural traits potentially affecting profitability of pecan production enterprises. These traits are June-drop, water-stage-fruit-split, structural strength of woody organs and developing shells, and pecan scab disease. Similar traits might be influenced in other crop species. An understanding of the "basic principals" for Ni, as discussed above, provides a basis for understanding how improving Ni nutrition might bring about many beneficial effects. Exactly how Ni, as an essential plant nutrient element, is best utilized in commercial crop production enterprises remains unknown; yet, the documented research noted in these Ni section clearly indicate that transitory Ni deficiencies exist; thus, preventing certain crops from performing at their physiological optimum. Ni usage potentially provides subtle, yet important, biological and economic benefits for many crop enterprises. The next section provides background information and guidelines pertaining to management of Ni nutrition.

Usage Guidelines

The previous sections briefly summarized known and likely influences of nickel (Ni) in certain Ni sensitive crops. As Ni is an essential nutrient element for plants, farmers, consultants, and extension specialists need to understand *when* Ni is likely limiting and *how* Ni technology is used when need arises. The following are general guidelines pertaining to Ni usage. These guidelines may change as additional data, crop type, and acquisition of experience.

1) Leaf Ni concentrations and visual deficiency symptoms. The concentration of Ni in spring foliage of pecan trees, and its relationship to *"visible"* Ni deficiency symptoms is such that leaf levels below roughly 0.85 ppm dw (dry weight) trigger

symptoms (Nyzcepir et al., 2006). The lower plant Ni concentrations drop below this threshold for expression of visible deficiency symptoms, the more severe the disruption of tree metabolism, physiology, growth, and development. We have measured foliar Ni concentrations as low as ≈ 0.001 ppm dw in severely deficient tissues of pecan.

Unpublished data from recent greenhouse and field studies on pecan indicate that this *"visible symptom threshold"* varies substantially, depending on the concentration of certain other elements (e.g., Zn and Cu; and probably Fe, Mn, and Co and P). This means that it is not possible to establish a *specific* leaf concentration threshold that triggers development of visual deficiency symptoms, but the 0.85 ppm concentration appears to be a reasonably approximation for most pecan orchard situations.

2) Leaf Ni concentration and hidden hunger. A previous section briefly reviewed evidence implying the existence of *"hidden hunger"* for Ni in certain situations. The following is background information about hidden hunger.

Concept of "hidden hunger". Many practitioners typically consider a nutrient element to be deficient if the crop exhibit visual deficiency symptoms. These symptoms usually encompass color changes, tissue death, or change in growth or morphology of a plant organ. For example, typical nutrient associated visible symptoms of foliage is usually either a) chlorosis (yellowing), b) necrosis (dead zones), c) abnormal growth (usually stunted growth), or d) anthocyanin accumulation (usually reddening). The presence of these visual symptoms usually means that irreparable damage has occurred to cells, tissues, and organs; thus, adding the nutrient only corrects future physiology and growth, and does not compensate for disruption of *past* growth and development.

In theory, deficiency thresholds involve establishment of a *"low critical"* and a *"high critical"* concentration tailored to specific crop species. The *"critical concentration"* is that concentration below which the relevant element brings about a suppression of yield. Because of the great difficulty involved in determining this concentration, *"range of sufficiency"* is used to characterize pecan nutrient element nutrition; and is sometime referred to as the *"luxury range"*. This range *varies* according to stage of plant growth, relative concentration of other elements, genotype or variety, and environmental conditions. This range of sufficiency is not absolute.

"Hidden hunger" occurs when the crop needs more of a particular nutrient, but does not show *visual* deficiency symptoms (Figure 4). *Invisible* deficiency symptoms can include biochemical, metabolic, and physiological symptoms. In the case of hidden hunger the nutrient element is below what is needed for optimum yield or a "*physiological optimum"* (Figure 4), but above that concentration triggering visible symptoms (see curves "A" and "B" of Figure 4). This means that a positive response comes from addition of the element (i.e., yield, quality, production, health, color, pest resistance, etc.) even though visible symptoms are absent. The measurement and characterization of hidden hunger is challenging, especially as practitioners increasingly employ excellent crop management and pursue higher yield goals. One way of identifying hidden hunger issues is to monitor carefully various plant responses after supplementing the nutrient in question. The concentration range of hidden hunger is typically very narrow for most of the essential micronutrients (e.g., Zn and Mn are 1-2 ppm dw (Figure 4, "A"), but B is an exception at 20-40 ppm dw (Figure 4, "B"); thus,

only a small concentration range in the micronutrient can lead to a transition from visible deficiency to sufficiency and the physiological optimum (Figure 4, "A").

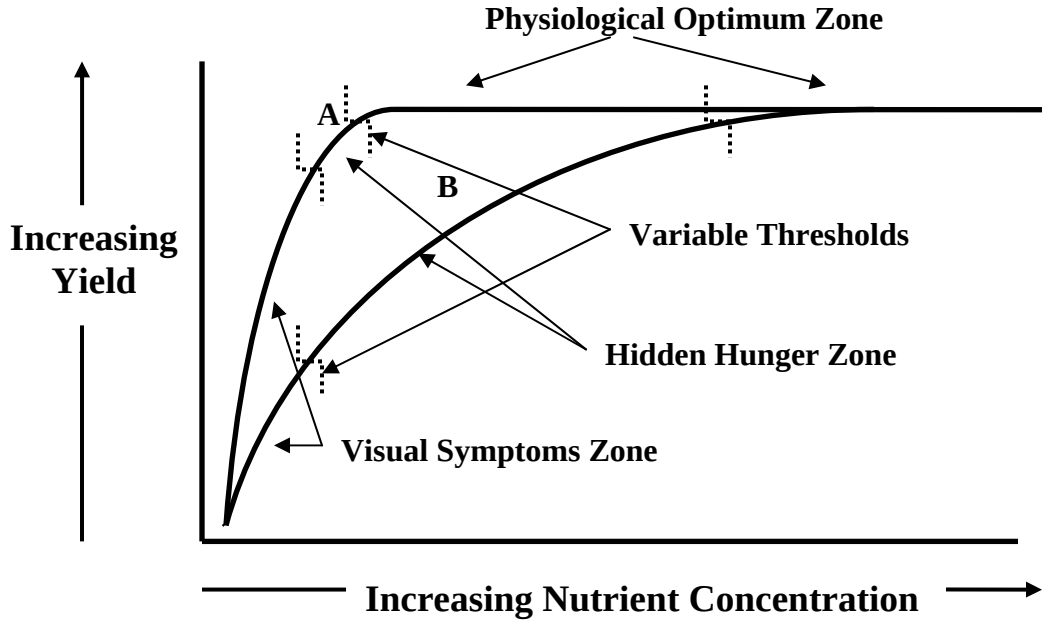


Figure 4. *Hidden hunger* occurs when the tree shows no obvious symptoms, yet nutrient content is insufficient for top profitable yield. Hidden hunger concentration range is difficult to determine and varies according to relative levels of other elements, variety, and environmental conditions. **"A"** refers to a narrow hidden hunger concentration range, usually 1-2 ppm dw (e.g., zinc), whereas **"B"** refers to a relatively wide concentration range of several ppm dw (e.g., boron).

An earlier section of this article presents cases where Ni deficiency is obvious (i.e., visual symptoms). Other sections present cases where Ni deficiency is not obvious (i.e., no visual symptoms), and therefore relate to the "hidden hunger" aspects of crop management. The more growers attempt to push for higher yield goals, the greater the potential for hidden hunger problems for Ni and other nutrient elements. It is presently unknown whether the nature of hidden hunger in crops, such as pecan, for Ni is more like "Curve A" (1-2 ppm range) or more like "Curve B" (several ppm range) (Figure 4).

Leaf Ni concentration and hidden hunger. July and August leaf analyses typically reveal Ni concentrations of several ppm in foliage absent of *visible* Ni deficiency symptoms; thus, far exceeding the 0.85 ppm dw threshold that roughly reflects the visible deficiency threshold concentration (Nyczepir et al., 2006; and unpublished data). This is also often true for slight or moderate Ni deficient trees. However, mid-

summer leaf analysis is potentially misleading for Ni, and possibly other metals (e.g., Cu), in that values do not necessarily reflect what was present in young expanding tissues and organs during the early season growth phase.

Evidence noted earlier indicates a likelihood that a short-term Ni deficiency occurs during early spring as new tissues and organs grow. Recall that Ni deficiency can cause substantial metabolic and physiological disruptions, as well as obvious morphologically apparent damage. Much of this physiological damage is *not correctable* once certain tissue or organ growth stages are reached; thus, foliage needs to be treated *before* deficiency arises. Stated in another way, regardless of whether it is a hidden hunger issue or the presence of severe deficiency with visible symptoms, evidence to date indicates that crop organs and tissues or high Ni requiring crops are probably Ni deficient within the context of "visible symptoms" for a period of less than roughly 20-40 days after growth begins in early spring. The <20-40 day period is most likely representative of the most severe cases of Ni deficiency. It is possible that the period of hidden hunger is considerably longer in pecan orchards, considering that Ni sprays appear to sometime influence June-drop, water-stage-fruit-split, and number of nuts per kilogram—all being growth processes taking place later into the growing season.

If there is indeed an early spring Ni deficiency occurring in developing foliage at a critical stage of development, there appears to be substantial potentially for permanent adverse influences on growth processes, such as leaflet area and impaired metabolism, just when key metabolic processes (especially those requiring organic reduced N) are determining the future photoassimilation capabilities of the leaflets and their future contribution to crop energy demands. This disruption of key aspects of primary and secondary metabolism has been demonstrated in a journal paper published (Bai, et al., 2006) in Plant Physiology. Additionally, Wood et al., (2004a) showed that the rate of net photosynthesis of foliage from Ni deficient trees (mouse-ear trees) can be considerably less than in foliage from trees not showing morphological symptoms of Ni deficiency (Wood et al., 2004a).

Hidden hunger types of micronutrient deficiency are usually difficult to recognize and quantify. For example, visible boron (B) deficiency in most higher plants is not apparent until tissue B concentrations are roughly 1 ppm dw, whereas hidden hunger type deficiency is thought to occur at levels ranging from roughly 1 to 20-40 ppm in most plant species. This concentration range for B hidden hunger is probably *much greater* than is the range for Ni, or other micronutrients, in pecan; yet, it illustrates what appears to be occurring in regards to the early season nutritional physiology of Ni. Field observations based on enhanced vegetative vigor of "early spring Ni treated" and "untreated" branches on the same trees (trees not showing Ni deficiency symptoms) is strong evidence of hidden hunger type Ni deficiency existing in at least certain orchards. It is unknown if this response occurs in all non-symptomatic orchards, but it is likely to do so in orchards with low foliar Ni concentrations during early spring.

3) Leaf Ni concentration and the range of sufficiency. The lower and upper thresholds for the "range of sufficiency" are presently unknown for crops and associated organs. Depending on concentration of certain other plant nutrient elements, these two thresholds vary. These observations are consistent with the Diagnosis and Recommendation Integrated System (DRIS) approach to plant analysis where the relative

balance of nutrients are taken into account when interpreting plant analysis reports. Within the context of most pecan orchard situations where mouse-ear symptoms do not appear, it appears that the lower threshold is ≈ 3 ppm dw whereas the high threshold is >15 ppm (but < ≈ 50 ppm). The range of sufficiency for Ni in pecan foliage is tentatively though to be a leaf Ni concentration of 3-15 ppm.

4) Potential impact of Ni on energy reserves of perennial crops. It is a physiological principal for plants that if carbon (C) assimilation (i.e., fixation, photoassimilation, photosynthesis) from the atmosphere is impaired due to a reduction in the leaf's ability to convert sunlight to chemical energy, then photoassimilation of nitrogen (N), sulfur (S) and phosphorus (P) are also likely impaired. The reduction, and associated energizing, of the four key non-metals are linked to net photosynthesis and associated photoassimilation processes. The energizing of C, S, N and P in plants requires tremendous amounts of chemical energy.

Deficiencies in Ni indirectly affects the rate of C fixation (i.e., production of carbohydrate based energy reserves), and by metabolic linkage, fixation of key energy carrying non-metals (i.e., N, S and P) (Wood et al. 2004a; Bai et al., 2006, 2007a, 2007b). Impairment of energized forms of these key non-metals has obvious potential for negatively impacting a multitude of metabolic and physiological processes that ultimately affect nut yields, return cropping, and orchard profitability. If one understands these linkages, then it becomes apparent that anything diminishing a plant's photoassimilation ability potentially impacts its ability to fix highly energized forms of the "Big Four" non-metals that are so critical for allowing plants to function at their physiological optimum and attain optimum productivity. This is true for any photoassimilation limiting nutrient element, not just Ni. It is within this context that it is highly probable that crops with sub-physiologically optimum levels of Ni will biologically benefit from Ni application. This benefit will likely include improved photoassimilation; thus, such plants will optimally respond to management inputs needed to ensure profitability.

5) Will crops, in general, benefit from spraying Ni on a regular basis? As with any new horticultural or pest management tool or strategy, there are unknowns about impact, utility, value, relevance, and profitability. The fiscal and human resources available for research has always been meager; yet, today it is, for most crops and many countries, only a small fraction of that available 30 years ago. The burden of assessing the *specific* relevance of new technology therefore lies with competent growers and extension specialists.

The only way to know if something works in any particular situation is to give it a *fair* test against a valid control, ensuring that significant confounding or interacting factors are controlled. The test must then be sufficiently replication in space and time to enable either a common-sense, or mathematical, assessment. However, due to the specific subtle nature of the potential benefits of Ni usage, effects on profitability of perennial crops may not be apparent until after 2-4 years of continued Ni usage coupled with careful observation.

It is presently unknown whether Ni application confers biological or economic benefit in perennial crops devoid of *morphological* deficiency symptoms (i.e., obvious visual mouse-ear)—i.e., those orchards showing a hidden hunger type of symptom. This

testing is underway, but will require at least 2-3 more years before firm conclusions are drawn. Ni needs to be properly tested against proper controls, and with substantial replication, within individual orchard settings across the pecan-belt. Even then, just as with any other macro- or micro-nutrient, conclusively proving that usage increases profits, unless studied over several years, and an untreated check and lots of replication is included, and potentially confounding and interacting factors are held constant, is theoretically problematic. Even with such rigorous testing, statistical analysis is incapable of yielding a 100% probability.

6) Usage of Ni. We want to emphasize that *Ni should probably not be sprayed, via multiple applications, on a regular basis.* The notion that commercial pecan orchards should receive multiple Ni applications during the early part of the growing season for correction of deficiency, similar to that presently done for Zn, is currently without a sound scientific. Such a practice would undoubtedly lead to nutritionally adverse side-effects after many decades of usage, much like those now appearing in certain old pecan orchards because of long-term Zn application. There is evidence that Zn application is excessive in many orchard enterprises. It is clear that excessive application of Zn has likely been a major cause of an increase in incidence and severity of Ni deficiency (mouse-ear) in southeastern U.S. orchards (Wood et al., 2004c, 2006), as trees exhibiting severe Ni deficiency are often tightly linked to high soil Zn and orchard sites where Zn has accumulated over decades of use. There is circumstantial evidence that excessive accumulation of soil Zn is also affecting early season Cu nutrition of trees.

There is no compelling evidence that *all* pecan orchards experience a short-term transitory Ni deficiency, nor is it suggested that the *profitability* of *all* Ni deficient orchards will increase because of Ni sprays. However, it is clear that many orchard operations are likely to be improved by wise usage of Ni. Within the context of foliar canopy development, there is no evidence that crops benefit biologically from more than two Ni sprays per growing season (i.e., early spring). An exception is severe Ni deficiency (usually observed in 2nd generation orchards) where a pre-leaf-fall autumn application was shown to be a good approach to preventing the reoccurrence of Ni deficiency in the following spring's foliage. However, within the context of effects on pecan scab disease, number of nuts per pound, and apparent effects on water-stage-fruit-split, there is evidence that mid-season application might potentially confer benefits capable of impacting orchard profitability. There is therefore need for more research on the merits of mid-season usage of Ni.

Foliar application of Ni should generally be at, or shortly after, the "parachute" stage of budbreak when young foliage is completing the unfolding step and are preparing for the rapid growth phase. If a second application is applied, then it should generally be applied about two to three weeks after the first. Nickel can be tank-mixed with other foliar micronutrient sprays (such as Zn, Cu, Mn, and Fe) and appears to be fully compatible with the vast majority of fungicides and insecticides. Rates should not exceed labeled rates. As with Zn, and other micronutrient metals, it is important to ensure that canopies are well covered by sprays, as Ni does not translocated far from the site of deposition. The timing of Ni application is important with regards to correction of visible deficiency symptoms (e.g., mouse-ear), as spraying does not restore deformed foliage to a normal shape, but only that of expanding foliage.

7) What about Ni toxicity? Pecan is a Ni loving species, possessing a relatively high Ni requirement (Wood et al., 2006, Bai et al., 2007), this being about 10-1000 fold greater than that of many non ureide-N transporting species. Observations to date indicate that pecan can withstand an endogenous foliar Ni concentration of roughly 60-70 ppm dw before visible toxicity symptoms appear. There are highly productive commercial pecan orchards with Ni concentrations of approximately 30 ppm dw (based on July leaf analysis). The absolute concentration of Ni that will cause toxicity substantially varies with relative concentrations of other elements, variety, and site conditions. Observations to data indicate that foliar sprays of pecan foliage with solutions exceeding 400 ppm Ni can potential harm young leaves. This threshold obviously varies with tissue, organ, variety, other elements, and soil conditions. To date, Ni foliar sprays $\leq$ 200 ppm Ni have not been observed to injure foliage or fruit. Sensitive species, such as tomato, can be injured with Ni sprays of roughly $\geq$ 50 ppm. Thus, because crops vary in their sensitivity to Ni, caution must be exercised to ensure against over exposure.

8) What about soil application of Ni? Almost all soils have plenty of Ni. Additionally, application of Ni to soils results with strong binding to soil particles and poor movement through the soil profile. The problem with Ni deficiency is not usually that soil content is a problem, but that there are soil chemistry issues that limit timely Ni uptake by roots. It is for this reason that foliar application of Ni is by far the preferred approach to meeting tree Ni needs.

9) What about Ni usage for organic certified operations? It was recently demonstrated that a certain species of a Ni hyperaccumulating plant can be used as an organic Ni fertilizer (Wood et al., 2006). An *Alyssum* species accumulates Ni to a level exceeding 3% dry weight while simultaneously removing Ni from Ni contaminated soils. This is such a high internal concentration of Ni that the plant's biomass can be harvested as hay, ashed, and smelted into Ni ingots. The farming of *Alyssum* potentially enables extraction and production of several pounds of Ni per acre. The relevance of this technology to pecan is such that it potentially provides "organic" pecan enterprises with a compatible Ni fertilizer.

10) What situations merit Ni usage? Generalizations are always dangerous as there are always plenty of exceptions. Within this context, one or two early spring foliar applications of Ni are likely to benefit plantings of woody perennials that 1) exhibit mouse-ear, 2) exhibit orchard replant malady, 3) have moderate to severe rootknot nematodes on roots, 4) have cold damage triggered mouse-ear, 5) are intensively managed to realize maximum yield goals, 6) are old, 7) are on soils with very high levels of either Fe, Mn, Zn, Cu, P, Ca, or Mg; or combination thereof, 8) are on soils with very low cation exchange capacities, 9) are on soils with substantially alkaline pH, 10) are on soils that are dry and/or cool at budbreak and the first few days thereafter, 11) receive large amounts of nitrogen fertilizer (especially urea), especially around budbreak, 12) have brittle limbs or shoots, 13) have excessive June-drop of fruit, 14) have a lot of water-stage-fruit-split of fruit, 15) have a lot of fungal disease, 16) have abnormally poor

seed, nut or kernel yields considering that other major stresses are properly managed, 17) are heavily root pruned, or 18) foliage with high concentrations of Fe, Mn, Cu, Zn, Co, or P; or combination thereof. While any one of these circumstances can trigger a need for Ni in certain extreme circumstances, the more of these "18" factors that are relevant to a specific crop situation, the greater the likelihood that proper usage of Ni will prove profitable. Lest one get the impression that Ni is a magic cure-all, it is important to recognize that many of the above circumstances equally apply to several other macro- and micronutrients.

Section summary. Failure to eliminate the *"most limiting"* factor places upper limits on the physiological optimum, yield optimum, or optimum profitability of commercial enterprises, regardless of how well crops are otherwise managed. The degree of success arising from expenditures of large sums of money and resources to raise yield goals and increase profitability are often regulated by subtle physiological stresses. A 1-2 ppm dw concentration deficit in an essential micronutrient at a critical physiological or growth juncture is one example of how a "small thing" frustrates realization of business goals. Ni, as well as any of the other micronutrient elements, functions in such a role in certain specific crop production situations. Just how widespread is the role of Ni as a profit limiting stressor remains for further research and experience, but the likelihood of Ni being the "most limiting" stressor greatly increases as operations are managed to optimize yields.

This section provides basic guidelines regarding use of Ni in crop enterprises. Careful application of these guidelines, taken within the context of the previous three sections, gives practitioners an understanding of *how* Ni potentially affects profitability and *whether* its use is likely to be relevant to their particular enterprise. Basic principals and tentative thresholds are identified, thus providing practitioners with a basis for Ni management. Additional research and experience will undoubtedly alter or refine usage guidelines and strategies.

The above discussion of Ni nutrition illustrates how pathological and non-pathological diseases might be affected by Ni. As for specific diseases, crop resistance is most likely to be enhanced if the crop genotype already possesses a degree of natural resistance to the pest. There is accumulating evidence that there exist considerably more Ni deficiency in agricultural/horticultural crops throughout the world than previously suspected. Most of this appears to be in ureide-N transporting species, and is manifested as a hidden hunger during the early stages of crop growth.

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